INTERVENTIONAL STRATEGIES FOR ENHANCING QUALITY OF LIFE AND HEALTH SPAN IN OLDER ADULTS

EDITED BY: Mario Bernardo-Filho, Michael George Bemben, Taiar Redha, Borja Sañudo, Trentham Furness and Brian C. Clark

PUBLISHED IN: Frontiers in Aging Neuroscience, Frontiers in Physiology and Frontiers in Medicine

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88966-141-1
DOI 10.3389/978-2-88966-141-1

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

INTERVENTIONAL STRATEGIES FOR ENHANCING QUALITY OF LIFE AND HEALTH SPAN IN OLDER ADULTS

Topic Editors:
Mario Bernardo-Filho, Rio de Janeiro State University, Brazil
Michael George Bemben, University of Oklahoma, United States
Taiar Redha, Université de Reims Champagne-Ardenne, France
Borja Sañudo, Sevilla University, Spain
Trentham Furness, Australian Catholic University, Australia
Brian C. Clark, Ohio University, United States

Citation: Bernardo-Filho, M., Bemben, M. G., Redha, T., Sañudo, B., Furness, T., Clark, B. C., eds. (2020). Interventional Strategies for Enhancing Quality of Life and Health Span in Older Adults. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88966-141-1

Table of Contents

frontiers in Aging Neuroscience

EDITORIAL
published: 11 September 2020
doi: 10.3389/fnagi.2020.00253

Editorial: Interventional Strategies for Enhancing Quality of Life and Health Span in Older Adults

Mario Bernardo-Filho[1]*, *Michael G. Bemben*[2], *Redha Taiar*[3], *Borja Sañudo*[4], *Trentham Furness*[5] and *Brian C. Clark*[6]

[1] Departamento de Biofísica e Biometria and Policlínica Piquet Carneiro, Rio de Janeiro State University, Rio de Janeiro, Brazil, [2] Department of Health and Exercise Science, University of Oklahoma, Norman, OK, United States, [3] Groupe de Recherche en Science pour l'Ingénieur (GRESPI), Université de Reims Champagne-Ardenne, Reims, France, [4] Departamento de Educación Física Y Deporte, Universidad de Sevilla, Seville, Spain, [5] Faculty of Health Sciences, Australian Catholic University, Sydney, NSW, Australia, [6] Department of Biomedical Sciences, Ohio Musculoskeletal and Neurological Institute, Ohio University, Athens, OH, United States

Keywords: older adults, interventional strategies, quality of life, health span, neuroscience of aging

Editorial on the Research Topic

Interventional Strategies for Enhancing Quality of Life and Health Span in Older Adults

Across our lifespan a wide range of factors can influence human health and well-being. Normal aging is associated with the accumulation of deleterious and undesirable changes, often resulting in an increased risk of co-morbid diseases and premature mortality. Geriatric syndromes, including frailty, sarcopenia, and cognitive impairment, are increasing worldwide and there is a growing need for cost-effective geriatric assessment and management strategies that can be implemented in a large number of settings. Technologies are playing an important role for the identification of certain geriatric syndromes, especially for healthcare professionals in the primary care setting. For example, rapid and feasible tools (e.g., mobile applications) such as the Rapid Geriatric Assessment (RGA) are now widely used for this purpose (Merchant et al.). While the development of pharmacological interventions have resulted in major advances in the management of numerous age-related conditions, there is still an urgent need to offer and develop simple and effective non-pharmacological interventions with known limited side effects to robustly improve quality of life (QoL) and independence in older adults. In particular, increased focus on interventional strategies that enhance both physical and cognitive function in the elderly are critically needed. It was historically believed that the neural substrates that determined motor and cognitive function were discrete; however, more recent conceptual frameworks, such as the concepts such as the "neural reuse theory" suggests it is quite common for neural circuits established for one purpose (e.g., movement) to be exploited, recycled, and/or redeployed during evolution or normal development and be put to different uses (e.g., cognition), often without losing their original functions (Anderson, 2007, 2010). Thus, the neural reuse theory offers an interesting perspective on the degree of localization of cognitive function, and suggest that portions of the brain that are involved in motor function are also involved in cognitive function (Anderson, 2007, 2010).

Physical exercise-based strategies have been established for the improvement of detrimental changes associated with aging, even in animal models (Macit et al.). Unfortunately, most older adults do not exercise regularly (Tavoian et al.) and some of them are reluctant to even initiate a program of increased physical activity; therefore, other strategies need be used to prevent the functional decline in the elderly (Valenzuela et al., 2019). Different exercise programs including

OPEN ACCESS

Edited and reviewed by:
Thomas Wisniewski,
New York University, United States

***Correspondence:**
Mario Bernardo-Filho
bernardofilhom@gmail.com

Received: *14 July 2020*
Accepted: *23 July 2020*
Published: *11 September 2020*

Citation:
Bernardo-Filho M, Bemben MG, Taiar R, Sañudo B, Furness T and Clark BC (2020) Editorial: Interventional Strategies for Enhancing Quality of Life and Health Span in Older Adults. Front. Aging Neurosci. 12:253. doi: 10.3389/fnagi.2020.00253

aerobic training (even at high intensities), strength training or mixed methods, have been suggested in order to improve both cardiorespiratory and muscle function in older adults (Tavoian et al.).

The functional decline described above can affect the ability to perform activities of daily living in older adults (Malmstrom et al., 2016; Sañudo et al., 2020) as a result of deterioration in the sensory systems (i.e., vestibular, visual, somatosensory), neurocognitive networks/system, and the musculoskeletal system. Consequently, many older adults are at risk of falling during daily life. One study in this issue (Dunsky) highlighted different exercise-based programs used in fall prevention. These programs challenge the sensory, cognitive, and musculoskeletal systems while addressing balance constraints, such as orientation in space, changes in direction, and the speed or height of the center of mass during static and dynamic situations resembling ADL. The author suggested that among older adults, programs that include a combination of dual-task, function-oriented challenges while controlling balance stimulates the sensory and neuromuscular control mechanisms, and have been found to improve static and dynamic stability, as well as a number of aspects in QoL (Dunsky). In another study, evidence was presented that traditional dance programs have the potential to improve the physical fitness and wellbeing of the elderly (Douka et al.). In this study, a 32 week dance intervention (2 times per week, for 75 min per session) using different intensities, was reported to improve physical fitness (e.g., handgrip strength, chair stand, and indices of flexibility) and static balance. Other articles in this issue: (1) assessed the effects of moderate-intensity treadmill aerobic training (8 weeks) on redox status and inflammatory biomarkers and motor performance in rats with knee osteoarthritis, and noted that motor performance in all joint function tests was improved and the inflammatory biomarkers were reduced with exercise (Martins et al.); and (2) evaluated the effects of adding whole body vibration to squat training on muscle strength and the plasma levels of brain-derived neurotrophic factor (BDNF) in elderly woman with knee osteoarthritis (Simão et al.). Here, improvements in lower limb muscle performance associated with neuromuscular adaptations (BDNF plasma levels) were observed. Lastly, one article described the protocol for an ongoing randomized control trial investigating the comparative effectiveness for three different pragmatic exercise intervention approaches (high-intensity interval cycle training vs. moderate, continues cycle training vs. progressive resistance exercise) to improve a comprehensive battery of physical fitness parameters (e.g., maximal oxygen consumption, muscular power and strength, 6-min walk distance, fatigue resistance) and body composition (e.g., fat mass and lean mass) (Tavoian et al.).

On the other hand, *cognitive training* was also reported to successfully promote healthy aging (Alnajjar et al.). Recent studies suggest that computer-based cognitive interventions could be effective at administering cognitive training for older adults (Alnajjar et al.). In the same line, Weng et al. explored the effects of cognitive training on working memory in older adults with mild cognitive impairment. The authors reported that the training effects on working memory could also be transferred to other untrained areas (such as executive function). Moreover, similar cognitive training strategies were also tested to improve car driving skills of older adults (Nouchi et al.). After only 6 weeks of training, cognitive functions such as processing speed, inhibition, and vigor–activity mood, were enhanced in healthy older people.

The acquisition and retention of motor skills is necessary for everyday functioning in the elderly and may be critical in the context of motor rehabilitation. Studies indicate that motor training closely followed by sleep may result in better engagement of procedural ("how to") memory consolidation processes in the elderly. Intriguing findings from Gal et al. suggest that evening (6–9 p.m.) training (multi-session motor practice program over 10 sessions across 3–4 weeks) enhances motor skill learning in older adults. The results are in line with the notion that motor training preceding a sleep interval may be better consolidated into long-term memory in the elderly, and thus result in lower forgetting rates (Gal et al.).

Other alternative strategies, such as acupuncture, were also reported to improve brain health, with both neural and vascular mechanisms being described (Sun et al.). Other newer strategies, such as exploratory behavior and responsiveness to novelty were reported to play an important role in maintaining cognitive function in older adults. In this study, Behforuzi et al. investigated the short-term test-retest reliability of event-related potential (ERP) and behavioral responses to novel stimuli in cognitively normal older adults. Their findings suggested that older adults may have a characteristic way of processing novelty that appears resistant to transient changes in their environment or internal states, which can be indexed during a single testing session. The establishment of reliable measures of novelty processing will allow investigators to determine whether proposed interventions have an impact on this important aspect of behavior (Behforuzi et al.).

In summary, in this special issue a broad range of non-pharmacologic, and relatively simple and pragmatically implementable approaches are presented that aim to increase life expectancy, promote health and independence, and/or improve QoL for the elderly via interventional strategies targeting, in part, the nervous system.

AUTHOR CONTRIBUTIONS

MB-F, BS and TF prepared the first version. All authors listed have made a substantial with direct and intellectual contribution to the final version that was approved for publication. In addition, MB and BC reviewed the English language.

FUNDING

BC's effort was supported, in part, by a grant from the National Institutes of Health (R01AG044424). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. MB-F had support from Brazilian Agencies (CNPq and FAPERJ).

REFERENCES

Anderson, M. L. (2007). Evolution of cognitive function via redeployment of brain areas. *Neuroscientist* 13, 13–21. doi: 10.1177/1073858406294706

Anderson, M. L. (2010). Neural reuse: a fundamental organizational principle of the brain. *Behav. Brain Sci.* 33, 245–266; discussion 266–313. doi: 10.1017/S0140525X10000853

Malmstrom, T. K., Miller, D. K., Simonsick, E. M., Ferrucci, L., Morley, J. E. (2016). SARC-F: a symptom score to predict persons with sarcopenia at risk for poor functional outcomes. *J. Cachexia. Sarcopenia Muscle* 7, 28–36. doi: 10.1002/jcsm.12048

Sañudo, B., de Hoyo, M., McVeigh, J. G. (2020). Improved muscle strength, muscle power, and physical function after flywheel resistance training in healthy older adults. *J. Strength Cond. Res.* doi: 10.1519/JSC.0000000000003428. [Epub ahead of print].

Valenzuela, P. L., Castillo-García, A., Morales, J. S., Izquierdo, M., Serra-Rexach, J. A., Santos-Lozano, A., et al. (2019). Physical exercise in the oldest old. *Compr. Physiol.* 9, 1281–1304. doi: 10.1002/cphy.c190002

Conflict of Interest: In the past 5-years, BC has received research funding from NMD Pharma, Regeneron Pharmaceuticals, Astellas Pharma Global Development, Inc., and RTI Health Solutions for contracted studies that involved aging and muscle related research. In the past 5-years, BC has received consulting fees from Regeneron Pharmaceuticals, Zev industries, and the Gerson Lehrman Group for consultation specific to age-related muscle weakness. BC is a co-founder with equity of AEIOU Scientific.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

ORIGINAL RESEARCH
published: 05 April 2019
doi: 10.3389/fnagi.2019.00075

Traditional Dance Improves the Physical Fitness and Well-Being of the Elderly

Styliani Douka[1], *Vasiliki I. Zilidou*[1,2]*, *Olympia Lilou*[1] *and Vasiliki Manou*[3]

[1]School of PE and Sport Science, Department of Physical Activity and Recreation, Aristotle University of Thessaloniki, Thessaloniki, Greece, [2]Laboratory of Medical Physics, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece, [3]School of PE and Sport Science, Department of Human Performance, Aristotle University of Thessaloniki, Thessaloniki, Greece

Regular physical activity is considered one of the most important factors for lifestyle, for maintaining good health in older ages and increasing life expectancy. Dance is considered an activity that involves coordinating movements with music, as well as brain activation because it is constantly necessary to learn and remember new steps. Dance as a musical-kinetics skill, requires the coordination of body movements with rhythmic stimuli, developing the adaptability of the movement. One-hundred-thirty (130) elderly people aged 60 years and over (mean age 67 years old) with an average of 8 years of education, attended Greek traditional dance sessions for 32 weeks. The frequency was 2 times per week, for 75 min per session. Dances were selected from all over Greece with moderate intensity initially. During the program, they had the opportunity to try with greater intensity dances. At the beginning and after the end of intervention all the participants were evaluated by the Fullerton Senior Fitness Test for their physical fitness, the Single Leg Balance and the Handgrip Strength Test. The results showed a significant improvement in their physical fitness (Chair Stand: $T = -5.459$, $p < 0.001$; Arm Curl: $T = -5.750$, $p < 0.001$; Back Scratch: $T = -4.648$, $p < 0.001$; Sit and Reach: $T = -4.759$, $p < 0.001$; 2 min Step: $T = -5.567$, $p < 0.001$; Foot Up and Go: $T = -8.599$, $p < 0.001$) and at their static balance with eyes open (Balance 1 leg: $T = -4.996$, $p < 0.001$) and Handgrip Strength (Handgrip: $T = -3.490$, $p < 0.001$). Elderly seem to enjoy dancing as an activity while maintaining their functionality. Probably the elderly in traditional dance cause prosperity in their lives by promoting active aging.

Keywords: greek traditional dance, elderly, physical health, physical function, well-being

OPEN ACCESS

Edited by:
Mario Bernardo-Filho,
Rio de Janeiro State University, Brazil

Reviewed by:
Ramesh Kandimalla,
Texas Tech University Health Sciences Center, United States
Danúbia Da Cunha De Sá Caputo,
Rio de Janeiro State University, Brazil

***Correspondence:**
Vasiliki I. Zilidou
vickyzilidou@gmail.com

Received: *01 September 2018*
Accepted: *14 March 2019*
Published: *05 April 2019*

Citation:
Douka S, Zilidou VI, Lilou O and Manou V (2019) Traditional Dance Improves the Physical Fitness and Well-Being of the Elderly. Front. Aging Neurosci. 11:75. doi: 10.3389/fnagi.2019.00075

INTRODUCTION

The percentage of people aged 60 and over is growing faster worldwide than any other age group, and the resulting aging population presents challenges and opportunities for all countries increased due to new social and economic demands. Countries adapting to this changing demographic, invest in healthy aging to enable people to live longer and have a healthy life. Healthy Ageing involves creating an environment that allows people to engage actively throughout their lives. Both the elderly and the environments in which they live

are diverse, dynamic, changing and playing an important role in determining the physical and mental ability throughout a person's life. In interaction with each other, they possess incredible possibilities to allow or limit healthy aging (World Health Organisation, 2018).

The advanced age besides changes in physical fitness, increases sensitivity to chronic diseases and disabilities, and reduces the quality of life (Wanderley et al., 2015). In many studies it has been shown that the combination of exercise with nutrition is considered effective intervention for elderly people. Improving or maintaining their nutritional status combined with exercise is associated with many benefits, including increased physical fitness and strength, reducing the incidence of sarcopenia, reducing functional loss and rehabilitation of musculoskeletal injuries, reducing the risk of falls and/or their frequency. Also, improving gait and balance, their quality of life and mortality and morbidity of diseases by 30% of all causes (Weening-Dijksterhuis et al., 2011; Cadore et al., 2013). The satisfaction of life is observed as a basic characteristic of well-being (Fugl-Meyer et al., 2002), constitutes a provision for physical health (Dominick et al., 2002) and has gradually entered a more central in healthcare systems (Fugl-Meyer et al., 2002; Daig et al., 2009).

Regular physical activity is considered one of the most important factors for lifestyle, maintaining good health in older ages and increasing life expectancy (Lee et al., 2012). In surveys with elderly people, it seems that physical exercise may have beneficial effects on cognitive (Lautenschlager et al., 2008) and the physical functions (Villareal et al., 2011). In addition, physical activity in the elderly is associated with increased survival (Manini et al., 2006; Stessman et al., 2009). In another study it seems that physical activity when done regularly, delaying the reduction of functional abilities associated with aging and sometimes reverses the loss and morbidity (Nied and Fraklin, 2002). The activities proposed for the elderly should lead to the improvement or maintenance of physical and mental health (Stathi et al., 2004).

Alternative categories of exercise programs have been performed in elderly people (Hui et al., 2009; Sofianidis et al., 2009). Dance of any type is being used for many years as a treatment modality. Dance involves elderly people and increases their motivation (Lima and Vieira, 2007). Furthermore, it has been shown in investigations that elderly people are excited when participating in dance programs, thus improving the quality of life, balance and mobility (Song et al., 2004; Federici et al., 2005).

Dance is considered an activity that offers the involvement of different senses and connects movement to music with self-expression and applies different aspects of personality (Kaufmann, 2011; Studer-Lüthi and Züger, 2012). Music, which is an important component of dance, improves physical performance. It's easier going to start moving, walking, dancing or to deal with any kind of exercise if some people choose their favorite music. Reduces fatigue and increases the levels of psychological stimulation during exercise (Jing and Xudong, 2008). Kattenstroth et al. (2011) showed that elderly people dancing on a regular basis have better balance, postural stability, flexibility and physical reaction time. Alpert et al. (2009) showed progressive balance enhancement in the sensory organization test (SOT) in their study for 13 women aged 52–88 years performing jazz dance that lasted 15 weeks. Also, Hui et al. (2009) shown that after 24 sessions for 52 adults aged 68 on average and trained in low-impact aerobic dancing, the dancers had improved their dynamic balance in Time Up-and-Go test, but not their static balance. Moreover, dance has been proposed as an actual promising program for the development and improvement of balance and to prevent falls in the elderly people (American Geriatrics Society, British Geriatrics Society, and American Academy of Orthopaedic Surgeons Panel on Falls Prevention, 2001; Judge, 2003).

Aweto et al. (2012) investigated the effects of dance programs on patients with hypertension with specific cardiac vascular conditions. Hackney and Bennett (2014), attempted to evaluate Parkinson's disease impact on dance participation as these patients had mainly mobility problems, with an increased risk of falls, reducing their quality of life as a result. Tsimaras et al. (2012) investigated the impact of a Greek dance program in 13 adults with hearing problems in their aerobic capacity and muscle tone. After 12 weeks of dance program observed significant progress in physiological peak parameters such as oxygen consumption and exhaustion time. Other research with Greek traditional dance, performed on people with breast cancer, showed improvement in their physical functioning, satisfaction with their lives as well as reducing depression symptoms (Kaltsatou et al., 2011).

The purpose of this study was to investigate the impact of a Greek traditional dances program on elderly people over the age of 60. Particularly, to investigate whether Greek traditional dance as a form of aerobic exercise, could improve the functional capacity and the well-being of the elderly people.

MATERIALS AND METHODS

Participants

One-hundred-thirty (130) Greek elderly people aged 60 years and over (mean age 67 years old) with an average span of education 8 years (Q2 = 8, IQR = 6), attended Greek traditional dance sessions for 32 weeks. The frequency of the intervention was twice a week, and each session lasted 75 min. The intervention was performed at the Greek Association of Alzheimer Disease and Relative Disorders (Alzheimer Hellas) and at the Day Care Centers of Municipality of Thessaloniki, Greece. During the program, they had the opportunity to try with greater intensity dances. In the period of 32 weeks, the maximum number of sessions to complete the intervention was 64 and the average presence of the participants was 51 sessions for completing the intervention.

Moreover, we recruited a control group (20 individuals) that it is matched to a smaller group of intervention participants (20 individuals) in demographic and baseline somatometric data. The control group did not receive any type of training and thus it was a waiting group.

For their participation in the research, they had to be in a good functional and emotional condition and to not participate in another dance program. Each of them was required to be examined by a doctor to ensure their participation in a mild intensity activity. In addition, elderly who have been diagnosed with hypertension, cardiac and respiratory failure were excluded from the intervention program. Also, the elderly who did not complete at least 80% of the total attendance hours of the program was rejected by this.

Written consent was requested for their participation in this study, after being given the required explanations for its purpose. At the beginning and after the end of intervention all the participants were evaluated for their fitness and functional capacity by a fitness instructor. Ethical and Scientific Committee of GAARD approved the protocol of this study. Also, the participants signed their acceptance of viewing videos and posting photos related to the traditional dance intervention program in online and/or print media for scientific purposes and public information.

Outcome Measures

Physical Assessments

All participants were evaluated both at the beginning of the intervention and after the end of this to investigate any improvement caused by the intervention. They were evaluated on their physical fitness and functional capacity by the Senior Fitness Fullerton Test. This test is safe, it does not require to use any special equipment and is used to assess six parameters such as strength, flexibility, coordination and endurance. Specifically, the test battery consists of: 30-s chair stand, arm curl, chair sit-and-reach, back scratch, 2-min step-in-place, and 8-foot (2.44-m) up and go (Rikli and Jones, 1999). Furthermore, they were evaluated on their static balance by the Stork Balance test (Johnson and Nelson, 1969).

Handgrip strength was measured using a hydraulic hand dynamometer holding the dynamometer in the dominant hand (Saehan Corp., Masan, Korea). Three trials from the dominant hand were calculated and used the best for the analysis. Handgrip strength is expressed in kilograms (kg). Furthermore, the jumping vertical ability was evaluated by free hand countermovement jump using OptoJump system (Microgate, Bolzano, Italy) and the jumping was calculated in centimeters. Lastly, the body mass index (BMI) was calculated following the measurement weight and height of each participant.

Greek Traditional Dances

Dances were selected for the intervention in the present study were from all over Greece (**Figure 1**) and ranked in three categories: mild, moderate and high intensity. Further classification of the dances was carried out in relation to the complexity and the number of steps, the position of hands, as well as the intensity of the rhythm. After the integration of 2–3 sessions, most of the dances were selected were considered, changing progressively and increasing, indicative of the age and physical abilities of the elderly. Initially, the dances had few and simple steps, for example, 6-step dances with minimal hand movement. The increasing difficulty, resulting in the higher intensity, was applied with dances of more steps, 8–16 steps, combined with alternations in the movements of the hands and body of the participants.

Statistical Analysis

Demographics

Demographic data (age, education level, etc.) was first tested for normality assumption using visual inspection of histograms, normal Q-Q plots and boxplots, in terms of skewness and kurtosis as well as using the normality tests (Shapiro-Wilk test) in order to calculate proper descriptive statistics. Since the age and the education level of participants were not approximately normally distributed median and interquartile range were calculated. The proportion of male/female participants was also computed. Similar procedures were followed when analyzing demographic data in a subgroup of intervention participants and matched controls.

Data

Participants underwent an evaluation of physical fitness both before and after the intervention. The assumptions of repeated measures analysis of variance (ANOVA) were not fulfilled and as such, score differences were computed in two-time conditions (post-pre) then explored for normality assumption. Subsequently, we performed Wilcoxon signed-rank tests as the score differences of the tested parameters were not approximately normally distributed. Statistical analysis was performed using the IBM SPSS Statistics (Version 23) and defining setting the significance level (a) to 0.05. Bonferroni correction was used to counteract the problem of alpha inflation due to multiple comparisons.

In the second analysis, we aimed to explore the within changes in each group as well as the between-group differences. Initially, we compared the performance of two groups in the baseline physical assessment performing Mann-Whitney *U* test or independent samples *t*-test depending on the normality assumption. Subsequently, the assumptions of Mixed Model ANOVA were not fulfilled and as such, an alternative analysis design was followed. More precisely, within-group changes were investigated using Wilcoxon signed-rank tests after grouping data by the group while between-groups score differences were compared using Mann-Whitney *U* test. Moreover, we performed Bonferroni correction to counteract the problem of multiple comparisons.

RESULTS

Demographics

Demographic data of 130 elderly participants are described in the following table (**Table 1**).

TABLE 1 | Demographic data of elderly participants as age, education years and gender.

Age Median, [Q1, Q3]	Education Median, [Q1, Q3]	Gender Male/Female
67.00, [63.00, 71.00]	8.00, [6.00, 12.00]	23/107 17.69%/82.31%

The subgroup of the intervention group did not significantly differ in age, education and BMI relatively to the matched controls [Age—Dance subgroup: 66.50, (62.00, 73.00), Controls: 65.50, (61.00, 69.50), $U = 181.50$, $p = 0.615$; Education—Dance subgroup: 6.00, (6.00, 11.25), Controls: 6.50, (6.00, 11.25), $U = 184.00$, $p = 0.643$; BMI—Dance subgroup: 29.10 (3.64), Controls: 27.76 (3.06), $t_{(38)} = 1.263$, $p = 0.214$].

Physical Assessments

Planned comparisons of physical assessments test scores in two-time points (before and after the training) showed that neither the height ($W = -0.258$, $p = 0.796$) and the weight ($W = -0.074$, $p = 0.941$) nor the BMI parameter ($W = -0.186$, $p = 0.852$) changed.

However, the participants' performance significantly altered in most of the physical tests tasks (Chair Stand: $W = -5.459$; $p < 0.001$; Sit and Reach: $W = -4.759$, $p < 0.001$; Foot Up and Go: $W = -8.599$, $p < 0.001$; Back Scratch: $W = -4.648$, $p < 0.001$; Arm Curl: $W = -5.750$, $p < 0.001$; 2 min Step: $W = -5.567$, $p < 0.001$; Balance 1 leg: $W = -4.996$, $p < 0.001$; Handgrip: $W = -3.490$, $p < 0.001$) apart from the jump ability ($W = -0.954$, $p = 0.340$; **Table 2**; **Figure 2**).

The intervention seems to promote significant improvement in Chair stand task as test scores after the training were increased compared to the baseline [pre-training: 16.00, (14.00, 18.00); post-training: 17.00, (15.00, 20.00)]. In more detail, 82 out of 130 participants increased their Chair Stand score, 28 participants did not show any significant change while 20 out of 130 decreased their performance at the aforestated task. Thus, a Wilcoxon signed-rank test determined that there was a statistically significant median increase of 1.00, [0.00, 3.00] in scores at Chair Stand task.

Similar findings were revealed in Sit and Reach task. A significant increase of 2.00, [0.00, 5.00] was observed when comparing test scores at the two-time points [pre-training: 2.00, (0.00, 5.00); post-training: 3.00, (0.00, 8.00)]. More particularly, 83 out of 130 showed enhanced scores at the post-training relative to the baseline, 30 participants decreased their scores while the scores of 17 participants preserved at the two-time points.

Moreover, a Wilcoxon signed-rank test determined that there was a statistically significant median decrease of 0.45, [−0.93, −0.19] in scores at Foot-Up-and-Go task [pre-training: 5.29, (4.64, 5.85); post-training: 4.71, (4.27, 5.17)]. In the Foot-Up-and-Go task 121, out of 130 participants decreased their scores and nine participants showed increased score after the training compared to the baseline. Significant improvement was observed at the Back and Scratch test as a median increase of 2.00, [−1.00, 4.00] [pre-training: −8.00, (−16.25, 2.00); post-training: −5.00, (−16.00, 3.25)]. Eighty-two participants enhanced their scores after training, 15 participants preserved their performance while 33 out of 130 participants showed a decreased in their scores at the Back and Scratch task.

A significant median increase of 2.00, [0.00, 4.00] was observed in Arm curl task when comparing scores both before and after the intervention [pre-training: 24.00, (21.75, 28.00); post-training: 26.50, (24.00, 30.00)]. Ninety-one participants increased their Arm Curl scores, 24 participants showed the opposite finding whereas 15 participants did not change their performance. Moreover, the intervention seems to promote gains in 2-min steps in the place as a median enhance of 6.00, [2.00, 13.25] [pre-training: 93.00, (80.75, 106.25); post-training: 99.50, (85.00, 114.00)]. More precisely, 104 out of 130 participants improved their performance at the 2-min steps in the place task, 25 participants decreased their scores and only one did not show any change.

Ninety-nine out of 130 participants improved their balance as indicated by their enhanced scores at the Balance-1-leg task after the intervention while 31 decreased their performance at the same task when comparing the scores at the two time-points. Furthermore, we observed a significant increase of 3.79, [0.29, 15.20] after the intervention compared to the baseline (pre-training: 22.40, [12.02, 56.87]; post-training: 32.44, [14.99, 60.28]). Additionally, the intervention given seems to induce positive gains in Handgrip task. Sixty-nine participants enhanced their performance after the training compared to the baseline evaluation, 34 had the opposite finding while 27 out of 130 participants remained stable. A significant increase of 1.00, [−1.00, 4.00] was found in Handgrip scores comparing participants' performance both before and after the training [pre-training: 24.50, (19.75, 30.25); post-training: 26.00, (20.00, 32.00)].

Although subjects showed an increase in their scores at the Jump ability [pre-training: 17.48, (8.87, 67.50); post-training: 20.00, (9.70, 61.25)], score change did not reach statistical significance.

A summary of the aforementioned results is displayed in **Table 2** and **Figure 2**.

TABLE 2 | The intervention provoked improvement in almost all the physical fitness parameters.

Physical fitness	Before training Median, [Q1, Q3]	After training Median, [Q1, Q3]	Score change Median, [Q1, Q3]	Test results
Chair Stand	16.00, [14.00, 18.00]	17.00, [15.00, 20.00]	1.00, [0.00, 3.00]	$W = -5.459$, $p < 0.001$
Sit and Reach	2.00, [0.00, 5.00]	3.00, [0.00, 8.00]	2.00, [0.00, 5.00]	$W = -4.759$, $p < 0.001$
Foot Up and Go	5.29, [4.64, 5.85]	4.71, [4.27, 5.17]	−0.45, [−0.93, −0.19]	$W = -8.599$, $p < 0.001$
Back Scratch	−8.00, [−16.25, 2.00]	−5.00, [−16.00, 3.25]	2.00, [−1.00, 4.00]	$W = -4.648$, $p < 0.001$
Arm Curl	24.00, [21.75, 28.00]	26.50, [24.00, 30.00]	2.00, [0.00, 4.00]	$W = -5.750$, $p < 0.001$
2-min step	93.00, [80.75, 106.25]	99.50, [85.00, 114.00]	6.00, [2.00, 13.25]	$W = -5.567$, $p < 0.001$
Balance-1-leg	22.40, [12.02, 56.87]	32.44, [14.99, 60.28]	3.79, [0.29, 15.20]	$W = -4.996$, $p < 0.001$
Handgrip	24.50, [19.75, 30.25]	26.00, [20.00, 32.00]	1.00, [−1.00, 4.00]	$W = -3.490$, $p < 0.001$
Jump ability	17.48, [8.87, 67.50]	20.00, [9.70, 61.25]	1.00, [−3.23, 3.00]	$W = -0.954$, $p = 0.340$

FIGURE 1 | Participants in a Greek traditional dance named: Litos and Zonaradikos. They are dancing in the regions of Macedonia and Thrace of Greece.

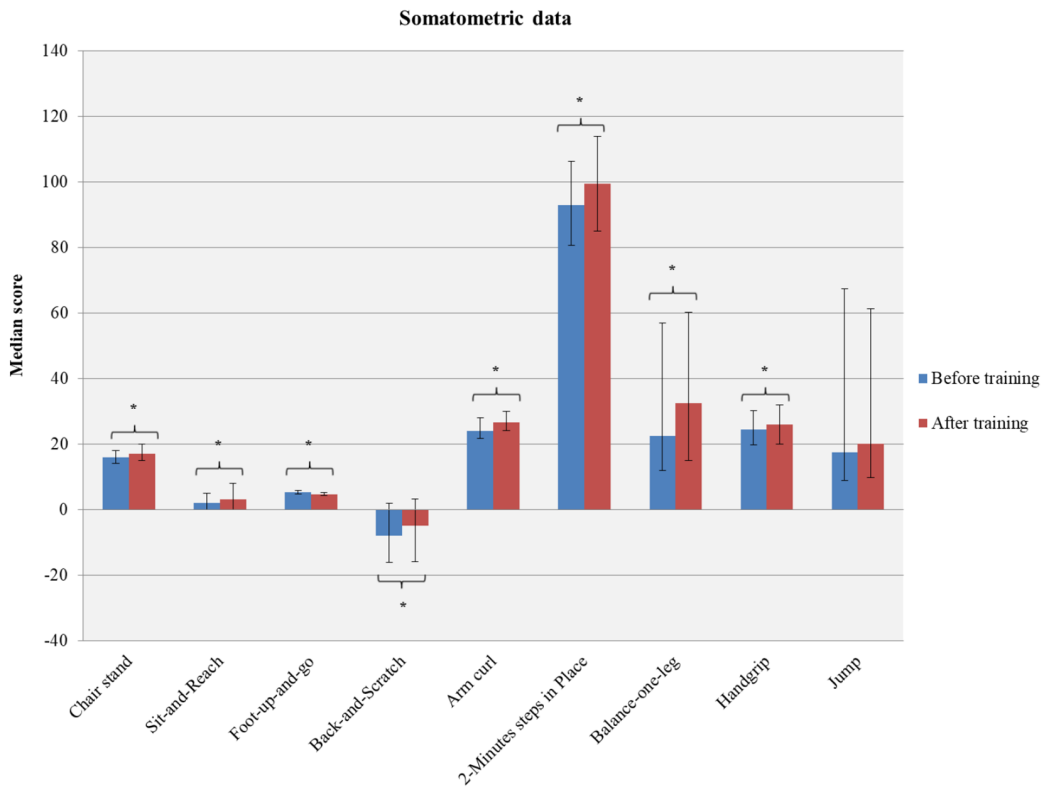


FIGURE 2 | A summary of intervention-induced changes in different physical fitness components. *indicates the significant improvement in the participants of these tests after the dance intervention.

In the second analysis we found that the two groups did not have significant differences in their baseline physical performance [all p-values (Bonferroni corrected) > 0.05]. Within-group changes were found only in the subgroup of dance group but not in the controls [in all tasks p-values (Bonferroni corrected) > 0.05]. In more detail, the intervention group showed significant improvement, when comparing their scores before and after training, in most tasks of physical assessment such as the Chair Stand [pre-training: 12.00, (11.00, 13.00); post-training: 15.00, (14.00, 16.00); $W = -3.737$; $p < 0.001$], Arm Curl [pre-training: 21.00, (19.00, 24.00); post-training: 25.50, (24.00, 27.00); $W = -3.881$; $p < 0.001$], 2-min Step [pre-training: 74.00, (55.25, 79.00); post-training: 80.00, (66.25, 87.50); $W = -3.119$; $p = 0.002$], Sit and Reach [pre-training: −1.50, (−7.50, 1.75); post-training: 3.00, (0.00, 5.50); $W = -3.163$; $p = 0.002$], Back Scratch [pre-training: −12.00, (−21.25, 2.75); post-training: −3.00, (−13.00, 4.75); $W = -3.431$; $p = 0.001$] and Foot Up and Go [pre-training: 6.03, (5.53, 6.73); post-training: 4.91, (4.70, 5.54); $W = -3.509$; $p < 0.001$; **Figures 3**, **4**].

Between-group analysis revealed significant differences in the aforementioned tasks in favor of the dance subgroup. More precisely, the intervention subgroup showed greater

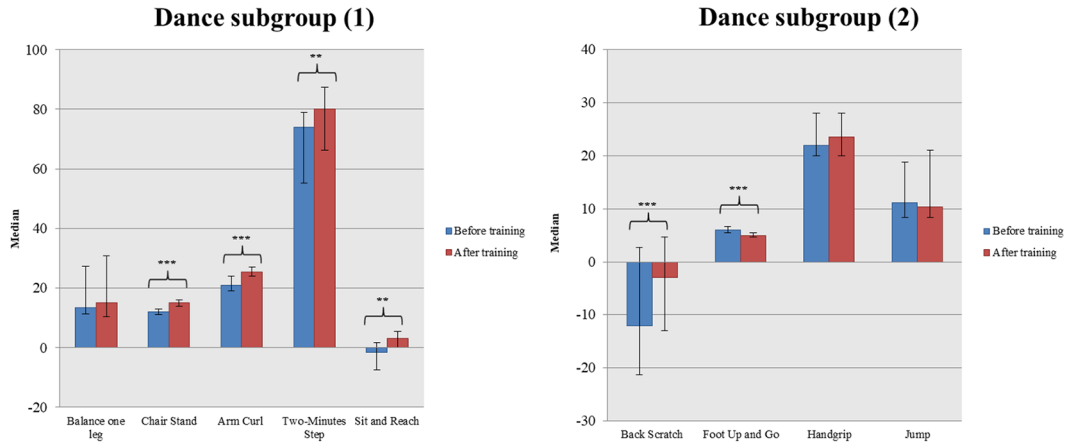


FIGURE 3 | Alterations in different physical assessment parameters in a dance subgroup (*** denotes $p \leq 0.001$, ** denotes $p \leq 0.010$).

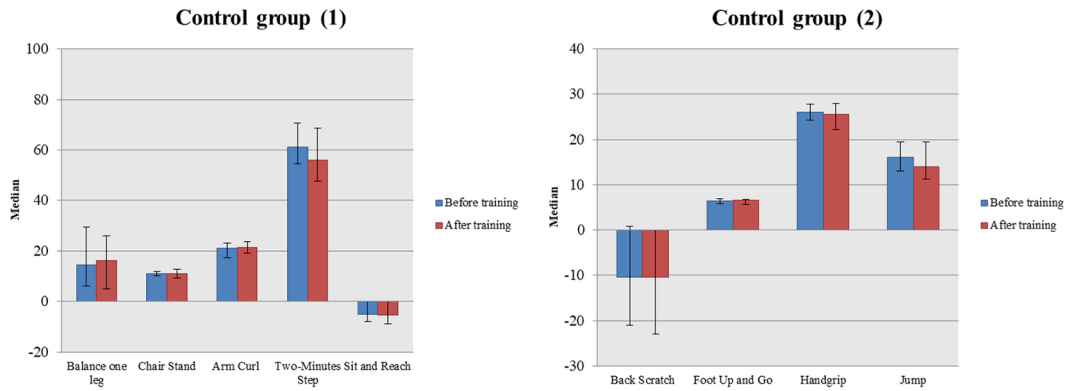


FIGURE 4 | Comparison in somatometric data of the control group in two-time conditions (before and after the waiting period).

changes in task scores compared to the control group in the following tasks: Chair Stand [Dance subgroup: 3.00, (2.00, 5.00); Controls: 0.00, (−1.00, 0.00); $U = 23.50$; $p < 0.001$], Arm Curl [Dance subgroup: 4.00, (2.00, 6.00); Controls: 1.00, (−1.00, 1.00); $U = 28.00$; $p < 0.001$], 2-min Step [Dance subgroup: 8.00, (2.50, 15.25); Controls: −3.50, (−9.75, 0.50); $U = 60.00$; $p < 0.001$], Sit and Reach [Dance subgroup: 4.50, (1.00, 8.75); Controls: 0.00, (−.75, 1.00); $U = 80.00$; $p = 0.001$], Back Scratch [Dance subgroup: 3.00, (1.25, 10.75); Controls: −1.00, (−2.00, 0.00); $U = 46.50$; $p < 0.001$] and Foot Up and Go [Dance subgroup: −0.88, (−1.34, −0.57); Controls: −0.08, (−0.38, 0.21); $U = 47.00$; $p < 0.001$].

DISCUSSION

In the present study, one-hundred-thirty (130) elderly people participated in an intervention program of Greek traditional dances to investigate how their physical fitness and functional capacity were affected, to enable them to have an independent and autonomous life improving their lifestyle. To assess the beneficial role of dance, we investigated the effect of an elderly dance team lasting 32 weeks with a frequency of two times a week for 75 min each.

The results of the post-intervention evaluations of the elderly compared to the initial pre-intervention evaluation showed significant statistical findings. A significant improvement was observed in the strength of the legs (Chair Stand test), a test that assesses the ease of climbing the stairs, walking speed and reduce the risk of falls. This result is consistent with the results in the Bohannon's (1995) study, where it is mentioned that the ability to stand up from the chair is important because of the correlation that exists with other performance variables such as balance and falls. Traditional dances with the different movements that perform the legs, enhance the strength of the lower limbs resulting in a better balance in the elderly.

Also, a significant improvement was found in the Sit and Reach test which is measure lower back and hamstring flexibility and is important as because tightness in this area is implicated in lumbar lordosis, lower back pain and forward pelvic tilt as well as in the Back-Scratch test which assesses the general shoulder

range of motion by measuring how close the hands can be brought together behind the back. In the Sit and Reach test have improved their flexibility by 2.48 cm and in Back-Scratch test have improved their flexibility by 2.07 cm. These significant results of our research are supported by the results of the research by Carvalho et al. (2009), where elderly participated in a program lasting 8 months and showed an improvement of 17.4% in the Sit and Reach test and 14.5% in the Back-Scratch test. It should be noted that an important measure for the prevention of abnormal abilities is considered to be the strength of the lower limbs, as well as the upper body for performing daily activities. The different positions of the hands and the combination of the movement of hands and the rest of the body that require the traditional dances, improved flexibility of participants.

Their aerobic endurance after dance intervention seems to have improved significantly. Some research shows that improvements in the 2-min test may correspond to improvements in cognitive function (Tanne et al., 2005; Stanek et al., 2011). At the Foot Up and Go test, which evaluated dynamic balance and agility, we found statistically significant results. Purath et al.'s (2009) study, showed that after an exercise program with aerobic activity, flexibility, balance and muscle strength, the time of this test decreased, resulting in improved dynamic balance, reaction time and the strength of the lower limbs. According to Duncan and Earhart's (2014) research, after a period of 24-months of dance intervention, participants had significantly reduced their time in this test, indicating that dance participants had improved over time. This is also observed in the Hamburg and Clair's (2004) research, where 36 adults aged 63–86 years increased their balance and standing toe/heel lifts as well as their speed and rhythm gait.

A statistically significant improvement was observed in strength by measuring hand grip (in a rate of about 5.5%), presumably in different gestures ranging from dance to dance, as well as in the same dance. The studies have reported the correlation of hand grip with the reduction of health in the elderly, mainly by linking it to functional disability (Onder et al., 2005) and mortality (Rantanen et al., 2003; Al Snih et al., 2004). A small number of studies indicate the correlation between muscle strength and cognition (Alfaro-Acha et al., 2006; Buchman et al., 2007). Participants in traditional dance have improved their time in the test that evaluate the static balance by about 20%. In a related study by Melzer et al. (2005), showed that the group who participated in the three-month balance training program improved its time by 64% compared to another group that participated in a strength program. According to Duncan and Earhart (2014), measurements of static balance showed a steady improvement in the elderly following dance interventions. Also, Sofianidis et al. (2009) reported that the Greek traditional dance seems to be effective in enhancing the static and dynamic balance in 10-weeks intervention.

Keogh et al. (2009) reported that dancing is a type of physical activity that indicates that this particular activity might improve older adults' lower body bone-mineral content and muscle strength, as well as reduce the prevalence of falls and cardiovascular health risks. Participating in dance may allow the elderly to improve their physical function, health and well-being. Another beneficial advantage is that they can significantly improve their aerobic capacity, lower body muscle endurance, strength and flexibility, balance, agility and gait through the dancing.

Physical activities for the elderly people include both regular and recreational actions in their daily social life (climbing stairs or walking) performing various tasks (for people who continue working), participating in different sports games as well as in specially designed exercise programs, such as the traditional dance. Dance beyond many benefits helps individuals to improve their body posture. Proper posture improves overall balance and generally there are positive effects on the body. Bones are well aligned, the vital organs are properly positioned, and the muscles, joints and ligaments can function in the way they should be. Also, good posture contributes to encouraging the normal functioning of the nervous system and is important for health and general well-being. Lima and Vieira (2007) at their research, showed that after a dance intervention, the elderly had improved skills such as flexibility, balance and coordination but also improved attitude and control of their movements.

In a previous research on people over 60 years, showed that social dance supported efforts to relieve physical and psychological degradation, provided a strong sense of pleasure and continuity as well as a vehicle for the changes required by aging. It also provided a strong sense of community as it allowed participants to showcase their "cultural heritage" (Cooper and Thomas, 2002). Another research with 24 older social dancers (average age 80), showed that after the intervention they had better balance, walked faster and had a longer mean step with a more stable pattern during walking with reduced stance time, longer swing time and shorter double support time (Verghese, 2006).

Furthermore, dance, in addition to physical activity, combines the emotion, social interaction, motor coordination and music, thus creating a thriving environmental condition for individuals. Through the revival of music and dance, the elderly have the opportunity to relive the past through the present. Individuals initiate to understand the meaning of these two, either individually or as a combination, and are benefiting from the positive effects on the body and mind. The importance of social interaction through music places them in a process, so the elderly can share their passion with others. This interaction with other people eliminates the feeling of loneliness and enhances their psychological status. Moreover, it is considered an important fact that their self-esteem and mood increase as they realized they can engage in new skills.

This study demonstrates the effects of traditional dance on people who choose to systematically participate in organized Greek traditional dance lessons as a means of exercise. In the analysis of the control group with a small intervention group, it was observed that in most tests and particularly in Fullerton's domains, there was an improvement in their performance after the intervention, such as the same was found for all of the 130 participants. However,

we should consider that one limitation of the study is the lack of randomization that strengthens the generalization of our results as well as the small number of control subjects recruited.

Greek traditional dance is a physical activity that contributes positively to many factors on the physical health of elderly people by enhancing the well-being outcomes for elderly people. Dance, specifically for the elderly, is a very interesting type of physical activity, because it carries less risk of injury than many other types of exercise. The Greek traditional dance for Greek elderly people is particularly important, because it relates to the tradition, culture, but also their lives. Thus, it is a physical activity more popular and hence readily selectable by the elderly, which can equally have beneficial effects of exercise.

Generally, dance offers a host of physical and mental benefits to individuals, especially when exercised to protect or improve their health. As well, exercise and participation in physical activities are associated with better performance in cognitive functions. Especially, in our previous study, the intervention involved dance training with adaptive difficulty and intensity. The results demonstrated the functional reorganization of cortical rest networks (Zilidou et al., 2018).

CONCLUSION

Through this research conducted on Greek traditional dances programs, it is believed that dancing could be defined as an important and effective tool for the prevention and the fight against the health problems of the elderly. The results of this study show that dancing contributes to the well-being of the elderly with a view of independent and quality living. Maintaining their physical fitness and functional capacity at satisfactory levels, lead them to a more qualitative and independent lifestyle while the risk of various diseases is reduced.

FUTURE DIRECTIONS

Dance is demonstrating greatly that it improves the elderly's functional ability and well-being. Therefore, it is suggested in a new study to investigate the physical effects of dance by combining nutritional education and psychological effects and comparing them with individuals of vulnerable groups such as patients with Parkinson disease. In Parkinson's disease, a healthy diet and exercise are considered important factors to stay healthy and active while maintaining the satisfactory levels their energy.

ETHICS STATEMENT

Ethical and Scientific Committee of GAARD approved the protocol of this study.

AUTHOR CONTRIBUTIONS

ZV: designed and implemented the dance program, collected the data, guided the analysis, prepared the initial draft of the manuscript, discussed the results and revised the manuscript. LO: implemented the dance program data and revised the manuscript. MV: contributed the physical assessments. DS: co-guided the study.

FUNDING

This study is partly supported by the project "Augmentation of the Support of Patients suffering from Alzheimer's Disease and their caregivers (ASPAD/2875)", which is materialized by the Special Account of the Research Committee at Aristotle University of Thessaloniki. The project is funded by the European Union (European Social Fund) and the Ministry of Education, Lifelong Learning and Religious Affairs in the context of the National Strategic Reference Framework (NSRF, 2007–2013).

REFERENCES

Al Snih, S., Markides, K. S., Ottenbacher, K. J., and Raji, M. A. (2004). Hand grip strength and incident ADL disability in elderly Mexican Americans over a seven-year period. *Aging Clin. Exp. Res.* 16, 481–486. doi: 10.1007/bf03327406

Alfaro-Acha, A., Al Snih, S., Raji, M. A., Kuo, Y.-F., Markides, S. A., and Ottenbacher, J. K. (2006). Handgrip strength and cognitive decline in older Mexican Americans. *J. Gerontol. A Biol. Sci. Med. Sci.* 61, 859–865. doi: 10.1093/gerona/61.8.859

Alpert, P. T., Miller, S. K., Wallmann, H., Havey, R., Cross, C., Chevalia, T., et al. (2009). The effect of modified jazz dance on balance, cognition, and mood in older adults. *J. Am. Acad. Nurse Pract.* 21, 108–115. doi: 10.1111/j.1745-7599.2008.00392.x

American Geriatrics Society, British Geriatrics Society, and American Academy of Orthopaedic Surgeons Panel on Falls Prevention. (2001). Guideline for the prevention of falls in older persons. American Geriatrics Society, British Geriatrics Society, and American Academy of Orthopaedic Surgeons Panel on Falls Prevention. *J. Am. Geriatr. Soc.* 49, 664–672.

Aweto, H. A., Owoeye, O. B., Akinbo, S. R., and Onabajo, A. A. (2012). Effects of dance movement therapy on selected cardiovascular parameters and estimated maximum oxygen consumption in hypertensive patients. *Nig. Q. J. Hosp. Med.* 22, 125–129.

Bohannon, R. W. (1995). Sit-to-stand test for measuring performance of lower extremity muscles. *Percept. Mot. Skills* 80, 163–166. doi: 10.2466/pms.1995.80.1.163

Buchman, A. S., Wilson, R. S., Boyle, P. A., Bienias, J. L., and Bennett, D. A. (2007). Grip strength and the risk of incident Alzheimer's disease. *Neuroepidimiology* 29, 66–73. doi: 10.1159/000109498

Cadore, E. L., Rodríguez-Mañas, L., Sinclair, A., and Izquierdo, M. (2013). Effects of different exercise interventions on risk of falls, gait ability, and balance in physically frail older adults: a systematic review. *Rejuvenation Res.* 16, 105–114. doi: 10.1089/rej.2012.1397

Carvalho, M. J., Marques, E., and Mota, J. (2009). Training and detraing effects on functional fitness after a multicomponent training in older women. *Gerontology* 55, 41–48. doi: 10.1159/000140681

Cooper, L., and Thomas, H. (2002). Growing old gracefully: social dance in the third age. *Ageing Soc.* 22, 689–708. doi: 10.1017/s0144686x02008929

Daig, I., Herschbach, P., Lehmann, A., Knoll, N., and Decker, O. (2009). Gender and age differences in domain-specific life satisfaction and the impact of depressive and anxiety symptoms: a general population survey from Germany. *Qual. Life Res.* 18, 669–678. doi: 10.1007/s11136-009-9481-3

Dominick, K. L., Ahern, F. M., Gold, C. H., and Heller, D. A. (2002). Relationship of health-related quality of life to health care utilization and mortality among older adults. *Aging Clin. Exp. Res.* 14, 499–508. doi: 10.1007/bf03327351

Duncan, R. P., and Earhart, G. M. (2014). Are the effects of community-based dance on Parkinson disease severity, balance and functional mobility reduced with time? A 2-year prospective pilot study. *J. Altern. Complement Med.* 20, 757–763. doi: 10.1089/acm.2012.0774

Federici, A., Bellagamba, S., and Rocchi, M. (2005). Does dance-based training improve balance in adult and young old subjects? A plot randomized controlled trial. *Aging Clin. Exp. Res.* 17, 385–389. doi: 10.1007/bf03324627

Fugl-Meyer, A. R., Melin, R., and Fugl-Meyer, K. S. (2002). Life satisfaction in 18-to 64-year-old Swedes: in relation to gender, age, partner and immigrant status. *J. Rehabil. Med.* 34, 239–246. doi: 10.1080/165019702760279242

Hackney, M., and Bennett, G. C. (2014). Dance therapy for individuals with Parkinson's disease: improving quality of life. *J. Parkinson. Rest. Legs Synd.* 4, 17–25. doi: 10.2147/jprls.s40042

Hamburg, J., and Clair, A. A. (2004). The effects of a laban-based movement program with music on measures of balance and gait in older adults. *Activ. Adapt. Aging* 28, 17–33. doi: 10.1300/j016v28n01_02

Hui, E., Chui, B., and Woo, J. (2009). Effects of dance on physical and psychological well-being in older persons. *Arch. Gerontol. Geriat.* 49, e45–e50. doi: 10.1016/j.archger.2008.08.006

Jing, L., and Xudong, W. (2008). Evaluation on the effects of relaxing music on the recovery from aerobic exercise-induced fatigue. *J. Sports Med. Phys. Fitness* 48, 102–106.

Johnson, B. L., and Nelson, J. K. (1969). *Practical Measurements for Evaluation in Physical Education.*Minneapolis, MN: Burgess.

Judge, J. O. (2003). Balance training to maintain mobility and prevent disability. *Am. J. Prev. Med.* 25, 150–156. doi: 10.1016/s0749-3797(03)00178-8

Kaltsatou, A., Mameletzi, D., and Douka, S. (2011). Physical and psychological benefits of a 24-week traditional dance program in breast cancer survivors. *J. Bodyw. Mov. Ther.* 15, 162–167. doi: 10.1016/j.jbmt.2010.03.002

Kattenstroth, J. C., Kalisch, T., Kolankowska, I., and Dinse, H. R. (2011). Balance, sensorimotor and cognitive performance in long-year expert senior ballroom dancers. *J. Aging Res.* 2011:176709. doi: 10.4061/2011/176709

Kaufmann, K. (2011). Movement as a metaphor: how persistence, the tao and the wisdom of the ostrich helped build school dance programs. *J. Phys. Educ. Recreat. Dance* 82, 37–45. doi: 10.1080/07303084.2011.10598627

Keogh, J. W., Kilding, A., Pidgeon, P., Ashley, L., and Gillis, D. (2009). Physical benefits of dancing for healthy older adults: a review. *J. Aging Phys. Act.* 17, 479–500. doi: 10.1123/japa.17.4.479

Lautenschlager, N. T., Cox, K. L., Flicker, L., Foster, J. K., van Bockxmeer, F. M., and Xiao, J. (2008). Effect of physical activity on cognitive function in older adults at risk for Alzheimer disease: a randomized trial. *JAMA* 300, 1027–1037. doi: 10.1001/jama.300.9.1027

Lee, I. M., Shiroma, E. J., Lobelo, F., Puska, P., Blair, S. N., and Katzmarzyk, P. T. (2012). Effect of physical inactivity on major non-communicable diseases worldwide: an analysis of burden of disease and life expectancy. *Lancet* 380, 219–229. doi: 10.1016/S0140-6736(12)61031-9

Lima, M. M., and Vieira, A. P. (2007). Ballroom dance as therapy for the elderly in Brazil. *Am. J. Dance Ther.* 29, 129–142. doi: 10.1007/s10465-007-9040-9

Manini, T. M., Everhart, J. E., Patel, K. V., Schoeller, D. A., Colbert, L. H., and Visser, M. (2006). Daily activity energy expenditure and mortality among older adults. *JAMA* 296, 171–179. doi: 10.1001/jama.296.2.171

Melzer, I., Benjuya, N., and Kaplanski, J. (2005). Effect of physical training on postural control of elderly. *Harefuah* 144, 839–844.

Nied, J. R., and Fraklin, B. (2002). Promoting and prescribing exercise for the elderly. *Am. Fam. Physician* 65, 419–426.

Onder, G., Penninx, B. W., Ferrucci, L., Fried, L. P., Guralnik, J. M., and Pahor, M. (2005). Measures of physical performance and risk for progressive and catastrophic disability: results from the Women's Health and Aging Study. *J. Gerontol. A Biol. Sci. Med. Sci.* 60, 74–79. doi: 10.1093/gerona/60.1.74

Purath, J., Buchholz, W. S., and Kark, L. D. (2009). Physical fitness assessment of older adults in the primary care setting. *J. Am. Acad. Nurse Pract.* 21, 101–107. doi: 10.1111/j.1745-7599.2008.00391.x

Rantanen, T., Volpato, S., Ferrucci, L., Heikkinen, E., Fried, L. P., and Guralnik, J. M. (2003). Handgrip strength and cause-specific and total mortality in older disabled women: exploring the mechanism. *J. Am. Geriatr. Soc.* 51, 636–641. doi: 10.1034/j.1600-0579.2003.00207.x

Rikli, R. E., and Jones, C. J. (1999). Functional fitness normative scores for community-residing older adults, ages 60–94. *J. Aging Phys. Activ.* 7, 162–181. doi: 10.1123/japa.7.2.162

Sofianidis, G., Hatzitaki, V., Douka, S., and Grouios, G. (2009). Effect of a 10-week traditional dance program on static and dynamic balance control in elderly adults. *J. Aging Phys. Act.* 17, 167–180. doi: 10.1123/japa.17.2.167

Song, R., June, K. J., Kim, C. G., and Jeon, M. Y. (2004). Comparisons of motivation, health behaviors, and functional status among elders in residential homes in Korea. *Public Health Nurs.* 21, 361–371. doi: 10.1111/j.0737-1209.2004.21410.x

Stanek, K. M., Gunstad, J., Spitznagel, M. B., Waechter, D., Hughes, J. W., and Luyster, F. (2011). Improvements in cognitive function following cardiac rehabilitation for older adults with cardiovascular disease. *Int. J. Neurosci.* 121, 86–93. doi: 10.3109/00207454.2010.531893

Stathi, A., McKenna, J., and Fox, K. (2004). The experiences of older people participating in exercise referral schemes. *J. R. Soc. Prom. Health* 124, 18–23. doi: 10.1177/146642400312400108

Stessman, J., Hammerman-Rozenberg, R., Cohen, A., Ein-Mor, E., and Jacobs, J. M. (2009). Physical activity, function, and longevity among the very old. *Arch. Intern. Med.* 169, 1476–1483. doi: 10.1001/archinternmed.2009.248

Studer-Lüthi, B., and Züger, B. (2012). Effects of dance intervention on body concept and cognitive abilities of normally developed children. *Musik, Tanz. Kunsttherapie* 23, 70–77. doi: 10.1026/0933-6885/a000077

Tanne, D., Freimark, D., Poreh, A., Merzeliak, O., Bruck, B., Schwammenthal, Y., et al. (2005). Cognitive functions in severe congestive heart failure before and after an exercise training program. *Int. J. Cardiol.* 103, 145–149. doi: 10.1016/j.ijcard.2004.08.044

Tsimaras, B., Giamouridou, G., Kokaridas, D., Sidiropoulou, M., and Patsiaouras, A. (2012). The effect of a traditional dance training program on dynamic balance of individuals with mental retardation. *J. Strength Cond. Res.* 26, 192–198. doi: 10.1519/jsc.0b013e31821c2494

Verghese, J. (2006). Cognitive and mobility Profile of older social dancers. *J. Am. Geriat. Soc.* 54, 1241–1244. doi: 10.1111/j.1532-5415.2006.00808.x

Villareal, D. T., Chode, S., Parimi, N., Hilton, T., Armamento-Villareal, R., Napoli, N., et al. (2011). Weight loss, exercise, or both and physical function in obese older adults. *N. Engl. J. Med.* 364, 1218–1229. doi: 10.1056/NEJMoa1008234

Wanderley, F., Oliveira, N., Marques, E., Moreira, P., and Carvalho, J. (2015). Aerobic versus resistance training effects on health-related quality of life, body composition and function of older adults. *J. Appl. Gerontol.* 34, NP143–NP165. doi: 10.1177/0733464812468502

Weening-Dijksterhuis, E., de Greef, M. H., Scherder, E. J., Slaets, J. P., and van der Schans, C. P. (2011). Frail institutionalized older persons: a comprehensive review on physical exercise, physical fitness, activities of daily living and quality-of-life. *Am. J. Phys. Med. Rehabil.* 90, 156–168. doi: 10.1097/phm.0b013e3181f703ef

World Health Organisation (2018). Ageing and Life Course. Available online at: http://www.who.int/ageing/en/. [Accessed on 14th August 2018]. doi: 10.1093/acprof:oso/9780199656516.003.0005

Zilidou, I. V., Frantzidis, A. C., Romanopoulou, D. E., Paraskevopoulos, E., Douka, S., and Bamidis, D. P. (2018). Functional re-organization of cortical networks of senior citizens after a 24-week traditional dance program. *Front. Aging Neurosci.* 10:422. doi: 10.3389/fnagi.2018.00422

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

CLINICAL TRIAL
published: 07 May 2019
doi: 10.3389/fnagi.2019.00099

Newly Developed TV-Based Cognitive Training Games Improve Car Driving Skills, Cognitive Functions, and Mood in Healthy Older Adults: Evidence From a Randomized Controlled Trial

Rui Nouchi[1,2]*, *Akiko Kobayashi*[3], *Haruka Nouchi*[3] and *Ryuta Kawashima*[2,3]

[1]Department of Cognitive Health Science, Institute of Development, Aging and Cancer (IDAC), Tohoku University, Sendai, Japan, [2]Smart Aging Research Center (S.A.R.C.), Tohoku University, Sendai, Japan, [3]Department of Functional Brain Imaging, Institute of Development, Aging and Cancer (IDAC), Tohoku University, Sendai, Japan

Background: Cognitive training in a laboratory improves car driving skills of older car drivers. However, it remains unclear whether other types of cognitive training at home have beneficial effects on driving skills. Using our developed cognitive training games that can be played on a television with a set-top box in a person's home, we investigated the effects of a 6-week cognitive training program on driving skills, which included on-road evaluation (primary outcome), and cognitive functions and emotional states (secondary outcome) in older people.

Methods: In this double-blinded randomized control trial (RCT), 60 older licensed drivers were randomly assigned into one of the two groups: a cognitive training game for car driving (CTCD) group and an active control cognitive training game (ACT) group. Participants in the CTCD group played the CTCD (processing speed, dual attention, and speed prediction) for 20 min in five sessions per week for 6 weeks. Participants in the ACT group played the ACT (selecting the larger number; selecting a number from largest to smallest; play a game of rock, article, scissors) for 20 min in five sessions per week for 6 weeks. We measured driving skills, various cognitive functions, and emotional states before and after the 6-week intervention period.

Results: Our main results showed that compared to the ACT group, the CTCD group demonstrated improved driving skills (adjusted $p = 0.034$). Moreover, the CTCD group demonstrated improved inhibition (stroop, adjusted $p = 0.042$: reverse Stroop, adjusted $p = 0.043$) and processing speed performance symbol search (SS), adjusted $p = 0.049$; digit symbol coding (adjusted $p = 0.047$), compared to the ACT group. The CTCD group scored higher on vigor–activity mood (adjusted $p = 0.041$) as measured using the Profile of Mood State.

OPEN ACCESS

Edited by:
Michael George Bemben, University of Oklahoma, United States

Reviewed by:
Enrico Mossello, University of Florence, Italy
Hannes Devos, University of Kansas, United States

***Correspondence:**
Rui Nouchi
rui@tohoku.ac.jp

Received: *16 January 2019*
Accepted: *10 April 2019*
Published: *07 May 2019*

Citation:
Nouchi R, Kobayashi A, Nouchi H and Kawashima R (2019) Newly Developed TV-Based Cognitive Training Games Improve Car Driving Skills, Cognitive Functions, and Mood in Healthy Older Adults: Evidence From a Randomized Controlled Trial. Front. Aging Neurosci. 11:99. doi: 10.3389/fnagi.2019.00099

Discussion: This randomized controlled trial provides scientific evidence for the benefits of the 6-week CTCD program on driving skills and cognitive functions, such as processing speed, inhibition, and vigor–activity mood, in healthy older people. Our results suggest that cognitive training is useful to improve the driving skills of older adults.

Trial registration: This trial was registered at The University Hospital Medical Information Network Clinical Trials Registry (UMIN 000029769). Registered 31 October 2017, https://upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000034010.

Keywords: car driving skill, cognitive training, processing speed, inhibition, positive mood

BACKGROUND

An increase in the aging population has led to an increase in the number of older car drivers worldwide. There is also an increasing risk of car accidents among older adults. Recent road safety studies have typically reported that younger and older car drivers have higher fatal and non-fatal crash risks than the middle-aged car drivers (McAndrews et al., 2013; Ma and Yan, 2014). In addition, previous studies have indicated that older car drivers experience a higher number of car accidents per unit distance traveled than other age groups (Li et al., 2003; Lombardi et al., 2017). Age-related cognitive and physical functional changes are a main factor of car accidents in the older adults (Anstey et al., 2012). Normal aging shows declines in several cognitive abilities needed for driving (Boot et al., 2014). Earlier reports have described that declines of processing speed, attention, and executive functions affect safe driving in older adults (Anstey et al., 2005; Horikawa et al., 2009; Adrian et al., 2011). In fact, car driving skills of older adults were lower than that of younger adults (Doroudgar et al., 2017). Nevertheless, driving is an important activity for older adults to maintain their mental health (Edwards et al., 2009a) and social relationships in a community (Dickerson et al., 2007). Older car drivers want to drive and possess a driver's license (Yassuda et al., 1997). Thus, researchers have shown great interest in developing ways to improve driving skills and reduce crash risks associated with older drivers.

There are several approaches, such as in-class and on-road education program, physical training, simulator-based training, and cognitive training, to improve the driving skills of older people (Karthaus et al., 2016). The first approach is a post-license educational program, by which older drivers receive in-call or on-road education (Stalvey and Owsley, 2003; Owsley et al., 2004). Such post-license educational programs can increase awareness and reduce exposure to risky driving situations (Boot et al., 2014). The second approach is a physical training program to improve car driving skills. A previous meta-analysis reported that physical functions are associated with driving skills in older adults (Mielenz et al., 2017). Therefore, it is expected that physical training would improve the car driving skills. For example, the 8-week joint-range-of-motion physical fitness training program was shown to improve car driving skills (Ostrow et al., 1992). Another study reported that a 12-week strength exercise training program improved car driving skills, measured by on-road evaluation (Marottoli et al., 2007). However, such education and physical training programs are not easy to conduct, because these training programs need specific trainers (e.g., car driving instructors and psychical therapist). The third approach is a simulator-based training. Several studies demonstrated that car driving simulator intervention improved car driving skills and cognitive functions in the older adults (Casutt et al., 2014b). The advantage of a simulator-based training is that participants can receive highly controlled, repeated training with immediate feedback. However, for older adults, a potential disadvantage of driving simulators is simulator sickness. A recent study revealed that older adults are at a high risk for simulator sickness (Matas et al., 2015). Simulator sickness is a risk factor for the dropout in studies. The fourth approach—cognitive training—involves cognitive training programs designed to enhance cognitive functions and daily behaviors through training tasks and games related to cognitive functions. Earlier studies have reported that a decline in cognitive abilities is associated with a decline in driving ability (Boot et al., 2014). For example, processing speed and executive function are important for the driving performance of older adults (Anstey et al., 2005; Horikawa et al., 2009; Adrian et al., 2011). Therefore, cognitive training is expected to enhance driving skills by improving cognitive abilities such as processing speed and executive functions. Earlier studies have demonstrated improved driving skills and cognitive functions in healthy older adults after cognitive training (Nozawa et al., 2015).

In this study, we focused on cognitive training because it is easy and safe to administer compared to the other approaches. Some previous studies have reported positive effects of cognitive training on driving skills (Roenker et al., 2003; Edwards et al., 2009a,b; Boot et al., 2014; Ross et al., 2018). Nevertheless, some unclear issues remain. First, cognitive training for driving skills has been conducted mainly with trainers (or experimenters) or specific devices or using simulators at laboratories (Casutt et al., 2014a, 2016; Nozawa et al., 2015; Hay et al., 2016; Haeger et al., 2018). To reduce costs related to cognitive training and to extend the outcomes of

the cognitive training to the society at large, it is necessary to develop new training methods that can be easily carried out at home. Second, cognitive training programs in driving studies have traditionally used a single-domain cognitive training such as visual processing speed training, like Useful Field of View (UFOV) training (Ball et al., 1988), and attention training (Casutt et al., 2016). Generalization of the effects of cognitive training on driving skill warrants an investigation of whether other types of cognitive training can improve driving skills. Third, previous cognitive training studies have usually used a no-intervention/no-training group for comparison (Ball et al., 2002; Haeger et al., 2018); alternatively, they have used other training programs with different devices (Nozawa et al., 2015; Casutt et al., 2016). Such a study design cannot exclude placebo effects or other potential factors such as learning new devices and meeting new people. Moreover, previous studies have not conducted an randomized control trial (RCT) based on the CONSORT guidelines (Schulz et al., 2010). An RCT with an active control group should be used for comparison in order to obtain evidence for the effects of cognitive training on driving skills.

In this study, to enhance driving skills, we developed cognitive training games that can be used with a television (TV)—the most popular home device. Older adults watch more TV programs than young adults (Mundorf and Brownell, 1990). In fact, watching TV is the most common leisure activity among older adults (Horgas et al., 1998; Strain et al., 2002). Previous cognitive training studies using a TV reported improved cognitive functions in older adults (Shatil et al., 2014). Participants can play all cognitive training games on their own TV at home. There were three cognitive training games (processing speed, dual attention, and speed prediction), because the results of the previous studies indicated that training for multiple domains or tasks had better benefits than did those that included a single domain or task training (Cheng et al., 2012; Nouchi et al., 2012a, 2013). In this study, we selected processing speed, dual attention, and speed prediction tasks as the cognitive training games for the following reasons. Driving is a complex and dynamic activity that involves various cognitive functions. It is difficult to conclude the core cognitive function involved in driving. However, existing evidence shows that processing speed, dual attention, and speed prediction are important for car driving performance. First, previous studies suggest that processing speed, divided attention, and speed prediction scores are correlated with driving skills (Maruyama and Kitamura, 1961; Anstey et al., 2005; Clay et al., 2005; Horikawa et al., 2009; Adrian et al., 2011; Cuenen et al., 2015). Second, cognitive training studies using processing speed, divided attention, and executive functions demonstrated improvements in driving skills and cognitive functions (Roenker et al., 2003; Nozawa et al., 2015; Ross et al., 2018). Based on these findings, we developed a cognitive training program, which included processing speed, dual attention, and speed prediction.

Additionally, we measured the emotional state of the participants before and after the intervention period. It has been reported that subjective emotional states are correlated with driving skills (Matthews et al., 1996; Garrity and Demick, 2001; Chliaoutakis et al., 2002). Furthermore, cognitive training can alter emotional states (Takeuchi et al., 2014; Nouchi et al., 2016a). Therefore, it is important to investigate whether cognitive training for driving skills can engender change in the emotional states and examine the relationship between change in emotional states and improvement in driving skills.

The main purpose of this trial was to investigate the benefits of TV-based cognitive training on driving skills in a healthy aging population. Therefore, we conducted a 6-week double-blinded RCT with two parallel groups: a cognitive training game for car driving skill (CTCD) group and an active control cognitive training game (ACT) group. To evaluate the effects of the developed cognitive training game, we assessed driving skills using an on-road evaluation test, cognitive functions, and emotional states. The primary outcome was driving skill performance. Based on previous studies (Wolinsky et al., 2013; Nozawa et al., 2015; Ball et al., 2002), we expected that the developed cognitive training games would engender improvements in driving skills, cognitive function, and emotional states of the CTCD group compared to the ACT group.

METHOD

Randomized Controlled Trial Design and Setting of This Trial

This RCT was conducted from November 2017 to January 2018 in Sendai, Shiogama, and Kurihama cities, Japan. The population of each city in 2018 was 1,086,377 in Sendai, 53,399 in Shiogama, and 67,566 in Kurihama[1]. The number of older drivers who possessed a car driver's license in each city was 113,839 in Sendai, 7,574 in Kurihama, and 12,473 in Shiogama[2]. The study protocol was approved by the Ethics Committee of Tohoku University Graduate School of Medicine. This RCT was registered at the University Hospital Medical Information Network (UMIN) Clinical Trial Registry (UMIN000029769).

To assess the benefits of cognitive training on driving performance of healthy older adults, we conducted a double-blinded RCT with an active control group. All participants and testers were blinded to the study hypothesis and the group membership of participants. The primary outcome was driving skills, which was assessed by an on-road evaluation. The secondary outcomes were cognitive functions and emotional states. The Consolidated Standards of Reporting Trials (CONSORT) statement[3] (see **Supplementary Table S1**), was used to report the study structure. The RCT design is presented in **Figure 1**.

Participants

First, advertisements were used to recruit 60 participants from each of the three cities. We displayed the advertisements at the entrance of the city hall and in small culture schools for 2 weeks. The inclusion and exclusion criteria were printed

[1]https://www.pref.miyagi.jp/uploaded/attachment/653161.pdf
[2]http://www.police.pref.miyagi.jp/hp/menkyo/mennkyo_%20toukei.pdf
[3]http://www.consort-statement.org/home/

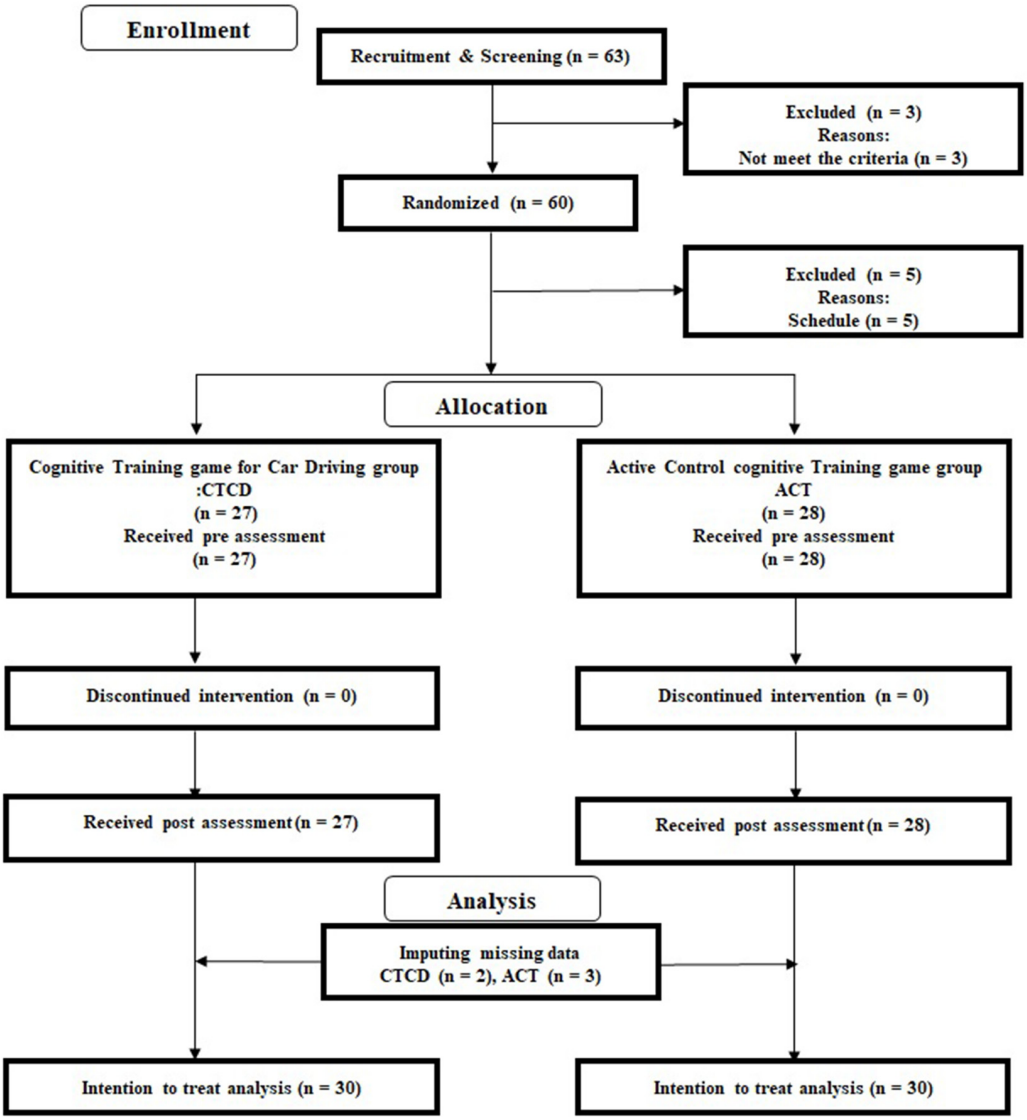


FIGURE 1 | Consolidated Standards of Reporting Trials (CONSORT) diagram.

on the flyers. Sixty-three interested participants contacted the research group by post or by phone (**Figure 1**) and participated in an orientation meeting. During the meeting, one researcher (RN) explained the study details. We received informed consent from each participant. The researcher then checked whether interested participants were eligible to participate in this study. All participants underwent a cognitive functional screening assessment using the Mini-Mental State Examination (MMSE; Folstein et al., 1975) and Frontal Assessment Battery at bedside (FAB; Dubois et al., 2000). No participant was excluded based on their MMSE and FAB scores. After excluding three participants who did not meet the inclusion criterion of medical history, 60 participants were randomly assigned to the CTCD or ACT group. After the randomization, five participants were excluded because of their schedule. Based on the intention and treatment analysis (ITT), we did not recruit another five participants but imputed the missing data using multiple imputation methods (please see "Analysis" section). **Table 1** presents the baseline characteristics of all participants [n = 55; 32 men, 23 women; average age = 72.40 years (SD = 3.80)]. There was no significant difference in the baseline data between the two groups (two-sample t-test).

Inclusion and Exclusion Criteria

Based on our previous studies (Nouchi et al., 2012a, 2016a,b; Nozawa et al., 2015), we used the following inclusion criteria: (1) right-handed; (2) native Japanese speakers; (3) 65–80 years of age; (4) not concerned about their own memory functions, not using medications known to interfere with cognitive functions (including benzodiazepines, antidepressants, or other

TABLE 1 | Age, education, driving history, and general cognitive function scores of both groups at baseline.

	CTCD group		ACT group			
	Mean	SD	Mean	SD	Effect size (*d*)	*p*-value
Age (years)	71.67	3.62	73.11	3.90	0.74	0.16
Education (years)	12.96	2.01	12.82	2.04	0.10	0.80
Driving license history (years)	44.54	6.11	45.26	6.26	0.29	0.67
Driving skills (on-road evaluation test score)	113.07	11.75	113.82	9.60	0.23	0.80
MMSE (score)	28.93	1.14	28.96	1.07	0.04	0.90
FAB (score)	15.04	1.45	14.89	1.68	0.12	0.74
JART (score)	113.07	11.75	113.82	9.60	0.23	0.80

Group comparison (two sample t-tests) of pre-training scores revealed no significant difference for any measure ($p > 0.10$). CTCD, Cognitive Training for Car driving Skills group; ACT, Active control group; MMSE, Mini Mental State Examination; FAB, Frontal Assessment Battery at bedside; JART, Japanese Reading Ability Test.

central nervous agents); (5) no history of diseases known to affect the central nervous system, including thyroid disease, multiple sclerosis, Parkinson disease, stroke, diabetes, and severe hypertension (systolic blood pressure over 180 mmHg, diastolic blood pressure over 110 mmHg); and (6) daily drivers who have been possessing a valid driver's license for over 10 years and drive more than thrice a week on average. The exclusion criteria were participants with an MMSE score of less than 26 and FAB score of less than 12. Participants who participated in other cognition-related intervention studies were also excluded.

Sample Size

We calculated the sample size using a software (Faul et al., 2009). The sample size was based on the change in driving skill score because the primary outcome in this RCT was driving performance. Previous studies applying cognitive training for vehicle operation (Nozawa et al., 2015) described a medium effect size $d = 0.47$ (vs. no intervention group) and $d = 0.55$ (vs. active control group) for driving skill. The pre-post changes in the cognitive training group revealed a large effect size ($d = 1.13$). Thus, we expected an effect size between medium and large ($f = 0.35$). To calculate the sample size, we used an analysis of covariance (ANCOVA) model with pre-intervention driving skill score, sex, and age as covariates, using a one-tailed test, $\alpha = 0.05$, and 0.85 power. The estimated sample size was 60.

Randomization

To randomly assign the 60 interested participants into the CTCD and ACT groups, we used an online randomization program[4]. We stratified participants based on sex because previous studies reported sex differences in car driving performance as the primary outcome (Ma and Yan, 2014) and cognitive function (McCarrey et al., 2016) and emotional states (Boyle, 1989; Masumoto et al., 2016) as the secondary outcomes. We used blocked randomization (block size: 4) with an allocation ratio of 1:1.

Overview of the Intervention

Participants were asked to execute the CTCD or ACT protocol at home for 20 min, at least 5 days per week, for a total of 6 weeks (at least 30 training days/sessions and at least a total of 10 h for training). The maximum number of training days was 42 training days/sessions (maximum training hours was 14 h). Previous studies have reported the effects on cognitive performance after self-administered training for 8–10 h on average (Wadley et al., 2006; Ball et al., 2013; Wolinsky et al., 2013).

We used an active control group that had the same training period and a similar training setting. To control for the effects of new experiences such as doing cognitive tasks on a new device, we also developed cognitive training games for an active control group. For the active control group, the difficulty levels of the games did not change during the intervention period. Participants simply completed the cognitive training program at the same level throughout the intervention period. Therefore, cognitive training for the active control group was not intensive adaptive training.

The CTCD and ACT training games were played on a TV with a set-top box at home. We provided the set-top box (Hikari BOX, NTT west) and controller, which can be connected to the Internet and enable the user to play a training game (**Figure 2A**[5]). Before the intervention period, the support staff visited each participant's home and set up the set-top box, which already had the CTCD or ACT program installed. Participants received instructions on how to use the set-top box and play the training game. All participants played the training game using their own TV. Training game scores and the training duration time were recorded by the set-top box, and the data were automatically transmitted over the internet to our data server using Secure Sockets Layer (SSL)/Transport Layer Security (TLS). Therefore, we were able to verify whether participants completed the training game according to the planned process. Participants were asked to play the training games alone for about 20 min, at least 5 days per week. They were not allowed to lend the set-box to anyone else. We examined the trajectory of the scores in each game to check whether the participant followed the rule. If someone else other than the participant played the game, a gap between the scores in the current play and that in the previous play was observed. We contacted and asked participants to follow the rules if they did not follow rules. At the end of the training period, participants reported their subjective feelings of satisfaction and enjoyment with the training game on a five-point Likert scale: 1, strongly disagree; 2, disagree; 3, neither agree nor disagree; 4, agree; and 5, strongly agree. They also confirmed that they followed the game rules during the intervention period.

[4]http://www.graphpad.com/quickcalcs/index.cfm

[5]https://www.ntt-west.co.jp/kiki/hikaribox/hb-2000/spec/

Then, we confirmed that none of the participants broke the rules. We administered cognitive tests and checked their emotional states 1 day before and after the 6-week intervention period. The set-top box and controller were returned immediately after the completion of the intervention period.

Cognitive Training Game for Car Driving (CTCD) Group

We developed three driving-related cognitive training games (processing speed, dual attention, and speed prediction) to be played on the TV and set-top box (**Figure 2A**). In the processing speed training, two signs with two numbers were presented on the TV screen. Participants were asked to select the sign with the larger number as quickly as possible (**Figure 2B**). In the case shown in **Figure 3A**, the sign on the right is the correct choice. In the dual attention training (**Figure 2C**), participants were asked to perform two tasks simultaneously. For the first task, the stimulus (pink colored musical notes) were moved along a circle. Participants were asked to push the button if the stimulus hid behind the mark (orange circle with yellow star). For the second task, when a target (human or obstacle, see **Figure 2D**) was approaching, participants were asked to push the button as quickly as possible if the target was human but not push the button if the target was an obstacle. In the speed prediction training (**Figure 2E**), a target (vehicle or non-vehicle, **Figure 2F**) moved through the wall from left to right on the TV screen. Participants were asked to push the button when the target came out from the wall but not push the button if the target was a non-vehicle. After finishing each training game, game performance and time were recorded automatically and transmitted to our data server. Therefore, we were able to check whether participants completed the training game based on the planned process. To increase the effects of CTCD, we used an intensive and adaptive training method. The difficulty (level) of each game increased from level 1 to level 20 based on participants' game performance. To enhance the processing speed elements, we asked the participants to complete all training games as quickly as possible. We developed the cognitive training games to resemble actual driving situations. Participants were asked to play the three training games equally.

Active Control Cognitive Training Game (ACT) Group (Active Control Group)

We developed three active control cognitive training games (select the larger number; draw a line from the largest to the smallest number; play a game of rock, article, scissors) to be played on the TV and set-top box. In the "select the large number" game (**Figure 3A**), participants were asked to select the larger number from two numbers displayed in different font sizes. In the case of **Figure 3A**, the number on the left is the correct answer (26). In the "draw a line from the largest to the smallest number" activity, five numbers were presented. Participants were asked to select the numbers in the descending order (**Figure 3B**). As shown in **Figure 3B**, participants drew a line through numbers 57, 52, 51, 50, to 47. In the game of rock, article, scissors, participants were asked to lose to the hand presented on the computer screen (**Figure 3C**). In the case shown in **Figure 3C**, the correct answer is scissors. To suppress the elements of processing speed in ACT, we set a fixed time for the answers. The game did not proceed if participants answered quickly. We also required the participants to give the correct answers without rushing. The difficulty level of each game did not increase during the intervention period. Participants simply completed the cognitive training at the same level. Therefore, the ACT was not an intensive adaptive training program. After finishing each game, the game performance and time were recorded automatically and were transmitted to our data server. Participants were asked to play the three training games equally. Consequently, each training game took approximately the same amount of time on each training day.

Driving Skill

We measured driving skills using an on-road evaluation test (Nozawa et al., 2015), which was conducted at a driving school in Sendai, Japan. All participants used the same car provided by the driving school. Participants drove a car with a driving school instructor for 20 min. Participants completed the driving routes twice. The driving school has a 1.7 km driving course, which includes several curves, signals, hills, and intersections. It was a non-public course. None of the participants were familiar with the routes. To reduce practice and order effects, we prepared four driving routes. The assignment of the driving routes to test orders (first test at pre-intervention, second test at pre-intervention, first test at post-intervention, and second test at post-intervention) was counterbalanced across participants. Participants did not drive the same route. The driving routes had eight driving goals, including a right turn, a left turn, passing a blind intersection (wherein the driver cannot see or had restricted visibility of the traffic coming down the intersecting road), an intersection requiring the driver to stop, change lanes, passing by a stopped car, curve, and other driving decisions and goals. For each driving route, participants drove three laps in the 1.7 km driving course (total 5.1 km). A driving school instructor was seated silently in the passenger seat during the on-roard evaluation test. During the driving task, the instructor checked participants' driving skill based on the eight driving goals. The on-road evaluation test had 27 checklist items, and the instructor counted behaviors related to the checklist items. The counts on each checklist item were converted into a five-point scale (ranging from 1 to 5). The maximum score was 135, and the minimum score was 27. A higher score represents better driving skills. The driving school instructor had enough experience to evaluate the driving performance of the older adults.

In the pilot study, 20 older adults (mean age = 68.4 years, SD = 2.1) completed the on-road evaluation test twice. The second test was conducted 4 weeks after the first test. The correlation between the two tests was 0.89. The procedure, route, and contents of the car driving evaluation were similar to those of the official car driving test in Japan, indicating that this on-road evaluation test has enough validity.

Cognitive Functions

To examine the effects of CTCD on cognitive functions, we assessed the scores of processing speed, attention, inhibition,

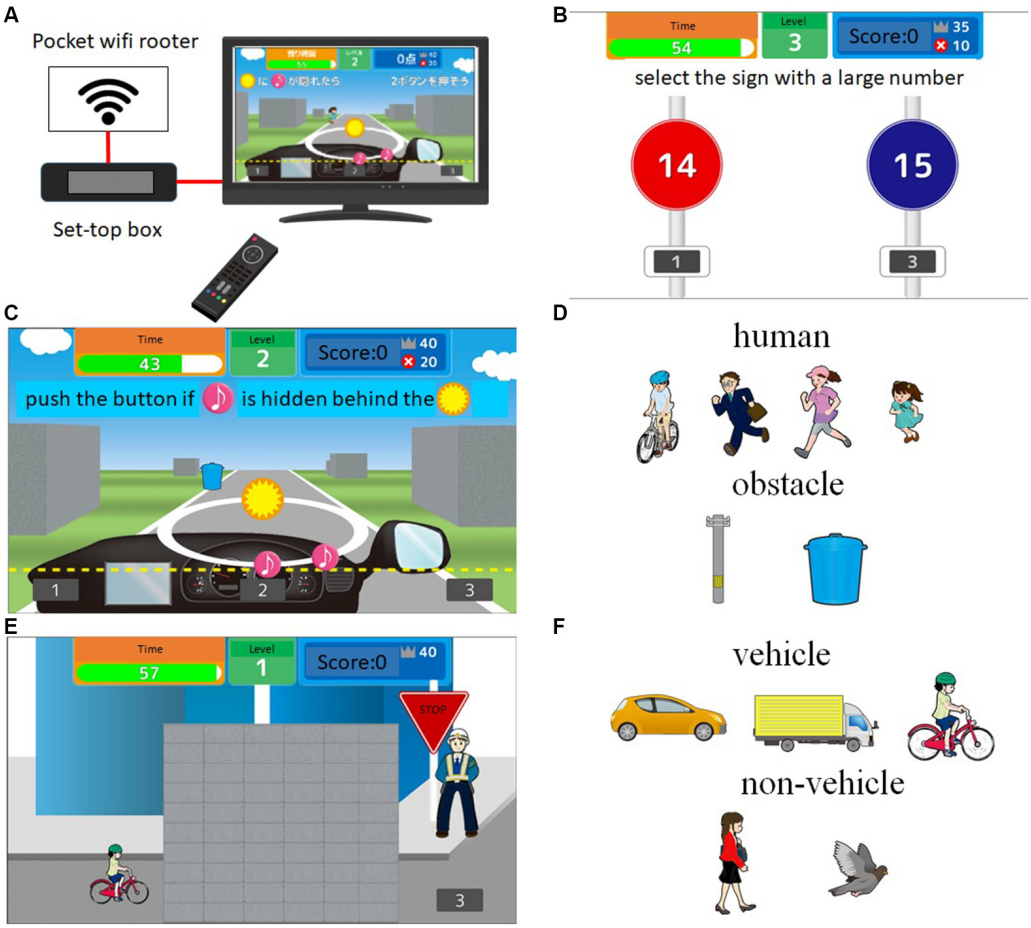


FIGURE 2 | Example of cognitive training games for car driving (CTCD). **(A)** Summary of the intervention setting using the TV and set-top box. (**B**; processing speed training) Two signs with two numbers are presented on the TV screen. Then, participants are asked to select the sign with the large number as quickly as possible **(B)**. In the case shown in **(B)**, the sign on the right is the correct choice. (**C,D**; dual attention training) Participants are asked to perform two tasks simultaneously. For the first task panel **(C)**, the stimulus (pink musical notes) moves along a circle. Then, participants are asked to push the button if the stimulus is hidden behind the mark (orange circle with a yellow star). For the second task, when a target (human or obstacle, see **D**) is approaching, participants are asked to push the button as quickly as possible if the target is human or not push the button if the target is an obstacle. In the case presented in **(C)**, participants do not push the button because the target is an obstacle. (**E**; speed prediction) A target (vehicle or non-vehicle, **F**) is moving through the wall from left to right on TV monitor. Participants are asked to push the button when the target comes out from the wall, but not push the button if the target is a non-vehicle.

short-term memory, working memory, and episodic memory. Cognitive status was measured using the MMSE and the JART. It took about 1.5 h to complete all the cognitive tests.

To briefly check the cognitive status, we used the MMSE, which measures memory, attention, language, and visuospatial abilities (Folstein et al., 1975). To ascertain participants' reading ability and IQ, we used the JART (Matsuoka et al., 2006), which is a Japanese version of the National Adult Reading Test (NART). The JART is a reading test consisting of 25 *Kanji* (Chinese characters) compound words. The reading stimuli were randomly printed for reading. Participants were asked to write the pronunciation of each Kanji compound word.

To assess processing speed, we used digit symbol coding (Cd) and symbol search (SS) from the WAIS-III (Wechsler, 1997). The following descriptions of Cd and SS were reproduced from our earlier report (Nouchi et al., 2012b). "For Cd, the participants were shown a series of symbols that were paired with numbers. Using a key within a 120 s time limit, participants drew each symbol under its corresponding number. The primary measure of this test was the number of correct answers. In SS, participants visually scanned two groups of symbols (a target group and a search group) and indicated whether either of the target symbols matched any symbol in the search group. Participants responded to as many items as possible within a 120 s time limit. The primary measure of this test was the number of correct answers."

To measure attention performance, we conducted the digit cancellation task (D-CAT). The following descriptions of the D-CAT are reproduced from our earlier report (Nouchi et al., 2013). "The test sheet consists of 12 rows of 50 digits. Each row contains five sets of numbers 0–9 arranged in random order. Thus, any one digit would appear five times in each row with randomly determined neighbors. The D-CAT consists of three

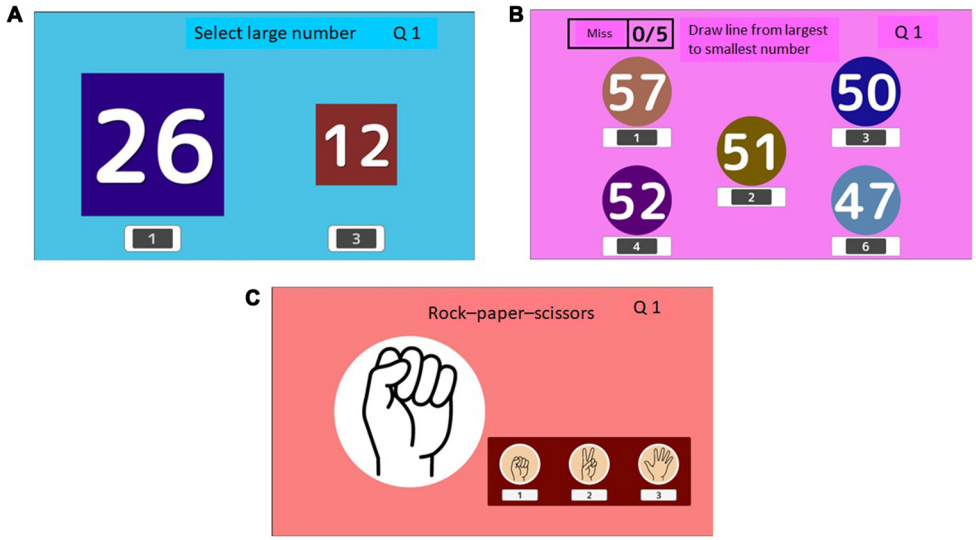


FIGURE 3 | Example of active control cognitive training (ACT). (**A**; select large number) Participants are asked to select the larger number from two numbers presented in different font sizes. In the case shown in **(A)**, the number on the left is the correct answer (26). (**B**; draw a line from the largest to the smallest number) Five numbers are presented. Participants selected numbers in the descending order panel **(B)**. In the case shown in **(B)**, the participant drew a line through numbers 57, 52, 51, 50, and 47. (**C**; rock, paper, scissors) Participants are asked to lose to the hand presented on the computer screen **(C)**. In the case shown in **(C)**, the correct answer is scissors.

such sheets. Participants were instructed to search for the target number(s) that had been specified to them and to delete each one with a slash mark as quickly and as accurately as possible until the experimenter sent a stop signal. Three trials were conducted, first with a single target number (6), second with two target numbers (9 and 4), and third with three (8, 3, and 7). Each trial was given 1 min. Consequently, the total time required for D-CAT was 3 min. For the second and third trials, it was emphasized that all instructed target numbers should be canceled without omission. The primary measure of this test was the number of hits (correct answers). We used only the number of hits in the first trial."

To measure inhibition ability of executive functions, we used a Stroop task (ST) and a reverse Stroop task [rST; Hakoda and Watanabe, 2004; "In the ST, in the leftmost of six columns, a word naming a color was printed in another color (e.g., 'red' was printed in blue letters)]; the other five columns contain words naming colors. Participants were required to check the column containing the word naming the color of the word in the leftmost column. In the reverse ST, in the leftmost of six columns, a word naming a color was printed in another color (e.g., 'red' was printed in blue letters); the other five columns were filled respectively, with five different colors, from which participants were required to check the column with the color matching the written word in the leftmost column. In each task, participants were instructed to complete as many of these exercises as possible in 1 min. The primary measure for this task was the number of correct items" (Nouchi et al., 2012b).

To measure short-term memory and working memory performance, we used the digit span forward (DS-F) and the digit span backward (DS-B) tasks, which are the subtests of the WAIS-III (Wechsler, 1997). The following descriptions of the DS-F and the DS-B are reproduced from our earlier report (Nouchi et al., 2012b). "For the DS-F, participants repeated numbers in the same order as they were read aloud by the examiner. For the DS-B, participants repeated numbers in the reverse order of that presented aloud by the examiner. In both tasks, the examiner read a series of number sequences which the participant was required to repeat in either forward or reverse order." The primary measures of this test were the digit number length. The maximum digit number length in the DS-F was 8, and that of the DS-B was 7.

To measure episodic memory, we used the logical memory (LM) subtest of the WMS-R (Wechsler, 1987): "LM consists of two short-paragraph-length stories (Story A and Story B). For the LM activity, participants were required memorize one of the two stories. The stories were scored in terms of the number of story units recalled, as specified in the WMS-R scoring protocol. We used either Story A or Story B. The primary measure for this task was the number of correct story units recalled" (Nouchi et al., 2012b). We checked the performances of immediate recall and delayed recall memory.

Emotional State Measure

To assess the change in emotional state, we used a short version of the Profile of Mood State Second Edition (POMS2; Heuchert and McNair, 2012; Yokoyama and Watanabe, 2015). The POMS2 has 35 items rated on a 5-point scale. These items are divided into POMS, which has seven subscales with 5-point scales (total 35 items). The POMS2 can measure the following emotional states in the prior week: Tension–Anxiety (T–A), Depression–Dejection (d),

Anger–Hostility (A–H), Vigor–Activity (V), Fatigue–Inertia (F–I), Confusion–Bewilderment (c), and Friendliness (F).

Analysis

All participants were included based on the intention to treat (ITT) principle. First, we calculated the change scores in driving skills, cognitive functions, and emotional states (post-intervention score minus pre-intervention score). Second, we imputed missing data using the multiple imputation method (predictive mean matching, $m = 20$). All variables of the pre-, post-, and change scores and participants' physical information (age, sex) were included in the data imputation process. We performed multiple imputations using the function of "mice" in the mice package (van Buuren and Groothuis-Oudshoorn, 2011). Third, using all 20 imputed datasets, we performed an ANCOVA with permutation tests for all change scores. We performed permutation tests because they are suitable for small sample analysis and are distributed freely. We used a one-tailed test because we had a strong hypothesis that the CTCD program would have more positive effects than would the ACT program. In the ANCOVA, the change score was considered as the dependent variable. The group was considered as the independent variable. The pre-scores in the dependent variable, MMSE, age, and sex were used as covariates. All ANCOVAs with the permutation test were performed using the "aovp" function in the lmPerm package[6]. Fourth, we combined/pooled the F values from all results of the 20 imputed datasets using the "micombine.F" function in the "miceadds" package[7]. The method of pooling the F Value was based on previous studies (van Buuren and Groothuis-Oudshoorn, 2011; Grund et al., 2016). Finally, we used false discovery rate (FDR) correction methods to adjust all pooled p values (Benjamini and Hochberg, 2000). All analyses were performed using software (R ver. 3.50).

For the additional analysis, if we detected improvement in driving skill, we performed a permutation multiple regression analysis of improvements in driving skills, cognitive functions, and mood. The additional analysis was intended to elucidate the relationship between changes in driving skill and changes in cognitive functions, and changes in moods. The pre-score in the dependent variable, MMSE score at baseline, age, and sex were used as covariates. After scaling all imputed datasets using the "mids2datlist" and "scale_datlist" functions in the "miceadds" package, we performed permutation multiple regression analyses using the "lmp" function in lmperm package. Finally, we combined/pooled all results from the 20 imputed datasets using Rubin's rule (Barnard and Rubin, 1999; Rubin, 2004). Significance was inferred for $p < 0.05$ for multiple comparison methods.

RESULTS

There was no significant difference in the average number of training days between CTCD ($M = 32.04$ days, SD = 2.25) and ACT ($M = 31.57$ days, SD = 1.93) groups and in the total training hours between CTCD ($M = 10.61$ h, SD = 1.19) and ACT ($M = 10.17$ h, SD = 1.03) groups. The maximum game levels for the CTCD group were Game 1 ($M = 18.46$, SD = 3.50), Game 2 ($M = 13.96$, SD = 6.42), and Game 3 ($M = 11.38$, SD = 3.63). We evaluated participants' satisfaction and enjoyment after the intervention using a five-point scale. There was no significant difference in the average satisfaction scores between CTCD ($M = 3.83$, SD = 0.54) and ACT ($M = 3.78$, SD = 0.43) groups or and in enjoyment scores between CTCD ($M = 3.92$, SD = 0.71) and ACT ($M = 3.88$, SD = 0.83) groups. There was no significant difference between the two groups with respect to the measures at baseline (**Table 1**). The cognitive functions and emotional states after the intervention period was shown in **Supplementary Table S2**.

Three participants in the CTCD group and two in the ACT group dropped out during the intervention period because of their respective schedules. By the intention-to-treat rule, we imputed missing values of the five participants (please see "Analysis" section). To check the benefits of CTCD on driving skills, cognitive functions, and emotional state, we performed a permutation test with ANCOVAs for the change scores (**Tables 2**, **3**). In terms of driving skill measures, the CTCD group showed a significant improvement ($F_{(1,469.88)} = 7.987$, $\eta^2 = 0.08$, *adjusted* $p = 0.034$). Regarding cognitive abilities, the CTCD group showed a significant improvement in processing speed performance with respect to the Cd score ($F_{(1,567.29)} = 5.161$, $\eta^2 = 0.10$, *adjusted* $p = 0.047$) and the SS score ($F_{(1,678.65)} = 6.63$, $\eta^2 = 0.09$, *adjusted* $p = 0.049$). Furthermore, the CTCD group showed a significant improvement in inhibition performance with respect to the rST score ($F_{(1,8006.18)} = 5.061$, $\eta^2 = 0.06$, *adjusted* $p = 0.043$) and the ST score ($F_{(1,2360.02)} = 3.945$, $\eta^2 = 0.06$, *adjusted* $p = 0.042$). Regarding the emotional state, the CTCD group showed improvement in the V–A score in POMS ($F_{(1,655.54)} = 4.46$, $\eta^2 = 0.06$, *adjusted* $p = 0.041$). There was no significant difference in other measures. In summary, the CTCD group demonstrated improvements in driving skills, two cognitive domains (processing speed and inhibition), and emotional state (vigor–activity).

Additionally, to investigate the relationships among these improved skills, we separately performed multiple regression analyses for the CTCD and ACT groups. The covariates were age, sex, MMSE at baseline, and the pre-score on the dependent variable. The results of the CTCD group showed a significant positive correlation between improved driving skill and improved Cd score (*standardized* $\beta = 0.55$, $t = 2.76$, $p = 0.01$) and between improvements in driving skills and SS score (*standardized* $\beta = 0.63$, $t = 3.65$, $p = 0.00$). There was no significant correlation between the improved driving skills or other improvements. Moreover, no significant result was found in the multiple regression analyses of the ACT group.

DISCUSSION

We developed new cognitive training games to improve the driving skills in older adults. The cognitive training games can be played on a TV using a set-top box. We investigated the benefits of CTCD on driving skills, cognitive functions, and emotional

[6]http://cran.r-project.org/web/packages/lmPerm/index.html
[7]https://github.com/alexanderrobitzsch/miceadds

TABLE 2 | Cognitive function and emotional states scores of both groups at baseline.

	CTCD group		ACT group			
	Mean	SD	Mean	SD	Effect size (*d*)	*p*-value
Processing speed						
Cd (number)	61.48	10.36	58.07	10.70	1.05	0.24
SS (number)	30.81	2.94	29.18	5.13	0.81	0.15
Executive functions (inhibition)						
rST (number)	38.85	5.43	36.14	5.75	1.15	0.08
ST (number)	27.41	7.63	23.93	8.02	1.24	0.11
Short-term memory						
DS-F (digit number)	5.30	1.03	5.18	1.12	0.11	0.69
Working memory						
DS-B (digit number)	4.04	1.13	3.89	0.83	0.15	0.59
Attention						
D-CAT (number)	4.04	1.13	3.89	0.83	0.15	0.59
Episodic memory						
LM immediate (score)	8.74	4.13	9.07	3.53	0.17	0.75
LM delay (score)	8.26	3.90	8.54	3.71	0.14	0.79
Emotional states						
T–A in POMS (score)	4.22	2.36	4.00	3.15	0.13	0.77
D in POMS (score)	1.67	1.98	1.93	2.16	0.18	0.64
A–H in POMS (score)	2.30	2.25	1.68	1.93	0.43	0.28
V in POMS (score)	8.59	4.00	7.64	3.49	0.49	0.35
F_I in POMS (score)	8.59	4.00	7.64	3.49	0.49	0.35
C in POMS (score)	2.41	1.97	2.50	2.40	0.06	0.88
F in POMS (score)	12.00	4.45	11.04	3.83	0.47	0.39

states of healthy older people. Our study revealed three main findings. First, the results of the on-road evaluation tests showed that the 6-week CTCD intervention improved participants' driving skills. Additionally, we found a significant positive correlation between the change scores of driving skills and the change scores of processing speed performance. Second, the CTCD group demonstrated improved cognitive performances in terms of processing speed measured by Cd and SS and inhibition performance measured by ST and rST compared to ACT. Third, CTCD showed improvement in vigor–activity mood, measured using the POMS. Taken together, the results of this study extend those of previous studies by demonstrating improvements in driving skills, cognitive functions, and emotional states. These findings are discussed below.

The first main finding is that the CTCD program improved the driving skills of healthy older adults. Our result is consistent with those of previous studies using cognitive training for driving skill (Edwards et al., 2009b; Haeger et al., 2018; Ross et al., 2018). For example, an 8-week cognitive training in a laboratory showed improvement in driving skills of the older adults (Nozawa et al., 2015). A unique aspect of the present study is that our cognitive training programs were conducted with a TV at home. Almost all cognitive training for driving skills was conducted at the laboratory with trainers or using specific devices (Casutt et al., 2014a, 2016; Nozawa et al., 2015; Haeger et al., 2018). This suggests that driving skills in older adults can be improved through cognitive training both at home and at the laboratory. It is important to note the significance of the change in scores of the car driving performance. Car driving skills were measured by an on-road evaluation test. A higher score indicates higher car driving skills. The average score was 113 (84%) out of 135, indicating that our participants had good car driving skills. The effect size (η^2) was 0.08, indicating that our intervention had a medium effect for improvements of car driving skills in healthy older adults. However, it does not mean that individuals who scored high in this car driving test are safe drivers. From the current result, it is difficult to conclude that the cognitive training can improve car driving safety. Therefore, in the future, it is important to examine whether cognitive training reduces car accidents. This study did not verify the long-term benefits after cognitive training. We measured driving skills immediately after the training period. Therefore, we demonstrated immediate training effects of cognitive training on driving skills. Our short-term cognitive training might not have long-term benefits for driving skills. To clarify the current results, future studies should assess long-term benefits using follow up assessments for several years. It is particularly interesting that we found a positive correlation between improvements of driving skills and improvements of cognitive functions, which suggest that changes in driving skills and cognitive functions share the same mechanism of performance improvement, which is discussed later.

The second main result of the present study is that the CTCD program improved the processing speed and inhibition of executive functions. For processing speed, previous studies of cognitive training for driving also showed significant improvement in processing speed, measured using Cd (Hay et al., 2016), simple reaction time (Casutt et al., 2014b), and the composite processing speed score, which combines the TMT-A and Cd scores (Nozawa et al., 2015). Supporting the existing evidence, we found improvement in processing speed as measured by Cd. Additionally, we expanded the existing evidence for improvement in processing speed,

TABLE 3 | Change scores of cognitive function and emotional states of both groups.

	CTCD group		ACT group				
	Mean	SD	Mean	SD	Effect size (η^2)	adjusted *p*-value	non-adjusted *p*-value
Car driving skill							
On-road evaluation	7.07	11.21	0.10	9.89	0.08	0.034	0.002
Processing speed							
Cd (number)	4.48	4.64	0.68	5.56	0.10	0.049	0.010
SS (number)	3.15	3.16	0.79	3.67	0.09	0.047	0.007
Executive functions (inhibition)							
rST (number)	4.00	4.88	0.89	5.01	0.06	0.043	0.015
ST (number)	2.70	3.23	0.29	4.48	0.06	0.042	0.012
Short-term memory							
DS-F (digit number)	0.00	1.39	0.29	1.01	0.02	0.273	0.156
Working memory							
DS-B (digit number)	0.33	0.96	0.21	0.99	0.00	0.323	0.369
Attention							
D-CAT (number)	12.37	25.13	11.14	17.79	0.00	0.314	0.381
Episodic memory							
LM immediate (score)	0.48	3.32	−0.18	2.84	0.01	0.293	0.209
LM delay (score)	0.33	3.06	−0.32	3.32	0.01	0.277	0.218
Emotional states							
T–A in POMS (score)	0.37	2.24	−0.04	2.20	0.01	0.290	0.269
D in POMS (score)	0.15	2.21	0.07	2.39	0.00	0.327	0.420
A–H in POMS (score)	0.30	1.49	−0.07	2.00	0.01	0.286	0.286
V in POMS (score)	2.59	3.20	0.71	3.22	0.06	0.041	0.018
F_I in POMS (score)	−0.19	2.62	−0.86	1.96	0.02	0.316	0.143
C in POMS (score)	0.44	1.91	−0.07	2.34	0.01	0.305	0.262
F in POMS (score)	−0.59	2.45	0.07	2.79	0.01	0.316	0.203

measured by SS. Nevertheless, it remains unclear whether cognitive training can improve other processing speed measures, such as UFOV. Previous studies have demonstrated that cognitive training for driving skills improves UFOV (Roenker et al., 2003). To generalize our findings, it is important to assess cognitive training improvements in UFOV, if any.

Regarding executive functions, previous studies using cognitive training for driving skills also showed improvements in shifting performance of executive functions, measured using Trail Making Test B (Hay et al., 2016). Our results are the first to demonstrate an improvement in the inhibition of executive functions, measured using ST and rST, after a 6-week cognitive training. It is noteworthy that a previous study using cognitive training for driving skills (Casutt et al., 2016) failed to demonstrate an improved inhibition performance, measured by ST. Several methodological differences exist between the current study and the previous study (Casutt et al., 2016). For example, although the previous study's cognitive training program focused on only a single cognitive domain such as attention, our cognitive training focused on multiple cognitive domains, such as processing speed, inhibition, and attention. previous studies have reported that multiple domain training has more benefits than does single-domain training (Cheng et al., 2012). The evaluation of the performance on the ST differed between the current study (the number of correct responses) and the previous study (reaction time). Additionally, we used the article version of the ST, whereas the previous study used the PC version of ST along with EEG recordings. These methodological differences might affect the inconsistent results. Future studies should use similar administration and scoring methods for the ST.

The CTCD group in the present study showed no significant improvement in attention or memory performance. These results are consistent with those of previous studies using short-term cognitive training for driving skills. For attention performance, previous studies reported no significant improvement in sustained, selective, or divided attention performance (Casutt et al., 2014b). However, a long-term intervention study using cognitive training for driving skills found improvement in the performance of choice reaction time (Roenker et al., 2003). One possibility is that the 6-week intervention period is insufficient to improve the attention performance. For memory performance, a previous study measured short-term memory using the Benton Visual Retention test, working memory using the spatial span test, and episodic memory using the Rey auditory–verbal learning test (Nozawa et al., 2015). The study found no significant improvement in the composite memory score included all memory performance (Nozawa et al., 2015). In keeping with the findings of previous studies, we found no significant improvements in other memory measures, such as the digit span for short-term and working memory performance and LM for episodic memory. An explanation for this finding is that memory was not targeted in our cognitive training. In addition, previous studies of cognitive training for driving skills did not specifically use memory measures. A few studies have measured memory performance. Therefore, we cannot infer that cognitive training for driving skills has no beneficial effects on memory performance. Future studies should be conducted to measure multiple memory performance better. It will be possible to verify

the benefits of cognitive training on driving skills in terms of memory performance.

Improvements in driving skills, processing speed, and inhibition are explained by the overlapping hypothesis (Nouchi and Kawashima, 2014; Nouchi et al., 2016a). The overlapping hypothesis assumes that driving skills and cognitive functions will improve with cognitive training when training tasks and untrained measures share common mental processes (Nouchi et al., 2016a). Based on the Cattel–Horn–Carroll (CHC) model (Schneider and McGrew, 2012), behavioral and mental processes can be divided into three levels, narrow, broad, and general abilities: "the broad ability of processing speed consists of several narrow abilities such as perceptual speed, rate of test-taking, number facility, reading speed, and writing speed" (Nouchi et al., 2016a). In fact, driving skill is a general ability that includes some narrow and broad abilities. Previous studies have reported that driving skills are correlated with several cognitive functions such as processing speed (Anstey et al., 2005; Horikawa et al., 2009; Adrian et al., 2011). Therefore, we infer that driving skills consist of several broad abilities, such as cognitive processing and executive functions. In this study, participants were asked to complete three training tasks (processing speed, dual attention, and speed prediction) during the 6-week intervention period. All training tasks involved processing speed and inhibition components. For example, we asked participants to complete the training games as quickly as possible. Therefore, processing speed is a common factor in the completion of all training tasks. Inhibition processes also play an important role in selecting appropriate targets of the processing speed game, by ignoring distractors in the dual attention game, pushing the button at an appropriate timing in the speed prediction game, and following the appropriate rules in each training game. Based on the overlapping hypothesis, improvements in driving skill, processing speed, and inhibition performance are sufficient to explain the following notion. Training games require mental processes including processing speed and inhibition components. The cognitive training game, the measurement of driving skills, and the measurements of cognitive functions shared similar mental process. Mental processes related to processing speed and inhibition were used in the training games. Although playing cognitive training games, these mental processes are expected to be facilitated and enhanced. Therefore, processing speed and inhibition were improved directly because all gaming tasks required processing speed and inhibition processes. Furthermore, driving skills improved because processing speed and inhibition processes were involved in the driving skills.

The third main finding of the present study is the improvement in vigor mood in POMS. A previous study demonstrated that long-term processing speed training for healthy older adults leads to reduction of depressive symptoms, measured by the Center for Epidemiologic Studies Depression Scale (CES-D; Wolinsky et al., 2009). A short-term cognitive training study of healthy older adults showed a change in depressive mood, measured by the POMS (Nouchi et al., 2016a). However, our study showed no depressive mood reduction. One explanation for this might be that our participants reported lower depressive scores at baseline (the average depression score was less than 2), indicating that our participants did not experience depressive symptoms. Therefore, we found no reduction in the depressive mood in this study. However, this study is the first to demonstrate an improvement in vigor mood in healthy older adults after a 6-week cognitive training for driving skills.

A potential mechanism underlying improved vigor mood might be that the cognitive training functioned as emotional regulation. A previous study demonstrated that cognitive tasks reduce negative emotions (Iida et al., 2011, 2012). A neuroimaging study using cognitive training also found that brain activity in the insula for emotional stimuli was reduced during the 4-week cognitive training (Takeuchi et al., 2014). In addition, several previous studies reported that playing video games can induce a positive mood (Russoniello et al., 2009; Granic et al., 2014; Pallavicini et al., 2018). Based on these findings, we infer that cognitive training might induce the person to ignore negative experiences and experience a positive mood in the cognitive training. Participants in this study experienced positive emotions during the training because the difficulties in the cognitive training games changed their game performance. Therefore, vigor emotion increased following the cognitive training intervention. To generalize these findings, we need to apply the same intervention to other populations (young adults or a clinical population). Moreover, if we could use a neuroimaging technique, such as functional magnetic resonance imaging (fMRI), we can provide new insights into the neural basis of emotional states after cognitive training.

Our study has some advantages over previous studies. First, to familiarize the general public with cognitive training, developing user-friendly cognitive training tools is a key step. In this study, we developed the cognitive training games to be played on a TV and then presented scientific evidence. Cognitive training using a TV is an effective and easy approach to conduct cognitive training for older people at any time because they usually own a TV at home and know how to operate it. Therefore, we believe that our study provides a new, useful, and effective tool for cognitive training. Second, we controlled for the effect of performing a regular cognitive task using active control games. In these active control games, participants were asked to do three cognitive training games that included some cognitive functions. However, the level of difficulty of the game did not change, suggesting that doing multiple cognitive training is not important to improve cognitive performance. An intensive and adaptive approach, whereby the task difficulty changes based on participants' performance, provides more key elements to improve the performance. To elucidate the key elements of the CTCD, future studies should be conducted with RCT using non-adaptive CTCD as an active control group.

This study has some limitations. First, this study investigated whether a 6-week short-term training can improve driving skills, cognitive functions, and emotional states of healthy older people. A cognitive training study of a large sample demonstrated that the benefits of 1-year processing speed training program were

observed for 5 years (Willis et al., 2006). It is, therefore, important to investigate whether the benefits of this intervention program are lasting. Second, we measured driving skills based on driving performance at a driving school. This method has several salient benefits. For example, it can readily control traffic conditions and measure basic driving skills. Therefore, we can measure driving skills in the same situation for all participants. However, it is also important to check driving behaviors in everyday situations. Future studies should measure driving skills during several situations, such as a traffic jam or a bad weather condition or during night-time driving. Generalizing the effects of a short-term cognitive training program on driving skills requires further verification of the benefits of the short-term processing speed training on long-lasting effects and everyday driving behaviors. Finally, we only evaluated the benefits of cognitive training on car driving skills, cognitive function, and emotional state. However, it remains unclear whether our cognitive training program can improve car driving safety in older adults, which can reduce car accidents among older adults. Future studies should investigate these important issues.

CONCLUSION

In conclusion, we developed a TV-based CTCD program and conducted an RCT to assess the benefits of a 6-week CTCD on driving skills, cognitive functions, and mood states in older car drivers. Our study provides scientific evidence that the CTCD program improves driving skills, processing speed, inhibition performance, and vigor–activity mood. Our results extend previous findings which demonstrated the benefits of cognitive training on driving skills, processing speed, inhibition, and vigor–activity mood in older people.

ETHICS STATEMENT

Ethical approval was provided by the Institutional Review Board of the Tohoku University Graduate School of Medicine (2017-2-209-1). This study was conducted according to the principles outlined in the Declaration of Helsinki. Written informed consent was received from each participant.

AUTHOR CONTRIBUTIONS

RN designed and developed the study protocol and analyzed all data. RN, AK, and HN conducted the study. RN wrote the manuscript with AK, HN, and RK. RK provided advice related to the study protocol. All authors have read and approved the final manuscript.

FUNDING

This study, supported by Sendai Television Incorporated, was an industry–academic collaboration of Tohoku University. This study is also supported by JSPS Leading Initiative for Excellent Young Researchers (LEADER) program and JSPS KAKENHI Grant Number 15H05366 [Grant-in-Aid for Young Scientists (A)], 17H06046 (Grant-in-Aid for Scientific Research on Innovative Areas) and 16KT0002 [Grant-in-Aid for Scientific Research (B)]. None of the funding sources had any involvement in the study design, collection, analysis, interpretation of data, or the writing of article.

ACKNOWLEDGMENTS

We are grateful to the instructors from Ohu Driving School for conducting the on-road driving evaluation and to Mr Ota, Mr Suzuki, and Ms Ishihara from Sendai Television Incorporated for supporting this project. They had no involvement in the study design, data collection, analysis, or reporting of the results.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2019.00099/full#supplementary-material

REFERENCES

Adrian, J., Postal, V., Moessinger, M., Rascle, N., and Charles, A. (2011). Personality traits and executive functions related to on-road driving performance among older drivers. *Accid. Anal. Prev.* 43, 1652–1659. doi: 10.1016/j.aap.2011.03.023

Anstey, K. J., Horswill, M. S., Wood, J. M., and Hatherly, C. (2012). The role of cognitive and visual abilities as predictors in the multifactorial model of driving safety. *Accid. Anal. Prev.* 45, 766–774. doi: 10.1016/j.aap.2011.10.006

Anstey, K. J., Wood, J., Lord, S., and Walker, J. G. (2005). Cognitive, sensory and physical factors enabling driving safety in older adults. *Clin. Psychol. Rev.* 25, 45–65. doi: 10.1016/j.cpr.2004.07.008

Ball, K. K., Beard, B. L., Roenker, D. L., Miller, R. L., and Griggs, D. S. (1988). Age and visual search: expanding the useful field of view. *J. Opt. Soc. Am. A* 5, 2210–2219. doi: 10.1364/josaa.5.002210

Ball, K. K., Berch, D. B., Helmers, K. F., Jobe, J. B., Leveck, M. D., Marsiske, M., et al. (2002). Effects of cognitive training interventions with older adults: a randomized controlled trial. *JAMA* 288, 2271–2281. doi: 10.1001/jama.288.18.2271

Ball, K. K., Ross, L. A., Roth, D. L., and Edwards, J. D. (2013). Speed of processing training in the ACTIVE study: how much is needed and who benefits? *J. Aging Health* 25, 65S–84S. doi: 10.1177/0898264312470167

Barnard, J., and Rubin, D. B. (1999). Miscellanea. Small-sample degrees of freedom with multiple imputation. *Biometrika* 86, 948–955. doi: 10.1093/biomet/86.4.948

Benjamini, Y., and Hochberg, Y. (2000). On the adaptive control of the false discovery rate in multiple testing with independent statistics. *J. Educ. Behav. Stat.* 25, 60–83. doi: 10.3102/10769986025001060

Boot, W. R., Stothart, C., and Charness, N. (2014). Improving the safety of aging road users: a mini-review. *Gerontology* 60, 90–96. doi: 10.1159/000354212

Boyle, G. J. (1989). Sex differences in reported mood states. *Pers. Individ. Dif.* 10, 1179–1183. doi: 10.1016/0191-8869(89)90083-4

Casutt, G., Martin, M., and Jäncke, L. (2016). Driving simulator training is associated with reduced inhibitory workload in older drivers. *Geriatrics* 1:16. doi: 10.3390/geriatrics1030016

Casutt, G., Martin, M., Keller, M., and Jäncke, L. (2014a). The relation between performance in on-road driving, cognitive screening and driving simulator in older healthy drivers. *Transp. Res. Part F Traffic Psychol. Behav.* 22, 232–244. doi: 10.1016/j.trf.2013.12.007

Casutt, G., Theill, N., Martin, M., Keller, M., and Jäncke, L. (2014b). The drive-wise project: driving simulator training increases real driving performance in healthy older drivers. *Front. Aging Neurosci.* 6:85. doi: 10.3389/fnagi.2014.00085

Cheng, Y., Wu, W., Feng, W., Wang, J., Chen, Y., Shen, Y., et al. (2012). The effects of multi-domain versus single-domain cognitive training in non-demented older people: a randomized controlled trial. *BMC Med.* 10:30. doi: 10.1186/1741-7015-10-30

Chliaoutakis, J. E., Demakakos, P., Tzamalouka, G., Bakou, V., Koumaki, M., and Darviri, C. (2002). Aggressive behavior while driving as predictor of self-reported car crashes. *J. Safety Res.* 33, 431–443. doi: 10.1016/s0022-4375(02)00053-1

Clay, O. J., Wadley, V. G., Edwards, J. D., Roth, D. L., Roenker, D. L., and Ball, K. K. (2005). Cumulative meta-analysis of the relationship between useful field of view and driving performance in older adults: current and future implications. *Optom. Vis. Sci.* 82, 724–731. doi: 10.1097/01.opx.0000175009.08626.65

Cuenen, A., Jongen, E. M. M., Brijs, T., Brijs, K., Lutin, M., Van Vlierden, K., et al. (2015). Does attention capacity moderate the effect of driver distraction in older drivers? *Accid. Anal. Prev.* 77, 12–20. doi: 10.1016/j.aap.2015.01.011

Dickerson, A. E., Molnar, L. J., Eby, D. W., Adler, G., Bédard, M., Berg-Weger, M., et al. (2007). Transportation and aging: a research agenda for advancing safe mobility. *Gerontologist* 47, 578–590. doi: 10.1093/geront/47.5.578

Doroudgar, S., Chuang, H. M., Perry, P. J., Thomas, K., Bohnert, K., and Canedo, J. (2017). Driving performance comparing older versus younger drivers. *Traffic InJ. Prev.* 18, 41–46. doi: 10.1080/15389588.2016.1194980

Dubois, B., Slachevsky, A., Litvan, I., and Pillon, B. (2000). The FAB: a frontal assessment battery at bedside. *Neurology* 55, 1621–1626. doi: 10.1212/wnl.55.11.1621

Edwards, J. D., Lunsman, M., Perkins, M., Rebok, G. W., and Roth, D. L. (2009a). Driving cessation and health trajectories in older adults. *J. Gerontol. A Biol. Sci. Med. Sci.* 64, 1290–1295. doi: 10.1093/gerona/glp114

Edwards, J. D., Myers, C., Ross, L. A., Roenker, D. L., Cissell, G. M., McLaughlin, A. M., et al. (2009b). The longitudinal impact of cognitive speed of processing training on driving mobility. *Gerontologist* 49, 485–494. doi: 10.1093/geront/gnp042

Faul, F., Erdfelder, E., Buchner, A., and Lang, A.-G. (2009). Statistical power analyses using G* Power 3.1: tests for correlation and regression analyses. *Behav. Res. Methods* 41, 1149–1160. doi: 10.3758/brm.41.4.1149

Folstein, M. F., Folstein, S. E., and McHugh, P. R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* 12, 189–198.

Garrity, R. D., and Demick, J. (2001). Relations among personality traits, mood states, and driving behaviors. *J. Adult Dev.* 8, 109–118. doi: 10.1023/A:1026446002317

Granic, I., Lobel, A., and Engels, R. C. M. E. (2014). The benefits of playing video games. *Am. Psychol.* 69, 66–78. doi: 10.1037/a0034857

Grund, S., Lüdtke, O., and Robitzsch, A. (2016). Pooling ANOVA results from multiply imputed datasets. *Methodology* 12, 75–88. doi: 10.1027/1614-2241/a000111

Haeger, M., Bock, O., Memmert, D., and Hüttermann, S. (2018). Can driving-simulator training enhance visual attention, cognition and physical functioning in older adults? *J. Aging Res.* 2018:7547631. doi: 10.1155/2018/7547631

Hakoda, Y., and Watanabe, M. (2004). *Manual for New Stroop Test II.* Fukuoka, Japan: Toyo Physical.

Hay, M., Adam, N., Bocca, M.-L., and Gabaude, C. (2016). Effectiveness of two cognitive training programs on the performance of older drivers with a cognitive self-assessment bias. *Eur. Transp. Res. Rev.* 8:20. doi: 10.1007/s12544-016-0207-7

Heuchert, J. P., and McNair, D. M. (2012). *POMS-2 Manual: A Profile of Mood States.* 2nd Edn. North Tonawanda, NY: Multi-Health Systems Inc.

Horgas, A. L., Wilms, H. U., and Baltes, M. M. (1998). Daily life in very old age: everyday activities as expression of successful living. *Gerontologist* 38, 556–568. doi: 10.1093/geront/38.5.556

Horikawa, E., Morizono, R., Koga, A., and Horie, J. (2009). Elderly driving behvior and cognitive functions. *IATSS Res.* 33, 18–26. doi: 10.1016/s0386-1112(14)60233-8

Iida, S., Nakao, T., and Ohira, H. (2011). Implicit attenuation of subsequent emotion by cognitive activity. *Cogn. Affect. Behav. Neurosci.* 11, 476–484. doi: 10.3758/s13415-011-0045-y

Iida, S., Nakao, T., and Ohira, H. (2012). Prior cognitive activity implicitly modulates subsequent emotional responses to subliminally presented emotional stimuli. *Cogn. Affect. Behav. Neurosci.* 12, 337–345. doi: 10.3758/s13415-012-0084-z

Karthaus, M., Falkenstein, M., Karthaus, M., and Falkenstein, M. (2016). Functional changes and driving performance in older drivers: assessment and interventions. *Geriatrics* 1:12. doi: 10.3390/geriatrics1020012

Li, G., Braver, E. R., and Chen, L.-H. (2003). Fragility versus excessive crash involvement as determinants of high death rates per vehicle-mile of travel among older drivers. *Accid. Anal. Prev.* 35, 227–235. doi: 10.1016/s0001-4575(01)00107-5

Lombardi, D. A., Horrey, W. J., and Courtney, T. K. (2017). Age-related differences in fatal intersection crashes in the United States. *Accid. Anal. Prev.* 99, 20–29. doi: 10.1016/j.aap.2016.10.030

Ma, L., and Yan, X. (2014). Examining the nonparametric effect of drivers' age in rear-end accidents through an additive logistic regression model. *Accid. Anal. Prev.* 67, 129–136. doi: 10.1016/j.aap.2014.02.021

Marottoli, R. A., Allore, H., Araujo, K. L. B., Iannone, L. P., Acampora, D., Gottschalk, M., et al. (2007). A randomized trial of a physical conditioning program to enhance the driving performance of older persons. *J. Gen. Intern. Med.* 22, 590–597. doi: 10.1007/s11606-007-0134-3

Maruyama, K., and Kitamura, S. (1961). Speed anticopation test: a test for discrimination of accident proneness in motor driver. *Tohoku Psychol. Folia* 20, 13–20.

Masumoto, K., Taishi, N., and Shiozaki, M. (2016). Age and gender differences in relationships among emotion regulation, mood, and mental health. *Gerontol. Geriatr. Med.* 2:2333721416637022. doi: 10.1177/2333721416637022

Matas, N. A., Nettelbeck, T., and Burns, N. R. (2015). Dropout during a driving simulator study: a survival analysis. *J. Safety Res.* 55, 159–169. doi: 10.1016/j.jsr.2015.08.004

Matsuoka, K., Uno, M., Kasai, K., Koyama, K., and Kim, Y. (2006). Estimation of premorbid IQ in individuals with Alzheimer's disease using Japanese ideographic script (Kanji) compound words: japanese version of National Adult Reading Test. *Psychiatry Clin. Neurosci.* 60, 332–339. doi: 10.1111/j.1440-1819.2006.01510.x

Matthews, G., Sparkes, T. J., and Bygrave, H. M. (1996). Attentional overload, stress, and simulate driving performance. *Hum. Perform.* 9, 77–101. doi: 10.1207/s15327043hup0901_5

McAndrews, C., Beyer, K., Guse, C. E., and Layde, P. (2013). Revisiting exposure: fatal and non-fatal traffic injury risk across different populations of travelers in Wisconsin, 2001–2009. *Accid. Anal. Prev.* 60, 103–112. doi: 10.1016/j.aap.2013.08.005

McCarrey, A. C., An, Y., Kitner-Triolo, M. H., Ferrucci, L., and Resnick, S. M. (2016). Sex differences in cognitive trajectories in clinically normal older adults. *Psychol. Aging* 31, 166–175. doi: 10.1037/pag0000070

Mielenz, T. J., Durbin, L. L., Cisewski, J. A., Guralnik, J. M., and Li, G. (2017). Select physical performance measures and driving outcomes in older adults. *InJ. Epidemiol.* 4:14. doi: 10.1186/s40621-017-0110-2

Mundorf, N., and Brownell, W. (1990). Media preferences of older and younger adults. *Gerontologist* 30, 685–691. doi: 10.1093/geront/30.5.685

Nouchi, R., and Kawashima, R. (2014). Improving cognitive function from children to old age: a systematic review of recent smart ageing intervention studies. *Adv. Neurosci.* 2014:235479. doi: 10.1155/2014/235479

Nouchi, R., Saito, T., Nouchi, H., and Kawashima, R. (2016a). Small acute benefits of 4 weeks processing speed training games on processing speed and inhibition performance and depressive mood in the healthy elderly people: evidence from a randomized control trial. *Front. Aging Neurosci.* 8:302. doi: 10.3389/fnagi.2016.00302

Nouchi, R., Taki, Y., Takeuchi, H., Nozawa, T., Sekiguchi, A., and Kawashima, R. (2016b). Reading aloud and solving simple arithmetic calculation intervention (learning therapy) improves inhibition, verbal episodic memory, focus attention and processing speed in healthy elderly people: evidence from a

randomized controlled trial. *Front. Hum. Neurosci.* 10:217. doi: 10.3389/fnhum.2016.00217

Nouchi, R., Taki, Y., Takeuchi, H., Hashizume, H., Akitsuki, Y., Shigemune, Y., et al. (2012a). Brain training game improves executive functions and processing speed in the elderly: a randomized controlled trial. *PLoS One* 7:e29676. doi: 10.1371/journal.pone.0029676

Nouchi, R., Taki, Y., Takeuchi, H., Hashizume, H., Nozawa, T., Sekiguchi, A., et al. (2012b). Beneficial effects of reading aloud and solving simple arithmetic calculations (learning therapy) on a wide range of cognitive functions in the healthy elderly: study protocol for a randomized controlled trial. *Trials* 13:32. doi: 10.1186/1745-6215-13-32

Nouchi, R., Taki, Y., Takeuchi, H., Hashizume, H., Nozawa, T., Kambara, T., et al. (2013). Brain training game boosts executive functions, working memory and processing speed in the young adults: a randomized controlled trial. *PLoS One* 8:e55518. doi: 10.1371/journal.pone.0055518

Nozawa, T., Taki, Y., Kanno, A., Akimoto, Y., Ihara, M., Yokoyama, R., et al. (2015). Effects of different types of cognitive training on cognitive function, brain structure, and driving safety in senior daily drivers: a pilot study. *Behav. Neurol.* 2015:525901. doi: 10.1155/2015/525901

Ostrow, A. C., Shaffron, P., and McPherson, K. (1992). The effects of a joint range-of-motion physical fitness training program on the automobile driving skills of older adults. *J. Safety Res.* 23, 207–219. doi: 10.1016/0022-4375(92)90003-r

Owsley, C., McGwin, G. Jr., Phillips, J. M., McNeal, S. F., and Stalvey, B. T. (2004). Impact of an educational program on the safety of high-risk, visually impaired, older drivers. *Am. J. Prev. Med.* 26, 222–229. doi: 10.1016/j.amepre.2003.12.005

Pallavicini, F., Ferrari, A., and Mantovani, F. (2018). Video games for well-being: a systematic review on the application of computer games for cognitive and emotional training in the adult population. *Front. Psychol.* 9:2127. doi: 10.3389/fpsyg.2018.02127

Roenker, D. L., Cissell, G. M., Ball, K. K., Wadley, V. G., and Edwards, J. D. (2003). Speed-of-processing and driving simulator training result in improved driving performance. *Hum. Factors* 45, 218–233. doi: 10.1518/hfes.45.2.218.27241

Ross, L. A., Edwards, J. D., O'Connor, M. L., Ball, K. K., Wadley, V. G., and Vance, D. E. (2018). The transfer of cognitive speed of processing training to older adults' driving mobility across 5 years. *J. Gerontol. B Psychol. Sci. Soc. Sci.* 71, 87–97. doi: 10.1093/geronb/gbv022

Rubin, D. B. (2004). *Multiple Imputation for Nonresponse in Surveys*. Hoboken, NJ: Wiley-Interscience.

Russoniello, C., O'Brien, K., and Parks, J. (2009). The effectiveness of casual video games in improving mood and decreasing stress. *J. Cyber Ther. Rehabil.* 2, 53–66.

Schneider, W. J., and McGrew, K. (2012). "The cattell-horn-carroll model of intelligence," in *Contemporary Intellectual Assessment: Theories, Tests, and Issues*, eds D. Flanagan and P. Harrison (New York, NY: Guilford), 99–144.

Schulz, K. F., Altman, D. G., and Moher, D. (2010). CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *Trials* 11:32. doi: 10.1186/1745-6215-11-32

Shatil, E., Mikulecká, J., Bellotti, F., and Bureš, V. (2014). Novel television-based cognitive training improves working memory and executive function. *PLoS One* 9:e101472. doi: 10.1371/journal.pone.0101472

Stalvey, B. T., and Owsley, C. (2003). The development and efficacy of a theory-based educational curriculum topromote self-regulation among high-risk older drivers. *Health Promot. Pract.* 4, 109–119. doi: 10.1177/1524839902250757

Strain, L. A., Grabusic, C. C., Searle, M. S., and Dunn, N. J. (2002). Continuing and ceasing leisure activities in later life: a longitudinal study. *Gerontologist* 42, 217–223. doi: 10.1093/geront/42.2.217

Takeuchi, H., Taki, Y., Nouchi, R., Hashizume, H., Sekiguchi, A., Kotozaki, Y., et al. (2014). Working memory training improves emotional states of healthy individuals. *Front. Syst. Neurosci.* 8:200. doi: 10.3389/fnsys.2014.00200

van Buuren, S., and Groothuis-Oudshoorn, K. (2011). mice: multivariate imputation by chained equations in R. *J. Stat. Softw.* 45, 1–67. doi: 10.18637/jss.v045.i03

Wadley, V. G., Benz, R. L., Ball, K. K., Roenker, D. L., Edwards, J. D., and Vance, D. E. (2006). Development and evaluation of home-based speed-of-processing training for older adults. *Arch. Phys. Med. Rehabil.* 87, 757–763. doi: 10.1016/j.apmr.2006.02.027

Wechsler, D. A. (1987). *Wechsler Memory Scale Revised*. San Antonio, TX: The Psychological Corporation.

Wechsler, D. A. (1997). *Wechsler Adult Intelligence Scale*. 3rd Edn. San Antonio, TX: The Psychological Corporation.

Willis, S. L., Tennstedt, S. L., Marsiske, M., Ball, K., Elias, J., Koepke, K. M., et al. (2006). Long-term effects of cognitive training on everyday functional outcomes in older adults. *JAMA* 296, 2805–2814. doi: 10.1001/jama.296.23.2805

Wolinsky, F. D., Vander Weg, M. W., Howren, M. B., Jones, M. P., and Dotson, M. M. (2013). A randomized controlled trial of cognitive training using a visual speed of processing intervention in middle aged and older adults. *PLoS One* 8:e61624. doi: 10.1371/journal.pone.0061624

Wolinsky, F. D., Vander Weg, M. W., Martin, R., Unverzagt, F. W., Ball, K. K., Jones, R. N., et al. (2009). The effect of speed-of-processing training on depressive symptoms in ACTIVE. *J. Gerontol. A Biol. Sci. Med. Sci.* 64A, 468–472. doi: 10.1093/gerona/gln044

Yassuda, M. S., Wilson, J. J., and von Mering, O. (1997). Driving cessation: the perspective of senior drivers. *Educ. Gerontol.* 23, 525–538. doi: 10.1080/0360127970230603

Yokoyama, K., and Watanabe, K. (2015). *Japanese Version POMS-2 Manual: A Profile of Mood States*. 2nd Edn.Tokyo: Kaneko Shobo.

Conflict of Interest Statement: RK developed the cognitive training games with Sendai Television Inc., which also provided a set-top box with training games and a portable Wi-Fi to participants. After this study, all devices were returned to Sendai Television Inc. This study was thereby supported to that extent by Sendai Television Inc. Nevertheless, sources of funding for this study had no involvement in the study design, collection, analysis, interpretation of data, or writing of the manuscript.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

CLINICAL TRIAL
published: 25 June 2019
doi: 10.3389/fphys.2019.00756

Whole Body Vibration Training on Muscle Strength and Brain-Derived Neurotrophic Factor Levels in Elderly Woman With Knee Osteoarthritis: A Randomized Clinical Trial Study

*Adriano Prado Simão[1], Vanessa Amaral Mendonça[2,3,4], Núbia Carelli Pereira Avelar[5], Sueli Ferreira da Fonseca[2,3], Jousielle Márcia Santos[2,3], Ana Carolina Coelho de Oliveira[2,4], Rosalina Tossige-Gomes[2,3], Vanessa Gonçalves César Ribeiro[2,3], Camila Danielle Cunha Neves[2,3], Cláudio Heitor Balthazar[2,3], Hércules Ribeiro Leite[2,3,4], Pedro Henrique Scheidt Figueiredo[2,3,4], Mário Bernardo-Filho[6] and Ana Cristina Rodrigues Lacerda[2,3,4]**

[1]Departamento de Fisioterapia, Universidade Federal de Alfenas (UNIFAL), Alfenas, Brazil, [2]Centro Integrado de Pós-Graduação e Pesquisa em Saúde (CIPq-Saúde), Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), Diamantina, Brazil, [3]Programa Multicêntrico de Pós-Graduação em Ciências Fisiológicas (PMPGCF), Sociedade Brasileira de Fisiologia (SBFis), Butanta, Brazil, [4]Programa de Pós-Graduação em Reabilitação e Desempenho Funcional (PPGReab), Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), Diamantina, Brazil, [5]Departamento de Ciências da Saúde, Universidade Federal de Santa Catarina (UFSC), Araranguá, Brazil, [6]Departamento de Biofísica e Biometria, Instituto de Biologia Roberto Alcântara Gomes, Universidade Federal do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

OPEN ACCESS

Edited by:
Michael George Bemben,
University of Oklahoma,
United States

Reviewed by:
Hamid Reza Marateb,
Universitat Politecnica de Catalunya,
Spain
Joyce McClendon Evans,
University of Kentucky, United States
Murat Karabulut,
University of Texas Rio Grande Valley
Brownsville, United States

***Correspondence:**
Ana Cristina Rodrigues Lacerda
lacerdaacr@gmail.com;
lacerda.acr@ufvjm.edu.br

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: 13 February 2019
Accepted: 31 May 2019
Published: 25 June 2019

Citation:
Simão AP, Mendonça VA, Avelar NCP, Fonseca SF, Santos JM, Oliveira ACC, Tossige-Gomes R, Ribeiro VGC, Neves CDC, Balthazar CH, Leite HR, Figueiredo PHS, Bernardo-Filho M and Lacerda ACR (2019) Whole Body Vibration Training on Muscle Strength and Brain-Derived Neurotrophic Factor Levels in Elderly Woman With Knee Osteoarthritis: A Randomized Clinical Trial Study. Front. Physiol. 10:756. doi: 10.3389/fphys.2019.00756

Background: Osteoarthritis of the knee (kOA) is a chronic, progressive, degenerative health condition that contributes to the imbalance between the synthesis and destruction of articular cartilage. Recently, whole body vibration (WBV) training has been recommended as an effective alternative for strength training in elderly people, and various physiological effects are obtained in response to exercise performed on a vibratory platform, such as an increase in muscle activation and improved muscle performance. However, the effects of WBV particularly on the strength of the quadriceps muscle and neuronal plasticity are unknown.

Objective: The aim of this study was to evaluate the effects of adding WBV to squat training on the isometric quadriceps muscle strength (IQMS) and the plasma levels of brain-derived neurotrophic factor (BDNF) in elderly woman with kOA.

Methods: Fifteen elderly women ≥65 years of age with kOA were randomized into two interventions: (1) the vibration group (VG), in which participants performed squat exercise training in association with WBV or (2) the exercise group (EG), in which participants performed squat exercise training without vibration, for 12 weeks 3×/week.

Results: Compared to the EG group, the VG group demonstrated a significantly greater delta (Δ) in IQMS values (IC95% 0.43–7.06; $p \leq 0.05$) and in Δ BDNF plasma levels (IC95% −32.51 to 4.217; $p \leq 0.05$) after the intervention period. There was an association between increase of Δ BDNF plasma levels and increase of Δ IQMS ($\beta = 0.57$; $R^2 = 0.32$; $p = 0.03$).

Conclusion: The addition of WBV to squat exercise training improves lower limb muscle performance in elderly women with kOA. These findings suggest that the improvement in muscle performance is related to neuromuscular adaptations induced by WBV.

Clinical Trial Registration: www.ClinicalTrials.gov, identifier NCT03918291.

Keywords: muscle strength, squat, brain-derived neurotrophic factor, osteoarthritis of knee, whole body vibration

INTRODUCTION

Osteoarthritis (OA) is a chronic, progressive, degenerative disease of multifactorial etiology involving biomechanical and genetic factors that contribute to the imbalance between the synthesis and destruction of articular cartilage (Goldring and Goldring, 2004) and characterized by arthralgia, stiffness, swelling, and decreased muscle strength and functionality (Zacaron et al., 2006; Michael et al., 2010). Osteoarthritis of the knee (kOA) is the most common form of OA, affecting approximately 7% of people aged 65–70 years and 11.2% of those aged over 80 years (Marx et al., 2006). The prevalence and incidence of OA differ by gender with females showing a significantly greater prevalence and severity of kOA (Srikanth et al., 2005).

The pain and the increase in intra-articular fluid, which are common in kOA, sensitize the capsular mechanoreceptors, which activate inhibitory interneurons in the spinal medulla and consequently decrease the muscle activation (e.g., the quadriceps). This phenomenon is called arthrogenic muscle inhibition and can lead to loss of muscle mass and impair performance of daily living activities such as walking, climbing stairs, and rising from a chair (McNair et al., 1996; Hautier and Bonnefoy, 2007). The reversal of this condition can be achieved by physical exercise. A study of our research group (Gomes et al., 2016) showed that the increased physical performance in elderly women with kOA may be related to the immunomodulatory effect of exercise. In addition, physical exercise induces a cascade of molecular and cellular processes that support brain plasticity. In this context, brain-derived neurotrophic factor (BDNF) appears to be the most susceptible to regulation induced by exercise and physical activity and could be linked to these mechanisms. The BDNF is a neurotrophin produced by several tissues, i.e., brain, muscle, and kidney, and has been involved with central and peripheral molecular processes of energy metabolism and homeostasis, development of immature neurons and survival of adult neurons, neuronal differentiation, and synaptic plasticity (Lindsay, 1994; Knaepen et al., 2010; Teixeira et al., 2010). Furthermore, it has been speculated about the role of BDNF as a myokine. Yu et al. (2017) demonstrated a time-dependent upregulation of BDNF in skeletal muscle of rats, which apparently is involved in the regeneration after exercise-induced muscle damage.

Recently, whole body vibration (WBV) training has been recommended as an effective alternative for strength training in elderly people (Roelants et al., 2004; Bogaerts et al., 2007), and various physiological effects are obtained in response to acute exercise performed on a vibratory platform, such as an increase in muscle activation (Abercromby et al., 2007), improved muscle performance (Cochrane et al., 2008, 2010). During this training modality, the individual stands on a platform that generates vertical sinusoidal vibrations. These mechanical stimuli are transmitted to the body where they stimulate the primary endings of the muscle spindles, which in turn activate α-motor neurons, resulting in muscle contractions known as tonic vibration reflex (Cardinale and Bosco, 2003). The WBV training is a safe, suitable, and effective training method and is a potentially feasible intervention for those patients who cannot participate in conventional strength training. In addition, WBV training could yield effects similar to regular strength training (Roelants et al., 2004; Trans et al., 2009), but with a lower load on the affected joint (Trans et al., 2009). Moreover, the time to perform the exercise is less, making it a faster form of training (Merriman and Jackson, 2009).

Studies demonstrated improvements on function, gait parameters, and quality of life in patients with kOA after a training program where WBV was added to squat exercise training (Wang et al., 2016a,b). Our research team also demonstrated improvements in the functionality and self-perception of disease status in elderly subjects with kOA after a 12-week training program in which WBV was added to squat exercise training (Avelar et al., 2011). However, to the best of our knowledge, only two studies have evaluated the effect of WBV training on muscle strength in elderly people with kOA (Trans et al., 2009; Bokaeian et al., 2016). In these studies, it was reported that the WBV-exercise regime on a platform increased muscle strength in subjects with kOA. Nevertheless, there is a gap in the literature regarding the possible mechanism related to the muscle performance improvement in this population. Studies suggest that adding WBV training to strengthening training may provide a better treatment effects for patients with kOA (Park et al., 2013; Bokaeian et al., 2016).

Because kOA in older adults contributes to a greater deterioration in muscle strength (Marx et al., 2006) and the reduction in muscle strength could be the result of changes in neuromuscular activation (McNair et al., 1996), it is believed that the addition of WBV to squat exercise training could promote an increase in muscle strength in older women with OA (Trans et al., 2009) Therefore, the present study was designed to investigate the effects of WBV in addition to squat exercise training in elderly women with kOA on the following parameters: (1) isometric strength of the quadriceps muscle (IQMS) and (2) BDNF plasma levels. Our hypothesis is that the addition of WBV to squat exercise training would increase the IQMS and the BDNF plasma levels in older women with kOA.

MATERIALS AND METHODS

Ethical Statement

This study was carried out in accordance with the recommendations of the ethical principles for research involving humans (Resolution 196-96 of the National Health Council of the Brazilian Ministry of Health) and Federal University of Jequitinhonha and Mucuri Valleys Ethics Committee. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Federal University of Jequitinhonha and Mucuri Valleys Ethics Committee (protocol No. 046/08) and was registered in ClinicalTrials.gov (protocol ID: NCT03918291).

Design

This was a randomized, controlled trial in which the variables were assessed 24 h before and after a 12-week training program. For the allocation of participants, a 1:1 ratio randomization was performed using opaque envelopes for allocation concealment. To minimize the chance of bias, we used the following methods: (1) opaque, sealed, and serial-numbered envelopes; (2) the envelopes were opened sequentially after the participant's name and details were written on the envelope; and (3) the envelopes were kept in a locked and secure place. The allocation sequence was concealed from the researcher enrolling and assessing participants. Only one researcher performing the randomization was aware of the group assignment.

Subjects

The volunteers were recruited from the physiotherapy clinic and medical referrals. To participate in the study, volunteers were required to meet the following inclusion criteria: females older than 65 years of age, not using hormone replacement therapy, and diagnosed with OA in at least one knee in accordance with of the American College of Rheumatology (Kellgren and Lawrence, 1957). The severity of the kOA was classified radiographically according to the Kellgren and Lawrence scale (grades 0–4, with 0 being normal and 4 representing severe OA). A Grade 2 classification (definite osteophytes and possible narrowing of joint space) was used as a cutoff to determine knee OA (Hinton et al., 2002; Schiphof et al., 2008). Exclusion criteria included the following: no recent knee injury, no walking aid requirement, and no rehabilitation procedures in the previous 3 months. Volunteers were also excluded if they had any orthopedic, neurological, respiratory, or acute cardiac diseases or if they had any cognitive deficit as determined by the Mini-Mental State Examination (Gomes et al., 2012).

Of the 125 elderly people screened for eligibility, 15 elderly women fulfilled the criteria, had blood sampling collected at rest (8:00 a.m.), and then participated in the IQMS test. Thereafter, the subjects were allocated into two groups: (1) the vibration group, in which participants performed squat exercise training in association with WBV (VG; n = 7) or (2) the exercise group, in which participants performed squat exercise training without vibration (EG; n = 8; **Figure 1**).

Procedures

The clinical and demographic data were collected from the participants using an evaluation chart. Prior to the initiation of the 12-week intervention program, a blood sample was collected at 8:00 a.m. from the volunteers of all groups followed by the IQMS evaluation. At 24 h after the intervention period, the volunteers of both groups were reassessed. These procedures were performed to avoid any circadian rhythm effects on hormonal status and the performance of IQMS. The tests were performed by an experienced examiner who was blind to the group allocation.

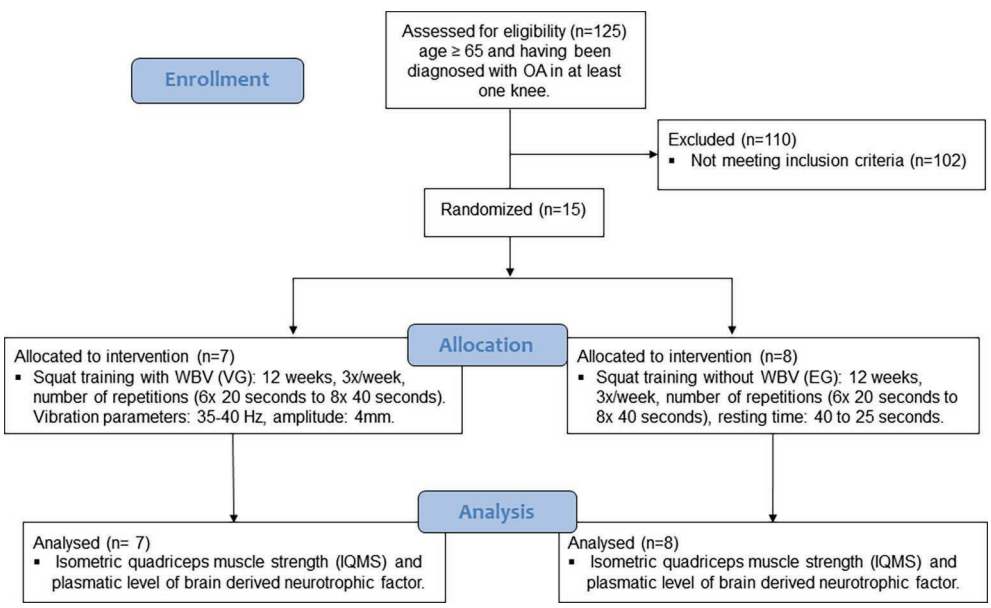


FIGURE 1 | The study flow chart. OA, osteoarthritis; VG, vibration group; EG, exercise group; WBV, whole body vibration.

The intervention program consisted of performing squat exercise training with or without WBV, three times a week on alternate days.

Warm-Up

Prior to each training session, the participants warmed up on a stationary cycle (Stone Fitness, 2001, Huntertown, United States) at 70% of the predicted maximum heart rate for each subject's age (220 – age) and were monitored using a Polar heart rate monitor (Polar F4, Kempele, Finland) for 10 min. Immediately afterwards, the participants of the VG group were placed in a position with their feet 28 cm apart (14 cm to the right and 14 cm to the left of the center of the vibration stimulus) to ensure that each of the lower limbs received the same amount of vibration stimulus and were ready to begin the squat exercises on the vibratory platform, while the participants of the EG group performed the same procedure without vibration.

Squat Exercises

The intensity of squat exercise training was systematically augmented in the VG and EG over the training period by increasing the number of repetitions (6 × 20 s to 8 × 40 s) and reducing the resting time (40–25 s). These parameters were based on the study by Avelar et al. The squat exercise was performed starting at approximately 10° of knee flexion and continuing until 60° of knee flexion was reached. The 60° angle was measured in each volunteer using a universal goniometer prior to initiating the exercise series, and a barrier was placed at the gluteal region to limit the degree of flexion of the knee. For temporal control during the squat, an examiner provided verbal encouragement to standardize the length of maintaining the semi full position (3 s) and the flexed position (3 s of isometric contraction) of the knees in each squat repetition. The participants of both groups were placed in position with their feet 28 cm apart.

Whole Body Vibration

For the volunteers in the VG group, a commercial model of a vibration platform was used (FitVibe, GymnaUniphy NV, Bilzen, Belgium). In this group, acceleration was also increased by varying the vibration frequency (35–40 Hz). The mechanical stimulation parameters of the vibration consisted of the following: frequency of 35–40 Hz, amplitude of 4 mm, and acceleration that ranged from 2.78 to 3.26 G. The platform provided a vertical sinusoidal vibration. Prior to initiating data collection, the platform acceleration values were verified using the Mega accelerometer (Acceleration Measuring Kit ZPP1-3D-147BC, Southampton, United Kingdom).

To measure acceleration on the horizontal and vertical axes, two accelerometers (Acceleration Measuring Kit ZPP1-3D-147BC, Southampton, United Kingdom) were fixed at a distance of 14 cm from the center of platform vibration. The signal was amplified electronically and was stored. This signal was obtained at a frequency of 1.000 Hz and was sent for computer analysis. Each accelerometer was calibrated using two calibration points, and applying zero and gravity, Earth's gravity being 1 G (9.81 m/s^2). To obtain the true acceleration values of the platform, the values of Earth's gravity were subtracted along the vertical axis from the total signal received so that the acceleration of the platform would begin at 0 m/s^2. The data were transferred to a computer using the Megawin software program, and the mean and maximum acceleration of each sample on each axis were analyzed using the Matlab software program. Each frequency used was measured over 60 s. In the pilot study, interexaminer reliability was found to be high, with a coefficient of variation of 1.05%.

The elderly underwent training on the vibratory platform with barefoot to avoid any damping effect due to different footwear (Marín et al., 2009). In addition, a predetermined distance from the feet (14 cm to the right and 14 cm to the left of the vibration center of the platform) was set to ensure that each of the lower limbs received the same amount of vibration stimulus. Moreover, with the aim of maintaining control of the body's center of gravity behind the base of the support, the positioning of the spine, arms, and head and the type of squat (simulating the motion of sitting in a chair) were standardized.

Isometric Quadriceps Muscle Strength

The IQMS was evaluated by the maximal voluntary isometric contraction of knee extensors (MVIC) measured using the load cell according to the method described by Neves et al. (2011). For this evaluation, the volunteer was positioned in flexion-extension chair (Home Sport, Master Top model, Belo Horizonte, Brazil) and assumed a sitting posture with a straight trunk and the hips flexed at 90°. The limb to be tested was positioned at 60° of knee flexion (assessed individually by goniometry of the knee), and the resistance lever was positioned on the distal leg.

The load cell was properly calibrated and positioned near the leg extension perpendicular to the ground for signal acquisition during the isometric muscle strength tests (Miotec - Biomedical Equipment, Porto Alegre, Brazil). The load cell was connected to the software MIOTOOL 400 that transmitted the isometric muscle strength values to a computer. The MVIC test was performed for 6 s with 1 min between sets and with repeating three sets per leg.

Before the test, the volunteers warmed up for 10 min on a stationary bicycle (Stone Fitness, 2001, Huntertown, United State), which increased the heart rate (HR) to 70% of the estimated maximum HR by age, as measured with the use of the Polar heart monitors (Polar F4, Kempele, Finland). This procedure was performed to reduce the risk of injury during the test (Neves et al., 2011). The intra-class correlation coefficient (ICC) for IQMS was 0.98 (95% CI: 0.94–0.99).

Analysis of Brain-Derived Neurotrophic Factor by ELISA

For plasma processing, 10 ml peripheral blood samples were collected from the antecubital vein using aseptic techniques and heparin as an anticoagulant. The blood was immediately centrifuged twice at 3.000 *g* for 10 min, and the plasma was kept frozen at −70°C until assayed. Plasma BDNF levels were measured in duplicate using ELISA kits for BDNF (BDNF DuoSet, R&D Systems) according to the manufacturer's instructions; the detection limit was 10 pg/ml.

Statistical Analysis

The SPSS statistical software program (IBM, Chicago, IL, USA) was used for the statistical analyses. All data were expressed as means (confidence interval 95%). The Shapiro–Wilk test was used to evaluate the normality of the data. Because the dependent variables were normally distributed, parametric tests were used for the statistical analyses. An independent *t*-test was performed to assess the differences between the means. Thus, we used the delta analysis (pretest-posttest control group design) because allowed to verify the magnitude of the variation of the effect of the intervention, i.e., how much the addition of WBV contributed to squat training (VG) compared to squat training without WBV (EG) in the outcomes. The pretest-posttest control group design involves the random assignment of units to either a treatment or a control group. Successful randomization of units to groups in this experimental design, as well as similar between-groups anthropomorphic characteristics and outcomes at baseline (pretest), ensures that any pre-existing differences between the units in the treatment and control groups are due to chance and do not reflect systematic differences (Valente and MacKinnon, 2017). The correlation between the variables was evaluated using the Pearson coefficient. Simple linear regression models were performed to predict the BDNF and IQMS variables. Effect size (*d*) was checked in the G*Power program. Effect size conventions for test family (*t* tests) and two independent means: $d = 0.20$ (small), $d = 0.50$ (medium), $d = 0.80$ large. The effect size analysis is an additional measure to the traditional statistical test of the null hypothesis and aims to verify the clinical significance of the effect found and is not limited to dichotomous (significant or not significant) results. Thus, with the effect size analysis, it is possible to identify whether the observed differences are small, moderate, or large. The level of significance was set at $p \leq 0.05$ for all the tests.

RESULTS

There were no significant differences between the groups regarding demographic and anthropometric data. No significant differences were found in the data collected prior to training between-groups, thus confirming the baseline homogeneity of the groups (**Table 1**).

TABLE 1 | Sample characterization before training.

Characteristics	VG (*N* = 7)	EG (*N* = 8)	*p*
Age (years)	75 (68.5–81.5)	71 (67.7–74.3)	0.06
Body mass (kg)	72.75 (62.2–83.3)	74.24 (65.9–82.6)	0.75
Height (m)	1.58 (1.5–1.6)	1.57 (1.5–1.6)	0.81
Severity of knee OA* (%)	2 3 4 29 14 57	2 3 4 50 13 37	–
IQMS before training (kg)	21.89 (19.5–24.3)	25.25 (21.8–28.7)	0.09
Plasma BDNF concentration before training (pg/ml)	4,778 (2952.4–6603.7)	3,043 (1623.4–4462.6)	0.06

*Classified radiographically according to Kellgren and Lawrence scale.
Data presented as means (confidence interval 95%). *p ≤ 0.05.
IQMS, isometric quadriceps muscle strength; BDNF, brain-derived neurotrophic factor; VG, vibration group; EG, exercise group.

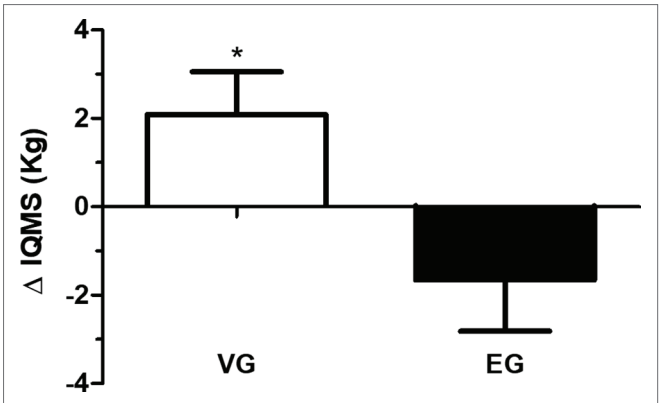


FIGURE 2 | The delta (Δ) in isometric quadriceps muscle strength (IQMS) in the vibration group (VG) and exercise group (EG). The data are presented as the mean and standard error. *VG is significantly different from EG ($p \leq 0.05$). Data are presented in delta (Δ), i.e., the variation between the values measured before and after the intervention period (independent *t*-test).

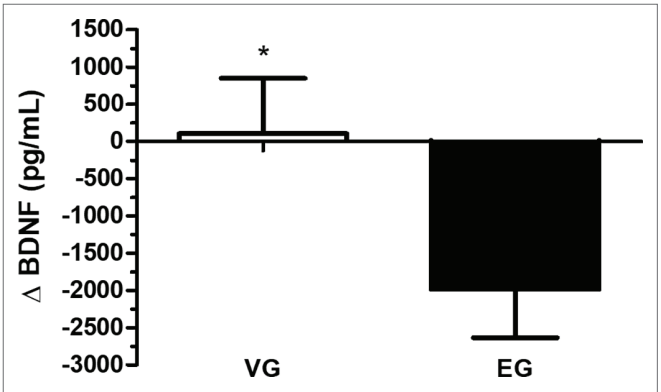


FIGURE 3 | The delta (Δ) in brain-derived neurotrophic factor (BDNF) in the vibration group (VG) and exercise group (EG). The data are presented as the mean and standard error. *VG is significantly different from EG ($p \leq 0.05$). Data are presented in delta (Δ), i.e., the variation between the values measured before and after the intervention period (independent *t*-test).

All participants in both intervention groups complied with the full intervention program. Overall compliance was 99.7% in the VG group and 98.6% in the EG group. There was no significant difference between the groups with respect to compliance ($p = 0.41$).

The IQMS data, which were analyzed through the variation between the values measured before and after the intervention period, i.e., delta (Δ), showed a significant increase in the VG group compared to the EG group (IQMS; $p \leq 0.05$; effect size = 1.3). After the intervention period, compared to the baseline of the study, the IQMS increased on average 2.17 kg (+13.48%) in the VG group and decreased on average 1.66 kg (−6.53%) in the EG group (**Figure 2**).

The Δ BDNF plasma levels demonstrated a significant increase in the VG group compared to the EG group (Δ BDNF; $p \leq 0.05$; effect size = 1.1) (**Figure 3**). There was a positive correlation between BDNF plasma levels and IQMS (**Figure 4**). The linear regression analysis showed the prediction between

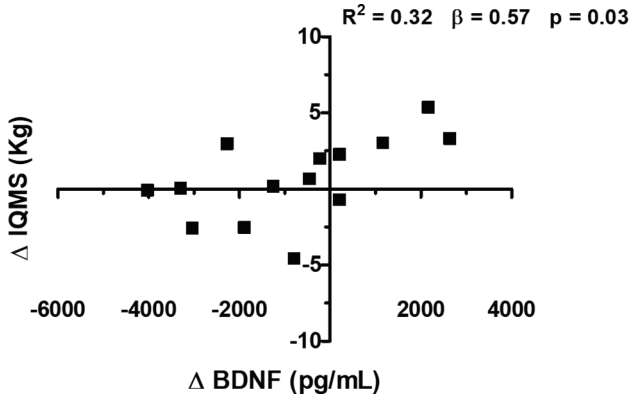


FIGURE 4 | Correlation between the delta (Δ) of the isometric quadriceps muscle strength (IQMS) and plasma concentration of brain-derived neurotrophic factor (BDNF) in older women with osteoarthritis (OA) of the knee. β, standardized regression coefficient; R^2, *R* square (Pearson correlation).

Δ BDNF and Δ IQMS, where the increase in Δ BDNF seems to account for 56.9% of the Δ IQMS. Thus, for each increase of 1 pg/ml in the Δ BDNF, there will be an increase of 0.32 kg in Δ IQMS (standardized regression coefficient = 0.57; *R* square = 0.32; $p = 0.03$).

DISCUSSION

To our knowledge, this was the first randomized study designed to determine whether the addition of WBV to squat training could improve IQMS and affect the BDNF plasma in elderly women with kOA. The results of this study indicated that the addition of WBV to squat exercise training improved IQMS values and increased plasma BDNF concentrations in the studied population.

The increase in IQMS observed when adding WBV to squat exercise training in elderly people is consistent with previous literature (Roelants et al., 2004; Bogaerts et al., 2007; Rees et al., 2008). The chronic effect of WBV training in IQMS is in accordance with the hypothesis that the tonic vibration reflex primarily affects the subject's ability to generate high firing rates in high-threshold motor units (Roelants et al., 2004). In addition, it has been suggested that the recruitment thresholds of the motor units during WBV is lower than with voluntary contractions, probably resulting in a more rapid activation and training of high-threshold motor units (Roelants et al., 2004).

However, it is important to mention that the studies cited above (Roelants et al., 2004; Bogaerts et al., 2007; Kean et al., 2010) were performed by elderly subjects with no history of joint damage. kOA appears to lead to a significant reduction in quadriceps muscle strength and, consequently, the ability to walk, climb stairs, and rise from a chair, especially in elderly people. Therefore, it is imperative to study the effect of the addition of WBV to squat exercise training in elderly people with kOA.

To our knowledge, there is only one study in the literature that investigated and demonstrated the effectiveness of WBV training on quadriceps muscle strength in individuals with kOA compared to a control group (without intervention; Trans et al., 2009). Although our results are in line with this prior study, some differences should be raised. In the study of Trans et al., the intervention group was subjected to solely WBV training, and the control group did not participate in any type of intervention. However, in the present study, the effects of adding WBV to squat training were evaluated. Moreover, in our study, there was a standardization of the knee flexion angle, and in the study of Trans et al., the participants flexed their knees until the position was self-perceived.

The increase in IQMS in the VG group has a significant clinical impact, as the literature indicates that clinically relevant minimum change is 2.25 Nm (Kean et al., 2010). Once 1 kg corresponds to 9.81 Nm, the increase of 2.17 kg in IQMS in the VG group corresponds to 21.29 Nm (a value close to the minimum clinically relevant). In EG group, there was a decrease in IQMS. The pain and the increase in intra-articular fluid, which are common in kOA, sensitize the capsular mechanoreceptors that send signals to medullary inhibitory interneurons. These interneurons inhibit alpha motor neurons and, consequently, reduce the signals that would be transmitted to the muscle groups, especially the quadriceps. This phenomenon, called arthrogenic muscle inhibition, is likely generated by the abnormal afferent information of the affected joint resulting in the decreased activation of the muscles that act there (McNair et al., 1996), progressing to a loss of muscle mass, decrease of muscle strength, and physical functioning (Sharma et al., 2003). Furthermore, age-related muscle loss is also a result of reductions in the size and number of muscle fibers, possibly due to a multifactorial process that involves physical inactivity, nutritional intake, oxidative stress, and hormonal changes corresponding to a condition known as sarcopenia. In addition, marked motoneuron loss and aberrant neuromuscular sprouting have been observed in aged mammals (Baumgartner et al., 1999). The reversal of this situation can be achieved by physical exercise that induces a cascade of molecular and cellular processes that support neuronal plasticity. However, the decrease in IQMS of the EG group demonstrated that the intensity of the exercise was not enough to preserve muscle mass.

The BDNF has been proposed as an essential neurotrophin related to neuronal plasticity (Knaepen et al., 2010; Teixeira et al., 2010) that plays an important role in old-age survival because of its role in preventing neuronal death during stress (Schabitz et al., 2007). The expression profiling of BDNF has shown that this neurotrophin is expressed differentially in skeletal muscle under various physiological and pathological conditions (Chevrel et al., 2006). Moreover, the expression of BDNF mRNA by human skeletal muscle increased after 2 h of cycle ergometer exercise (Matthews et al., 2009).

Therefore, we proposed in the present study to investigate the effect of adding WBV to squat exercise training on BDNF plasma concentrations. As a result, a significant increase in the VG group compared with the EG group was observed,

supporting a possible neuromuscular adaptation hypothesis arising from this type of intervention in the studied population, as neurotrophins and neurotrophin receptors play a role in the coordination of muscle innervation and functional differentiation of neuromuscular junctions (Chevrel et al., 2006). This hypothesis is supported by the positive correlation found between plasma level of BDNF and IQMS.

Several studies have demonstrated that BDNF is a protein that is produced in skeletal muscle cells, and its expression is increased by contraction to enhance fat oxidation in an AMPK-dependent fashion, most likely by acting in an autocrine and/or paracrine manner within skeletal muscles (Bogaerts et al., 2007; Krabbe et al., 2009). Thus, an increase in BDNF plasma could play an important role in old-age survival because of its role in preventing neuronal death during stress (Schabitz et al., 2007). Thus, it seems plausible that the improvement in isometric quadriceps muscle strength in the vibration group could be related to an increase in BDNF level.

In the EG group, plasma BDNF levels showed a decrease after the intervention period. BDNF is a member of the neurotrophic factor family that plays key roles in regulating survival, growth, and maintenance of neurons in differentiation (Lindsay, 1994) and synaptic plasticity and synaptic transmission efficacy (Knaepen et al., 2010), and it is involved in muscle regeneration (Sakuma and Yamaguchi, 2011). Of the neurotrophins, BDNF, which is considered a contraction-inducible protein in skeletal muscle (Matthews et al., 2009), appears to be the most susceptible to regulation by exercise and physical activity (Knaepen et al., 2010; Coelho et al., 2012). However, it seems that performing only squatting exercises has not induced changes in BDNF plasma levels, preventing the degeneration of motoneurons and muscle fiber innervations in the elderly with knee OA. Inevitably, this study had some inherent limitations. First, the study was performed as a single-center trial with a relatively small number of participants. However, statistical analyses demonstrated a "large" effect size for IQMS ($p \leq 0.05$; effect size = 1.1) and BDNF ($p \leq 0.05$; effect size = 1.3). Moreover, considering the variability in the BDNF response to exercise and the influence of the BDNF polymorphism variant on such response, the analysis of only seven plasma BDNF samples should be interpreted with caution. Moreover, because specific frequency and amplitude were used, the findings of this study cannot be extrapolated to other parameters of vibration and cannot be generalized to the elderly population since the volunteers were female healthy and sedentary. Last, as we aimed to investigate the addition of WBV to squat exercise training, we have decided not to use a sound that mimicked the noise of the vibratory platform in the control group.

CONCLUSION

The addition of WBV to squat exercise training improves lower limb muscle performance in elderly women with kOA, likely by increasing BDNF, suggestive of modulation in neuromuscular plasticity. Future studies should be designed to investigate the specific mechanism, including joint analyses.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the ethical principles for research involving humans (Resolution 196-96 of the National Health Council of the Brazilian Ministry of Health) and Federal University of Jequitinhonha and Mucuri Valleys Ethics Committee. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Federal University of Jequitinhonha and Mucuri Valleys Ethics Committee (protocol no. 046/08) and was registered in ClinicalTrials.gov (protocol ID: NCT03918291).

AUTHOR CONTRIBUTIONS

The specific contributions of the authors are as follows: AS, VM, NA, and AL contributed to the conception and design of the study. AS, VM, NA, SF, RT-G, CN, CB, and AL contributed to analysis and interpretation of the data. AS, VM, NA, SF, RT-G, CN, CB, JS, AO, and AL contributed to drafting of the article. AS, VM, NA, SF, JS, AO, VR, HL, PF, MB-F, and AL involved in the critical revision of the article for important intellectual content. AS, VM, NA, SF, RT-G, CN, CB, JS, AO, VR, HL, PF, MB-F, and AL contributed to the final approval of the article. AS, NA, VR, HL, PF, and AL provided statistical expertise. AS, VM, NA, SF, RT-G, CN, CB, MB-F, and AL contributed to provision of study materials. AS, VM, NA, CB, MB-F, and AL contributed to administrative, technical, or logistic support.

FUNDING

We thank the Brazilian Society of Physiology (SBFis), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) (number APQ-02503-16), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (number 408721/2016-2, and number 303746/2018-1), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Finance code 001.

ACKNOWLEDGMENTS

We would like to acknowledge the Centro Integrado de Pós-Graduação e Pesquisa em Saúde at the Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, MG, for providing equipment and technical support for experiments.

REFERENCES

Abercromby, A. F., Amonette, W. E., Layne, C. S., McFarlin, B. K., Hinman, M. R., and Paloski, W. H. (2007). Variation in neuromuscular responses during acute whole-body vibration exercise. *Med. Sci. Sports Exerc.* 39, 1642–1650. doi: 10.1249/mss.0b013e318093f551

Avelar, N. C., Simão, A. P., Tossige-Gomes, R., Neves, C. D., Rocha-Vieira, E., Coimbra, C. C., et al. (2011). The effect of adding whole-body vibration to squat training on the functional performance and self-report of disease status in elderly patients with knee osteoarthritis: a randomized, controlled clinical study. *J. Altern. Complement. Med.* 17, 1149–1155. doi: 10.1089/acm.2010.0782

Baumgartner, R. N., Waters, D. L., Gallagher, D., Morley, J. E., and Garry, P. J. (1999). Predictors of skeletal muscle mass in elderly men and women. *Mech. Ageing Dev.* 107, 123–136. doi: 10.1016/S0047-6374(98)00130-4

Bogaerts, A., Delecluse, C., Claessens, A. L., Coudyzer, W., Boonen, S., and Verschueren, S. M. (2007). Impact of whole-body vibration training versus fitness training on muscle strength and muscle mass in older men: a 1-year randomized controlled trial. *J. Gerontol. A Biol. Sci. Med. Sci.* 62, 630–635. doi: 10.1093/gerona/62.6.630

Bokaeian, H. R., Bakhtiary, A. H., Mirmohammadkhani, M., and Moghimi, J. (2016). The effect of adding whole body vibration training to strengthening training in the treatment of knee osteoarthritis: a randomized clinical trial. *J. Bodyw. Mov. Ther.* 20, 334–340. doi: 10.1016/j.jbmt.2015.08.005

Cardinale, M., and Bosco, C. (2003). The use of vibration as an exercise intervention. *Exerc. Sport Sci. Rev.* 31, 3–7. doi: 10.1097/00003677-200301000-00002

Chevrel, G., Hohlfeld, R., and Sendtner, M. (2006). The role of neurotrophins in muscle under physiological and pathological conditions. *Muscle Nerve* 33, 462–476. doi: 10.1002/mus.20444

Cochrane, D. J., Sartor, F., Winwood, K., Stannard, S. R., Narici, M. V., and Rittweger, J. (2008). A comparison of the physiologic effects of acute whole-body vibration exercise in young and older people. *Arch. Phys. Med. Rehabil.* 89, 815–821. doi: 10.1016/j.apmr.2007.09.055

Cochrane, D. J., Stannard, S. R., Firth, E. C., and Rittweger, J. (2010). Acute whole-body vibration elicits post-activation potentiation. *Eur. J. Appl. Physiol.* 108, 311–319. doi: 10.1007/s00421-009-1215-2

Coelho, F. M., Pereira, D. S., Lustosa, L. P., Silva, J. P., Dias, J. M., Dias, R. C., et al. (2012). Physical therapy intervention (PTI) increases plasma brain-derived neurotrophic factor (BDNF) levels in non-frail and pre-frail elderly women. *Arch. Gerontol. Geriatr.* 54, 415–420. doi: 10.1016/j.archger.2011.05.014

Goldring, S. R., and Goldring, M. B. (2004). The role of cytokines in cartilage matrix degeneration in osteoarthritis. *Clin. Orthop. Relat. Res.* 427, 27–36. doi: 10.1097/01.blo.0000144854.66565.8f

Gomes, W. F., Lacerda, A. C., Brito-Melo, G. E., Fonseca, S. F., Rocha-Vieira, E., Leopoldino, A. A., et al. (2016). Aerobic training modulates T cell activation in elderly women with knee osteoarthritis. *Braz. J. Med. Biol. Res.* 49:e5181. doi: 10.1590/1414-431x20165181

Gomes, W. F., Lacerda, A. C., Mendonca, V. A., Arrieiro, A. N., Fonseca, S. F., Amorim, M. R., et al. (2012). Effect of aerobic training on plasma cytokines and soluble receptors in elderly women with knee osteoarthritis, in response to acute exercise. *Clin. Rheumatol.* 31, 759–766. doi: 10.1007/s10067-011-1927-7

Hautier, C., and Bonnefoy, M. (2007). Training for older adults. *Ann. Readapt. Med. Phys.* 50, 469–474. doi: 10.1016/j.annrmp.2007.04.018

Hinton, R., Moody, R. L., Davis, A. W., and Thomas, S. F. (2002). Osteoarthritis: diagnosis and therapeutic considerations. *Am. Fam. Physician* 65, 841–848.

Kean, C. O., Birmingham, T. B., Garland, S. J., Bryant, D. M., and Giffin, J. R. (2010). Minimal detectable change in quadriceps strength and voluntary muscle activation in patients with knee osteoarthritis. *Arch. Phys. Med. Rehabil.* 91, 1447–1451. doi: 10.1016/j.apmr.2010.06.002

Kellgren, J. H., and Lawrence, J. S. (1957). Radiological assessment of osteo-arthrosis. *Ann. Rheum. Dis.* 16, 494–502.

Knaepen, K., Goekint, M., Heyman, E. M., and Meeusen, R. (2010). Neuroplasticity - exercise-induced response of peripheral brain-derived neurotrophic factor: a systematic review of experimental studies in human subjects. *Sports Med.* 40, 765–801. doi: 10.2165/11534530-000000000-00000

Krabbe, K. S., Mortensen, E. L., Avlund, K., Pedersen, A. N., Pedersen, B. K., Jørgensen, T., et al. (2009). Brain-derived neurotrophic factor predicts mortality risk in older women. *J. Am. Geriatr. Soc.* 57, 1447–1452. doi: 10.1111/j.1532-5415.2009.02345.x

Lindsay, R. M. (1994). Neurotrophic growth factors and neurodegenerative diseases: therapeutic potential of the neurotrophins and ciliary neurotrophic factor. *Neurobiol. Aging* 15, 249–251. doi: 10.1016/0197-4580(94)90124-4

Marín, P. J., Bunker, D., Rhea, M. R., and Ayllón, F. N. (2009). Neuromuscular activity during whole-body vibration of different amplitudes and footwear conditions: implications for prescription of vibratory stimulation. *J. Strength Cond. Res.* 23, 2311–2316. doi: 10.1519/JSC.0b013e3181b8d637

Marx, F. C., Oliveira, L. M. D., Bellini, C. G., and Ribeiro, M. C. C. (2006). Translation and cultural validation of the Lequesne's algofunctional questionnaire for osteoarthritis of knee and hip for Portuguese language. *Rev. Bras. Reumatol.* 46, 253–260.

Matthews, V. B., Astrom, M. B., Chan, M. H., Bruce, C. R., Krabbe, K. S., Prelovsek, O., et al. (2009). Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase. *Diabetologia* 52, 1409–1418. doi: 10.1007/s00125-009-1364-1

McNair, P. J., Marshall, R. N., and Maguire, K. (1996). Swelling of the knee joint: effects of exercise on quadriceps muscle strength. *Arch. Phys. Med. Rehabil.* 77, 896–899. doi: 10.1016/S0003-9993(96)90277-4

Merriman, H., and Jackson, K. (2009). The effects of whole-body vibration training in aging adults: a systematic review. *J. Geriatr. Phys. Ther.* 32, 134–145. doi: 10.1519/00139143-200932030-00009

Michael, J. W., Schluter-Brust, K. U., and Eysel, P. (2010). The epidemiology, etiology, diagnosis, and treatment of osteoarthritis of the knee. *Dtsch. Arztebl. Int.* 107, 152–162. doi: 10.3238/arztebl.2010.0152

Neves, C. D., Tossige-Gomes, R., Avelar, N. C. P., and Lacerda, A. C. (2011). Assessing the reliability of isometric strength of knee extensors by using load cell. *Ter. Man* 9, 16–21.

Park, Y. G., Kwon, B. S., Park, J. W., Cha, D. Y., Nam, K. Y., Sim, K. B., et al. (2013). Therapeutic effect of whole body vibration on chronic knee osteoarthritis. *Ann. Rehabil. Med.* 37, 505–515. doi: 10.5535/arm.2013.37.4.505

Rees, S. S., Murphy, A. J., and Watsford, M. L. (2008). Effects of whole-body vibration exercise on lower-extremity muscle strength and power in an older population: a randomized clinical trial. *Phys. Ther.* 88, 462–470. doi: 10.2522/ptj.20070027

Roelants, M., Delecluse, C., and Verschueren, S. M. (2004). Whole-body-vibration training increases knee-extension strength and speed of movement in older women. *J. Am. Geriatr. Soc.* 52, 901–908. doi: 10.1111/j.1532-5415.2004.52256.x

Sakuma, K., and Yamaguchi, A. (2011). The recent understanding of the neurotrophin's role in skeletal muscle adaptation. *J. Biomed. Biotechnol.* 2011:201696. doi: 10.1155/2011/201696

Schabitz, W. R., Steigleder, T., Cooper-Kuhn, C. M., Schwab, S., Sommer, C., Schneider, A., et al. (2007). Intravenous brain-derived neurotrophic factor enhances poststroke sensorimotor recovery and stimulates neurogenesis. *Stroke* 38, 2165–2172. doi: 10.1161/STROKEAHA.106.477331

Schiphof, D., De Klerk, B. M., Koes, B. W., and Bierma-Zeinstra, S. (2008). Good reliability, questionable validity of 25 different classification criteria of knee osteoarthritis: a systematic appraisal. *J. Clin. Epidemiol.* 61, 1205–1215. doi: 10.1016/j.jclinepi.2008.04.003

Sharma, L., Cahue, S., Song, J., Hayes, K., Pai, Y. C., and Dunlop, D. (2003). Physical functioning over three years in knee osteoarthritis: role of psychosocial, local mechanical, and neuromuscular factors. *Arthritis Rheum.* 48, 3359–3370. doi: 10.1002/art.11420

Srikanth, V. K., Fryer, J. L., Zhai, G., Winzenberg, T. M., Hosmer, D., and Jones, G. (2005). A meta-analysis of sex differences prevalence, incidence and severity of osteoarthritis. *Osteoarthr. Cartil.* 13, 769–781. doi: 10.1016/j.joca.2005.04.014

Teixeira, A. L., Barbosa, I. G., Diniz, B. S., and Kummer, A. (2010). Circulating levels of brain-derived neurotrophic factor: correlation with mood, cognition and motor function. *Biomark. Med* 4, 871–887. doi: 10.2217/bmm.10.111

Trans, T., Aaboe, J., Henriksen, M., Christensen, R., Bliddal, H., and Lund, H. (2009). Effect of whole body vibration exercise on muscle strength and

proprioception in females with knee osteoarthritis. *Knee* 16, 256–261. doi: 10.1016/j.knee.2008.11.014

Valente, M. J., and MacKinnon, D. P. (2017). Comparing models of change to estimate the mediated effect in the pretest-posttest control group design. *Struct. Equ. Modeling* 24, 428–450. doi: 10.1080/10705511.2016.1274657

Wang, P., Yang, L., Li, H., Lei, Z., Yang, X., Liu, C., et al. (2016a). Effects of whole-body vibration training with quadriceps strengthening exercise on functioning and gait parameters in patients with medial compartment knee osteoarthritis: a randomized controlled preliminary study. *Physiotherapy* 102, 86–92. doi: 10.1016/j.physio.2015.03.3720

Wang, P., Yang, L., Liu, C., Wei, X., Yang, X., Zhou, Y., et al. (2016b). Effects of whole body vibration exercise associated with quadriceps resistance exercise on functioning and quality of life in patients with knee osteoarthritis: a randomized controlled trial. *Clin. Rehabil.* 30, 1074–1087. doi: 10.1177/0269215515607970

Yu, T., Chang, Y., Gao, X. L., Li, H., and Zhao, P. (2017). Dynamic expression and the role of BDNF in exercise-induced skeletal muscle regeneration. *Int. J. Sports Med.* 38, 959–966. doi: 10.1055/s-0043-118343

Zacaron, K., Dias, J., Abreu, N., and Dias, R. (2006). Physical activity levels, pain and swelling and their relationships with knee muscle dysfunction in elderly people with osteoarthritis. *Braz. J. Phys. Ther.* 10, 279–284. doi: 10.1590/S1413-35552006000300005

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

ORIGINAL RESEARCH
published: 28 June 2019
doi: 10.3389/fnagi.2019.00165

Markers of Novelty Processing in Older Adults Are Stable and Reliable

Hura Behforuzi[1], Nicole C. Feng[1], Adam R. Billig[1], Eliza Ryan[1], Erich S. Tusch[1], Phillip J. Holcomb[2], Abdul H. Mohammed[3,4] and Kirk R. Daffner[1]*

[1]Laboratory of Healthy Cognitive Aging, Department of Neurology, Center for Brain/Mind Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, United States, [2]Department of Psychology, San Diego State University, San Diego, CA, United States, [3]Department of Psychology, Linnaeus University, Växjö, Sweden, [4]Department of Neurobiology, Care Sciences and Society, Division of Neurogeriatrics, Karolinska Institutet, Stockholm, Sweden

Exploratory behavior and responsiveness to novelty play an important role in maintaining cognitive function in older adults. Inferences about age- or disease-related differences in neural and behavioral responses to novelty are most often based on results from single experimental testing sessions. There has been very limited research on whether such findings represent stable characteristics of populations studied, which is essential if investigators are to determine the result of interventions aimed at promoting exploratory behaviors or draw appropriate conclusions about differences in the processing of novelty across diverse clinical groups. The goal of the current study was to investigate the short-term test-retest reliability of event-related potential (ERP) and behavioral responses to novel stimuli in cognitively normal older adults. ERPs and viewing durations were recorded in 70 healthy older adults participating in a subject-controlled visual novelty oddball task during two sessions occurring 7 weeks apart. Mean midline P3 amplitude and latency, mean midline amplitude during successive 50 ms intervals, temporospatial factors derived from principal component analysis (PCA), and viewing duration in response to novel stimuli were measured during each session. Analysis of variance (ANOVA) revealed no reliable differences in the value of any measurements between Time 1 and 2. Intraclass correlation coefficients (ICCs) between Time 1 and 2 were excellent for mean P3 amplitude (ICC = 0.86), the two temporospatial factors consistent with the P3 components (ICC of 0.88 and 0.76) and viewing duration of novel stimuli (ICC = 0.81). Reliability was only fair for P3 peak latency (ICC = 0.56). Successive 50 ms mean amplitude measures from 100 to 1,000 ms yielded fair to excellent reliabilities, and all but one of the 12 temporospatial factors identified demonstrated ICCs in the good to excellent range. We conclude that older adults demonstrate substantial stability in ERP and behavioral responses to novel visual stimuli over a 7-week period. These results suggest that older adults may have a characteristic way of processing novelty that appears resistant to transient changes in their environment or internal states, which can be indexed during a single testing session. The establishment of reliable measures of novelty processing will allow investigators to determine whether proposed interventions have an impact on this important aspect of behavior.

Keywords: ERP, test-retest reliability, aging, novelty processing, visual modality

OPEN ACCESS

Edited by:
Brian C. Clark,
Ohio University, United States

Reviewed by:
Robert J. Barry,
University of Wollongong, Australia
Richard B. Reilly,
Trinity College Dublin, Ireland

****Correspondence:***
Hura Behforuzi
hurabehforuzi@gmail.com

Received: *21 March 2019*
Accepted: *13 June 2019*
Published: *28 June 2019*

Citation:
Behforuzi H, Feng NC, Billig AR, Ryan E, Tusch ES, Holcomb PJ, Mohammed AH and Daffner KR (2019) Markers of Novelty Processing in Older Adults Are Stable and Reliable. Front. Aging Neurosci. 11:165. doi: 10.3389/fnagi.2019.00165

INTRODUCTION

Participating in cognitively stimulating activities has been associated with a reduced risk of cognitive decline and dementia (Wilson et al., 2002; Gates et al., 2011; Najar et al., 2019). There has been a growing number of intervention studies aimed at engaging individuals in cognitively demanding activities. Curiosity/exploratory behavior and novelty seeking have been shown to be one of the driving forces that play an important role in maintaining cognitive function, learning, and even longevity in aging populations (Swan and Carmelli, 1996; Galli et al., 2018; Sakaki et al., 2018). Prior work in our laboratory has demonstrated that increased responsiveness to novelty is associated with successful cognitive aging (Daffner et al., 2006b; Riis et al., 2008). It is critical to establish reliable measures of novelty processing that will allow investigators to determine whether proposed interventions have an impact on this important aspect of behavior.

The process of orienting to and actively exploring novel events facilitates new learning and is an integral part of adapting to a rapidly changing environment (Sokolov, 1963; Daffner et al., 1998; Mesulam, 1998). The neural and behavioral underpinnings of novelty processing have been investigated using functional imaging [PET and functional magnetic resonance imaging (fMRI; Tulving et al., 1996; Opitz et al., 1999; Downar et al., 2000, 2002; Kiehl et al., 2001a,b; Bunzeck and Düzel, 2006; Bunzeck et al., 2007, 2010, 2012; Strobel et al., 2008; Blackford et al., 2010)], magnetoencephalography (Bunzeck et al., 2009; Naeije et al., 2016), and especially high temporal resolution event-related potentials (ERPs) that are often measured during different kinds of oddball paradigms (Näätänen, 1990; Fabiani and Friedman, 1995; Daffner et al., 1998, 2001, 2003; Friedman et al., 2001; Polich and Comerchero, 2003; Schomaker and Meeter, 2014; Kaufman et al., 2016b). Although the N1, P2, and N2 ERP components have been shown to be elicited by novel stimuli (Courchesne et al., 1975; Beck et al., 1980; Chong et al., 2008; Riis et al., 2008; Friedman et al., 2011; Tarbi et al., 2011; Barry et al., 2013; Schomaker et al., 2014), the novelty P3 component remains the most commonly employed ERP marker of novelty processing (Friedman et al., 2001).

The impact of normal aging and different neurological conditions on novelty processing has been an area of active investigation (Knight, 1984; Kaipio et al., 1999; Daffner et al., 2000a,b, 2001, 2003, 2006b; Stevens et al., 2007; Sokhadze et al., 2009; Ischebeck et al., 2011; Schott et al., 2015; Kaufman et al., 2016a; Sanjuan et al., 2018). Of note, inferences about age- or disease-related differences in neural and behavioral activity are most often based on results from single experimental testing sessions. There has been very limited research on whether such findings represent stable characteristics of the populations studied, which is essential if investigators are to draw appropriate conclusions about differences in response to novelty across diverse clinical groups or to determine the result of interventions aimed at promoting exploratory behaviors. The current study focuses on the stability and reliability of behavioral and ERP responses to novel stimuli in a sample of older adults who participated in a subject-controlled novelty oddball paradigm, as described below.

In the traditional version of the novelty oddball task, deviant stimuli are most commonly used to assess the degree to which participants are distracted from their assigned task, which is to identify (and often respond to) designated target stimuli (Fabiani and Friedman, 1995; Friedman et al., 2001; Polich and Comerchero, 2003; Kaufman et al., 2016b). Stimulus durations are fixed. By contrast, in the subject-controlled visual novelty oddball paradigm, participants determine viewing duration of stimuli by a button press (Daffner et al., 2006b; Chong et al., 2008). Viewing duration is used as an index of visual attention/exploratory behavior, and the P3 amplitude serves as an index of resources allocated to attentional processing (Berlyne, 1960; Daffner et al., 1994, 1998, 2000b). In this version of the paradigm, novel stimuli do not primarily serve as task-irrelevant distracters, but as potential "invitations" to explore interesting or salient aspects of one's environment (Chong et al., 2008).

Based on investigations of patients with focal neurological lesions (Daffner et al., 2000a,b, 2003) who participated in a subject-controlled novelty oddball task, we have proposed that the prefrontal cortex and posterior parietal cortex reflect two nodes of a neuroanatomical network for responding to and processing of novelty (Daffner et al., 2003). The prefrontal cortex regulates the allocation of attentional resources to potentially significant events in the environment (Daffner et al., 2000a,b,d, 2003). The posterior parietal cortex is involved in updating one's internal model of the environment to account for novel events (Daffner et al., 2003), a hypothesis consistent with Mesulam's schema (Mesulam, 1981, 1990) of the posterior parietal cortex as a gateway to integrating information to develop a dynamic internal representation of the environment. Injury to this frontoparietal network is indexed by disruption of the novelty P3, which has been strongly linked to diminished attention to novel stimuli as measured by viewing duration (Daffner et al., 1998, 2000b,c, 2001). Also of note, we have shown that the P3 amplitude to novel visual stimuli in this paradigm inversely correlates with the degree of apathy in neurological patients, as measured by informant ratings (Daffner et al., 2000b, 2001). In addition, we have found that cognitively high performing older adults generate larger novelty P3 responses and spend more time attending to novel events than their cognitively average performing peers (Daffner et al., 2006b). Moreover, cognitively high performing older adults produce a larger P3 response to novel stimuli than their younger, matched cognitively high performing counterparts (Daffner et al., 2006a,b), which we have suggested represents successful compensatory activity adopted by these older adults.

In summary, the subject-controlled novelty oddball paradigm has provided an opportunity to examine the relationship between neural and behavioral responses to novel visual stimuli. Additionally, results in the lab have been associated with meaningful real-world behavior, specifically the degree of apathy displayed by neurological patients. Thus, it appears to be a promising paradigm to investigate the stability of the response to novelty in older adults.

ERP measures exhibit variability that can be due to a variety of sources (Segalowitz and Barnes, 1993) including biological and state factors such as arousal (Koshino et al., 1993), circadian rhythms and seasonal cycles (Deldin et al., 1994; Huang et al., 2006), exercise and fatigue (Yagi et al., 1999), sleep deprivation (Morris et al., 1992), and mood (Pierson et al., 1996; Cavanagh and Geisler, 2006). However, a fundamental tenant of research in this area is that ERP components are reliable markers of underlying cognitive operations and processes (Kappenman and Luck, 2012) that may differ across clinical populations. If so, ERP results should demonstrate relative consistency over time. Research in this area has tended to focus on test-retest reliability of the P3 response of young adults to target stimuli in the auditory modality (Sinha et al., 1992; Segalowitz and Barnes, 1993; Kinoshita et al., 1996; Sandman and Patterson, 2000; Walhovd and Fjell, 2002; Lew et al., 2007), with fewer studies examining this issue using paradigms in the visual modality (Sinha et al., 1992; Cassidy et al., 2012; Brunner et al., 2013; Huffmeijer et al., 2014). These studies have varied in terms of paradigms used and the intervals between test and retest. In general, the investigations have demonstrated that P3 latency and P3 amplitude values in normal individuals are relatively stable, with no significant differences between test and retest values at follow-up intervals that have varied between 2 days and 36 months. Test-retest reliability [as measured by Pearson's r or intraclass correlation coefficient (ICC)] has ranged from 0.50 to 0.86 for P3 amplitude measures and from 0.40 to 0.88 for P3 latencies (Segalowitz and Barnes, 1993; Kinoshita et al., 1996; Sandman and Patterson, 2000; Walhovd and Fjell, 2002; Hall et al., 2006; Lew et al., 2007; Cassidy et al., 2012). Investigations of test-retest reliability of ERPs in older adults are particularly sparse (Sandman and Patterson, 2000; Walhovd and Fjell, 2002). These studies have reported lower reliability of latency in older individuals than young adults and greater reliability of amplitude than latency measures across all ages. No investigations seem to have highlighted novelty processing.

MATERIALS AND METHODS

Participants

Healthy older subjects were recruited through community announcements in the Boston metropolitan area. All subjects provided written informed consent approved by the Partners Human Research Committee. Brigham and Women's Hospital, where the study took place, is part of the Partners Healthcare system. To be included in this study, participants were required to be age 65 or older, English speaking, have a Mini-Mental State Exam (MMSE) (Folstein et al., 1975) score $\geq$26, an estimated IQ on the American National Adult Reading Test (AMNART) (Ryan and Paolo, 1992) $\geq$90, and score within 2 SDs of age-appropriate means on the short form of the Boston Naming Test (Kaplan et al., 1983) and on the Logical Memory Subtest of the Wechsler Memory Scale—Third Edition (Wechsler, 1997).

Subjects were excluded if they had a history of central nervous system (CNS) diseases or major psychiatric disorders based on the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) criteria (American Psychiatric Association, 1994), Geriatric Depression Scale (GDS) (Yesavage et al., 1982) score $\geq$10, corrected visual acuity worse than 20/50 (as tested using a Snellen eye chart), severe hearing impairment that would interfere with their ability to participate in the experiment or complete neuropsychological testing, a history of medical conditions that would limit their ability to participate in a physical exercise program, focal abnormalities on neurological examination consistent with a CNS lesion or a Clinical Dementia Rating Scale (Morris, 1993) score of 0.5 or above, based on interview questions and completion of a questionnaire by an informant who knew the subject well. See **Table 1** for subject demographic information and neuropsychological test performance.

Experimental Procedure

The experiment consisted of a subject-controlled visual novelty oddball task that has previously been used to study normal aging (Daffner et al., 2003; Riis et al., 2009) as well as patients with focal neurological injury due to a cerebral infarction (Daffner et al., 2000b, 2003) and patients with mild Alzheimer's disease (Daffner et al., 2001). Alternate versions of the task were presented during two sessions approximately 7 weeks apart, the order of which varied randomly across subjects. Stimuli were presented using E-Prime software (E-Prime 2.0, 2012). There were three categories of visual stimuli: frequent standard stimuli (a triangle)-70% frequency, rare target stimuli (upside down triangle)-15% frequency, and rare novel stimuli (randomly drawn from a set of unfamiliar line drawings many of which came from the collection of drawings that have been used by Kosslyn et al., 1994 and Kroll and Potter, 1984)-15% frequency (each shown only once). Two-hundred and forty line drawings, white on black background, were presented in four blocks of 60, each at the center of a high-resolution computer monitor. Visual stimuli appeared one at a time within a fixation box, subtending a visual angle of $\sim$3.5 $\times$ 3.5°, which remained on the screen at all times. Visual stimuli subtended an angle of $\sim$2.75° along their longest dimension.

TABLE 1 | Subject characteristics.

	Age (years)	Sex (Male/Female)	Education (years)	MMSE	AMNART	GDS
N = 70 Subjects	75.2 (6.5)	18/52	17.7 (2.9)	29.1 (1.2)	122.3 (5.6)	2.8 (2.2)

Values are given as mean (standard deviation). MMSE, Mini Mental State Examination; AMNART, American Adult Reading Test; GDS, Geriatric Depression Scale.

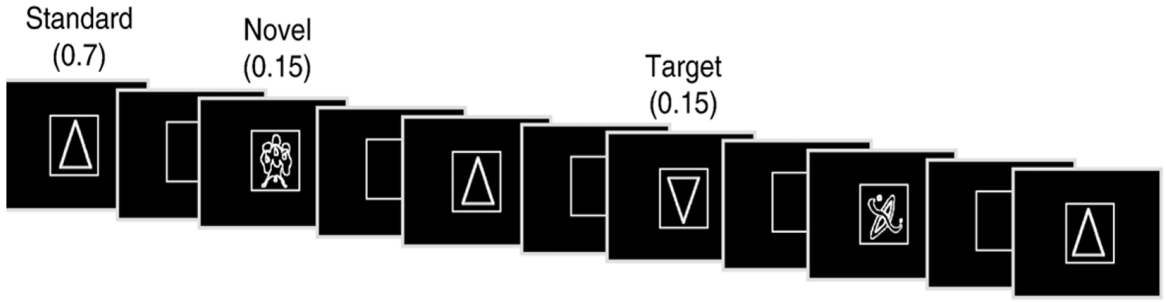


FIGURE 1 | Illustration of an experimental run.

Subjects controlled viewing duration of stimuli by space bar press that triggered the onset of the next stimulus. Subjects also responded to designated targets with a mouse click. All stimuli were displayed for a minimum duration of 600 ms, regardless of when the subject pressed the space bar to ensure that each stimulus was visible when pertinent ERP components (e.g., P3) were elicited. The interstimulus interval ranged between 800 and 1,200 ms. Stimuli were presented in a pseudorandom order with the extra constraints that no more than two novel stimuli were shown successively, and that each block of 60 stimuli had the same number of standard stimuli and approximately the same number of target and novel stimuli. Each subject started the experiment after a series of practice runs that did not include novel stimuli. See **Figure 1** for an illustration of the experiment.

Between sessions, subjects participated in one of four randomly assigned, structured programs involving adaptive or non-adaptive computerized cognitive training (CCT), physical exercise, or mindfulness meditation. Prior research suggests that CCT, physical exercise and mindfulness meditation may have a beneficial impact on cognitive functioning in older adults (Gates et al., 2011; Gard et al., 2014; Cheng, 2016; Tusch et al., 2016; Simon et al., 2018). Note that the purpose of these kinds of interventions has been to influence cognition, not novelty processing. Each intervention was structurally similar and conducted in subjects' homes using interactive, web-based software over the course of 5 weeks (five sessions per week, ∼35 min/session). The timing was based on the computerized Cogmed® (Pearson Education, Inc., Fort Worth, TX, USA) program that offered an adaptive and non-adaptive training format. In the adaptive cognitive training program, task difficulty increased as training proceeded over time. In the non-adaptive cognitive training program, individuals participated in the same computerized program but with the same low-level task difficulty throughout the training period. In the mindfulness program, subjects participated in a series of mindfulness training and exercises where the tasks became increasingly more self-directed over the 5-week period. In the physical exercise training program participants were involved in a structured physical exercise program that aimed to progressively increase their level of activity over the training period. There was an approximately 1 week delay between the first ERP session and the start of each intervention and between the end of the intervention and the second ERP session. Thus, the duration between the experimental testing that took place at Time-1 and Time-2 was about 7 weeks (M = 7.2, SD = 1.2).

ERP Recordings

An ActiveTwo electrode cap (Behavioral Brain Sciences Center, Birmingham, UK) was used to hold to the scalp a full array of 128 Ag-AgCl BioSemi (Amsterdam, Netherlands) "active" electrodes, whose locations were based on a pre-configured montage. Electrodes were arranged in equidistant concentric circles from 10 to 20 system electrode site Cz. In addition to the 128 electrodes on the scalp, six mini bio-potential electrodes were placed over the left and right mastoids (and used as references), beneath each eye and next to the outer canthi of the eyes to check for eye blinks and vertical and horizontal eye movements. EEG activity was digitized at a sampling rate of 512 Hz and filtered offline with a bandwidth of 0.016–100 Hz.

Data Analysis

The focus of this report is on ERP and behavioral responses to novel visual stimuli.

Behavioral Data

E-Prime software was used to collect the behavioral data. Viewing durations were calculated by subtracting the stimulus onset time from the space bar press time. This measure served as an index of visual attention and exploratory behavior (Daffner et al., 1992, 2000b).

Average Waveforms

EEG data were analyzed using ERPLAB (Lopez-Calderon and Luck, 2014) and EEGLAB (Delorme and Makeig, 2004) toolboxes that operate within the MATLAB framework. Raw EEG data were resampled to 256 Hz and referenced off-line to the algebraic average of the right and left mastoids. EEG signals were filtered using an IIR bandpass filter with a bandwidth of 0.03–40 Hz (12 dB/octave roll-off). Eye artifacts were removed through an independent component analysis. Individual channels that upon visual inspection revealed a consistently different pattern of activity from surrounding channels were corrected with the EEGLAB interpolation function. EEG epochs for novel stimuli

were averaged separately at three midline sites Fz, Cz, and Pz. The sampling epoch for each trial lasted for 1,200 ms, including a 200 ms pre-stimulus period that was used to baseline correct the ERP epochs. Trials were discarded from the analyses if they contained baseline drift or movement artifacts greater than 90 μV. Only trials with correct responses were included in the analyses. One of the 71 participants was excluded from further analyses because of excessively noisy ERP data, leaving a total of 70 participants.

P3 latency was measured as the local positive peak between 400 and 600 ms at midline electrodes Fz, Cz, and Pz in response to novel and target stimuli. P3 amplitude was measured as the average voltage between 400 and 600 ms at midline electrodes Fz, Cz, and Pz. Although the emphasis of this article is on the P3 response, a time course analysis to novel stimuli also was carried out by measuring the mean amplitude at Fz, Cz, and Pz for twenty 50 ms intervals across the entire 1,000 ms information processing period.

Statistical analysis of averaged ERP and behavioral data was carried out using IBM SPSS 25.0. In general, ERP dependent measures for novel and target stimuli were analyzed using repeated measures analysis of variance (ANOVA), with time (Time-1 vs. Time-2) and electrode site (Fz, Cz, and Pz) as the within-subjects variables and intervention condition (non-adaptive cognitive training, adaptive cognitive training, physical exercise, mindfulness training) as the between-subjects variable. The Greenhouse-Geisser correction was applied to all repeated measures with greater than 1 degree of freedom.

Principal Component Analysis

In addition to measuring average waveforms at midline electrodes, we performed a principal component analysis (PCA) of the data collected at Time-1 and Time-2 to identify and disentangle the constituent temporal and/or spatial components for further analysis of stability of ERP data over time. We used temporospatial PCA, following a method developed by Dien (2012). PCA is a data-driven method that decomposes ERP waveforms into their underlying components and is particularly useful in separating spatially and/or temporally overlapping components. Temporospatial PCA takes advantage of this method's ability to parse components both temporally and spatially by breaking down each temporal principal component into a series of spatially distinct components.

Following the recommendation of Dien (2012), a temporospatial PCA was conducted on averaged trials for each individual subject at all 134 electrode sites at Time-1 and Time-2. ERPs to novel, target, and standard stimuli were included in the analysis to augment variance. Each dataset consisted of 307 time points between −200 and 1,000 ms. Utilizing the ERP PCA toolkit 2.38 (Dien, 2010), temporal PCA followed by a spatial PCA (on each identified temporal factor) was performed. A parallel test was used to restrict the number of factors generated for each PCA. The covariance matrix was used as input, with Kaiser normalization, followed by Promax rotation.

Intraclass Correlation

ICC represents the consistency of a measure with the time of testing introduced into the error variance (Shrout and Fleiss, 1979). ICC was used in the analysis of viewing duration, averaged ERP waves, and PCA components at the two time points. Per the descriptions and guidelines of different models of ICC (Shrout and Fleiss, 1979; Koo and Li, 2016), test-retest reliability was calculated by ICC method using a two-way mixed effect model with the setting of absolute agreement in SPSS. Since the values studied represented the average of multiple trials, average rather than single value ICC measurements are reported. As per classification of Cicchetti (Cicchetti, 1994), values less than 0.4 are indicative of poor reliability, values between 0.4 and 0.59 indicate fair reliability, values between 0.6 and 0.74 denote good reliability, and values greater than 0.75 are considered excellent reliability.

RESULTS

Viewing Duration

Figure 2 illustrates the mean viewing durations in response to novel stimuli at Time-1 vs. Time-2. Repeated measures ANOVA

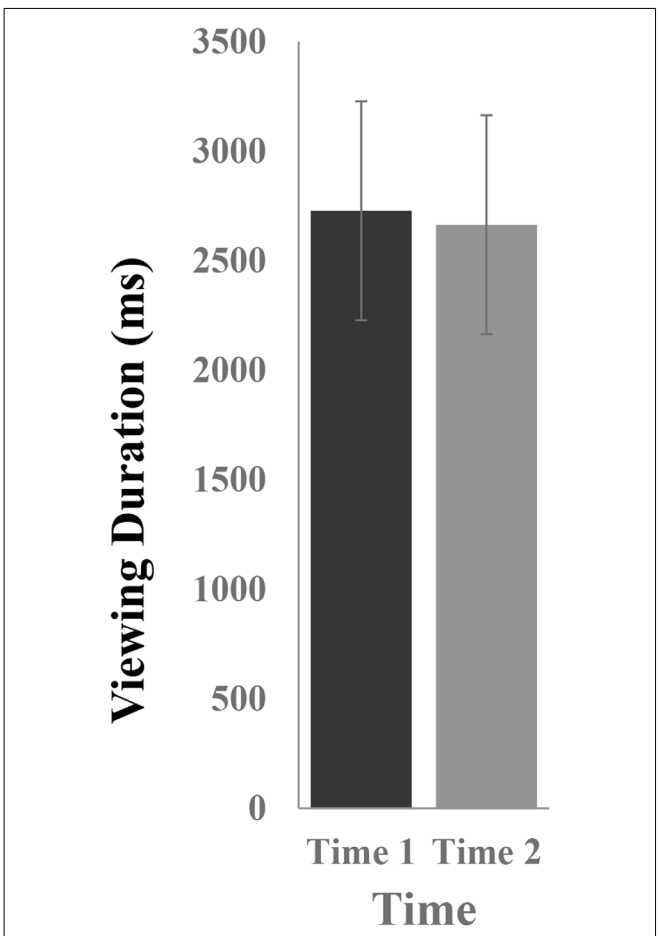


FIGURE 2 | Mean [± standard error of the mean (SEM)] viewing duration (in ms) in response to novel stimuli.

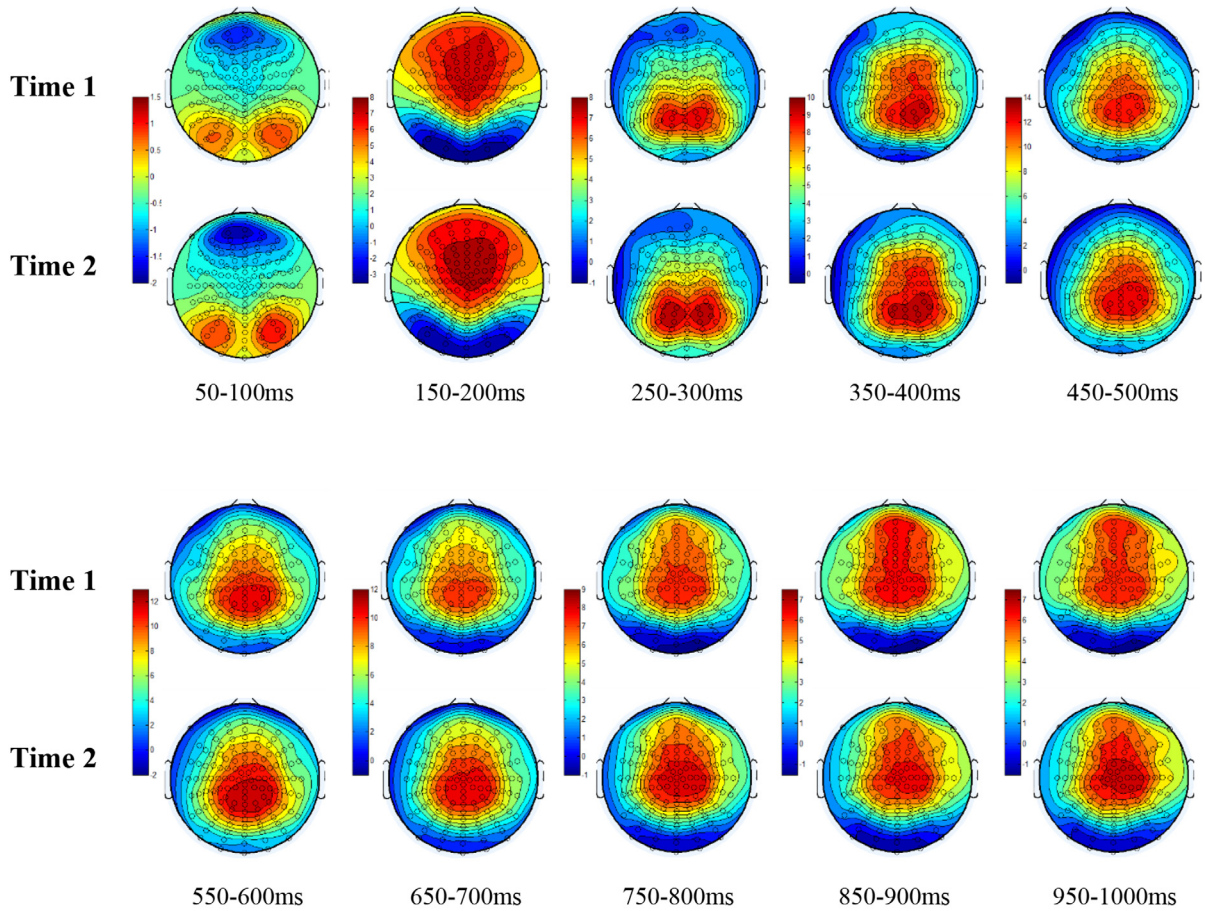


FIGURE 3 | Topographic maps of the mean amplitude for every other 50 ms interval (beginning 50–100 ms) of novelty processing at Time-1 vs. Time-2 (Note that the scales are different across the time frames).

was performed for the effect of time (Time-1, Time-2) on viewing duration. It demonstrated no effect of time, $F_{(1,66)} = 0.06$, $p = 0.80$, partial $\eta^2 = 0.0001$. The average measure ICC between viewing duration at Time-1 and Time-2 was 0.81, with a 95% confidence interval from 0.69 to 0.88, $F_{(69,69)} = 5.13$, $p < 0.001$.

Grand Average Waveforms

Novel Stimuli

All the results are collapsed across the four structured programs since none of the findings were modulated by this between-subject variable. **Figure 3** presents topographic surface potential maps in response to novel stimuli for Time-1 vs. Time-2 at 50 ms intervals. Note that the pattern of electrophysiologic response is very similar across the two time points.

The grand average ERP plots for novel stimuli (Time-1 and Time-2) at midline electrode sites Fz, Cz, and Pz are presented in **Figure 4**. **Figure 5** illustrates a bar graph of the mean P3 amplitude data at midline sites for novel stimuli. Repeated measures ANOVA for the P3 mean amplitude demonstrated no effect of time, $F_{(1,69)} = 1.19$, $p = 0.28$, partial $\eta^2 = 0.02$; and no time × electrode site interaction, $F_{(2,138)} = 1.68$, $p = 0.19$, partial $\eta^2 = 0.02$. There was an effect of electrode site on P3 mean amplitude, $F_{(2,138)} = 41.6$, $p < 0.001$, partial $\eta^2 = 0.38$. *Post hoc* comparisons using the LSD test indicated that the P3 mean amplitude at Fz ($M = 9.30$ μV, SE = 0.65) was smaller than at Cz ($M = 11.02$ μV, SE = 0.73), which in turn was smaller than at Pz ($M = 12.40$ μV, SE = 0.71). The ICC between P3 mean amplitude collapsed across midline sites at Time-1 and Time-2 was 0.86, with a 95% confidence interval from 0.78 to 0.92, $F_{(69,69)} = 7.31$, $p < 0.001$.

Repeated measures ANOVA for P3 peak latency demonstrated no effect of time, $F_{(1,69)} = 1.11$, $p = 0.30$, partial $\eta^2 = 0.02$; and no time × electrode site interaction, $F_{(2,138)} = 0.18$, $p = 0.80$, partial $\eta^2 = 0.003$. There was an effect of electrode site on P3 peak latency, $F_{(2,138)} = 5.00$, $p = 0.01$, partial $\eta^2 = 0.07$. *Post hoc* comparisons using the LSD test indicated that the mean P3 peak latency was longer at Cz ($M = 510$ ms, SE = 5.55) than Fz ($M = 497$ ms, SE = 5.24). There was no difference between Pz ($M = 504$ ms, SE = 5.52) and the other two electrode sites. The ICC between the average midline P3 peak latency at Time-1 and Time-2 was 0.56, with a 95% confidence interval from 0.30 to 0.73, $F_{(69,69)} = 2.29$, $p < 0.001$.

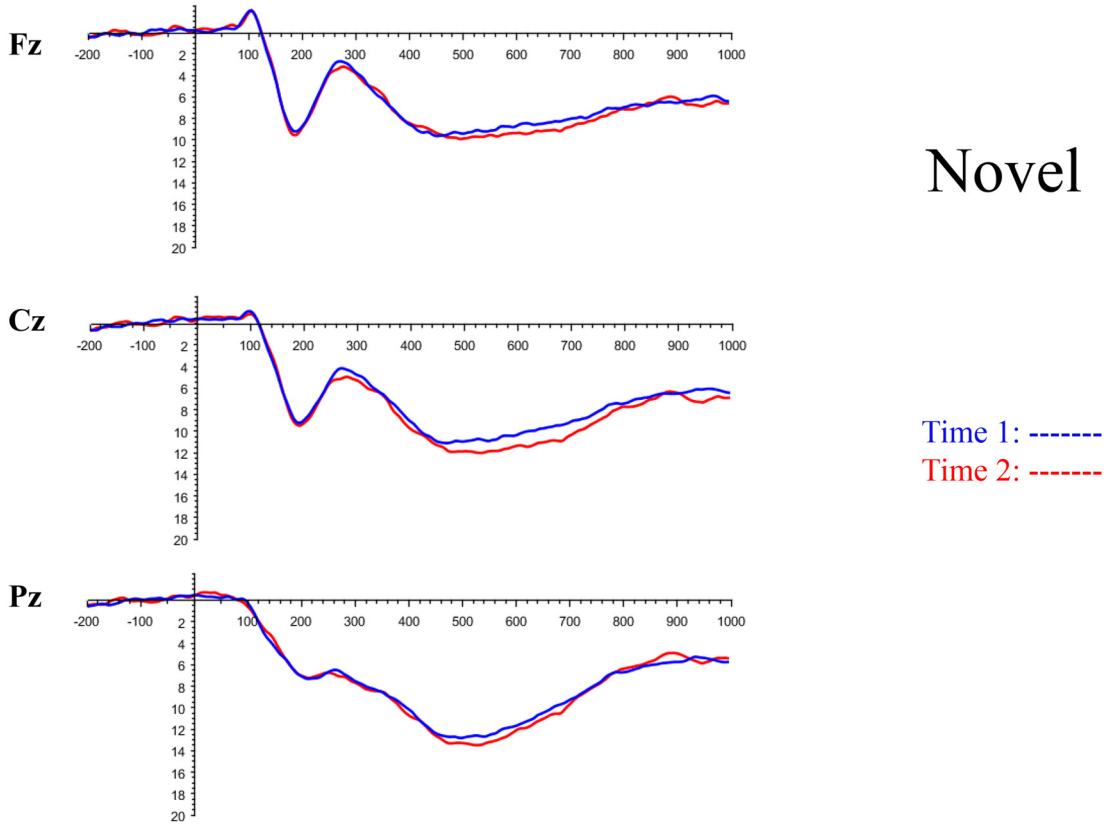


FIGURE 4 | Grand average event-related potential (ERP) plots in response to novel stimuli at Fz, Cz, and Pz at Time-1 and Time-2.

Figure 6 depicts the ICCs between the average amplitudes at Time-1 vs. Time-2 for each 50 ms interval in response to novel stimuli at Fz, Cz, and Pz. Except for two time frames (0–50 ms at Cz and 50–100 ms at Pz) the ICCs were significant throughout the 1,000 ms temporal epoch at midline electrodes. The ICC reliability ranged from 0.53 to 0.91 between 100 ms and 1,000 ms, with the very high reliabilities between 200 ms and 600 ms (ICC range 0.80–0.91).

Target Stimuli

Although the focus of this study was on novelty processing, data for target stimuli were also analyzed to help determine the consistency of response to non-novel visual stimuli. The grand average ERP plots for target stimuli (Time-1 and Time-2) at midline electrode sites Fz, Cz, and Pz are presented in **Figure 7**. Repeated measures ANOVA for target P3 amplitude demonstrated no effect of time, $F_{(1,69)} = 3.29$, $p = 0.07$, partial $\eta^2 = 0.05$; and no time × electrode site interaction, $F_{(2,138)} = 0.57$, $p = 0.55$, partial $\eta^2 = 0.008$. There was an effect of electrode site, $F_{(2,138)} = 4.40$, $p = 0.02$, partial $\eta^2 = 0.06$. *Post hoc* comparisons using the LSD test indicated that the mean P3 mean amplitude at Fz ($M = 12.65$ μV, SE = 0.59) was smaller than at Cz ($M = 13.43$ μV, SE = 0.74) and Pz ($M = 13.88$ μV, SE = 0.72), with no difference between the latter two electrode sites. The ICC between P3 mean amplitude collapsed across midline sites at Time-1 and Time-2 was 0.90, with a 95% confidence interval from 0.84 to 0.94, $F_{(69,69)} = 10.63$, $p < 0.001$.

For P3 latency, repeated measures ANOVA revealed no effect of time, $F_{(1,69)} = 1.22$, $p = 0.27$, partial $\eta^2 = 0.01$; and no time × electrode site interaction, $F_{(2,138)} = 0.21$, $p = 0.76$, partial $\eta^2 = 0.003$. There was an effect of electrode site on P3 peak latency, $F_{(2,138)} = 7.54$, $p = 0.002$, partial $\eta^2 = 0.09$. *Post hoc* comparisons using the LSD test indicated that the mean P3 peak latency at Pz ($M = 493$ ms, SE = 5.30) was longer than at Fz ($M = 483$ ms, SE = 5.61) or Cz ($M = 480$ ms, SE = 5.27), with no difference between the latter two electrode sites. The ICC between P3 peak latency collapsed across midline sites at Time-1 and Time-2 was 0.78, with a 95% confidence interval from 0.65 to 0.86, $F_{(69,69)} = 4.59$, $p < 0.001$.

Novel vs. Target

To determine whether responses to novels differed from those to targets, the stimuli were compared to each other. Differences in viewing duration of novel vs. target stimuli were examined. Repeated measures ANOVA was performed with stimulus type (novel, target) and time (Time-1, Time-2) as within-subject variables. There was an effect of stimulus type, $F_{(1,66)} = 8.11$, $p = 0.006$, partial $\eta^2 = 0.11$, due to longer mean viewing durations on novels ($M = 2,725$ ms, SE = 326) than targets ($M = 1,852$ ms,

SE = 93.5). There was no effect of time, $F_{(1,3)} = 0.63$, $p = 0.43$, partial $\eta^2 = 0.01$, and no time × stimulus type interaction, $F_{(1,66)} = 0.08$, $p = 0.78$, partial $\eta^2 = 0.001$.

To assess P3 amplitude, repeated measures ANOVA was performed, with stimulus type (novel, target), time (Time-1, Time-2) and electrode site (Fz, Cz, and Pz) as within-subject variables. There was an effect of stimulus type, $F_{(1,69)} = 23.4$, $p < 0.001$, partial $\eta^2 = 0.25$, due to the mean P3 mean amplitude to targets ($M = 13.32$ μV, SE = 0.64) being larger than to novels ($M = 10.91$ μV, SE = 0.67). There was no effect of time, $F_{(1,69)} = 3.42$, $p = 0.07$, partial $\eta^2 = 0.05$, and no time × stimulus type interaction, $F_{(1,69)} = 0.09$, $p = 0.77$, partial $\eta^2 = 0.001$. There was a stimulus type × electrode site interaction, $F_{(2,138)} = 22.9$, $p < 0.001$, partial $\eta^2 = 0.25$. As noted above, the P3 mean amplitude to novel stimuli at Fz ($M = 9.30$ μV, SE = 0.65) was smaller than at Cz ($M = 11.02$ μV, SE = 0.72), which in turn was smaller than at Pz ($M = 12.40$ μV, SE = 0.71). In contrast, the P3 mean amplitude to target stimuli at Fz ($M = 12.65$ μV, SE = 0.59) was smaller than at Cz ($M = 13.43$ μV, SE = 0.73) and Pz ($M = 13.88$ μV, SE = 0.72), with no difference between the latter two electrode sites.

P3 latency was examined using repeated measures ANOVA, with stimulus type (novel, target), time (Time-1, Time-2) and electrode site (Fz, Cz, and Pz) as within-subject variables. There was an effect of stimulus type, $F_{(1,69)} = 9.27$, $p = 0.003$, partial $\eta^2 = 0.12$, with P3 latency to novels ($M = 504$ ms, SE = 4.87) being longer than to targets ($M = 486$ ms, SE = 5.0). There was no effect of time, $F_{(1,69)} = 0.04$, $p = 0.84$, partial $\eta^2 = 0.001$. There was also significant electrode × stimulus type interaction, $F_{(2,138)} = 6.61$, $p = 0.003$, partial $\eta^2 = 0.09$. The mean P3 peak latency in response to novel stimuli was longer at Cz ($M = 510$ ms, SE = 5.55) than Fz ($M = 497$ ms, SE = 5.16). There was no difference between Pz ($M = 504$ ms, SE = 5.52) and the other two electrode sites. In contrast, the mean P3 peak latency in response to target at Pz ($M = 493$ ms, SE = 5.30) was longer than at Fz ($M = 483$ ms, SE = 5.61) or Cz ($M = 480$ ms, SE = 5.26), with no difference between the latter two electrode sites.

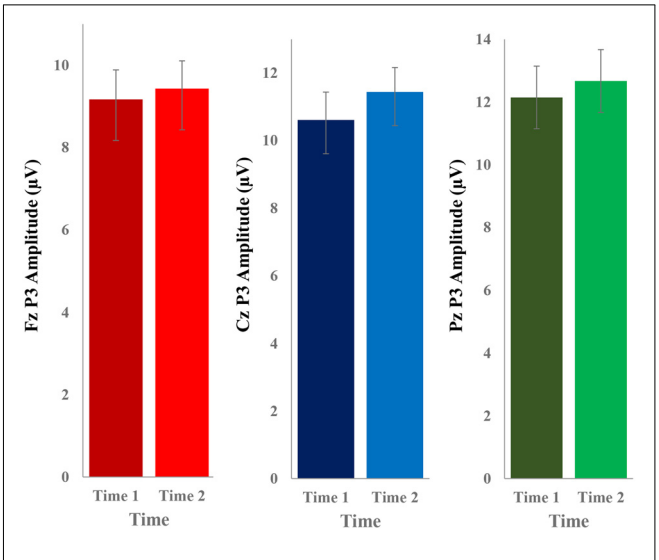


FIGURE 5 | Bar graph of the mean (± SEM) P3 amplitude (in μV) in response to novel stimuli at Fz, Cz, and Pz at Time-1 and Time-2.

PCA

A temporospatial PCA of the whole data set yielded 132 factor combinations [12 temporal factors (TFs), each with 11 spatial factors (SFs)]. **Table 2** illustrates temporospatial factors that each accounted for >1% of the variance, ordered by the amount of variance explained by each factor. The table includes the factor name, peak latency, percentage of the total variance accounted for, topography at Time-1 and Time-2 in response to novel stimuli, and ICC (and *p*-values) between Time-1 and Time-2. One-hundred and twenty of the factor combinations were not analyzed further because each accounted for <1% of the total variance.

Based on visual inspection of the timing and topographic distribution of temporospatial factors, two factors, TF2SF1 (389 ms peak) and TF3SF1 (573 ms peak) likely contributed to the P3 component (Spencer et al., 1999, 2001; Goldstein et al., 2002; Dien et al., 2003). Both demonstrated excellent test-retest stability, with ICCs of 0.89 and 0.77 respectively. TF1SF1 (819 ms peak) was consistent with the late positive slow wave (Spencer et al., 2001; Alperin et al., 2015). TF4SF1 (167 ms peak) was suggestive of an early anterior P2, and

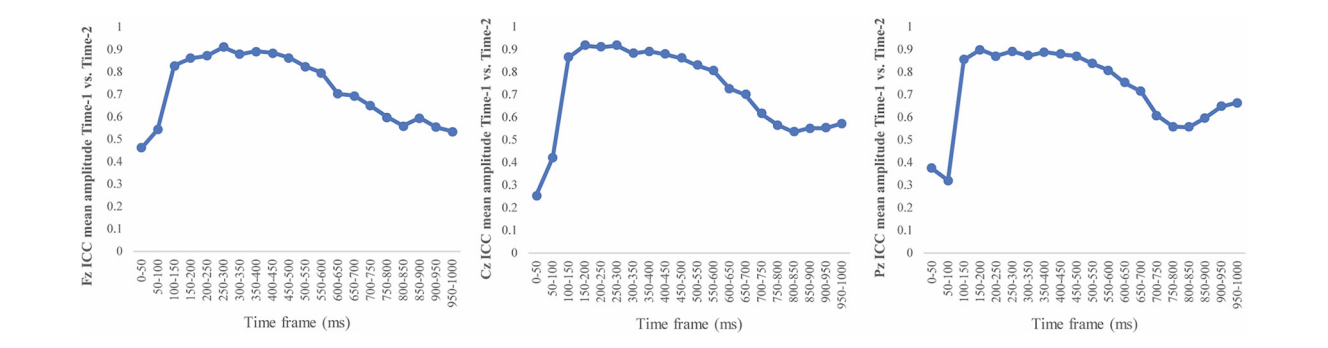


FIGURE 6 | Intraclass correlation coefficient (ICC) between Time-1 and Time-2 of the mean amplitude for each 50 ms post novel stimuli at Fz, Cz, and Pz.

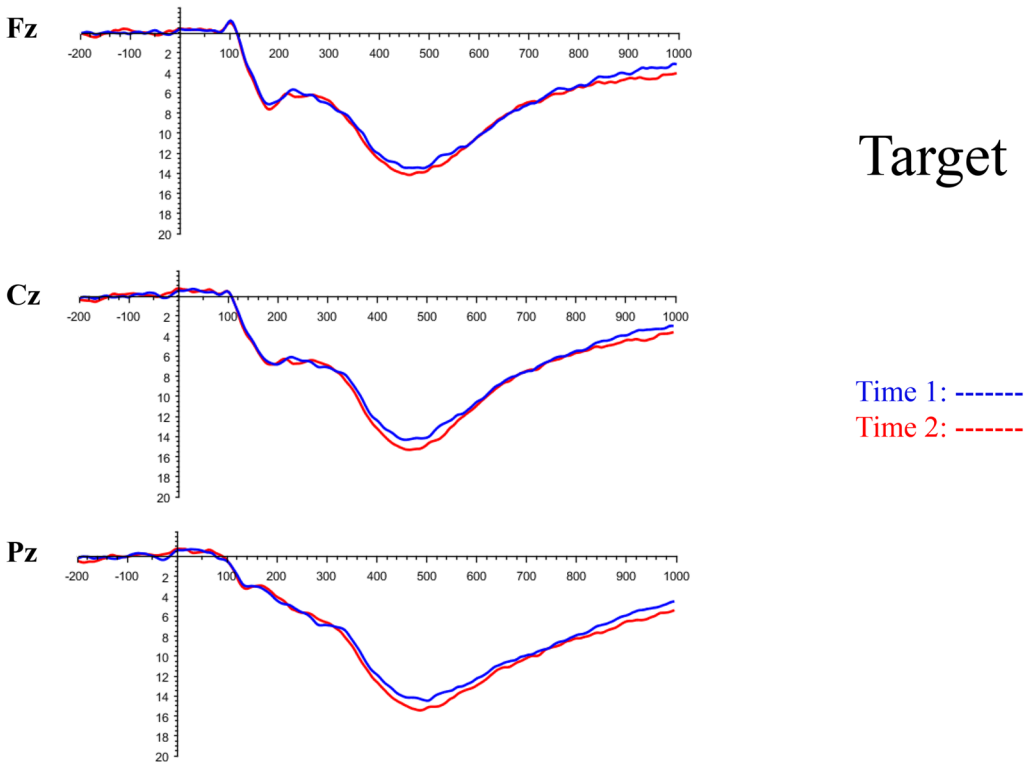


FIGURE 7 | Grand average ERP plots in response to target stimuli at Fz, Cz, and Pz at Time-1 and Time-2.

TF5SF1 (221 ms peak) was consistent with late anterior P2 (Riis et al., 2009; Alperin et al., 2015). All but one temporospatial factor demonstrated good to excellent test-retest stability. TF1SF1 showed fair stability (see **Table 2**).

DISCUSSION

Attention to novel stimuli plays a critical role in adaptation, learning, and the maintenance of cognitive functions as adults grow older (Sokolov, 1963; Daffner et al., 1994, 2006b; Riis et al., 2008). ERPs have helped to track neurophysiological changes associated with novelty processing across different age groups and neurological conditions (Daffner et al., 2000a,b, 2003, 2006a,b). Using ERPs to characterize differences between clinical populations or to assess the impact of interventions on promoting engagement with one's environment requires a demonstration of the reliability of the measures themselves. Much more research has been directed at investigating the consistency of ERP responses across testing sessions among young than old adults, and in response to target rather than novel events. The current study aimed to evaluate the test-retest reliability of electrophysiological and behavioral responses to novel stimuli in cognitively normal older adults.

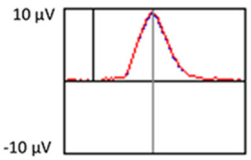
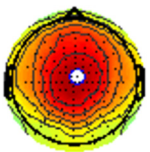
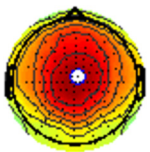
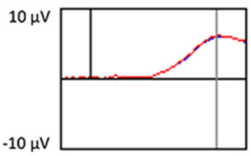
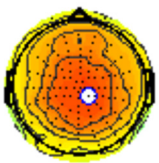
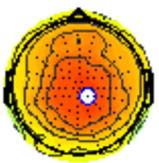
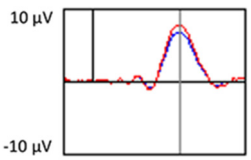
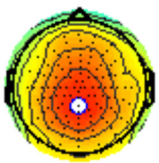
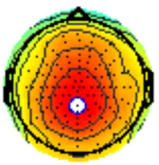
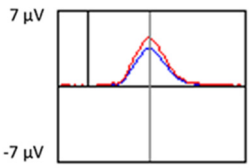
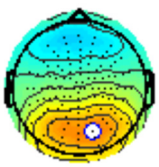
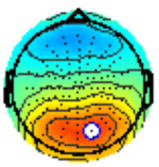
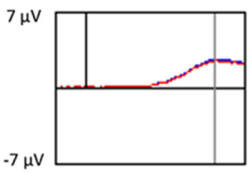
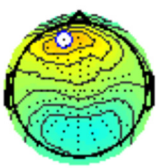
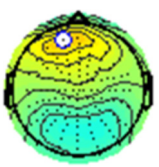
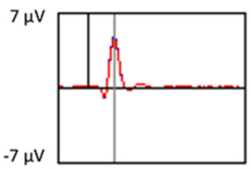
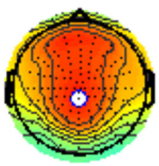
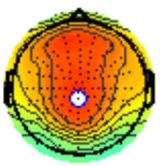
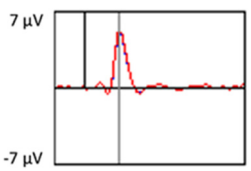
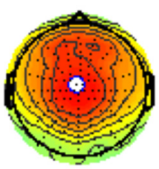
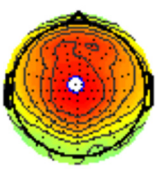
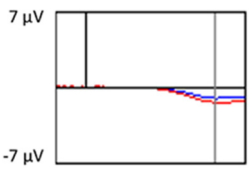
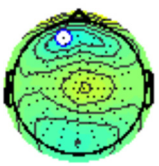
Novel visual stimuli were infrequent and highly unusual/unfamiliar figures. Because participants in this subject-controlled novelty oddball paradigm had to determine the duration of each stimulus, the novel events were not task-irrelevant, as they are in traditional novelty oddball paradigms. In our study, there was electrophysiological and behavioral evidence that novel stimuli were processed differently from target ones. Viewing duration was much longer on novel than target events; P3 mean amplitude was larger in response to target than to novel stimuli; and P3 peak latency was longer to novel than target stimuli.

ANOVA yielded no reliable differences in the electrophysiologic and behavioral responses to novel visual stimuli between test sessions approximately 7 weeks apart, with *p*-values ranging from 0.27 (mean P3 amplitude) to 0.8 (viewing duration). These results point to the stability of the measures used. However, confirming the null hypothesis (i.e., no differences between sessions) is not possible statistically. Thus, we used ICC as a measure of test-retest reliability.

Our findings indicate that the mean P3 amplitude response to novel visual stimuli, as measured on average waveforms at midline sites, exhibits excellent reliability (ICC of 0.86). Converging evidence for the stability of the P3 to novel stimuli was derived from PCA, a data-driven method. The amplitude of the temporospatial factors consistent with the P3 components (TF2SF1 and TF3SF1) also demonstrated excellent reliability (ICC of 0.88 and 0.76, respectively).

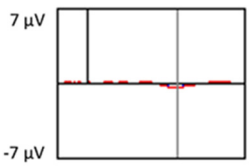
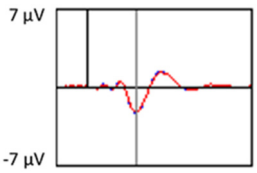
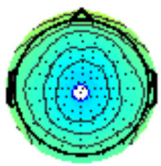
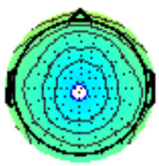
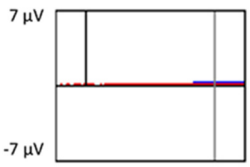
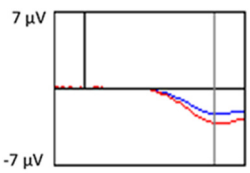
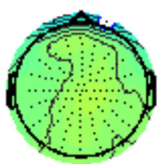
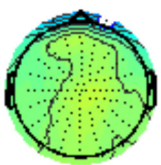
In keeping with other reports in the literature on ERP latencies, P3 peak latency in response to novel visual stimuli demonstrated only fair reliability (ICC of 0.56) across the 7-week interval. P3 latency, a marker of processing speed, may

TABLE 2 | Temporospatial factors accounting for >1% of variance.

Temporal spatial factor	Amount of variance explained	Peak latency	Waveform	Topography Time-1	Topography Time-2	Intraclass correlation *P*-value
TF02SF01	22.5%	389 ms				0.88 $P < 0.001$
TF01SF01	16.4%	819 ms				0.49 $P < 0.01$
TF03SF01	9.0%	573 ms				0.76 $P < 0.001$
TF02SF02	4.24%	389 ms				0.91 $P < 0.001$
TF01SF02	4.06%	819 ms				0.74 $P < 0.001$
TF04SF01	3.00%	167 ms				0.90 $P < 0.001$
TF05SF01	2.27%	221 ms				0.76 $P < 0.001$
TF01SF03	2.1%	819 ms				0.68 $P < 0.001$

(Continued)

TABLE 2 | Continued

Temporal spatial factor	Amount of variance explained	Peak latency	Waveform	Topography Time-1	Topography Time-2	Intraclass correlation *P*-value
TF03SF02	1.99%	573 ms				0.73 $P < 0.001$
TF06SF01	1.8%	303 ms				0.79 $P < 0.001$
TF01SF04	1.49%	819 ms				0.67 $P < 0.001$
TF01SF05	1.21%	819 ms				0.70 $P < 0.001$

(Note that the amplitude scales used differ across factors)

be more sensitive than P3 amplitude to a variety of state functions, including level of arousal, variation in sleep, or changes in mood (Bruder et al., 1991; Polich and Kok, 1995; Polich, 2004). Latency measures are often reported to have lower test-retest reliability than amplitude measures regardless of age group (Sinha et al., 1992; Sandman and Patterson, 2000; Walhovd and Fjell, 2002; Olvet and Hajcak, 2009; Weinberg and Hajcak, 2011; but see Segalowitz and Barnes, 1993; Brunner et al., 2013 for conflicting evidence). Walhovd and Fjell (2002) found in a two-stimulus auditory oddball task that test-retest reliability of P3 latency was lower in older than younger adults. In contrast, these investigators and others (Hämmerer et al., 2013) who have used tasks in the visual modality have reported no differences in the reliability of P3 amplitude across age groups.

Our results also strongly point to stability in the electrophysiologic response to novel stimuli throughout the 1,000 ms temporal epoch studied and not only the interval containing the P3 component. Inspection of the surface potential maps for Time-1 vs. Time-2 (**Figure 3**) suggests considerable overlap in the appearance of scalp voltage distributions from 100 to 1,000 ms. This impression was validated by assessing the mean amplitude at midline electrode sites using time course analysis during sequential 50 ms intervals. This evaluation demonstrated fair to excellent reliability (ICC range 0.53–0.91) between 100 ms and 1,000 ms time range, with very high reliability between 200 ms and 600 ms (ICC range 0.80–0.91), which includes the temporal interval of the P3 component (see **Figure 6**). Moreover, with only one exception the 12 temporospatial factors analyzed (all of which peaked between 167 and 819 ms) demonstrated ICCs in the good to excellent range. Thus, we provide strong converging evidence in older adults for the stability of electrophysiological responses to novel stimuli throughout the measured information processing stream. These results of our study are consistent with findings reported by Walhovd and Fjell in their study using a two-stimulus auditory oddball task (Walhovd and Fjell, 2002). They investigated the reliability of successive 15 ms time window measurements across 0–705 ms post-stimulus and observed high reliability, especially during the temporal windows in which ERP components (N1, P2, P3) are conventionally measured. They suggest that these results may provide further validation of established ERP components as reflecting stable cerebral responses to different stimulus types.

In the current study, viewing duration was used as an index of visual attention and exploratory behavior (Daffner et al., 1994). Viewing duration of novel stimuli demonstrated excellent test-retest reliability (ICC of 0.81) over the 7-week period. This result suggests that an older individual may exhibit a characteristic degree of engagement by novel visual stimuli that

remain stable over time. Both P3 amplitude and viewing duration can be understood in terms of resources being allocated in response to a presented stimulus (Daffner et al., 2000c). Both experimental measures appear to be consistent and reliable, a result that has notable implications for future research. The finding suggests that if a clinical intervention (behavioral or pharmacologic) is associated with a significant alteration in P3 amplitude or viewing duration in response to novel stimuli, it is unlikely that such changes would be simply due to chance. This idea is important because of interest in developing interventions to help older adults become more engaged by the novel aspects of their environment as a means of promoting healthy cognitive/brain aging (Wilson et al., 2002; Daffner et al., 2006b; Veyrac et al., 2009). Objective laboratory measurements of such engagement can serve as a valuable component of the research.

The generalizability of our findings remains uncertain. The participants in our study were well educated and had above average intellectual capacity. Further research is necessary to determine whether similar stability of electrophysiologic and behavioral responses to novelty would be observed in older adults with different demographic characteristics. It would be informative for future studies to include a sample of younger adults to help determine if there are age-related differences in test-retest reliability in response to novel visual events. Additional studies are also needed to address the reliability of ERP measures over periods longer than 7 weeks and across multiple testing sessions. The limited number of studies that have investigated the test-retest reliability over more than two sessions, with inter-session intervals ranging from days to months (Kinoshita et al., 1996) or even years (Sandman and Patterson, 2000) have provided additional support for the stability of ERP measurements.

CONCLUSION

Older adults exhibit considerable stability in their electrophysiological and behavioral responses to novel visual events over a 7-week period. These results suggest older adults may have a characteristic way of processing novelty that appears resistant to transient changes in their environment or internal states, such as level of arousal, and that can be indexed during a single testing session.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Human Research Committee of the Partners Health Care system, with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Partners Human Research Committee.

AUTHOR CONTRIBUTIONS

HB analyzed the data, wrote the initial manuscript, prepared the figures. NF analyzed the data, helped prepare the figures, and edited the manuscript. AB analyzed the data and edited the manuscript. ER worked with participants and helped collect the data. ET collected the data and assisted with analysis. PH helped design the experiment and interpret the ERP data. AM helped design the overall study. KD was responsible for the overall design of the experiment, the data analysis, and the final manuscript.

FUNDING

This study was funded by the Kamprad Family Foundation, Vaxjo, Sweden. In addition, the Laboratory of Healthy Cognitive Aging at Brigham and Women's Hospital has been supported by the Wimberly family, the Muss family, and the Mortimer/Grubman family.

ACKNOWLEDGMENTS

We would like to thank Brittany McFeeley for her administrative assistance.

REFERENCES

Alperin, B. R., Tusch, E. S., Mott, K. K., Holcomb, P. J., and Daffner, K. R. (2015). Investigating age-related changes in anterior and posterior neural activity throughout the information processing stream. *Brain Cogn.* 99, 118–127. doi: 10.1016/j.bandc.2015.08.001

American Psychiatric Association. (1994). *Diagnostic and Statistical Manual of Mental Disorders.* Washington, DC: American Psychological Association.

Barry, R. J., MacDonald, B., De Blasio, F. M., and Steiner, G. Z. (2013). Linking components of event-related potentials and autonomic measures of the orienting reflex. *Int. J. Psychophysiol.* 89, 366–373. doi: 10.1016/j.ijpsycho.2013.01.016

Beck, E. C., Swanson, C., and Dustman, R. E. (1980). Long latency components of the visually evoked potential in man: effects of aging. *Exp. Aging Res.* 6, 523–545. doi: 10.1080/03610738008258385

Berlyne, D. E. (1960). *Conflict, Arousal and Curiosity.* New York, NY: McGraw-Hill.

Blackford, J. U., Buckholtz, J. W., Avery, S. N., and Zald, D. H. (2010). A unique role for the human amygdala in novelty detection. *Neuroimage* 50, 1188–1193. doi: 10.1016/j.neuroimage.2009.12.083

Bruder, G. E., Towey, J. P., Stewart, J. W., Friedman, D., Tenke, C., and Quitkin, F. M. (1991). Event-related potentials in depression: influence of task, stimulus hemifield and clinical features on P3 latency. *Biol. Psychiatry* 30, 233–246. doi: 10.1016/0006-3223(91)90108-x

Brunner, J. F., Hansen, T. I., Olsen, A., Skandsen, T., Haberg, A., and Kropotov, J. (2013). Long-term test-retest reliability of the P3 NoGo wave and two independent components decomposed from the P3 NoGo wave in a visual Go/NoGo task. *Int. J. Psychophysiol.* 89, 106–114. doi: 10.1016/j.ijpsycho.2013.06.005

Bunzeck, N., Dayan, P., Dolan, R. J., and Duzel, E. (2010). A common mechanism for adaptive scaling of reward and novelty. *Hum. Brain Mapp.* 31, 1380–1394. doi: 10.1002/hbm.20939

Bunzeck, N., Doeller, C. F., Dolan, R. J., and Duzel, E. (2012). Contextual interaction between novelty and reward processing within the mesolimbic system. *Hum. Brain Mapp.* 33, 1309–1324. doi: 10.1002/hbm.21288

Bunzeck, N., Doeller, C. F., Fuentemilla, L., Dolan, R. J., and Duzel, E. (2009). Reward motivation accelerates the onset of neural novelty signals in humans to 85 milliseconds. *Curr. Biol.* 19, 1294–1300. doi: 10.1016/j.cub.2009.06.021

Bunzeck, N., and Düzel, E. (2006). Absolute coding of stimulus novelty in the human substantia nigra/VTA. *Neuron* 51, 369–379. doi: 10.1016/j.neuron.2006.06.021

Bunzeck, N., Schutze, H., Stallforth, S., Kaufmann, J., Duzel, S., Heinze, H. J., et al. (2007). Mesolimbic novelty processing in older adults. *Cereb. Cortex* 17, 2940–2948. doi: 10.1093/cercor/bhm020

Cassidy, S. M., Robertson, I. H., and O'Connell, R. G. (2012). Retest reliability of event-related potentials: evidence from a variety of paradigms. *Psychophysiology* 49, 659–664. doi: 10.1111/j.1469-8986.2011.01349.x

Cavanagh, J., and Geisler, M. W. (2006). Mood effects on the ERP processing of emotional intensity in faces: a P3 investigation with depressed students. *Int. J. Psychophysiol.* 60, 27–33. doi: 10.1016/j.ijpsycho.2005.04.005

Cheng, S. T. (2016). Cognitive reserve and the prevention of dementia: the role of physical and cognitive activities. *Curr. Psychiatry Rep.* 18:85. doi: 10.1007/s11920-016-0721-2

Chong, H., Riis, J. L., McGinnis, S. M., Williams, D. M., Holcomb, P. J., and Daffner, K. R. (2008). To ignore or explore: top-down modulation of novelty processing. *J. Cogn. Neurosci.* 20, 120–134. doi: 10.1162/jocn.2008.20.1.120

Cicchetti, D. V. (1994). Guidelines, criteria, and rules of thumb for evaluating normed and standardized assessment instruments in psychology. *Psychol. Assess.* 6, 284–290. doi: 10.1037/1040-3590.6.4.284

Courchesne, E., Hillyard, S. A., and Galambos, R. (1975). Stimulus novelty, task relevance and the visual evoked potential in man. *Electroencephalogr. Clin. Neurophysiol.* 39, 131–143. doi: 10.1016/0013-4694(75)90003-6

Daffner, K. R., Mesulam, M. M., Holcomb, P. J., Calvo, V., Acar, D., Chabrerie, A., et al. (2000a). Disruption of attention to novel events after frontal lobe injury in humans. *J. Neurol. Neurosurg. Psychiatry* 68, 18–24. doi: 10.1136/jnnp.68.1.18

Daffner, K. R., Mesulam, M. M., Scinto, L. F., Acar, D., Calvo, V., Faust, R., et al. (2000b). The central role of the prefrontal cortex in directing attention to novel events. *Brain* 123, 927–939. doi: 10.1093/brain/123.5.927

Daffner, K. R., Mesulam, M. M., Scinto, L. F., Calvo, V., Faust, R., and Holcomb, P. J. (2000c). An electrophysiological index of stimulus unfamiliarity. *Psychophysiology* 37, 737–747. doi: 10.1017/s0048577200990814

Daffner, K. R., Scinto, L. F., Calvo, V., Faust, R., Mesulam, M. M., West, W. C., et al. (2000d). The influence of stimulus deviance on electrophysiologic and behavioral responses to novel events. *J. Cogn. Neurosci.* 12, 393–406. doi: 10.1162/089892900562219

Daffner, K. R., Mesulam, M. M., Scinto, L. F., Cohen, L. G., Kennedy, B. P., West, W. C., et al. (1998). Regulation of attention to novel stimuli by frontal lobes: an event-related potential study. *Neuroreport* 9, 787–791. doi: 10.1097/00001756-199803300-00004

Daffner, K. R., Rentz, D. M., Scinto, L. F., Faust, R., Budson, A. E., and Holcomb, P. J. (2001). Pathophysiology underlying diminished attention to novel events in patients with early AD. *Neurology* 56, 1377–1383. doi: 10.1212/wnl.56.10.1377

Daffner, K. R., Ryan, K. K., Williams, D. M., Budson, A. E., Rentz, D. M., Wolk, D. A., et al. (2006a). Age-related differences in attention to novelty among cognitively high performing adults. *Biol. Psychol.* 72, 67–77. doi: 10.1016/j.biopsycho.2005.07.006

Daffner, K. R., Ryan, K. K., Williams, D. M., Budson, A. E., Rentz, D. M., Wolk, D. A., et al. (2006b). Increased responsiveness to novelty is associated with successful cognitive aging. *J. Cogn. Neurosci.* 18, 1759–1773. doi: 10.1162/jocn.2006.18.10.1759

Daffner, K. R., Scinto, L. F., Weintraub, S., Guinessey, J. E., and Mesulam, M. M. (1992). Diminished curiosity in patients with probable Alzheimer's disease as measured by exploratory eye movements. *Neurology* 42, 320–328. doi: 10.1212/wnl.42.2.320

Daffner, K. R., Scinto, L. F., Weintraub, S., Guinessey, J., and Mesulam, M. M. (1994). The impact of aging on curiosity as measured by exploratory eye movements. *Arch. Neurol.* 51, 368–376. doi: 10.1001/archneur.1994.00540160062009

Daffner, K. R., Scinto, L. F., Weitzman, A. M., Faust, R., Rentz, D. M., Budson, A. E., et al. (2003). Frontal and parietal components of a cerebral network mediating voluntary attention to novel events. *J. Cogn. Neurosci.* 15, 294–313. doi: 10.1162/089892903321208213

Deldin, P. J., Duncan, C. C., and Miller, G. A. (1994). Season, gender, and P300. *Biol. Psychol.* 39, 15–28. doi: 10.1016/0301-0511(94)90054-x

Delorme, A., and Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J. Neurosci. Methods* 134, 9–21. doi: 10.1016/j.jneumeth.2003.10.009

Dien, J. (2010). The ERP PCA Toolkit: an open source program for advanced statistical analysis of event-related potential data. *J. Neurosci. Methods* 187, 138–145. doi: 10.1016/j.jneumeth.2009.12.009

Dien, J. (2012). Applying principal components analysis to event-related potentials: a tutorial. *Dev. Neuropsychol.* 37, 497–517. doi: 10.1080/87565641.2012.697503

Dien, J., Spencer, K. M., and Donchin, E. (2003). Localization of the event-related potential novelty response as defined by principal components analysis. *Cogn. Brain Res.* 17, 637–650. doi: 10.1016/s0926-6410(03)00188-5

Downar, J., Crawley, A. P., Mikulis, D. J., and Davis, K. D. (2000). A multimodal cortical network for the detection of changes in the sensory environment. *Nat. Neurosci.* 3, 277–283. doi: 10.1038/72991

Downar, J., Crawley, A. P., Mikulis, D. J., and Davis, K. D. (2002). A cortical network sensitive to stimulus salience in a neutral behavioral context across multiple sensory modalities. *J. Neurophysiol.* 87, 615–620. doi: 10.1152/jn.00636.2001

Fabiani, M., and Friedman, D. (1995). Changes in brain activity patterns in aging: the novelty oddball. *Psychophysiology* 32, 579–594. doi: 10.1111/j.1469-8986.1995.tb01234.x

Folstein, M. F., Folstein, S. E., and McHugh, P. R. (1975). "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* 12, 189–198. doi: 10.1016/0022-3956(75)90026-6

Friedman, D., Cycowicz, Y. M., and Gaeta, H. (2001). The novelty P3: an event-related brain potential (ERP) sign of the brain's evaluation of novelty. *Neurosci. Biobehav. Rev.* 25, 355–373. doi: 10.1016/s0149-7634(01)00019-7

Friedman, D., Nessler, D., Kulik, J., and Hamberger, M. (2011). The brain's orienting response (novelty P3) in patients with unilateral temporal lobe resections. *Neuropsychologia* 49, 3474–3483. doi: 10.1016/j.neuropsychologia.2011.08.023

Galli, G., Sirota, M., Gruber, M. J., Ivanof, B. E., Ganesh, J., Materassi, M., et al. (2018). Learning facts during aging: the benefits of curiosity. *Exp. Aging Res.* 44, 311–328. doi: 10.1080/0361073X.2018.1477355

Gard, T., Hölzel, B. K., and Lazar, S. W. (2014). The potential effects of meditation on age-related cognitive decline: a systematic review. *Ann. N Y Acad. Sci.* 1307, 89–103. doi: 10.1111/nyas.12348

Gates, N. J., Sachdev, P. S., Fiatarone Singh, M. A., and Valenzuela, M. (2011). Cognitive and memory training in adults at risk of dementia: a systematic review. *BMC Geriatr.* 11:55. doi: 10.1186/1471-2318-11-55

Goldstein, A., Spencer, K. M., and Donchin, E. (2002). The influence of stimulus deviance and novelty on the P300 and novelty P3. *Psychophysiology* 39, 781–790. doi: 10.1017/s004857720201048x

Hall, M. H., Schulze, K., Rijsdijk, F., Picchioni, M., Ettinger, U., Bramon, E., et al. (2006). Heritability and reliability of P300, P50 and duration mismatch negativity. *Behav. Genet.* 36, 845–857. doi: 10.1007/s10519-006-9091-6

Hämmerer, D., Li, S. C., Völkle, M., Müller, V., and Lindenberger, U. (2013). A lifespan comparison of the reliability, test-retest stability and signal-to-noise ratio of event-related potentials assessed during performance monitoring. *Psychophysiology* 50, 111–123. doi: 10.1111/j.1469-8986.2012.01476.x

Huang, J., Katsuura, T., Shimomura, Y., and Iwanaga, K. (2006). Diurnal changes of ERP response to sound stimuli of varying frequency in morning-type and evening-type subjects. *J. Physiol. Anthropol.* 25, 49–54. doi: 10.2114/jpa2.25.49

Huffmeijer, R., Bakermans-Kranenburg, M. J., Alink, L. R., and van Ijzendoorn, M. H. (2014). Reliability of event-related potentials: the influence of number of trials and electrodes. *Physiol. Behav.* 130, 13–22. doi: 10.1016/j.physbeh.2014.03.008

Ischebeck, M., Endrass, T., Simon, D., and Kathmann, N. (2011). Auditory novelty processing is enhanced in obsessive-compulsive disorder. *Depress. Anxiety* 28, 915–923. doi: 10.1002/da.20886

Kaipio, M. L., Alho, K., Winkler, I., Escera, C., Surma-aho, O., and Naatanen, R. (1999). Event-related brain potentials reveal covert distractibility in closed head injuries. *Neuroreport* 10, 2125–2129. doi: 10.1097/00001756-199907130-00024

Kaplan, E., Goodglass, H., and Weintraub, S. (1983). *The Boston Naming Test.* Philadelphia, PA: Lea and Febiger.

Kappenman, E. S., and Luck, S. J. (2012). "ERP components: the ups and downs of brainwave recordings," in *Oxford Handbook of Event-Related Potential Components*, eds S. J. Luck and E. S. Kappenman (New York, NY: Oxford University Press), 3–30.

Kaufman, D. A., Bowers, D., Okun, M. S., Van Patten, R., and Perlstein, W. M. (2016a). Apathy, novelty processing, and the P3 potential in Parkinson's disease. *Front. Neurol.* 7:95. doi: 10.3389/fneur.2016.00095

Kaufman, D. A., Keith, C. M., and Perlstein, W. M. (2016b). Orbitofrontal cortex and the early processing of visual novelty in healthy aging. *Front. Aging Neurosci.* 8:101. doi: 10.3389/fnagi.2016.00101

Kiehl, K. A., Laurens, K. R., Duty, T. L., Forster, B. B., and Liddle, P. F. (2001a). An event-related fMRI study of visual and auditory oddball tasks. *J. Psychophysiol.* 15, 221–240. doi: 10.1027/0269-8803.15.4.221

Kiehl, K. A., Laurens, K. R., Duty, T. L., Forster, B. B., and Liddle, P. F. (2001b). Neural sources involved in auditory target detection and novelty processing: an event-related fMRI study. *Psychophysiology* 38, 133–142. doi: 10.1017/s0048577201981867

Kinoshita, S., Inoue, M., Maeda, H., Nakamura, J., and Morita, K. (1996). Long-term patterns of change in ERPs across repeated measurements. *Physiol. Behav.* 60, 1087–1092. doi: 10.1016/0031-9384(96)00130-8

Knight, R. T. (1984). Decreased response to novel stimuli after prefrontal lesions in man. *Electroencephalogr. Clin. Neurophysiol.* 59, 9–20. doi: 10.1016/0168-5597(84)90016-9

Koo, T. K., and Li, M. Y. (2016). A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J. Chiropr. Med.* 15, 155–163. doi: 10.1016/j.jcm.2016.02.012

Koshino, Y., Nishio, M., Murata, T., Omori, M., Murata, I., Sakamoto, M., et al. (1993). The influence of light drowsiness on the latency and amplitude of P300. *Clin. Electroencephalogr.* 24, 110–113. doi: 10.1177/155005949302400307

Kosslyn, S. M., Alpert, N. M., Thompson, W. L., Chabris, C. F., Rauch, S. L., and Anderson, A. K. (1994). Identifying objects seen from different viewpoints. *Brain* 117, 1055–1071. doi: 10.1093/brain/117.5.1055

Kroll, J. F., and Potter, M. C. (1984). Recognizing words, pictures and concepts: a comparison of lexical, object, and reality decisions. *J. Verbal Learning Verbal Behav.* 23, 39–66. doi: 10.1016/s0022-5371(84)90499-7

Lew, H. L., Gray, M., and Poole, J. H. (2007). Temporal stability of auditory event-related potentials in healthy individuals and patients with traumatic brain injury. *J. Clin. Neurophysiol.* 24, 392–397. doi: 10.1097/wnp.0b013e318 14a56e3

Lopez-Calderon, J., and Luck, S. J. (2014). ERPLAB: an open-source toolbox for the analysis of event-related potentials. *Front. Hum. Neurosci.* 8:213. doi: 10.3389/fnhum.2014.00213

Mesulam, M. M. (1981). A cortical network for directed attention and unilateral neglect. *Ann. Neurol.* 10, 309–325. doi: 10.1002/ana.410100402

Mesulam, M. M. (1990). Large-scale neurocognitive networks and distributed processing for attention, language, and memory. *Ann. Neurol.* 28, 597–613. doi: 10.1002/ana.410280502

Mesulam, M. M. (1998). From sensation to cognition. *Brain* 121, 1013–1052. doi: 10.1093/brain/121.6.1013

Morris, J. C. (1993). The clinical dementia rating (CDR): current version and scoring rules. *Neurology* 43, 2412–2414. doi: 10.1212/wnl.43.11.2412-a

Morris, A. M., So, Y., Lee, K. A., Lash, A. A., and Becker, C. E. (1992). The P300 event-related potential. The effects of sleep deprivation. *J. Occup. Med.* 34, 1143–1152.

Näätänen, R. (1990). The role of attention in auditory information processing as revealed by event-related potentials and other brain measures of cognitive function. *Behav. Brain Sci.* 13, 201–233. doi: 10.1017/s0140525x00078407

Naeije, G., Vaulet, T., Wens, V., Marty, B., Goldman, S., and De Tiege, X. (2016). Multilevel cortical processing of somatosensory novelty: a magnetoencephalography study. *Front. Hum. Neurosci.* 10:259. doi: 10.3389/fnhum.2016.00259

Najar, J., Östling, S., Gudmundsson, P., Sundh, V., Johansson, L., Kern, S., et al. (2019). Cognitive and physical activity and dementia: a 44-year longitudinal population study of women. *Neurology* 92, e1322–e1330. doi: 10.1212/wnl.0000000000007021

Olvet, D. M., and Hajcak, G. (2009). Reliability of error-related brain activity. *Brain Res.* 1284, 89–99. doi: 10.1016/j.brainres.2009.05.079

Opitz, B., Mecklinger, A., Friederici, A. D., and von Cramon, D. Y. (1999). The functional neuroanatomy of novelty processing: integrating ERP and fMRI results. *Cereb. Cortex* 9, 379–391. doi: 10.1093/cercor/9.4.379

Pierson, A., Ragot, R., Van Hooff, J., Partiot, A., Renault, B., and Jouvent, R. (1996). Heterogeneity of information-processing alterations according to dimensions of depression: an event-related potentials study. *Biol. Psychiatry* 40, 98–115. doi: 10.1016/0006-3223(95)00329-0

Polich, J. (2004). Clinical application of the P300 event-related potential. *Phys. Med. Rehabil. Clin. N. Am.* 15, 133–161. doi: 10.1016/s1047-9651(03)00109-8

Polich, J., and Comerchero, M. D. (2003). P3a from visual stimuli: typicality, task, and topography. *Brain Topogr.* 15, 141–152. doi: 10.1023/A:1022637732495

Polich, J., and Kok, A. (1995). Cognitive and biological determinants of P300: an integrative review. *Biol. Psychol.* 41, 103–146. doi: 10.1016/0301-0511(95)05130-9

Riis, J. L., Chong, H., McGinnnis, S., Tarbi, E., Sun, X., Holcomb, P. J., et al. (2009). Age-related changes in early novelty processing as measured by ERPs. *Biol. Psychol.* 82, 33–44. doi: 10.1016/j.biopsycho.2009.05.003

Riis, J. L., Chong, H., Ryan, K. K., Wolk, D. A., Rentz, D. M., Holcomb, P. J., et al. (2008). Compensatory neural activity distinguishes different patterns of normal cognitive aging. *Neuroimage* 39, 441–454. doi: 10.1016/j.neuroimage.2007.08.034

Ryan, J., and Paolo, A. (1992). A screening procedure for estimating premorbid intelligence in the elderly. *Clin. Neuropsychol.* 6, 53–62. doi: 10.1080/13854049208404117

Sakaki, M., Yagi, A., and Murayama, K. (2018). Curiosity in old age: a possible key to achieving adaptive aging. *Neurosci. Biobehav. Rev.* 88, 106–116. doi: 10.1016/j.neubiorev.2018.03.007

Sandman, C. A., and Patterson, J. V. (2000). The auditory event-related potential is a stable and reliable measure in elderly subjects over a 3 year period. *Clin. Neurophysiol.* 111, 1427–1437. doi: 10.1016/s1388-2457(00)00320-5

Sanjuan, P. M., Andrews, C., and Claus, E. D. (2018). Abnormal target detection and novelty processing neural response in posttraumatic stress disorder. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 85, 54–61. doi: 10.1016/j.pnpbp.2018.04.003

Schomaker, J., and Meeter, M. (2014). Novelty detection is enhanced when attention is otherwise engaged: an event-related potential study. *Exp. Brain Res.* 232, 995–1011. doi: 10.1007/s00221-013-3811-y

Schomaker, J., Roos, R., and Meeter, M. (2014). Expecting the unexpected: the effects of deviance on novelty processing. *Behav. Neurosci.* 128, 146–160. doi: 10.1037/a0036289

Schott, B. H., Voss, M., Wagner, B., Wustenberg, T., Düzel, E., and Behr, J. (2015). Fronto-limbic novelty processing in acute psychosis: disrupted relationship with memory performance and potential implications for delusions. *Front. Behav. Neurosci.* 9:144. doi: 10.3389/fnbeh.2015.00144

Segalowitz, S. J., and Barnes, K. L. (1993). The reliability of ERP components in the auditory oddball paradigm. *Psychophysiology* 30, 451–459. doi: 10.1111/j.1469-8986.1993.tb02068.x

Shrout, P. E., and Fleiss, J. L. (1979). Intraclass correlations: uses in assessing rater reliability. *Psychol. Bull.* 86, 420–428. doi: 10.1037/0033-2909.86.2.420

Simon, S. S., Tusch, E. S., Feng, N. C., Hakansson, K., Mohammed, A. H., and Daffner, K. R. (2018). Is computerized working memory training effective in healthy older adults? Evidence from a multi-site, randomized controlled trial. *J. Alzheimers Dis.* 65, 931–949. doi: 10.3233/jad-180455

Sinha, R., Bernardy, N., and Parsons, O. A. (1992). Long-term test-retest reliability of event-related potentials in normals and alcoholics. *Biol. Psychiatry* 32, 992–1003. doi: 10.1016/0006-3223(92)90060-d

Sokhadze, E., Baruth, J., Tasman, A., Sears, L., Mathai, G., El-Baz, A., et al. (2009). Event-related potential study of novelty processing abnormalities in

autism. *Appl. Psychophysiol. Biofeedback* 34, 37–51. doi: 10.1007/s10484-009-9074-5

Sokolov, E. N. (1963). Higher nervous functions; the orienting reflex. *Annu. Rev. Physiol.* 25, 545–580. doi: 10.1146/annurev.ph.25.030163.002553

Spencer, K. M., Dien, J., and Donchin, E. (1999). A componential analysis of the ERP elicited by novel events using a dense electrode array. *Psychophysiology* 36, 409–414. doi: 10.1017/s0048577299981180

Spencer, K. M., Dien, J., and Donchin, E. (2001). Spatiotemporal analysis of the late ERP responses to deviant stimuli. *Psychophysiology* 38, 343–358. doi: 10.1017/s0048577201000324

Stevens, M. C., Pearlson, G. D., and Kiehl, K. A. (2007). An FMRI auditory oddball study of combined-subtype attention deficit hyperactivity disorder. *Am. J. Psychiatry* 164, 1737–1749. doi: 10.1176/appi.ajp.2007.06050876

Strobel, A., Debener, S., Sorger, B., Peters, J. C., Kranczioch, C., Hoechstetter, K., et al. (2008). Novelty and target processing during an auditory novelty oddball: a simultaneous event-related potential and functional magnetic resonance imaging study. *Neuroimage* 40, 869–883. doi: 10.1016/j.neuroimage.2007.10.065

Swan, G. E., and Carmelli, D. (1996). Curiosity and mortality in aging adults: a 5-year follow-up of the Western Collaborative Group Study. *Psychol. Aging* 11, 449–453. doi: 10.1037/0882-7974.11.3.449

Tarbi, E. C., Sun, X., Holcomb, P. J., and Daffner, K. R. (2011). Surprise? Early visual novelty processing is not modulated by attention. *Psychophysiology* 48, 624–632. doi: 10.1111/j.1469-8986.2010.01129.x

Tulving, E., Markowitsch, H. J., Craik, F. E., Habib, R., and Houle, S. (1996). Novelty and familiarity activations in PET studies of memory encoding and retrieval. *Cereb. Cortex* 6, 71–79. doi: 10.1093/cercor/6.1.71

Tusch, E. S., Alperin, B. R., Ryan, E., Holcomb, P. J., Mohammed, A. H., and Daffner, K. R. (2016). Changes in neural activity underlying working memory after computerized cognitive training in older adults. *Front. Aging Neurosci.* 8:255. doi: 10.3389/fnagi.2016.00255

Veyrac, A., Sacquet, J., Nguyen, V., Marien, M., Jourdan, F., and Didier, A. (2009). Novelty determines the effects of olfactory enrichment on memory and neurogenesis through noradrenergic mechanisms. *Neuropsychopharmacology* 34, 786–795. doi: 10.1038/npp.2008.191

Walhovd, K. B., and Fjell, A. M. (2002). One-year test-retest reliability of auditory ERPs in young and old adults. *Int. J. Psychophysiol.* 46, 29–40. doi: 10.1016/s0167-8760(02)00039-9

Wechsler, D. (1997). *Wechsler Memory Scale III: Administration and Scoring Manual*. Orlando: Psychological Corporation.

Weinberg, A., and Hajcak, G. (2011). Longer term test-retest reliability of error-related brain activity. *Psychophysiology* 48, 1420–1425. doi: 10.1111/j.1469-8986.2011.01206.x

Wilson, R. S., Mendes De Leon, C. F., Barnes, L. L., Schneider, J. A., Bienias, J. L., Evans, D. A., et al. (2002). Participation in cognitively stimulating activities and risk of incident Alzheimer disease. *JAMA* 287, 742–748. doi: 10.1001/jama.287.6.742

Yagi, Y., Coburn, K. L., Estes, K. M., and Arruda, J. E. (1999). Effects of aerobic exercise and gender on visual and auditory P300, reaction time, and accuracy. *Eur. J. Appl. Physiol. Occup. Physiol.* 80, 402–408. doi: 10.1007/s004210050611

Yesavage, J. A., Brink, T. L., Rose, T. L., Lum, O., Huang, V., Adey, M., et al. (1982). Development and validation of a geriatric depression screening scale: a preliminary report. *J. Psychiatr. Res.* 17, 37–49. doi: 10.1016/0022-3956(82)90033-4

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

BRIEF RESEARCH REPORT
published: 28 June 2019
doi: 10.3389/fnagi.2019.00164

Long Term Cosmetic Application Improves Tactile Discrimination in the Elderly; a New Psychophysical Approach

*Jean-Marc Aimonetti[1], Claire Deshayes[1], Marcel Crest[2], Pierre-Henri Cornuault[3], Benjamin Weiland[3] and Edith Ribot-Ciscar[1]**

[1]Aix Marseille University, CNRS, Laboratoire de Neurosciences Sensorielles et Cognitives (LNSC), SFR3C, Marseille, France, [2]Aix Marseille University, CNRS, LNC, SFR3C, Marseille, France, [3]Femto-ST Institute, Department of Applied Mechanics, University Bourgogne-Franche-Comte, CNRS/ENSMM/UFC/UTBM, Besançon, France

Introduction: Tactile sensitivity is impaired in older adults, which contributes to the loss of manual dexterity and mobility function. The reliability of classical psychophysical tests, such as two-point gap discrimination, has been questioned. Here we tested a new method to determine tactile acuity during dynamic touch, which is more functional than static touch. The aim was to validate a method providing a high level of discrimination of tactile acuity in the elderly.

Methods: We tested the ability of subjects to evaluate the distance between bands printed on poly-methyl-methacrylate (PMMA) sheets. Pairs of sheets were compared in two groups of participants aged from 60 to 74 years; the test group was required to apply a cosmetic foam with an active ingredient on both their hands twice a day for 1 month, the control group had an identical task but used the same cosmetic foam without any active ingredient. The tests were run in a double-blind, placebo-controlled study.

Results: The tactile discrimination threshold decreased by 83 μm after 1 month of cosmetic application in the group using the active ingredient, while it was unchanged in the control group.

Discussion: The test presented here provided highly accurate results and should be useful to determine tactile performance. It allows the monitoring of tactile rehabilitation and/or skin treatments used to restore tactile acuity in the elderly.

Keywords: tactile discrimination, haptic touch, skin aging, psychophysics, cosmetic

OPEN ACCESS

Edited by:
Borja Sañudo,
University of Seville, Spain

Reviewed by:
Hakan Olausson,
Linköping University, Sweden
Jonathan Tong,
York University, Canada

****Correspondence:***
Edith Ribot-Ciscar
edith.ribot-ciscar@univ-amu.fr

Received: *22 March 2019*
Accepted: *13 June 2019*
Published: *28 June 2019*

Citation:
Aimonetti J-M, Deshayes C, Crest M, Cornuault P-H, Weiland B and Ribot-Ciscar E (2019) Long Term Cosmetic Application Improves Tactile Discrimination in the Elderly; a New Psychophysical Approach. Front. Aging Neurosci. 11:164. doi: 10.3389/fnagi.2019.00164

INTRODUCTION

Tactile sensitivity relies on the sensory information provided by low-threshold mechanoreceptors located in the dermis of the glabrous skin, such as Merkel's disks, Meissner, Pacinian, and Ruffini's corpuscles. These mechanoreceptors are preferentially activated by deformation of the skin such as pressure, vibration, and tension. These mechanical events are transduced into sensory messages going through the peripheral and then the central nervous system, where their processing give rise to tactile sensations (Johnson, 2001).

Tactile sensitivity is known to decline with age, resulting from mechanoreceptor loss (Bruce, 1980; Iwasaki et al., 2003; Skedung et al., 2018), but also from changes in the mechanical properties of the skin itself such as reduced elasticity (Farage et al., 2013; Skedung et al., 2018), that may result from a decrease in hydration (Verrillo et al., 1998; Skedung et al., 2018). Thus, both tactile detection threshold and tactile acuity are impaired with age, where the feet and hands are the most affected, as a result of a reduced blood flow and/or greater physical wear on the contact surfaces (Thornbury and Mistretta, 1981; Stevens and Choo, 1996; Bowden and McNulty, 2013; da Silva et al., 2014; Franco et al., 2015). This has important functional implications for older adults, as it has been linked to impairment in manual control (Wickremaratchi and Llewelyn, 2006), but also deficits in balance and walking ability with an increased risk of injurious falls, which contributes to disability and death in older adults (Soriano et al., 2007). More tragically, many older adults are unaware of their peripheral neurological impairment and are therefore unlikely to seek preemptive intervention (Cruz-Almeida et al., 2014). In a previous study conducted in aged people, we demonstrated that tactile acuity increased 30 min after the application of a moisturizing cream (Lévêque et al., 2000). This short-term beneficial effect of skin hydration on tactile sensitivity has been confirmed in older men (Bowden and McNulty, 2013). These results suggest that, contrary to what has been claimed previously (Woodward, 1993), changes in the mechanical properties of the skin may affect tactile perception.

Tactile sensitivity is classically characterized by analyzing the detection threshold, with calibrated monofilaments, or by spatial discrimination, such as the two-point gap discrimination test (Bell-Krotoski et al., 1993). However, these tests have been criticized due to great variations within subjects, between subjects, and between studies (Levin et al., 1978; Lundborg and Rosén, 2004). For instance, calibrated monofilaments are simple to use, but they are fragile and can be distorted after multiple uses, as well as being sensitive to temperature and humidity (Haloua et al., 2011). This probably accounts for the unreliability of the tests notably those performed with the five smallest monofilaments, i.e., 0.008–0.16 g (Massy-Westropp, 2002). The results obtained with the two-point gap discrimination test are also extremely variable because they depend upon the way the test is performed, notably the amount of pressure applied to the skin and the synchronicity of application of the two stimulation points (Johnson and Phillips, 1981; Lundborg and Rosén, 2004). Recently, a test where the participant has to discriminate the orientation (horizontal vs. vertical) of two points of contact has been recommended as a better measure of tactile spatial acuity (Tong et al., 2013).

In the present study, we chose an active touch test, which is more functional than a static test, and evaluated a psychophysical method to assess spatial discrimination threshold. We designed a double-blind, placebo-controlled study in healthy elderly subjects to determine whether any beneficial effect of a cosmetic substance may be found, as compared to a placebo substance, by means of this psychophysical method. The demonstration of a difference between the two groups of participants in tactile acuity should validate the present method as a useful tool to track the beneficial effects of sensory training and/or treatment in older people.

MATERIALS AND METHODS

Participants

The experiments were performed in 42 healthy, right-handed volunteers aged from 60 to 74 years. They were recruited in the retirement communities in the Marseille area of France. The exclusion criteria were: a history of neurological, psychiatric, or dermatological disorders, or clinically significant peripheral neuropathy, such as diabetes. No ethical review process was required for the present study because the French Conseil d'Etat ruled in an order dated 8 February 2017 that studies using cosmetic products, even for scientific investigation, do not require an application for approval from an ethics committee. Our study nevertheless conformed with the ethical guidelines set out by the Declaration of Helsinki and all participants gave their written informed consent.

Design and Procedure

Participants were tested in a quiet room, with a constant temperature of ∼22°C. They were asked to attend an initial test to evaluate their tactile acuity. The participants were seated comfortably in an armchair with their right hand positioned in a cushioned groove, which allowed a standardized and relaxed position. The participants were asked to close their eyes and they wore noise-canceling headphones (Bose; Framingham, MA, USA).

Participants were asked to explore pairs of poly-methyl-methacrylate (PMMA) sheets specifically designed for the experiment, using the distal phalanx of their right index finger. These sheets, of 5 mm thickness and 50 × 80 mm^2 surface, included regularly-spaced grooves with a depth of ∼0.07 mm, which were oriented perpendicularly to the finger displacement (**Figure 1A**). The space between the grooves (the "inter-band space") varied from 3.6 to 6 mm between test sheets. The sheet with a median spacing of 4.8 mm was defined as the reference. On each side of this reference, four test sheets with spacing varying in 0.2 mm steps were used. At each extreme, the sheets presented a change in the spacing of 0.4 mm. For convenience, the sheets were coded as follows: the shortest spacing (3.6 mm) was coded as 6; then the sheets with spacings of 4, 4.2, 4.4, 4.6 mm were coded as 7, 7.5, 8, 8.5, respectively; the reference (spacing of 4.8 mm) was coded as 9; then the sheets with spacings of 5, 5.2, 5.4, 5.6 mm were coded as 9.5, 10, 10.5, 11, respectively; finally the largest spacing (6 mm) was coded as 12 (**Figure 1B**). The method consisted of presenting pairs of sheets, where the reference was always presented together with one of the 10 test sheets, and the order of presentation was randomized for each pair. The participants were instructed to explore each sheet once, from top to bottom, at about 20 mm/s, which corresponds to the speed classically chosen to explore

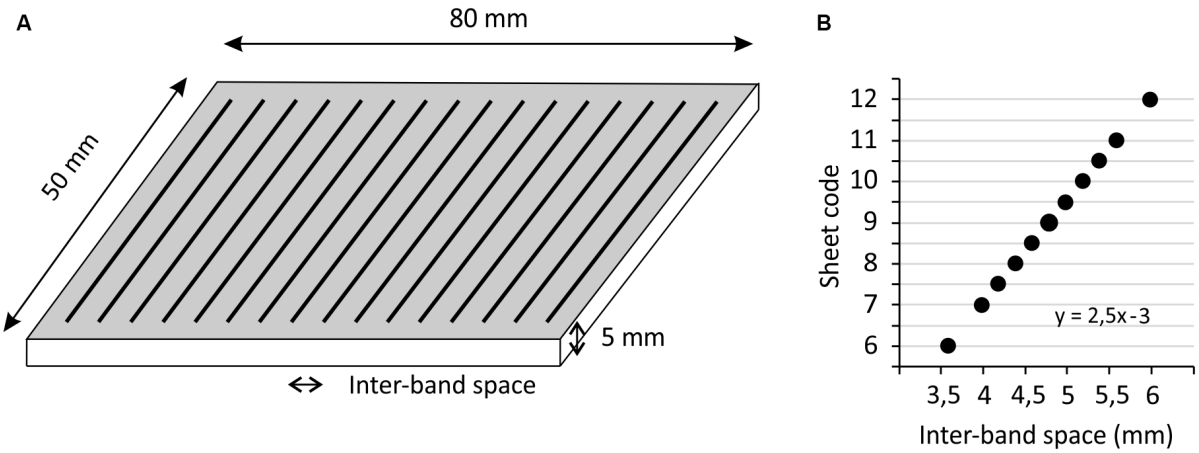


FIGURE 1 | The subjects were required to explore pairs of poly-methyl-methacrylate (PMMA) sheets printed with regular grooves **(A)**, the inter-band space was constant in each sheet and varied around a reference sheet (large point in **B**).

textured surfaces (Vega-Bermudez et al., 1991). They were familiarized with this instruction before the test session by moving their finger over the sheet during two audible beeps that were spaced 4 s apart.

The test was a two-alternative forced choice discrimination task, where the participants had to determine whether the first or the second sheet had the larger inter-band spacing. The eight sheets with a 0.2 mm-spacing difference were presented 15 times and the two extreme sheets with a 0.4 mm-spacing difference six times; the lower number of presentations for the two extreme sheets was justified by the ease in differentiating these sheets from the reference. In total, the participants had to compare 132 reference/test pairs. The whole psychophysical examination lasted about 1.5 h, including rest time of 5 min every 15 min.

After this initial tactile test (called "pre"), the participants received cosmetic foam (Laboratoires Chemineau, Anjac Health and Beauty Inc.). The participants were asked to apply the foam on their both hands (palm, fingers, and dorsum) each morning in their daily routine and each evening before sleeping, for 1 month. They were instructed not to wash their hands in the hour following the foam application.

The 42 participants were divided into two groups: "active" and "placebo." The repartition was done in a random manner, taking into account the participant's age and gender. Participants of the active group (n = 21, six men) received the foam with an active ingredient (IP patent in process n° BFF170321IMA, Laboratoires Chemineau, Anjac Health and Beauty Inc.). In the placebo group, participants received a foam with exactly the same formulation, apart from the active ingredient (n = 21, six men). The participants and the experimenter (CD) were blinded to who received the active formulation as both treatments were placed in similar packages only identified as A and B; the participants were even blinded as to the existence of any placebo, in order to enhance commitment with the experiment (Bang, 2016). The participants were then asked to attend a second time, exactly 30 days after application of the foam, to evaluate their tactile sensitivity during the "post" test that was run in exactly the same conditions as the pre-test.

Data Analysis

In order to evaluate and compare participants' performances across the two tests (Pre/Post), the psychometric data (i.e., the proportion of correct answers, corresponding to which sheet was of larger inter-band space) were fitted by the following cumulative Gaussian function:

$$P(x) = \lambda + (1 - 2\lambda)\frac{1}{\sigma_\psi\sqrt{2\pi}}\int_{-\infty}^{x} e^{-\frac{(y-\mu\psi)^2}{2\sigma_\psi^2}}\,dy$$

Here, x represents the sheet code; $\mu\psi$ is the mean of the Gaussian, i.e., the point of subjective equality (PSE), that corresponds to the stimulation intensity (here, the inter-band space) leading the participant to perceive no difference between the reference and the test sheet; and $\sigma\psi$ is the standard deviation of the curve (discrimination threshold), which is inversely related to the participant's discrimination sensitivity. The $\sigma\psi$ value (also called the uncertainty range) is given by the difference between the projection onto the X-axis of 75% and 50% of response (see **Figure 2**). A smaller $\sigma\psi$ value corresponds to higher discrimination sensitivity in the task. The PSE is given by the projection at 50% of the response. The two indices, PSE and $\sigma\psi$, characterize the participant's performance, and λ accounts for stimulus-independent errors (e.g., due to participant lapses) and was restricted to small values ($0 < \lambda < 0.06$, Wichmann and Hill, 2001). This parameter is not informative about the perceptual decision, thus we disregarded it for the subsequent analyses. Psignifit toolbox, implemented in MATLAB software (The Mathworks, Natick, MA, USA) was used to fit the psychometric curves.

Differences in the PSE and uncertainty range between the groups, before cosmetic treatment, were evaluated with an unpaired Student's t-test. The impact of cosmetic application upon tactile sensitivity was estimated within each group with a paired Student's t-test. Effect sizes were calculated according

to Cohen's instructions where $d < 0.2$ corresponds to a small effect, $0.2 < d < 0.5$ a medium effect, and $d > 0.5$ a strong effect (Cohen, 1992).

RESULTS

Basic Demographics and Clinical Characteristics

Among the 42 participants, a total of eight participants (four in each group) were excluded because their responses in the pre- and/or post-tests were almost indistinguishable from chance level. These results were explained either by an inability to perform the test both in pre- and post-tests (four subjects) or for medical reasons that interfered with the post-test after the 30 days of foam application (four subjects, lesion of the index, sickness, temperature etc.). Details of the basic demographics and clinical characteristics of the 34 participants finally included in the study are given in **Table 1**. As can be seen, the two groups of participants were similar, with regard to age and gender because the groups were established on the basis of these parameters, but also with regard to their socio-professional characteristics and medical treatments (no significant difference).

Psychophysical Results

Figure 2 illustrates the results obtained in two participants during the pre-test. The results reported in **Figure 2A** shows that the subject answered correctly for the sheets coded 6–7.5, where none of these had an inter-band space larger than that of the reference, and the percentage of responses deemed "larger" was close to 0%. The responses are less certain for sheets 8–10, where the inter-band space was close to that of the reference and the percentage of response is around 50%. The performance improved with sheets 10.5–12, where, for most of the time, the participant answered correctly that the test sheet had larger inter-band spacing than the reference, and the percentages of responses reached 100%. From this psychometric curve, we extracted the PSE, which here equaled 9.1, and the uncertainty range was 1.25. Therefore, for this participant, the minimal inter-band space for a sheet to be statistically differentiated from the reference was at least 0.5 mm. The results reported in **Figure 2B** are those of a participant who performed the task better, as shown by the psychometric curve, which had a steeper slope than that in **Figure 2A**. More precisely, their responses were uncertain only for sheets 9.5 and 10. The PSE remained close to the reference (9.4) and the uncertainty range was smaller (0.81), meaning that only 0.32 mm of difference between two sheets was necessary for these to be differentiated by this participant, who thus presented a better tactile discrimination.

At the population level, the PSE was not found to differ during the pre-test between the active and placebo groups (9.3 ± 0.4, 9.2 ± 0.3, respectively, $t = 0.85$, $p = 0.4$). After 1 month of cosmetic application, the PSE calculated in each group during the post-test was not found to differ with that calculated during the pre-test, either in the active group (9.2 ± 0.3, $t = 0.98$, $p = 0.34$) or in the placebo group (9.4 ± 0.5, $t = 1.67$, $p = 0.11$). In other words, in both groups, applying cosmetic foam for 1 month did not affect the tactile evaluation of the reference sheet.

With regard to the uncertainty range (**Figure 3**), it was not found to differ between the two groups in the pre-test ($t = 1.14$, $p = 0.26$). However, in the post-test, the uncertainty range was significantly smaller than that measured during the pre-test, in the active group (**Figure 3A**, $t = 2.41$, $p = 0.03$, $d = 0.57$) and remained unchanged in the placebo group ($p = 0.54$). This means that, in the active group, a minimal difference of 0.48 mm in the inter-band space, that was necessary for a significant discrimination, fell to 0.40 mm after cosmetic application. In other words, a decrease of 83 μm in spatial discrimination followed 1 month of cosmetic application.

TABLE 1 | Basic and clinical characteristics of the two groups of subjects tested (n = number).

	Active	Placebo	Mann-Whitney test Z limit = 1.96
Basic demographics			
Age (years)	69.0 ± 2.9	66.4 ± 3.9	$Z = 1.60$, $p = 0.11$
Men (n)	6	5	$Z = 0.29$, $p = 0.77$
Right-handers (n)	17	17	$Z = 0$, $p = 1$
Senior executive, teacher (n)	3	5	
Technician (n)	7	4	Not applicable
Workers (n)	7	8	
Clinical characteristics			
No treatment (n)	8	9	$p = 1.06$
Hypertension (n)	5	5	
Cholesterol (n)	3	6	
Thyropenia (n)	3	1	
Arrythmia (n)	2	1	
Other drug therapies (n)	5	4	

DISCUSSION

The present study aimed at evaluating the reliability of a new psychophysical method to assess tactile acuity in the elderly. We compared the impact of 1-month application twice a day of a cosmetic substance, which did or did not include an active ingredient favoring skin hydration. The spatial discrimination threshold was assessed in a double-blind, placebo-controlled manner, where we tested the ability of participants at discriminating a test surface with grooves either more distant or closer than those of a reference surface, during active touch. The results showed that the spatial discrimination acuity was significantly improved in the group of participants treated with the active ingredient, while it was unchanged in the placebo group. Importantly, the two groups of participants were very similar in terms of their spatial tactile acuity in the pre-test, eliminating any group differences on the observed improvement. This must be noted, since people are not equal regarding skin aging and their related decline in tactile acuity, as some elderly remains as high tactile performers, comparable to younger people (Skedung et al., 2018). In the present population of participants, there was a variability in tactile abilities and therefore were also included the high performers, but their presence was counterbalanced in both groups.

Tactile sensitivity is classically characterized by analyzing the detection threshold, with calibrated monofilaments, or the

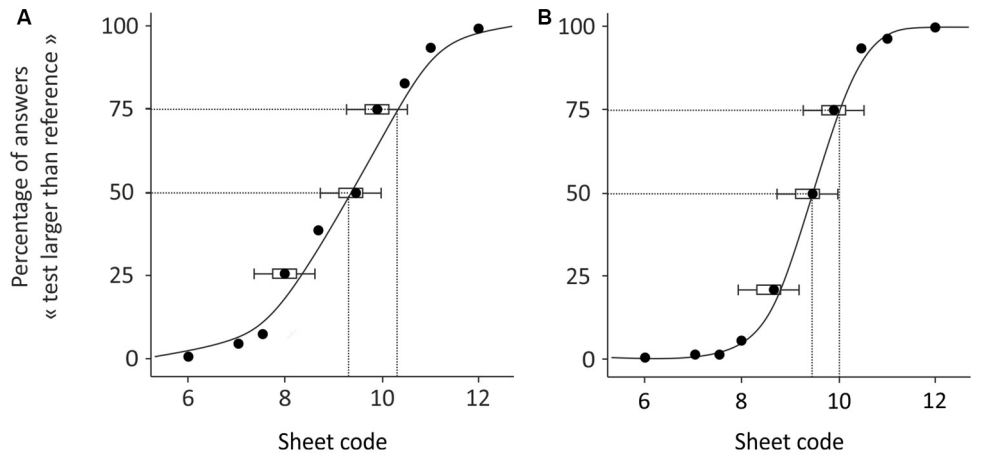


FIGURE 2 | Examples of psychometric curves obtained from two subjects during the pre-test. The participant in **(B)** performed better than the participant in **(A)**.

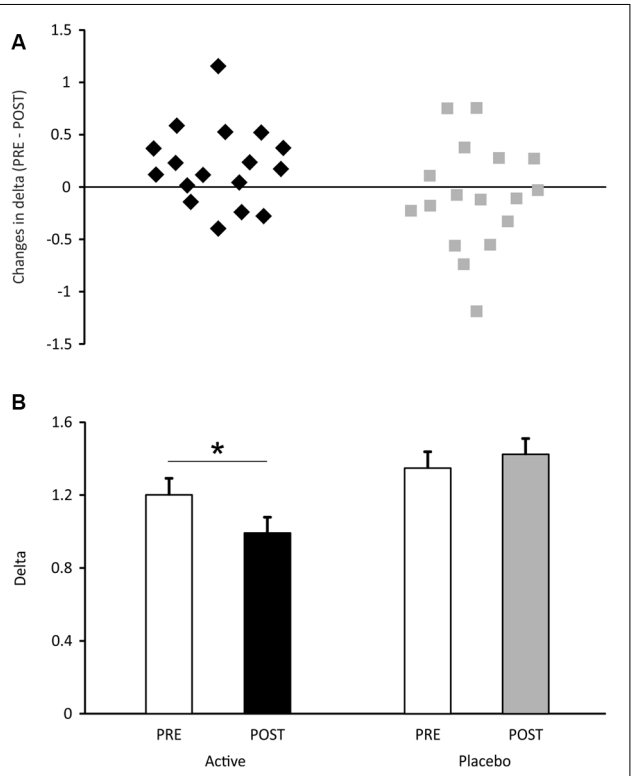


FIGURE 3 | Uncertainty range measured in the active and placebo groups of participants. In **(A)**, the mean data are presented (±SEM). In **(B)**, the individual subject data are expressed as the difference between uncertainty ranges measured during pre- and post-tests. Positive values express an increase in tactile discrimination. $^*p < 0.05$.

spatial discrimination, using the two-point gap discrimination test. It has been reported that, after moisturizing the skin in seniors, the former decreases by 50 mg and the latter by about 3 mm (Lévêque et al., 2000; Bowden and McNulty, 2013). In both studies, the effects of skin hydration were evaluated only 30 min after cosmetic application. Besides methodological issues, such as the unreliability of force with the smallest monofilaments or the synchronization of application with the two-point gap discrimination test (Levin et al., 1978; Lundborg and Rosén, 2004), these tests evaluate only static touch and not dynamic touch. Presently, we focused on the more functional characteristics (i.e., dynamic touch), because during active touch, proprioceptive inputs implement cutaneous information from the hand and fingers (Gibson, 1962).

It is known that skin hydration improves tactile discrimination ability, and this has been demonstrated during both static (Lévêque et al., 2000) and dynamic touch (Skedung et al., 2018). We also knew that the active ingredient included here in the foam increases skin hydration and induces vasodilatation, as compared to the same cosmetic foam without this ingredient (Anjac, personal communication). We demonstrate that the new psychophysical method we tested here found differences between the active and placebo groups, in terms of tactile acuity, and thus provides a high level of discrimination.

Recently, a similar method has been published where the short-term effects of applying skin moisturizing were assessed during dynamic touch, by analyzing subjects' tactile perception ability using a test of texture discrimination (Skedung et al., 2018). In this study, participants had to judge a surface as being different from the reference one and/or to judge the reference to be the same when presented against itself, and authors quantified the percentage of correct responses. The fact that this study and ours was performed almost at the same time shows that there is a need nowadays to improve the old, classical tests to better evaluate tactile sensitivity, notably to assess the effects of treatments or remediation by training. The present method appears more sophisticated, since not only did the participant have to judge that the test texture was different from reference,

but also to say in which way, i.e., with farther or closer grooves. Therefore, one should expect closer differences to be detected by the present method, which also allowed the extraction of statistical parameters that gave additional information, including the PSE and the uncertainty range. Therefore, the two methods constitute a real step towards a better estimation of tactile acuity and one may choose one or the other depending on the requirements and sensitivity of the experiment.

About the mechanisms responsible for the improvement in tactile discrimination, we can exclude an effect of protocol learning for two reasons: this should have impacted the participants from the placebo group, and more importantly, the participants were tested only two times, with a 1-month interval. Tactile learning of complex tasks requires a training based on multiple seances over a couple of weeks (Debowska et al., 2016), which was not the case here. The most reasonable hypothesis would have initially been that the improved performance in the active group was related to changes in skin mechanical properties, which we did not test here, but that has been largely documented, where moisturizing the stratum corneum has been shown to promote recovery of skin elasticity associated with improvement in tactile sensitivity (Lévêque et al., 2000; Bowden and McNulty, 2013; Skedung et al., 2018). However, in their recent study, Skedung et al. (2018) report a great variability in tactile sensitivity in elderly people, where high and low performers exhibited almost the same skin mechanical properties. In the present study, the two groups of subjects performed similarly before the month of cosmetic application and they were all exposed to the same amount of humectant or softening product, so that we can reasonably think that the mechanical properties of the skin were similar between the two groups of subjects after the month of cosmetic substance application. Interestingly, the same authors (Skedung et al., 2018) found that among the elderly participants, the higher performing group had a statistically higher density of Meissner corpuscles, which are cutaneous mechanoreceptors strongly involved in dynamic touch, such as in the present active task. All these considerations lead us to suggest that the active product used here may have promoted peripheral sensory regeneration after 1 month of application, as previously demonstrated to occur with various drugs such as hydrogen peroxide (Rieger and Sagasti, 2011) or neuropoietic cytokines (Feld et al., 2016), but this remains speculative and further studies are required to establish the properties of the present active product. Changes in tactile discrimination after 1 month of cosmetic substance application have been documented in elderly people using a new psychophysical method, to evaluate alterations in the range of a few micrometers. We suggest that this high level of discrimination validates our new test as being particularly useful for studying the effects of tactile rehabilitation and/or skin treatments in the elderly.

DATA AVAILABILITY

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

All participants gave their written, informed consent and the investigation was carried out in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

This study was designed by ER-C, J-MA, and MC. Data collection and analysis were conducted by CD. PMMA sheets were designed and provided by P-HC and BW. The content of the manuscript was prepared by J-MA and ER-C.

FUNDING

This study was supported by grants from Laboratoires Chemineau, Anjac Health and Beauty Inc.

ACKNOWLEDGMENTS

We are grateful to Grégoire Huet, Estelle Dessables and Benoit Agnus from Laboratoires Chemineau. We are grateful to Dr Rochelle Ackerley for improving the English.

REFERENCES

Bang, H. (2016). Random guess and wishful thinking are the best blinding scenarios. *Contemp. Clin. Trials Commun.* 3, 117–121. doi: 10.1016/j.conctc.2016.05.003

Bell-Krotoski, J., Weinstein, S., and Weinstein, C. (1993). Testing sensibility, including touch-pressure, two-point discrimination, point localization and vibration. *J. Hand Ther.* 6, 114–123. doi: 10.1016/s0894-1130(12)80292-4

Bowden, J. L., and McNulty, P. A. (2013). Age-related changes in cutaneous sensation in the healthy human hand. *Age Dordr.* 35, 1077–1089. doi: 10.1007/s11357-012-9429-3

Bruce, M. F. (1980). The relation of tactile thresholds to histology in the fingers of elderly people. *J. Neurol. Neurosurg. Psychiatry* 43, 730–734. doi: 10.1136/jnnp.43.8.730

Cohen, J. (1992). A power primer. *Psychol. Bull.* 112, 155–159. doi: 10.1037/0033-2909.112.1.155

Cruz-Almeida, Y., Black, M. L., Christou, E. A., and Clark, D. J. (2014). Site-specific differences in the association between plantar tactile perception and mobility function in older adults. *Front. Aging Neurosci.* 6:68. doi: 10.3389/fnagi.2014.00068

da Silva, L. A., Lin, S. M., Teixeira, M. J., de Siqueira, J. T. T., Jacob Filho, W., and de Siqueira, S. R. D. T. (2014). Sensorial differences according to sex and ages. *Oral Dis.* 20, e103–110. doi: 10.1111/odi.12145

Debowska, W., Wolak, T., Nowicka, A., Kozak, A., Szwed, M., and Kossut, M. (2016). Functional and structural neuroplasticity induced by short-term tactile training based on braille reading. *Front. Neurosci.* 10:460. doi: 10.3389/fnins.2016.00460

Farage, M. A., Miller, K. W., Elsner, P., and Maibach, H. I. (2013). Characteristics of the Aging Skin. *Adv. Wound Care (New Rochelle)* 2, 5–10. doi: 10.1089/wound.2011.0356

Feld, M., Garcia, R., Buddenkotte, J., Katayama, S., Lewis, K., Muirhead, G., et al. (2016). The pruritus- and TH2-associated cytokine IL-31 promotes growth of

sensory nerves. *J. Allergy Clin. Immunol.* 138, 500–508. doi: 10.1016/j.jaci.2016.02.020

Franco, P. G., Santos, K. B., and Rodacki, A. L. (2015). Joint positioning sense, perceived force level and two-point discrimination tests of young and active elderly adults. *Braz. J. Phys. Ther.* 19, 304–310. doi: 10.1590/bjpt-rbf.2014.0099

Gibson, J. J. (1962). Observations on active touch. *Psychol. Rev.* 69, 477–491. doi: 10.1037/h0046962

Haloua, M. H., Sierevelt, I., and Theuvenet, W. J. (2011). Semmes-weinstein monofilaments: influence of temperature, humidity and age. *J. Hand Surg. Am.* 36, 1191–1196. doi: 10.1016/j.jhsa.2011.04.009

Iwasaki, T., Goto, N., Goto, J., Ezure, H., and Moriyama, H. (2003). The aging of human Meissner's corpuscles as evidenced by parallel sectioning. *Okajimas Folia Anat. Jpn.* 79, 185–189. doi: 10.2535/ofaj.79.185

Johnson, K. O., and Phillips, J. R. (1981). Tactile spatial resolution. I. Two-point discrimination, gap detection, grating resolution, and letter recognition. *J. Neurophysiol.* 46, 1177–1192. doi: 10.1152/jn.1981.46.6.1177

Johnson, K. O. (2001). The roles and functions of cutaneous mechanoreceptors. *Curr. Opin. Neurobiol.* 11, 455–461. doi: 10.1016/s0959-4388(00)00234-8

Lévêque, J. L., Dresler, J., Ribot-Ciscar, E., Roll, J. P., and Poelman, C. (2000). Changes in tactile spatial discrimination and cutaneous coding properties by skin hydration in the elderly. *J. Invest. Dermatol.* 115, 454–458. doi: 10.1046/j.1523-1747.2000.00055.x

Levin, S., Pearsall, G., and Ruderman, R. J. (1978). Von Frey's method of measuring pressure sensibility in the hand: an engineering analysis of the Weinstein-Semmes pressure aesthesiometer. *J. Hand Surg.* 3, 211–216. doi: 10.1016/S0363-5023(78)80084-7

Lundborg, G., and Rosén, B. (2004). The two-point discrimination test–time for a re-appraisal? *J. Hand Surg. Br.* 29, 418–422. doi: 10.1016/j.jhsb.2004.02.008

Massy-Westropp, N. (2002). The effects of normal human variability and hand activity on sensory testing with the full Semmes-Weinstein monofilaments kit. *J. Hand Ther.* 15, 48–52. doi: 10.1053/hanthe.2002.v15.01548

Rieger, S., and Sagasti, A. (2011). Hydrogen peroxide promotes injury-induced peripheral sensory axon regeneration in the zebrafish skin. *PLoS Biol.* 9:e1000621. doi: 10.1371/journal.pbio.1000621

Skedung, L., El Rawadi, C., Arvidsson, M., Farcet, C., Luengo, G. S., Breton, L., et al. (2018). Mechanisms of tactile sensory deterioration amongst the elderly. *Sci. Rep.* 8:5303. doi: 10.1038/s41598-018-23688-6

Soriano, T. A., DeCherrie, L. V., and Thomas, D. C. (2007). Falls in the community-dwelling older adult: a review for primary-care providers. *Clin. Interv. Aging* 2, 545–554. doi: 10.2147/cia.s1080

Stevens, J. C., and Choo, K. K. (1996). Spatial acuity of the body surface over the life span. *Somatosens. Mot. Res.* 13, 153–166. doi: 10.3109/08990229609051403

Thornbury, J. M., and Mistretta, C. M. (1981). Tactile sensitivity as a function of age. *J. Gerontol.* 36, 34–39. doi: 10.1093/geronj/36.1.34

Tong, J., Mao, O., and & Goldreich, D. (2013). Two-point orientation discrimination versus the traditional two-point test for tactile spatial acuity assessment. *Front. Hum. Neurosci.* 7:579. doi: 10.3389/fnhum.2013.00579

Vega-Bermudez, F., Johnson, K. O., and Hsiao, S. S. (1991). Human tactile pattern recognition: active versus passive touch, velocity effects, and patterns of confusion. *J. Neurophysiol.* 65, 531–546. doi: 10.1152/jn.1991.65.3.531

Verrillo, R. T., Bolanowski, S. J., Checkosky, C. M., and McGlone, F. P. (1998). Effects of hydration on tactile sensation. *Somatosens Mot. Res.* 15, 93–108.

Wichmann, F. A., and Hill, N. J. (2001). The psychometric function: I. Fitting, sampling, and goodness of fit. *Percept. Psychophys.* 63, 1293–1313. doi: 10.3758/bf03194544

Wickremaratchi, M. M., and Llewelyn, J. G. (2006). Effects of ageing on touch. *Postgrad. Med. J.* 82, 301–304. doi: 10.1136/pgmj.2005.039651

Woodward, K. L. (1993). The relationship between skin compliance, age, gender, and tactile discriminative thresholds in humans. *Somatosens. Mot. Res.* 10, 63–67. doi: 10.3109/08990229309028824

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

CLINICAL TRIAL
published: 14 August 2019
doi: 10.3389/fnagi.2019.00212

The Transfer Effects of Cognitive Training on Working Memory Among Chinese Older Adults With Mild Cognitive Impairment: A Randomized Controlled Trial

*Wenqi Weng†, Jiaming Liang†, Jiang Xue, Tingfei Zhu, Yuxing Jiang, Jiayu Wang and Shulin Chen**

Department of Psychology, Zhejiang University, Zhejiang, China

Objectives: To explore the transfer effects of cognitive training on working memory among older Chinese adults with mild cognitive impairment (MCI).

Methods: Sixty-two MCI participants aged more than 60 years old were recruited by holding recruitment sessions in communities in China [33 for cognitive training, and 29 for mental leisure activities (MLA) control]. Cognitive functions, including working memory, execution function, reasoning ability, verbal ability, ability of daily living, were measured at three time-points (baseline, post-training and 3 months after training).

Results: Compared to the MLA control, the cognitive training group showed significant effects in both the trained (working memory) and untrained (execution function and ability of daily living) domains. The effects of cognitive training on overall cognitive function, working memory and daily life ability of daily living of MCI could be maintained for at least 3 months, even without the cognitive training. Besides, complete mediating effects of cognitive training were found in executive function through working memory and working memory in ability of daily living though executive function, which suggests the presence of transfer effect of cognitive training.

Conclusions: The present study supported that cognitive training could effectively improve working memory in elders with MCI. The training effects on working memory could transfer to other untrained areas (such as executive function), which also improved the comprehensive ability (ability of daily living). And the effects of training could largely persist for 3 months.

Keywords: mild cognitive impairment, cognitive training, working memory, transfer effects, maintaining effect

OPEN ACCESS

Edited by:
Mario Bernardo-Filho,
Rio de Janeiro State University, Brazil

Reviewed by:
Helena Carvalho,
Virginia Tech Carilion, United States
Laisa Liane Paineiras-Domingos,
Rio de Janeiro State University, Brazil

****Correspondence:***
Shulin Chen
chenshulin@zju.edu.cn

†These authors have contributed equally to this work

Received: *27 March 2019*
Accepted: *26 July 2019*
Published: *14 August 2019*

Citation:
Weng W, Liang J, Xue J, Zhu T, Jiang Y, Wang J and Chen S (2019) The Transfer Effects of Cognitive Training on Working Memory Among Chinese Older Adults With Mild Cognitive Impairment: A Randomized Controlled Trial. Front. Aging Neurosci. 11:212. doi: 10.3389/fnagi.2019.00212

INTRODUCTION

The average age of populations is rising—an irreversible trend all over the world, along with which comes the increasing mental health needs of the elderly. And the subsequent increasing number of Alzheimer's and related dementias cause a heavier burden to society, which is a significant problem. It is estimated that dementia cases have reached

nearly 50 million globally, and the incidence of dementia is predicted to rise to 50%, while the number will reach 75 million by 2030 (Prince et al., 2015). Due to its extremely low rate of successful treatment and great social burden, researchers around the world are eager to find new treatment methods for dementia.

As mild cognitive impairment (MCI) is a pre-phase of Alzheimer's disease (AD) in most cases (Albert et al., 2011; Vos et al., 2015; Klimova and Maresova, 2017), elderly people and their families are seeking effective ways to delay or ameliorate MCI in order to prevent AD. Cognitive training is considered a promising way to delay the decline of cognitive function, and indeed, promising results have been observed for MCI (Jean et al., 2010; Hyer et al., 2016; Lin et al., 2017). Recent meta-analyses have identified its significant cognitive outcomes in multidomain and lifestyle approaches for individuals with MCI (Sherman et al., 2017). Also, cognitive training is a more effective and economical method compared to pharmacological approaches (Dresler et al., 2013).

Cognitive training is a set of training programs designed by researchers, including standardized training instructions and training tasks, and each task is targeted at improving one or several specific cognitive functions. Since the 1990s, researchers have attempted to improve the cognitive function of elder people by using cognitive training. The initial training mode is mainly based on the paper-pencil intelligence tests and the techniques of enhancing memory. For instance, researchers provide the participants with some scattered numbers and letters and ask them to connect the numbers and letters in alphabetical order by drawing lines (Jobe et al., 2001). With the popularity of electronic devices, standardized computer-based cognitive training programs have been gradually developed. Computer-based cognitive training has advantages of high efficiency and high sensitivity, which means its training materials can be coded in the computer and automatically presented to the participants, and the degree of training difficulty can be self-adaptive according to the performance of the participants (Dresler et al., 2013). Also, computer-based cognitive training can contribute to the moderation of cognitive disorders in dementia, particularly in the early stages of AD, as have been confirmed by several researches (Preece and Maloney-Krichmar, 2003; Savitch and Zaphiris, 2006).

Although the cognitive trainings are able to improve some specific cognitive functions, training every domain of cognition is difficult and time-consuming. Thereby, cognitive decline in the elderly is still widespread. In this case, the effect of training on non-trained cognitive functions (i.e., the transfer effect) should be discussed. The transfer effect refers to the ability that individuals can use the knowledge and skills learned in one scenario to achieve different goals in other scenarios. And it can be differentiated into near-transfer effects (post-training improvement in tasks similar to the training tasks) and far-transfer effects (post-training improvement in tasks that are different from the training tasks in nature or in appearance; Barnett and Ceci, 2002). Far-transfer effects occur when two different tasks share an underlying processing component and neuroanatomical areas or neural circuits (Jonides, 2004).

According to the transfer effects, researchers attempted to transfer the effects of cognitive training to other cognitive abilities to help MCI elder people adapt to their daily lives and solve corresponding problems. Researchers trained hearing processing speed of the MCI elderly and found that the participants not only showed faster processing speed but also had a higher accuracy rate in the auditory signal detection task. Besides, the participants also showed enhancement in memory (Anderson et al., 2013). More surprisingly, cognitive training also showed a transfer effect in physical activities (Verghese et al., 2010; Smith-Ray et al., 2014, 2015). For instance, after the Stroop training, MCI participants not only showed enhancement in working memory and executive functions but also had better performance in terms of physical balance (Li et al., 2011). However, there are also studies that did not find transfer effect after training cognitive functions (Dosher and Lu, 1998). And the sustainability of transfer effects after cognitive training is barely discussed.

There is no doubt that to explore the transfer effects in cognitive training is meaningful and imperative. On one hand, if the transfer effects are accessible and sustainable, the efficiency of cognitive training will be greatly improved. In other words, elderly people with MCI only need to receive a small amount of training to get multi-domain benefits. On the other hand, the transfer effects of cognitive training can overcome many training obstacles, such as disability and physical aging. That means researchers are able to help the bedridden elder people (e.g., people with stroke) improve their abilities which are hard to be trained (e.g., ability of daily living) by the transfer effects.

Aim of Study

The main objective of this study is to examine whether single-domain cognitive training (working memory training) has a transfer effect on un-trained cognitive functions, based on the effectiveness of cognitive training. The reason of choosing working memory is because it is a fundamental cognitive function that underpins other complex cognitive functions (Morrison and Chein, 2010). And the cognitive functions might be affected as follows (see **Figure 1**).

Hypothesis 1 (training effect): working memory would significantly improve after cognitive training.

Hypothesis 2 (transfer effect): the un-trained cognitive function (executive function, verbal ability and reasoning ability) and comprehensive ability (ability of daily living) would significantly improve after training.

Hypothesis 3 (sustainability): the training effect and the transfer effect can maintain for at least 3 months.

METHOD

Participants

The present study was approved by the Human Subjects Review Committee of Zhejiang University. Participants consisted of MCI elderly, over 60 years old, living in Nanxing Street, Shangcheng District, Hangzhou City, and participants were

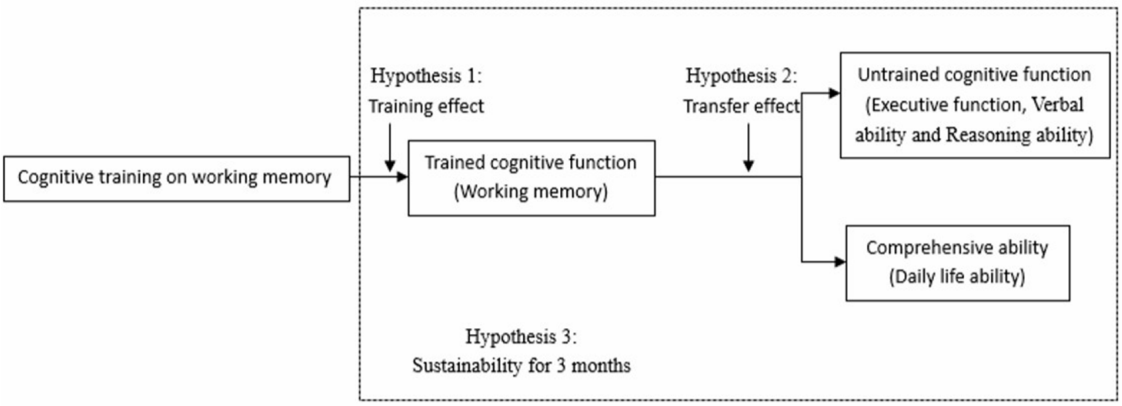


FIGURE 1 | Hypothesis of cognitive training model.

recruited *via* holding recruitment sessions at older adult communities.

The inclusion criteria were as follows:

1. Age ≥60 years old.
2. With no significant visual or auditory impairment.
3. The Montreal Cognitive Assessment (MoCA, <26 when education level >12 years or MoCA <25 when education level ≤12 years).
4. Meeting the MCI diagnostic criteria of the National Institute of Neurological Disorders and Stroke Alzheimer Disease and Related Disorders (NINCDS-ADRDA; McKhann et al., 1984).
5. Noticed and freely to give informed consent.

The exclusion criteria were as follows:

1. Meeting the dementia diagnostic criteria of the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-V; American Psychiatric Association, 2013) and NINCDS-ADRDA.
2. Taking antipsychotics, or stopping for less than 3 months.
3. Participating in other cognitive training projects.
4. Refusing to participate in the study.

After recruitment, there were 96 individuals who met the criteria while 31 participants were declined to participate because of lack of time for training or fulfilling 3-months follow-up. The enrolled participants (N = 65) were randomly assigned to a cognitive training group (N = 33) or a control group (N = 32). All participants had signed up the informed consent and committed not to attend any other cognitive training activity during the present study. If an individual was on medication (i.e., memantine or cholinesterase inhibitors), it was required to have no changes in dosage from the 3 months prior to recruitment to the end of the present study except in extreme circumstances. During the cognitive training, one participant fell off (lost contact), and two participants in the control group discontinued the study due to death (see in **Figure 2**).

According to the results of independent-sample t-test and χ^2 test, there was no significant difference in the population and sociological variables between the two groups (see **Table 1**).

Procedures

The cognitive training group received a cognitive training of 8 weeks, 2 times/week for 40–60 min (Ross et al., 2018). The control group was treated with mental leisure activities (MLA) to simulate participants' everyday mental activities and entertain them to prevent dropping out. Each time the cognitive training group received training, the researchers or community workers organized the control group to conduct activities (Lin et al., 2016). The activities mainly included watching TV dramas, watching news, blood pressure measurement, and chatting, etc. The present study collected cognitive evaluation data at three time-points, before cognitive training (T1), after cognitive training (T2), and 3-month follow-up (T3). The flowchart is presented in **Figure 2**.

Cognitive Training

The present study used computer programs for cognitive training and mainly focused on the training of working memory, including "graphic/image/information delay matching," "object tracking," "memory/attention composite task." All games were developed by Zhejiang University and Nanjing Zhihui Education Technology Company, Limited. The cognitive training contains four main tasks:

1. The graphic/image delay matching task: first, participants were asked to remember the picture(s) presented (one picture or more) on the screen (including simple shapes, landscape pictures). Then in the recognition phase, participants were asked to determine whether the new image has been studied before.
2. The information delay matching task: it required the participant to remember the photos of the characters presented on the screen, as well as the basic information related, and in the subsequent testing phase participants were

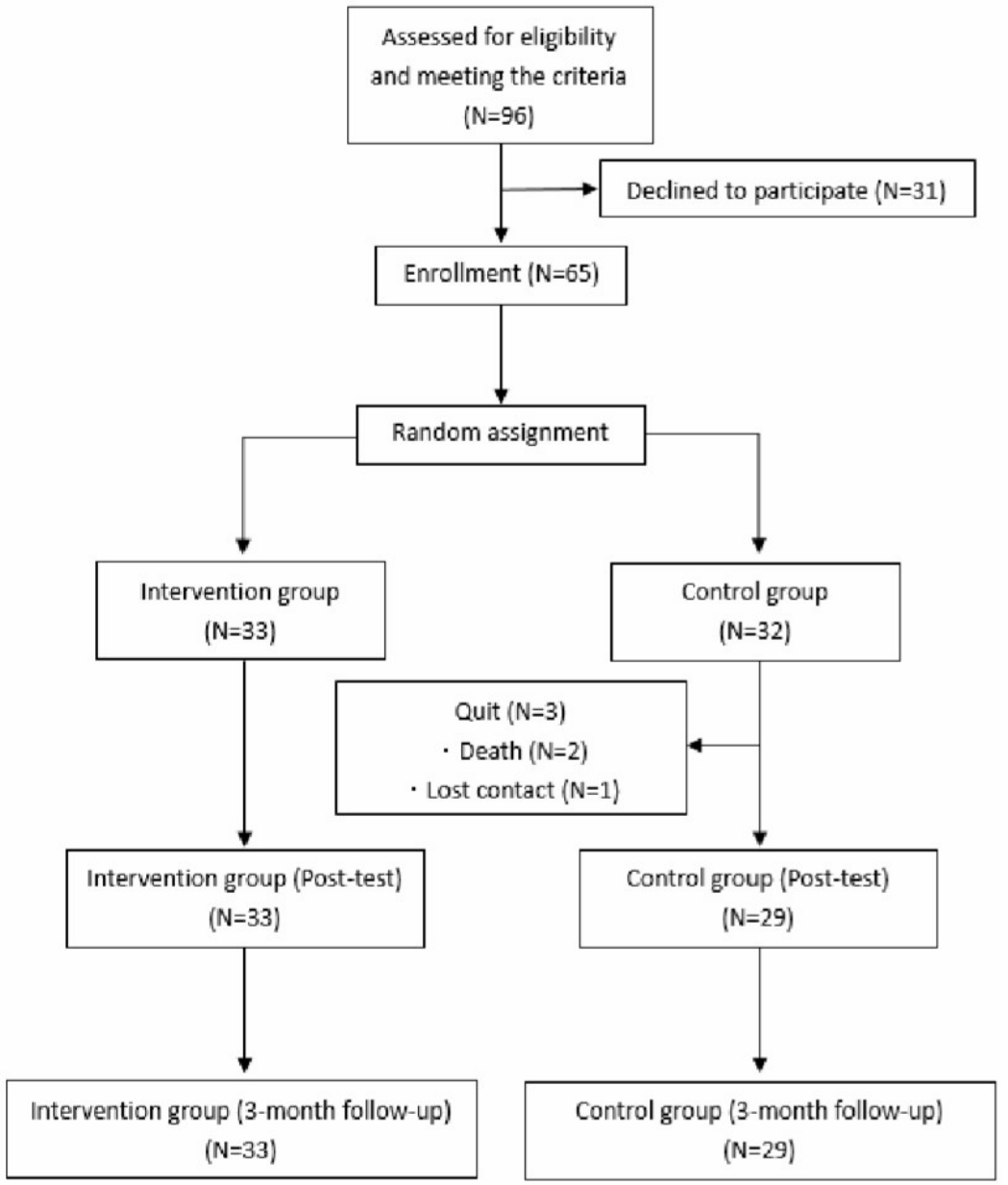


FIGURE 2 | Research procedure.

asked to match the person's photo and the corresponding individual information;

3. The object tracking task: it required participants to pay attention to the trajectory of one or more targets (the target can be a simple graphic or a physical picture) moving on the screen. After a period of time, the object disappears, and participants were asked to select the previous position of target object before it disappears;
4. Memory/Attention Composite task: it presented a scene containing one object 1 and multiple objects 2 and required participants to pay attention and click on the position of object 1 on the screen while remembering the number of objects 2. Both object 1 and objects 2 are simple graphic or physical objects. After clocking object 1, objects 2 immediately disappeared, then participants were required to report the number of objects 2.

Measures

Basic demographic information was recorded, including gender (male/female), age and education level (1 = illiterate; 2 = primary school degree; 3 = junior high school degree; 4 = high school degree and above; **Appendix 1**).

TABLE 1 | Demographic information of cognitive training group and control group.

Variables	Cognitive training group (n = 33)		Control group (n = 29)		t/χ^2
	M/%	*SD*	*M*/%	*SD*	*p*
Age	81.82	11.28	80.72	9.91	0.69
65–69	21.21%		20.69%		0.62
70–79	21.21%		20.69%		
80–89	30.30%		44.83%		
90–	27.27%		13.79%		
Gender (female)	87.88%		96.55%		0.22
Education (year)					
Illiteracy	42.42%		41.38%		0.79
1–6	18.18%		13.79%		
7–9	21.21%		24.14%		
10–	18.18%		20.69%		

Cognitive assessments focused on working memory, executive function, reasoning ability, verbal ability, ability of daily living. In the present study, three subtests of digit span, digital symbol conversion and similarity test in the Wechsler Adult Intelligence Scale-Fourth Edition of Chinese version (WAIS–IV; Wang et al., 2013) were used to evaluate working memory, executive function (conversion ability, untrained) and reasoning ability (untrained; **Appendices 4–6**). The Weng and Huang's (2014) study was used to evaluate the ability of daily living (**Appendix 3**). In addition, Montreal Cognitive Assessment (MoCA; Hongji et al., 2014; Razali et al., 2014; Gan et al., 2017) was used to evaluate comprehensive cognitive function, and its subtest (i.e., the verbal fluency test, VFT) was used to evaluate the verbal ability (**Appendix 2**).

In order to avoid the impact of operational familiarity caused by computer training program, all cognitive assessments were conducted in the form of paper and pencil.

Analyses

The data for the present study were analyzed by IBM SPSS 21.0 and Mplus Version 8.2.

1. The independent-sample t-test and χ^2 test were used to compare the baseline data of the cognitive training group with that of the control group.
2. The analysis of covariance (ANCOVA) was used to test the training effect. The data of cognitive assessments (T2) was the dependent variable, and the grouping condition was the independent variable while controlling for age, gender and baseline data (T0).
3. Mediating effect model (bootstrap analyses, bootstrap = 5,000) was used to test the transfer effect of cognitive training on un-trained cognitive functions and comprehensive ability, with working memory as the mediator.
4. Paired sample t-tests (T2-T3) were used to test the maintenance of training effect. And the ANCOVA was also used to test the long-term effect. The data of cognitive assessments (T3) was the dependent variable, and the grouping condition was the independent variable, while age, gender and baseline data (T1) were controlled for.
5. The value of α is 0.05, and the value of significance p is a two-sided probability.

RESULTS

Training Effect

By independent-sample t-test and χ^2 test, there were no significant differences in comprehensive cognitive function (MoCA), working memory (forward and backward digit span), executive function (digital symbol conversion), verbal ability (VFT), logic reasoning ability (the similarity reasoning test), ADL between the cognitive training group and the control group at baseline (see **Table 2A**).

The ANCOVA was used to control the baseline, age, gender and education level of the participants. After the cognitive training (T2), forward digit span ($F_{(1,56)}$ = 12.36, $p < 0.01$, η^2 = 0.18), backward digit span ($F_{(1,56)}$ = 6.93, $p < 0.05$, η^2 = 0.11) and digital symbol conversion test ($F_{(1,56)}$ = 17.38, $p < 0.001$, η^2 = 0.24) showed significant grouping differences. The difference in MoCA scores between the two groups was not significant after cognitive training ($F_{(1,56)}$ = 3.79, p = 0.058, η^2 = 0.06). VFT, similarity reasoning test, and ADL scores were not significantly different (see **Table 2B**).

Maintenance of Training Effect

The scores of cognitive function and life ability at three times (T1, T2 and T3) can be seen in **Figure 3A**. Among them, cognitive ability, such as working memory and executive function, had a decline trend after the cognitive training.

TABLE 2A | Three time-points (baseline, post-training and 3 months after training) data of cognitive training group and control group.

Variables	Baseline					Post-training				3-months follow-up			
	Cognitive training group		Control group		t/χ^2	Cognitive training group		Control group		Cognitive training group		Control group	
	M/%	*SD*	*M*/%	*SD*	*p*	*M*/%	*SD*	*M*/%	*SD*	*M*/%	*SD*	*M*/%	*SD*
MoCA	17.45	4.65	18.41	3.40	0.36	18.09	4.71	17.86	3.32	18.12	4.79	17.38	3.42
Digit span													
Forward	4.42	1.25	4.03	1.12	0.20	4.55	1.23	3.76	1.12	4.33	1.24	3.79	1.15
Backward	2.45	0.94	2.69	0.97	0.34	2.73	0.91	2.52	0.91	2.67	0.82	2.41	1.02
Digital symbol conversion	19.03	8.12	21.45	7.78	0.24	21.48	6.70	20.10	8.03	20.48	6.28	20.17	8.12
VFT	8.94	3.03	9.59	2.71	0.38	9.06	2.68	9.66	2.76	9.15	2.46	9.52	2.59
Reasoning based on similarity	10.61	4.96	11.17	3.96	0.62	11.06	4.44	11.55	4.37	10.79	5.01	11.24	3.99
ADL	24.06	11.72	22.72	11.58	0.65	23.79	11.31	23.07	11.16	24.00	11.81	23.41	11.74

TABLE 2B | Analysis of covariance (ANCOVA) data between cognitive training group and controlled group in T2.

Variables	*F*	*p*	η^2
MoCA	3.79	0.058	0.063
Digit span			
Forward	12.363	0.001	0.181
Backward	6.929	0.011	0.110
Digital symbol conversion	17.381	0.000	0.237
VFT	0.158	0.693	<0.01
Reasoning based on similarity	<0.01	0.998	<0.01
ADL	1.585	0.213	0.028

TABLE 2C | Training effect in T2 and T3 (paired sample *t*-tests, cognitive training group).

Variables	Score in T2 (*M* ± *SD*)	Score in T3 (*M* ± *SD*)	*p*
MoCA	18.09 ± 4.71	18.12 ± 4.79	0.94
Digit span			
Forward	4.55 ± 1.23	4.33 ± 1.24	0.09
Backward	2.73 ± 0.91	2.67 ± 0.82	0.33
Digital symbol conversion	21.38 ± 7.70	20.48 ± 6.28	0.01
ADL	23.79 ± 11.31	24.00 ± 11.81	0.68

However, the results of the paired sample *t*-tests showed that, except for the executive function (conversion; $p = 0.01$), there was no significant change in other cognitive abilities and life abilities at 3-month follow-up (T3) compared to the end of cognitive training (T2; see **Table 2C**). It is worth mentioning that the MoCA and ADL scores of the cognitive training group remained at a specific level from T2 to T3, but the MoCA and ADL (reverse scoring) scores of the control group continued to decrease. These results indicate that the cognitive training mainly focused on working memory did not improve global cognitive function and ability of daily living, but there might be a long-term transfer effect. In order to explore the delay effect, the ANCOVA was used to control the baseline, age, gender and education level of the participants. Comparing the data at 3-months follow-up (T3) with baseline (T1), MoCA ($F_{(1,56)} = 17.164$, $p < 0.001$, $\eta^2 = 0.24$), backward digit span ($F_{(1,56)} = 13.894$, $p < 0.001$, $\eta^2 = 0.199$), digital sign conversion ($F_{(1,56)} = 12.821$, $p < 0.01$, $\eta^2 = 0.186$) and ADL ($F_{(1,56)} = 9.30$, $p < 0.01$, $\eta^2 = 0.142$) showed grouping differences. Compared with the post-cognitive training (T2) data, MoCA and ADL showed a grouping effect (see **Table 2D**).

Transfer Effect

It is difficult for cognitive training to train only one cognitive ability, for example, memory training must involve the use of attention resources, and computer operations also train exclusive function. In this case, the results of the general linear model cannot distinguish between the transfer effect and the training itself. Therefore, a mediating effect model (bootstrap analyses) was used to explore the mediating effect of working memory on un-trained cognitive functions (executive function) and comprehensive ability (ability of daily living) after cognitive training, in order to verify the transfer effect. The results showed that cognitive training had a significant training effect on working memory ability ($\beta = 0.320$, $p < 0.01$), and the effect of working memory ability on ability of daily living ($\beta = 0.311$, $p < 0.01$) and executive function (conversion; $\beta = 0.735$, $p < 0.001$) was significant. The effects of cognitive training on ability of daily living ($\beta = -0.125$, $p = 0.245$) and executive function (conversion; $\beta = -0.141$, $p = 0.147$) were not significant (see **Figure 3B**). The results indicated that cognitive training had no significant direct impact on ability of daily living and executive function.

TABLE 2D | ANCOVA data between cognitive training group and controlled group in T3.

Variables	*F*	*p*	η^2
MoCA	17.164	0.000	0.235
Digit span			
Forward	3.382	0.071	0.057
Backward	13.894	0.000	0.199
Digital symbol conversion	12.821	0.001	0.186
VFT	0.491	0.486	<0.01
Reasoning based on similarity	0.034	0.854	<0.01
ADL	9.298	0.003	0.142

In the above model, there was a significant correlation between DLA and EF, which suggested that WM may not directly affect DLA, with EF as the mediator in the process. Thus, another bootstrap analysis was used to explore the mediating effect of EF on the relation between WM and DLA (see in **Figure 3C**). The results showed that cognitive training had a significant effect on working memory ability ($\beta = 0.320$, $p < 0.01$), and the effect of WM on EF ($\beta = 0.735$, $p < 0.001$) and EF on DLA ($\beta = 0.607$, $p < 0.001$) was significant. The effects of cognitive training on DLA ($\beta = -0.040$, $p = 0.713$), cognitive training on EF ($\beta = -0.141$, $p = 0.147$) and WM on DLA ($\beta = -0.135$, $p = 0.412$) were not significant.

DISCUSSION

The present study used cognitive training on the community MCI elderly, mainly focusing on working memory. Then cognitive abilities and ability of daily living were evaluated to test the cognitive training effect, its maintenance and transfer effect of the cognition training. The results showed that cognition training had a high degree of acceptance in the in-home MCI elderly population in urban communities, and the compliance in the cognitive training process was satisfactory. And the results also indicated that cognition training not only enhanced the performance of MCI elderly people in working memory tests but also had a significant transfer effect in executive function and ability of daily living.

In addition, the MCI elderly people in communities showed varying degrees of decline in memory, attention, executive function, verbal ability, and abstract thinking ability, among which the decline of memory function was the most notable. For instance, in the delayed recall test, even after the relevant cue clues were given by the tester, all MCI participants could not fully recall the five words previously studied. Therefore, the

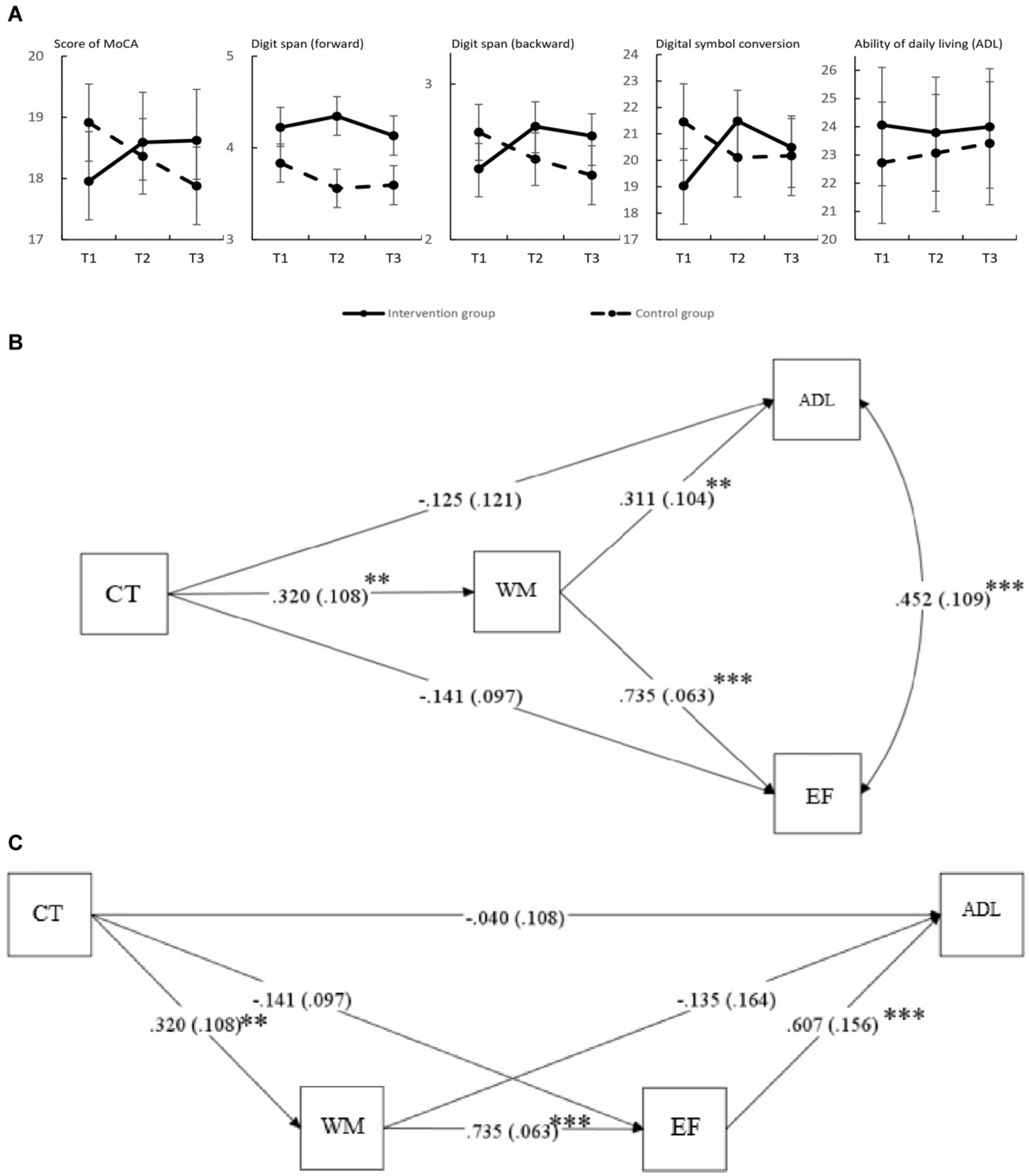


FIGURE 3 | (A) Evaluation score in T1, T2 and T3. From left to right, the ordinates represent the score of Montreal Cognitive Assessment (MoCA), digit span (forward), digit span (backward), digital symbol conversion and ability of daily living (ADL). **(B)** Mediating effect model of cognitive training (CT represents cognitive training, WM represents working memory, EF represents excusive function and ADL represents ability of daily living; $^{**}p < 0.01$, $^{***}p < 0.001$). **(C)** Mediating effect model of cognitive training (considering the relationship between executive function and ability of daily living; $^{**}p < 0.01$, $^{***}p < 0.001$).

researchers believe that the decline of memory function is a vital risk factor of the transformation from MCI into dementia (Gao et al., 2011).

The present study supports the theory of "cognitive plasticity" (Willis and Schaie, 2009), which embodies in the cognition improvement brought by the training. In the digit span task (forward and backward), the cognitive training group and the control group showed significant differences after the cognitive training. The above two tasks (forward and backward) mainly examined working memory ability of the participants, which

was also the cognitive ability that the training mainly focused on. The results indicated that the training not only improved the performance of participants in the same type of test but also improved other cognitive function of the participants to complete relevant tasks. It also showed that, for the working memory capacity of MCI elderly people, cognitive training has played a role in delaying the decline, which was also proved by the previous studies (Dannhauser et al., 2014; Ross et al., 2018).

According to the results of the MoCA, MoCA is a very credible measurement of the cognitive functions for MCI elderly people, therefore it can effectively reflect the changes in the overall cognitive functions before and after the cognitive training (Nasreddine et al., 2005). The cognitive training used in the present study could effectively delay the decline of overall cognitive ability for MCI elderly people, which was supported by the result that the MoCA score tends to be stable. However, the improving effect on overall cognitive ability was not significant. The MoCA scores of the training group did not exceed the critical points of cognitive health standards in the present study.

The mediation model of the research results mostly excluded the possibility that training directly enhanced executive function, and supported the hypothesis that cognitive training has a transfer effect. Cognitive training has a significant transfer effect on executive function (conversion ability; Smith-Ray et al., 2015). The transfer effect of the participants on the digital symbol conversion task in the present study not only existed after cognitive training but also remained significant after 3-months follow-up. In addition to examining the executive functions (conversion), the digital symbol conversion task also examined the memory ability of the participants. For example, during the task, the subject needed to repeatedly compare the digital symbol conversion example, and then memorize the converted symbol and then wrote it on the answer sheet. And the memory ability was the training cognitive function of the present study, so it was more likely that the transfer effects showed up (Hausdorff et al., 2005). At the same time, some researchers believe that visual cognitive functions are mainly dominated by the frontal and parietal-related visual cortex, while tasks related to task execution and outcome feedback are mainly dominated by the prefrontal cortex (Thorpe et al., 1996). There is a large crossover between the two regions, so the use of visual cognitive training also has an impact on executive function. This sort of transfer effect may be called the "close-transfer effect" (Yogev et al., 2005).

The two tasks of verbal fluency and similarity reasoning examined the extraction of long-term memory and the ability to summarize abstract things. They belong to complex advanced cognitive functions and are more dependent on the crystal intelligence of the participants. In the present study, no relevant training transfer effect was found. Some researchers have found that through the inference game training, the summary ability of the participants was improved, so as the letter fluency (Ross et al., 2018). It has also been found that training for reasoning ability can improve the execution function in conversion (Schulz and Jobe, 2001). Combined with the results of this study, this effect may be one-way irreversible.

Apart from improvement in the untrained cognitive ability, the present study also found that cognitive training on working memory can also improve the ability of daily living of MCI elderly people. A significant difference in ADL scores was found at T3 (3-month follow-up). As shown in **Figure 3A**, the cognitive training group had a small downward trend after cognitive training, with the overall level remained stable. At three time points, scores of the control group showed an upward trend. The effect of cognitive training was not significant in the post-cognitive training test, which might be caused by insufficient training (Edwards et al., 2005; Seidler et al., 2010). Also, the present study found the cognitive training on WM improved ADL through the mediation of EF (see in **Figure 3C**). The ability to enhance physical activity through cognitive training (including *in situ* rotation, grip strength, etc.,) had a mediating effect on executive function, which was called "far-transfer effect" (Adams et al., 2005). However, the ADL assessed by this study is a complex comprehensive capability, so the existence of "far-transfer effect" in this process remains to be further verified.

Participants in the cognitive training group showed a maintenance effect on the MoCA and digit span (forward and

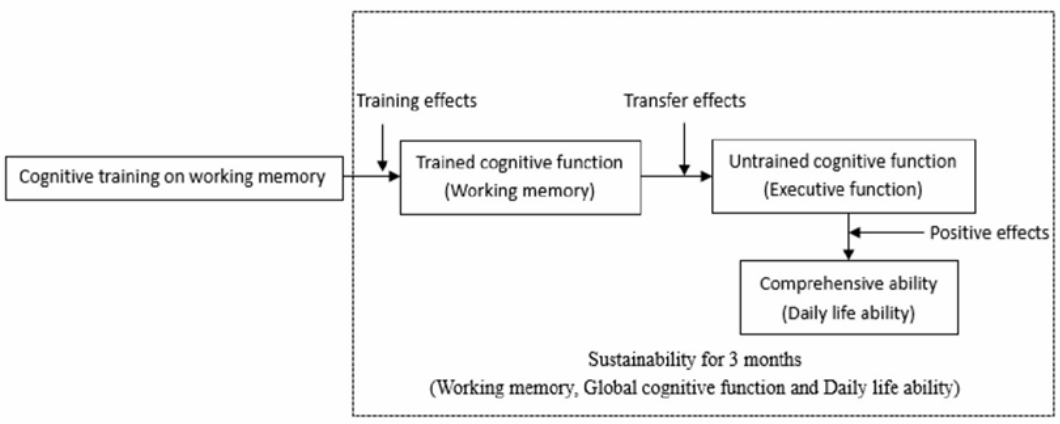


FIGURE 4 | Possible mechanism of cognitive training.

backward) tasks, and there was no significant difference between post-cognitive training and 3-month follow-up (paired sample *t*-tests). In the data of 3-months follow-up (T3), the scores of the cognitive training group's digital symbol conversion task decreased significantly, which reflected a decline in executive function. The effect of memory loss over time was significant in MCI (Nittrouer and Lowenstein, 2015). Thus, the reason which can interpret the results might be that executive function (conversion) became more pronounced as memory declines. Compared to the digit span (forward), the decline in the digit span (backward) score was slower. That might be due to the fact that digit span (backward) task not only examines the working memory capacity but also examines the spatial psychological rotation ability which requires visual space ability. And visual space ability was one of the main contents of visual cognitive training adopted in the present study. Some researchers have found that the training of spatial attention ability can improve the trainee's working memory (digit span test score; Nasreddine et al., 2005). Therefore, the backward memory maintained better. Some researchers also believe that the abilities of forward and backward digit span are not developed simultaneously in childhood, because the backward task is relatively more complex. Thus, the decline of the forward memory ability is more significant in elderly people (Myerson et al., 2003).

There are still some limitations in the present study. First, due to research resource constraints, the total sample size of the study was small, and most of the participants were female (*N* female = 57). According to a 2017 review of cognitive trainings (Shah et al., 2017), only 11 of the 26 studies had a total sample size of less than 62 (the present study). Although the conclusions of the present study are basically consistent with previous research results, and the assumptions of the effectiveness and transfer effects of cognitive training on community MCI elderly are validated, future researches need to expand the sample size in order to eliminate the interference of irrelevant factors. Second, the present study only discussed conversion in executive function, however, the execution function is a complex cognitive function which also contains refresh and suppression. Future research can further explore the transfer effect on execution function more comprehensively. Third, the previous researches were based on theories of neural plasticity, but the results of the present study did not explain the impact of visual cognition training on the brain structure of MCI elderly. That is, in-depth study in neuroimaging is required to explore whether there are structural changes in synapses and neural networks, or changes in gray matter density and blood oxygen balance in related brain regions. Finally, the research used objective indicators such as standard cognitive psychology tests. A study in Hong Kong, China, pointed out that cognitive trainings can affect the subjective feelings of the elderly on their own health status (Kwok et al., 2013), and subjective well-being is an important evaluation index in the construction of home-based care environment. Therefore, it is necessary to add relevant subjective psychological rating scales or interviews in order to more comprehensively evaluate the effects of cognitive trainings.

CONCLUSION

The present study acquires a schematic diagram of the revised model of the mechanism of visual cognition training (see in **Figure 4**). The specific conclusions are as follows:

1. Cognitive training on working memory is able to delay the decline of MCI's working memory, which effect will transfer to other untrained cognitive functions (execution functions);
2. The effect of cognitive training can not only transfer to other untrained cognitive functions (execution functions) but also affect the comprehensive ability (ability of daily living) positively;
3. The effect of cognitive training on comprehensive cognitive function, working memory and ability of daily living of MCI can be maintained for at least 3 months.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the **Supplementary Files**.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the Human Subjects Review Committee of Zhejiang University with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Human Subjects Review Committee of Zhejiang University.

AUTHOR CONTRIBUTIONS

WW and JL provided the idea of the article. WW was responsible for writing and editing this article. SC directed the entire article as the corresponding author. JX, TZ, YJ and JW helped with experimental training and data processing.

FUNDING

The project was supported by the Natural Science Foundation of Zhejiang Province (LY16H090011).

ACKNOWLEDGMENTS

We thank all the MCI participants in this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2019.00212/full#supplementary-material

REFERENCES

Albert, M. S., DeKosky, S. T., Dickson, D., Dubois, B., Feldman, H. H., Fox, N. C., et al. (2011). The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the national institute on aging-Alzheimer's association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.* 7, 270–279. doi: 10.1016/j.jalz.2011.03.008

American Psychiatric Association. (2013). *Diagnostic and Statistical Manual of Mental Disorders.* 5th Edn. Washington, DC: American Psychiatric Association.

Anderson, S., Whiteschwoch, T., Parberyclark, A., and Kraus, N. (2013). Reversal of age-related neural timing delays with training. *Proc. Natl. Acad. Sci. U S A* 110, 4357–4362. doi: 10.1073/pnas.1213555110

Barnett, S. M., and Ceci, S. J. (2002). When and where do we apply what we learn? a taxonomy for far transfer. *Psychol. Bull.* 128, 612–637. doi: 10.1037/0033-2909.128.4.612

Dannhauser, T. M., Cleverley, M., Whitfield, T. J., Fletcher, B. C., Stevens, T., and Walker, Z. (2014). A complex multimodal activity intervention to reduce the risk of dementia in mild cognitive impairment—ThinkingFit: pilot and feasibility study for a randomized controlled trial. *BMC Psychiatry* 14:129. doi: 10.1186/1471-244x-14-129

Dosher, B. A., and Lu, Z.-L. (1998). Perceptual learning reflects external noise filtering and internal noise reduction through channel reweighting. *Proc. Natl. Acad. Sci. U S A* 95, 13988–13993. doi: 10.1073/pnas.95.23.13988

Dresler, M., Sandberg, A., Ohla, K., Bublitz, C., Trenado, C., Mroczko-Wąsowicz, A., et al. (2013). Non-pharmacological cognitive enhancement. *Neuropharmacology* 64, 529–543. doi: 10.1016/j.neuropharm.2012.07.002

Edwards, J. D., Wadley, V., Vance, D., Wood, K., Roenker, D., and Ball, K. (2005). The impact of speed of processing training on cognitive and everyday performance. *Aging Ment. Health* 9, 262–271. doi: 10.1080/13607860412331336788

Gan, L., Liu, T., Wang, S. H., and Zheng, J. G. (2017). Progress in the clinical application of the Chinese version of the concise mental state scale and the montreal cognitive assessment scale. *Chin. J. Rehabil. Med.* 32, 842–845. doi: 10.3969/j.issn.1001-1242.2017.07.026

Gao, Z. B., Wang, W., Zhao, X. L., Shang, Y. C., Bai, X. J., and Wu, W. P. (2011). A follow-up study on cognitive changes of mild cognitive impairment in the elderly. *Zhonghua Yi Xue Za Zhi* 91, 37–39. doi: 10.3760/cma.j.issn.0376-2491.2011.01.010

Hausdorff, J. M., Yogev, G., Springer, S., Simon, E. S., and Giladi, N. (2005). Walking is more like catching than tapping: gait in the elderly as a complex cognitive task. *Exp. Brain Res.* 164, 541–548. doi: 10.1007/s00221-005-2280-3

Hongji, S., Yue, X., Xiaohong, Z., Hengge, X., and Weiping, W. (2014). Item in montreal cognitive assessment. *Chin. J. Geriatr. Heart Brain Vessel Dis.* 16, 387–390. doi: 10.3969/j.issn.1009-04.015

Hyer, L., Scott, C., Atkinson, M. M., Mullen, C. M., Lee, A., Johnson, A., et al. (2016). Cognitive training program to improve working memory in older adults with mci. *Clin. Gerontol.* 39, 410–427. doi: 10.1080/07317115.2015.1120257

Jean, L., Bergeron, M.-E., Thivierge, S., and Simard, M. (2010). Cognitive intervention programs for individuals with mild cognitive impairment: systematic review of the literature. *Am. J. Geriatr. Psychiatry* 18, 281–296. doi: 10.1097/JGP.0b013e3181c37ce9

Jobe, J. B., Smith, D. M., Ball, K., Tennstedt, S. L., Marsiske, M., Willis, S. L., et al. (2001). ACTIVE: a cognitive intervention trial to promote independence in older adults. *Control. Clin. Trials* 22, 453–479. doi: 10.1016/S0197-2456(01)00139-8

Jonides, J. (2004). How does practice makes perfect? *Nature Neuroscience* 7, 10–11. doi: 10.1038/nn0104-10

Klimova, B., and Maresova, P. (2017). Computer-based training programs for older people with mild cognitive impairment and/or dementia. *Neuroimage Clin.* 11:262. doi: 10.3389/fnhum.2017.00262

Kwok, T., Wong, A., Chan, G., Shiu, Y., Lam, K.-C., Young, D., et al. (2013). Effectiveness of cognitive training for Chinese elderly in Hong Kong. *Clin. Interv. Aging* 8, 213–219. doi: 10.2147/cia.s38070

Li, H., Li, J., Li, N., Li, B., Wang, P., and Zhou, T. (2011). Cognitive intervention for persons with mild cognitive impairment: a meta-analysis. *Ageing Res. Rev.* 10, 285–296. doi: 10.1016/j.arr.2010.11.003

Lin, F., Heffner, K. L., Ren, P., and Tadin, D. (2017). A role of the parasympathetic nervous system in cognitive training. *Curr. Alzheimer Res.* 14, 784–789. doi: 10.2174/1567205014666170203095128

Lin, F., Heffner, K. L., Ren, P., Tivarus, M. E., Brasch, J., Chen, D. G., et al. (2016). Cognitive and neural effects of vision-based speed-of-processing training in older adults with amnestic mild cognitive impairment: a pilot study. *J. Am. Geriatr. Soc.* 64, 1293–1298. doi: 10.1111/jgs.14132

McKhann, G., Drachman, D. A., Folstein, M., Katzman, R., Price, D. L., and Stadlan, E. M. (1984). Clinical diagnosis of Alzheimer's disease—report of the NINCDS-ADRDA work group under the auspices of department of health and human services task force on Alzheimer's disease. *Neurology* 34, 939–944. doi: 10.1212/wnl.34.7.939

Morrison, A. B., and Chein, J. M. (2010). Does working memory training work? The promise and challenges of enhancing cognition by training working memory. *Psychon. Bull. Rev.* 18, 46–60. doi: 10.3758/s13423-010-0034-0

Myerson, J., Emery, L., White, D. A., and Hale, S. (2003). Effects of age, domain and processing demands on memory span: evidence for differential decline. *Aging Neuropsychol. Cogn.* 10, 20–27. doi: 10.1076/anec.10.1.20.13454

Nasreddine, Z. S., Phillips, N. A., Bédirian, V., Charbonneau, S., Whitehead, V., Collin, I., et al. (2005). The montreal cognitive assessment, MoCA: a brief screening tool for mild cognitive impairment. *J. Am. Geriatr. Soc.* 53, 695–699. doi: 10.1111/j.1532-5415.2005.53221.x

Nittrouer, S., and Lowenstein, J. H. (2015). Working memory problems of the elderly arise in the central processor, not the phonological loop. *J. Acoust. Soc. Am.* 138, 1924–1925. doi: 10.1121/1.4934057

Preece, J., and Maloney-Krichmar, D. (2003). "Online communities: focusing on sociability and usability," in *The Human-Computer Interaction Handbook*, eds J. Jacko and A. Sears (Mahwah, NJ: Lawrence Erlbaum Associates), 596–620.

Prince, M., Wimo, A., Guerchet, M., Ali, G., Wu, Y., and Prina, M. (2015). *World Alzheimer Report 2015—The Global Impact of Dementia: An Analysis of Prevalence, Incidence, Cost and Trends.* London: Alzheimer's Disease International.

Razali, R., Jean-Li, L., Jaffar, A., Ahmad, M., Shah, S. A., Ibrahim, N., et al. (2014). Is the bahasa malaysia version of the montreal cognitive assessment (moca-bm) a better instrument than the malay version of the mini mental state examination (m-mmse) in screening for mild cognitive impairment (mci) in the elderly? *Compr. Psychiatry* 55, S70–S75. doi: 10.1016/j.comppsych.2013.04.010

Ross, L. A., Sprague, B. N., Phillips, C. B., O'Connor, M. L., and Dodson, J. E. (2018). The impact of three cognitive training interventions on older adults' physical functioning across 5 years. *J. Aging Health* 30, 475–498. doi: 10.1177/0898264316682916

Savitch, N., and Zaphiris, P. (2006). "Accessible websites for people with dementia: a preliminary investigation into information architecture," in *Computers Helping People with Special Needs. ICCHP 2006. Lecture Notes in Computer Science*, eds K. Miesenberger, J. Klaus, W. L. Zagler and A. I. Karshmer (Berlin, Heidelberg: Springer), 144–151.

Schulz, M., and Jobe, L. A. (2001). Codification and tacitness as knowledge management strategies: an empirical exploration. *J. High Technol. Manag. Res.* 12, 139–165. doi: 10.1016/s1047-8310(00)00043-2

Seidler, R. D., Bernard, J. A., Burutolu, T. B., Fling, B. W., Gordon, M. T., Gwin, J. T., et al. (2010). Motor control and aging: links to age-related brain structural, functional and biochemical effects. *Neurosci. Biobehav. Rev.* 34, 721–733. doi: 10.1016/j.neubiorev.2009.10.005

Shah, T. M., Weinborn, M., Verdile, G., Sohrabi, H. R., and Martins, R. N. (2017). Enhancing cognitive functioning in healthly older adults: a systematic review of the clinical significance of commercially available computerized cognitive training in preventing cognitive decline. *Neuropsychol. Rev.* 27, 62–80. doi: 10.1007/s11065-016-9338-9

Sherman, D. S., Mauser, J., Nuno, M., and Sherzai, D. (2017). The efficacy of cognitive intervention in mild cognitive impairment (mci): a meta-analysis of outcomes on neuropsychological measures. *Neuropsychol. Rev.* 27, 440–484. doi: 10.1007/s11065-017-9363-3

Smith-Ray, R. L., Hughes, S. L., Prohaska, T. R., Little, D. M., Jurivich, D. A., and Hedeker, D. (2015). Impact of cognitive training on balance and

gait in older adults. *J. Gerontol. B Psychol. Sci. Soc. Sci.* 70, 357–366. doi: 10.1093/geronb/gbt097

Smith-Ray, R. L., Makowski-Woidan, B., and Hughes, S. L. (2014). A randomized trial to measure the impact of a community-based cognitive training intervention on balance and gait in cognitively intact black older adults. *Health Educ. Behav.* 41, 62S–69S. doi: 10.1177/1090198114537068

STAR Collaboration, Adams, J., Aggarwal, M. M., Ahammed, Z., Amonett, J., Anderson, B. D., et al. (2005). Experimental and theoretical challenges in the search for the quark-gluon plasma: the STAR Collaboration's critical assessment of the evidence from RHIC collisions. *Nucl. Phys. A* 757, 102–183. doi: 10.1016/j.nuclphysa.2005.03.085

Thorpe, S., Fize, D., and Marlot, C. (1996). Speed of processing in the human visual system. *Nature* 381, 520–522. doi: 10.1038/381520a0

Verghese, J., Mahoney, J., Ambrose, A. F., Wang, C., and Holtzer, R. (2010). Effect of cognitive remediation on gait in sedentary seniors. *J. Gerontol. A Biol. Sci. Med. Sci.* 65, 1338–1343. doi: 10.1093/gerona/glq127

Vos, S. J., Verhey, F., Frölich, L., Kornhuber, J., Wiltfang, J., Maier, W., et al. (2015). Prevalence and prognosis of Alzheimer's disease at the mild cognitive impairment stage. *Brain* 138, 1327–1338. doi: 10.1093/brain/awv029

Wang, J., Zou, Y. Z., Cui, J. F., Fan, H. Z., Chen, N., Yao, J., et al. (2013). Reliability and construct validity of the Chinese version of the WECHSLER adult intelligence scale-fourth edition. *China Ment. Health J.* 27, 692–697. doi: 10.3969/j.issn.1000-6729.2013.09.011

Weng, Y. H., and Huang, J. H. (2014). Evaluation of the reliability and validity of Alzheimer's disease rating scale—cognitive part and ability of daily life scale (Chinese version). *Chin. J. Gerontol.* 34. doi: 10.3969/j.issn.1005-9202.2014.07.009

Willis, S. L., and Schaie, K. W. (2009). Cognitive training and plasticity: theoretical perspective and methodological consequences. *Restor. Neurol. Neurosci.* 27, 375–389. doi: 10.3233/RNN-2009-0527

Yogev, G., Giladi, N., Peretz, C., Springer, S., Simon, E. S., and Hausdorff, J. M. (2005). Dual tasking, gait rhythmicity, and Parkinson's disease: which aspects of gait are attention demanding? *Eur. J. Neurosci.* 22, 1248–1256. doi: 10.1111/j.1460-9568.2005.04298.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

MINI REVIEW
published: 13 September 2019
doi: 10.3389/fnagi.2019.00240

Can Acupuncture Treatment of Hypertension Improve Brain Health? A Mini Review

*Jongjoo Sun, John Ashley and J. Mikhail Kellawan**

Human Circulation Research Laboratory, Department of Health and Exercise Science, University of Oklahoma, Norman, OK, United States

With age, cerebrovascular and neurodegenerative diseases (e.g., dementia and Alzheimer's) are some of the leading causes of death in the United States. Related to these outcomes is the increased prevalence of hypertension, which independently increases the development of cerebrovascular and neurodegenerative diseases. While a direct mechanistic link between hypertension and poor brain health is unknown, many hypothesize that the etiology stems from poor blood pressure (BP) and cerebrovascular regulation. This dysfunction fosters hypoperfusion of the brain, causing stress to the tissue through a nutrient mismatch, subtly damaging the brain over many years. Current Western medical treatment relies on pharmacological treatment (mainly beta-blockers, angiotensin-converting enzyme inhibitors, or a combination of the two). However, Western treatments have not been successful in mitigating brain health outcomes and are burdened with unwanted side effects and non-adherence issues. Alternatively, traditional East Asia medicine has used acupuncture as a treatment for hypertension and may offer a promising approach in response to the limitations of conventional therapy. While detailed clinical and mechanistic experimental evidence is lacking, acupuncture has been observed to reduce BP and improve endothelial function in hypertensive adults. Further, acupuncture has been shown to have specific cerebrovascular effects, increasing cerebrovascular reactivity in healthy adults, highlighting possible neuroprotective properties. Therefore, our review is aimed at evaluating acupuncture as a treatment for hypertension and the potential impact on brain health. We will interrogate the current literature as well as discuss the proposed neural and vascular mechanisms by which acupuncture acts.

Keywords: acupuncture, hypertension, cerebral blood flow, cerebrovascular disease, neurodegenerative disease, blood pressure

OPEN ACCESS

Edited by:
Mario Bernardo-Filho, Rio de Janeiro State University, Brazil

Reviewed by:
Bernardo Diniz Coutinho, Universidade Federal do Ceará, Brazil
Chao-Qun Yan, Dongzhimen Hospital, Beijing University of Chinese Medicine, China

****Correspondence:***
J. Mikhail Kellawan
kellawan@ou.edu

Received: *27 June 2019*
Accepted: *19 August 2019*
Published: *13 September 2019*

Citation:
Sun J, Ashley J and Kellawan JM (2019) Can Acupuncture Treatment of Hypertension Improve Brain Health? A Mini Review. Front. Aging Neurosci. 11:240. doi: 10.3389/fnagi.2019.00240

INTRODUCTION

Cerebrovascular diseases and neurodegenerative diseases are both leading causes of death in the United States (Xu et al., 2018). Subsequently, with an aging population, the prevalence of cerebrovascular disease and neurodegenerative disease is projected to dramatically increase, raising concerns regarding quality of life and cost of care (i.e., medical expenses, elderly care, nursing fees, etc.) for the American population (Khavjou et al., 2016; Benjamin et al., 2019).

Hypertension independently increases the development of cerebrovascular disease and neurodegenerative disease (Baumgart et al., 2015; Benjamin et al., 2019). Although a direct mechanistic link between hypertension and poor brain health is unknown, having hypertension predicts lower cognitive function (Kilander et al., 1998) and increased risk for dementia with age (Sharp et al., 2011). Many hypothesize that the etiology stems from poor blood pressure (BP) and cerebrovascular regulation. This dysfunction fosters hypoperfusion, stressing the brain through a nutrient mismatch, subtly causing damage over many years (de la Torre, 2012). Animal models indicate that increased systolic BP (SBP) and cerebrovasculature remodeling in hypertension ultimately reduce brain blood flow, leading to behavioral and cognitive impairments (Pires et al., 2013; Wiesmann et al., 2017). In hypertensive adults, a diminished cerebral perfusion may accelerate the development of Alzheimer's *via* decreased oxygen delivery in ischemia-sensitive brain regions like the hippocampus, inducing neurodegeneration and subsequent cognitive decline (de la Torre, 2000).

Treatment of hypertension has shown benefits in neurodegenerative disease development and overall brain health. Most observational studies have suggested that improved SBP control reduces the risk of Alzheimer's and other dementias (Qiu et al., 2005; Hughes and Sink, 2016). Additionally, treating hypertension is among the most effective strategies to prevent a stroke (Meschia et al., 2014). Each year, approximately 795,000 US adults suffer a new or recurrent stroke (Benjamin et al., 2019). Therefore, effective treatment of hypertension has tremendous far-reaching impacts on brain health.

However, pharmacological treatment of hypertension faces challenges such as unwanted side effects, limited adherence, and difficulty individualizing treatment for such a diverse population. Up to 97% of patients taking antihypertensive medications experience adverse side effects (Toyoshima et al., 1997; Bardage and Isacson, 2000), which can reduce future adherence (Tedla and Bautista, 2016). Moreover, 25% of patients do not fill their initial antihypertensive prescription (Holland et al., 2008; Franklin et al., 2012; Berra et al., 2016). As a result, four out of five patients do not sufficiently adhere to antihypertensive treatment, failing to control their BP (Petrilla et al., 2005; Gwadry-Sridhar et al., 2013). Additionally, only two out of the six classes of antihypertensive drugs are independently associated with decreased risk of dementia (van Middelaar et al., 2017). These circumstances result in patients maintaining an elevated dementia and stroke risk. Therefore, there is a dire need to identify adequate antihypertensive treatments to improve cerebral blood flow (CBF) that circumvents the limitations presented.

Acupuncture is regarded as a promising complementary and integrative antihypertensive approach that does not share many of the limitations of medical interventions. It is a practice of traditional East Asia medicine in which specific points on the body are stimulated, most often by inserting disposable thin stainless-steel needles through the skin. Acupuncture has documented benefits easing various types of pain (e.g., low back, neck, osteoarthritis, headache, etc.) and conditions including cardiovascular diseases (World Health Organization, 2002; McDonald and Janz, 2017). Western cultures have become more welcoming of acupuncture; a 2017 clinical practice guideline from the American College of Physicians included acupuncture among the nondrug treatment options for management of both acute and chronic back low-back pain (Qaseem et al., 2017). However, acupuncture's effects on the cardiovascular system are still under-researched, preventing its utilization as a therapeutic option in the Western world.

While detailed clinical and mechanistic experimental evidence is lacking, acupuncture has been observed to reduce BP (Li et al., 2015; Liu et al., 2015) and improve endothelial function in hypertensive adults (Park et al., 2010). Further, acupuncture has been shown to have specific cerebrovascular effects in healthy adults, highlighting possible neuroprotective properties (Byeon et al., 2011; Hyun et al., 2014; Im et al., 2014). However, it is yet to be determined if the literature is in support of using antihypertensive acupuncture prescriptions to improve brain health *via* enhanced cerebrovascular control. Therefore, our review is aimed at evaluating the acupuncture literature; as a treatment for hypertension, its effects on cerebrovascular control, the evidence for specific acupuncture hypertension treatment improving brain health outcomes, and the mechanisms by which acupuncture can affect BP and cerebrovascular control.

EVIDENCE SUPPORTING ACUPUNCTURE AS A TREATMENT OF HYPERTENSION

Recent randomized control trials (RCTs) indicate that acupuncture is effective at lowering BP in humans. One of the largest RCTs involving more than 400 mostly non-hypertensive adults found reductions in SBP (122–113 mmHg) and DBP (68–65 mmHg) following 6 weeks of biweekly acupuncture compared to no change in sham and auricular acupuncture (Abdi et al., 2017). However, it should be noted that the auricular acupuncture and the two sham groups had lower initial SBP (110, 116, and 111 mmHg, respectively). This leads to speculation that acupuncture only impacts individuals with higher BP, specifically higher SBP. Therefore, the hypotensive effect of acupuncture may be best represented in hypertensive individuals.

Four weeks of acupuncture treatment (20-minutes/treatment, 2 treatments/week) reduced SBP and DBP ∼ 7mmHg in hypertensive adults. After 8 weeks SBP and DBP remained lower (∼6.5 and ∼4.9mmHg, respectively) than initial values. However, 4 weeks after treatment was ceased, SBP and DBP remained ∼5 mmHg lower, with only DBP being significantly different. The time-matched control did not have a change in BP at any time point (Liu et al., 2015). Similarly, Li et al. (2015) compared 8 weeks of acupuncture in hypertensive adults at acupoints shown to have a BP-lowering effect vs. control acupoints (sites that do not alter BP). Acupuncture

reduced SBP by 6 mmHg and DBP by 4 mmHg. Subsets of the subjects participated in crossover assessment with the active treatment lowering SBP ∼7 mmHg and DBP ∼4 mmHg compared to control. A further subset of subjects stopped all treatments and were assessed at 1 and 2 months post-treatment. At 1 month following cessation of treatment, SBP remained lower and DBP reverted to pre-treatment values. Two months after treatment, BP was not different from pre-treatment values. From these studies, it is clear that acupuncture can have an effect on hypertension, and this effect can be sustained for up to 1 month, with diminishing effects thereafter.

The promising results presented above highlight the growing research interest in acupuncture and are only a fraction of the large volume of research that is currently being generated. This volume has further spurred many recent systematic reviews on acupuncture and hypertension. Interestingly, few determine that acupuncture alone can manage BP in hypertensive adults (Wang et al., 2013). Rather, the consensus is that acupuncture, when combined with pharmacological therapy, lowers BP than either treatment alone (Li et al., 2014; Zhao et al., 2015, 2019; Chen et al., 2018; Yang et al., 2018; Niu et al., 2019). Therefore, acupuncture may best be used as an adjunctive treatment for hypertension.

Although the recent results seem promising, it is not unanimous. The above listed reviews were meticulous in limiting bias and incorporating the most robust research, yet there are still methodological concerns with antihypertensive acupuncture RCTs. There is enormous heterogeneity of study design among the RCTs, with no consensus for how to properly provide control/placebo for acupuncture. Control types used in the RCTs include a time-matched group, a sham acupuncture group, and an inactive acupoint acupuncture group. The use of sham acupuncture has been argued as the best means for control (Yang et al., 2018), mainly because the use of "inactive" acupoints raises concerns of possible spillover effects. Meaning, stimulation of an acupoint believed to have no effect on cardiovascular parameters could have a minor effect, limiting statistical determination of active acupoint stimulation. Additionally, many RCTs contain small sample sizes and do not always utilize a crossover design. For instance, in Li et al. (2015), *post hoc* power analysis determined that at least five more participants per group would be needed to achieve the appropriate power. These limitations cause skepticism about the effectiveness, efficacy, and safety of acupuncture treatment in hypertension.

Overall, while it seems that acupuncture's effect on lowering BP is minimal, as an adjunctive treatment to conventional therapies, acupuncture is a promising avenue in the quest to control hypertension and limit its damaging effects throughout the body. Although meaningful issues are present in most of the RCTs examined, acupuncture appears to be effective in lowering BP in hypertensive adults. However, the lack of mechanistic integrative human research elucidating a link between acupuncture and BP regulation and experimental design concerns justify the need to critically evaluate acupuncture as a reliable treatment option.

THE EFFECT OF ACUPUNCTURE TREATMENT ON CEREBRAL HEMODYNAMICS

Attempts to determine acupuncture's effect on brain health outcomes have steadily increased over the years. However, true assessment of these outcomes requires longitudinal studies that have yet to be undertaken. Assessing brain hemodynamics and outcomes is more immediate and has been researched using various imaging modalities [e.g., transcranial Doppler (TCD), near-infrared spectroscopy (NIRS), functional magnetic resonance imaging (fMRI), etc.] that have various strengths and weakness that can complicate interpretation. However, each provides valuable information of the brain environment.

Lower CBF, measured using TCD, is associated with cognitive decline and neurodegenerative disease (Ruitenberg et al., 2005). Therefore, it is theorized that treatments that improve or restore CBF may attenuate or possibly prevent the onset of these conditions. Interestingly, TCD studies present evidence that acupuncture can improve CBF and that there is acupoint-cerebral vessel specificity. Acupuncture of GB20 point has been shown to improve CBF regulation in posterior (Vertebral and Basilar) arteries but not the middle cerebral arteries (MCAs; Yuan et al., 1998; Im et al., 2014). Similarly, in healthy adults, a single 20-min acupuncture treatment at acupoint ST36 improved flow and CO_2 reactivity in the basilar artery but only reactivity in the MCA (Hyun et al., 2014), whereas 20-min acupuncture of GV20 increased CBF and CO_2 reactivity in both middle and anterior cerebral arteries (basilar not measured; Byeon et al., 2011).

As previously stated, the effects of acupuncture are best exemplified using diseased individuals. Applying acupuncture to stroke patients at acupoints LV3, LV4, SJ5, and GB34 significantly increased CBF (in MCAs). This is accompanied by a decreased SBP; however, the sham acupuncture group also saw a decreased DBP (Ratmansky et al., 2016). Using NIRS, acupuncture intervention on stroke patients showed a significant increase in regional cerebral blood volume or oxyhemoglobin parameters (Li et al., 2011). A systemic review of NIRS studies indicates that acupuncture varies wildly in healthy adults but seems to be more appropriate for disordered populations such as stroke patients (Lo et al., 2015).

Experiments using fMRI have been completed to determine acupuncture's effect on specific brain regions and the brain network. Acupuncture (Ll11 and ST36) in both healthy and stroke patients improved brain activity in various areas of the brain (Cho et al., 2013). Further, increased activity and connectivity across hemispheres and various portions of the brain following acupuncture has also been observed in patients with mild cognitive impairment (Feng et al., 2012), Alzheimer's disease (Wang et al., 2014), and stroke (Chen et al., 2014).

Single-photon emission computerized tomography (SPECT) and position emission tomography–computed tomography (PET-CT) allow for a depiction of regional brain perfusion. In healthy subjects, acupuncture (Ll4 and Ll11) increased both regional CBF and glucose metabolism in both frontal regions (An et al., 2009) and specifically at important regions regarding the

limbic system, middle cingulum, and medial orbitofrontal gyrus (Jung et al., 2011).

The literature advocates for acupuncture having positive effects on brain hemodynamics in an acupoint-specific manner. However, many of these studies have: (a) been conducted in healthy populations and (b) suffered from similar methodological concerns raised in our discussion of acupuncture on BP regulation. What remains unclear is a mechanistic link between acupoints and brain-specific responses as well as if there is a connection between acupoints used in the treatment of hypertension and cerebral responses. Garnering further understanding of these acupoints and if acupuncture treatment of hypertension actually improves cerebrovascular responses could have a significant biomedical impact as it would provide further information on the mechanistic links between hypertension and poor brain health, as well as offer a greater number of treatment options.

EFFECTS OF ACUPUNCTURE TECHNIQUES AIMED AT TREATING HYPERTENSION ON BRAIN HEALTH OUTCOMES

As alluded to earlier, hypertension is a strong risk factor in the development of cerebrovascular diseases and neurodegenerative diseases. As such, few studies have focused on acupuncture's effect on stroke *via* regulating hypertension. Work in hypertensive animals support hypertensive-acupuncture treatment that prevents stroke through several pathways related to the nervous system, oxidative stress, the endocrine system, cardiovascular function, and hemorheology (Zheng et al., 2018). However, the human data are unclear. Meta-analyses have challenged the efficacy of acupuncture for the treatment of hypertension as a risk factor for stroke (Sibbritt et al., 2018). However, controlling BP *via* acupuncture as an additive treatment is an effective secondary prevention of stroke (Du et al., 2017). In stroke patients complicated with hypertension, antihypertensive acupuncture improved the National Institutes of Health stroke scale and Barthel index, both of which are indicative of stroke-related neurologic deficit. Further, the treatment reduced morning BP (esp. DBP) and improved SBP and DBP load (Guo and Shi, 2019), therefore suggesting antihypertensive acupuncture as a post-stroke treatment option rather than just a preventative measure.

Studies investigating the possible underlying molecular mechanisms of acupuncture in the treatment of neurodegenerative diseases, specifically dementia, have found that acupuncture can alter neurotrophin regulation (Hwang et al., 2010; Lee et al., 2012; Lin et al., 2016), reduce oxidative damage (Liu et al., 2006), and modulate apoptotic signaling (Chen et al., 2012; Xue et al., 2014) in a manner that promotes positive health outcomes. However, there are few studies that have investigated the effect of acupuncture on dementia directly related to hypertension despite the strong human epidemiological evidence linking the two. Such a study in spontaneous hypertensive rats found that acupuncture at DU20 (also referred to as GV20) and ST36 acupoints reduced BP, increased microvessel dilation, increased CBF, attenuated neuron injury, and restored cognitive impairment (Tian et al., 2013). In hypertensive humans, acupuncture at LR3 activated anterior cingulated gyrus (measured with fMRI) to lower BP through modulation of parasympathetic nervous activity. Additionally, through anterior cingulated gyrus activation, the connection with the surrounding areas was strengthened to improve cognitive impairment caused by long-term hypertension (Sun et al., 2014).

Currently, the evidence of acupuncture's favorable effect on brain health outcomes directly *via* regulating hypertension is still scant. However, the data are promising and justify more experimental and clinical studies into acupuncture's reliability as a possible treatment/prevention strategy for brain health outcomes related to hypertension.

POTENTIAL MECHANISM(S) OF ACUPUNCTURE IMPROVING HYPERTENSION AND VASCULAR CONTROL

There are many different proposed mechanisms linking acupuncture to positive BP and vascular outcomes; however, most fail at drawing a logical link from acupuncture stimulus to observed outcome. Acupuncture involves sticking needles just below the skin surface, twisting the needle, and expecting a change in a corresponding system such as the cardiovascular system (**Figure 1**). Accumulating evidence indicates that the hypotensive effect is mediated by a reduction in sympathetic outflow (Sato et al., 1993; Michikami et al., 2006) and an increase in endorphin release (Guo and Longhurst, 2007). Increases in various endorphins has independently been linked to reduced BP in hypertension (Bądzyńska et al., 2016; Li et al., 2016). This seems to be a paradox, as previous research shows venipuncture increases catecholamine release, synonymous with a hypertensive effect (Frankenhaeuser et al., 1976). The difference must be attributed to the depth of the needle (venous vs. just below the skin surface) and the twisting of the needle, which promotes "de qi," a key component of acupuncture. Langevin et al. (2001) describe that, during de qi, the connective tissue is wrapped around the needle, promoting tension. The authors speculate that this tension could be activating various sensory organs within the skin, increasing afferent nerve firing. Zhou et al. (2005a) specifically looked at responses to acupoints that reduced BP and observed increased afferent sensory neuronal firing. When afferent nerves are severed, the hypotensive effect and sympathetic nerve inhibition are eliminated (Sato et al., 1993). In healthy humans, the data are not definitive. Stimulation of acupoints PC6 and HT7 have been argued to elicit hypotensive effects through alterations in sympathetic (Jung et al., 2006) and parasympathetic nerve activity (Jung et al., 2007). Conversely, in patients with post-stroke insomnia, intradermal acupuncture at PC6 and HT7 greatly decreased the number of non-dippers possibly by lowering LF/HF ratio (Lee et al., 2009). Taken

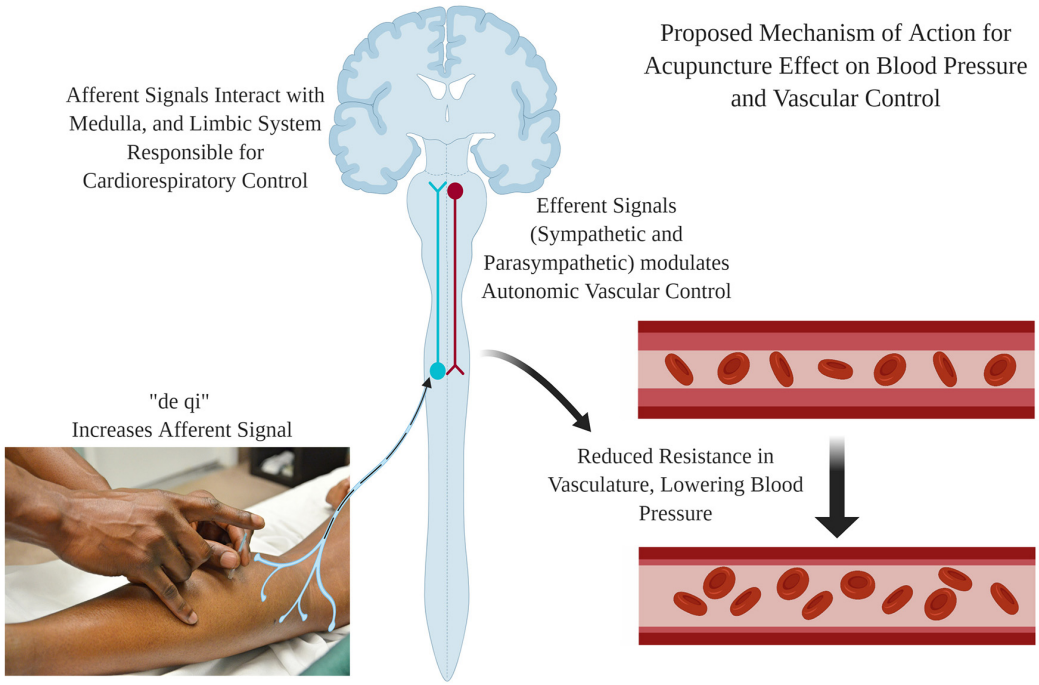


FIGURE 1 | Proposed mechanism of action for the hypotensive effect of acupuncture treatment. Needle insertions and stimulation causes "de qi," which increases the afferent neuron firing. The afferent signal increases activity in areas of the brain responsible for cardiorespiratory control (Medulla and Limbic System). This activity results in the modulation of autonomic vascular control, reducing vascular resistance and blood pressure (BP). Created using BioRender.

together, acupuncture works through a feedback loop where afferent nerves initiate a correction in autonomic nerve outflow, properly regulating BP. Insight into the mechanisms by which acupuncture is reducing sympathetic outflow come from elegant animal studies investigating the activity in the rostral ventrolateral medulla (RVLM) where sympathetic nerve activity is controlled. In normotensive rats, acupuncture (PC6) may alter the baroreflex. PC6 stimulation increased afferent neuron firing, which was associated with a decrease in RVLM activity, resulting in a reduction of sympathetic outflow and a lowering of BP (Zhou et al., 2005b). Similarly, Wang et al. (2018) performed a highly controlled study proving that acupuncture (LR3) reduces oxidative stress in the RVLM and that the observed antihypertensive effect was directly tied to NADPH oxidase activity and the REDOX environment of the RVLM in spontaneously hypertensive rats. Again, linking reduced BP and sympathetic outflow *via* RVLM. Alternatively, in humans, acupuncture increases firing in the gracile nucleus, frontal lobe, cerebellum insula, hypothalamus, and many other areas of the brain related to maintenance of BP (Chen and Ma, 2003; Chen et al., 2013; Zheng et al., 2016).

While the cited research has provided a general mechanistic outline for acupuncture to modulate BP, these data are far from conclusive. However, the promising results provide the foundation for future research to delineate physiological mechanistic responses to acupuncture. Until these mechanisms are well understood and verifiable, mass adoption of acupuncture as an additive treatment for hypertension cannot be recommended.

LIMITATIONS

This review is not without some limitations. First, this review prioritized human RCTs. Thus, the volume of studies reviewed and the sample size within each reviewed article are limited. However, we contest that RCTs provide the best evidence of the effectiveness of acupuncture (Sibbald and Roland, 1998). Further, this choice limits review of articles using animal models especially outside our "mechanisms" section. Thus, some well-designed studies were omitted. However, these restrictions allow for the most rigorous and translational review regarding acupuncture's effect on BP and cerebrovascular control. Finally, mostly English language articles were cited. Acupuncture literature is written in many other languages and we have included several non-English articles (Li et al., 2011; Sun et al., 2014; Zheng et al., 2018; Guo and Shi, 2019). However, there may be a few relevant articles that our study team was unable to access.

DISCUSSION

Evaluation of the literature suggests that acupuncture, at best, can be used as a co-treatment option for hypertension. Further, acupuncture has been found to improve cerebrovascular control and brain activation in regions consistent with positive health outcomes *via* the maintenance of BP and cognition. The

mechanisms that link acupuncture to positive health outcomes are still poorly understood, but they appear to be linked to improvements in autonomic cardiovascular control. Regardless, the evidence is in support of safe and efficacious use of acupuncture in human hypertension. Yet, there is a profound need for tightly controlled mechanistic human research to determine the validity and the physiological underpinnings of acupuncture before wider adoption of acupuncture as a treatment option for improved cardiovascular and brain health can be recommended.

AUTHOR CONTRIBUTIONS

JS, JA, and JK contributed equally to the writing and editing of the manuscript.

FUNDING

Open access fees were provided from the University of Oklahoma Libraries Open Access Fund.

REFERENCES

Abdi, H., Tayefi, M., Moallem, S. R., Zhao, B., Fayaz, M., Ardabili, H. M., et al. (2017). Abdominal and auricular acupuncture reduces blood pressure in hypertensive patients. *Complement. Ther. Med.* 31, 20–26. doi: 10.1016/j.ctim.2017.01.003

An, Y.-S., Moon, S.-K., Min, I.-K., and Kim, D.-Y. (2009). Changes in regional cerebral blood flow and glucose metabolism following electroacupuncture at LI 4 and LI 11 in normal volunteers. *J. Altern. Complement. Med.* 15, 1075–1081. doi: 10.1089/acm.2009.0257

Bądzyńska, B., Lipkowski, A. W., Olszyński, K. H., and Sadowski, J. (2016). Different blood pressure responses to opioids in 3 rat hypertension models: role of the baseline status of sympathetic and renin-angiotensin systems. *Can. J. Physiol. Pharmacol.* 94, 1159–1169. doi: 10.1139/cjpp-2016-0111

Bardage, C., and Isacson, D. G. (2000). Self-reported side-effects of antihypertensive drugs: an epidemiological study on prevalence and impact on health-state utility. *Blood Press.* 9, 328–334. doi: 10.1080/080370500300000905

Baumgart, M., Snyder, H. M., Carrillo, M. C., Fazio, S., Kim, H., and Johns, H. (2015). Summary of the evidence on modifiable risk factors for cognitive decline and dementia: a population-based perspective. *Alzheimers Dement.* 11, 718–726. doi: 10.1016/j.jalz.2015.05.016

Benjamin, E. J., Muntner, P., Alonso, A., Bittencourt, M. S., Callaway, C. W., Carson, A. P., et al. (2019). Heart disease and stroke statistics—2019 update: a report from the American Heart Association. *Circulation* 139, e56–e528. doi: 10.1161/CIR.0000000000000659

Berra, E., Azizi, M., Capron, A., Høieggen, A., Rabbia, F., Kjeldsen, S. E., et al. (2016). Evaluation of adherence should become an integral part of assessment of patients with apparently treatment-resistant hypertension. *Hypertension* 68, 297–306. doi: 10.1161/hypertensionaha.116.07464

Byeon, H., Moon, S., Park, S., Jung, W., Park, J., Ko, C., et al. (2011). Effects of GV20 acupuncture on cerebral blood flow velocity of middle cerebral artery and anterior cerebral artery territories and CO_2 reactivity during hypocapnia in normal subjects. *J. Altern. Complement. Med.* 17, 219–224. doi: 10.1089/acm.2010.0232

Chen, H., Dai, J., Zhang, X., Wang, K., Huang, S., Cao, Q., et al. (2013). Hypothalamus-related resting brain network underlying short-term acupuncture treatment in primary hypertension. *Evid. Based Complement. Altern. Med.* 2013:808971. doi: 10.1155/2013/808971

Chen, A., Lin, Z., Lan, L., Xie, G., Huang, J., Lin, J., et al. (2012). Electroacupuncture at the Quchi and Zusanli acupoints exerts neuroprotective role in cerebral ischemia-reperfusion injured rats *via* activation of the PI3K/Akt pathway. *Int. J. Mol. Med.* 30, 791–796. doi: 10.3892/ijmm.2012.1074

Chen, S., and Ma, S.-X. (2003). Nitric oxide in the gracile nucleus mediates depressor response to acupuncture (ST36). *J. Neurophysiol.* 90, 780–785. doi: 10.1152/jn.00170.2003

Chen, H., Shen, F., Tan, X., Jiang, W., and Gu, Y. (2018). Efficacy and safety of acupuncture for essential hypertension: a meta-analysis. *Med. Sci. Monit.* 24, 2946–2969. doi: 10.12659/msm.909995

Chen, J., Wang, J., Huang, Y., Lai, X., Tang, C., Yang, J., et al. (2014). Modulatory effect of acupuncture at Waiguan (TE5) on the functional connectivity of the central nervous system of patients with ischemic stroke in the left basal ganglia. *PLoS One* 9:e96777. doi: 10.1371/journal.pone.0096777

Cho, S.-Y., Kim, M., Sun, J. J., Jahng, G.-H., Kim, H. J., Park, S.-U., et al. (2013). A comparison of brain activity between healthy subjects and stroke patients on fMRI by acupuncture stimulation. *Chin. J. Integr. Med.* 19, 269–276. doi: 10.1007/s11655-013-1436-4

de la Torre, J. C. (2000). Critically attained threshold of cerebral hypoperfusion: the CATCH hypothesis of Alzheimer's pathogenesis. *Neurobiol. Aging* 21, 331–342. doi: 10.1016/s0197-4580(00)00111-1

de la Torre, J. C. (2012). Cardiovascular risk factors promote brain hypoperfusion leading to cognitive decline and dementia. *Cardiovasc. Psychiatry Neurol.* 2012:367516. doi: 10.1155/2012/367516

Du, Y., Gao, X., Wang, C.-T., Zheng, H., Lei, Y., Wu, M., et al. (2017). Acupuncture lowering blood pressure for secondary prevention of stroke: a study protocol for a multicenter randomized controlled trial. *Trials* 18:428. doi: 10.1186/s13063-017-2171-5

Feng, Y., Bai, L., Ren, Y., Chen, S., Wang, H., Zhang, W., et al. (2012). FMRI connectivity analysis of acupuncture effects on the whole brain network in mild cognitive impairment patients. *Magn. Reson. Imaging* 30, 672–682. doi: 10.1016/j.mri.2012.01.003

Frankenhaeuser, M., Dunne, E., and Lundberg, U. (1976). Sex differences in sympathetic-adrenal medullary reactions induced by different stressors. *Psychopharmacology* 47, 1–5. doi: 10.1007/bf00428693

Franklin, S. S., Thijs, L., Hansen, T. W., Li, Y., Boggia, J., Kikuya, M., et al. (2012). Significance of white-coat hypertension in older persons with isolated systolic hypertension: a meta-analysis using the international database on ambulatory blood pressure monitoring in relation to cardiovascular outcomes population. *Hypertension* 59, 564–571. doi: 10.1161/hypertensionaha.111.180653

Guo, Z.-L., and Longhurst, J. C. (2007). Expression of c-Fos in arcuate nucleus induced by electroacupuncture: relations to neurons containing opioids and glutamate. *Brain Res.* 1166, 65–76. doi: 10.1016/j.brainres.2007.06.042

Guo, Y.-P., and Shi, X.-M. (2019). [Effect of " *Huoxue Sanfeng* acupuncture" auxiliary therapy on morning blood pressure and blood pressure load in elderly patients with stroke complicated with hypertension]. *Zhongguo Zhen Jiu* 39, 349–354. doi: 10.13703/j.0255-2930.2019.04.002

Gwadry-Sridhar, F. H., Manias, E., Lal, L., Salas, M., Hughes, D. A., Ratzki-Leewing, A., et al. (2013). Impact of interventions on medication adherence and blood pressure control in patients with essential hypertension: a systematic review by the ISPOR medication adherence and persistence special interest group. *Value Health* 16, 863–871. doi: 10.1016/j.jval.2013.03.1631

Holland, N., Segraves, D., Nnadi, V. O., Belletti, D. A., Wogen, J., and Arcona, S. (2008). Identifying barriers to hypertension care: implications for quality improvement initiatives. *Dis. Manag.* 11, 71–77. doi: 10.1089/dis.2008.1120007

Hughes, T. M., and Sink, K. M. (2016). Hypertension and its role in cognitive function: current evidence and challenges for the future. *Am. J. Hypertens.* 29, 149–157. doi: 10.1093/ajh/hpv180

Hwang, I. K., Chung, J. Y., Yoo, D. Y., Yi, S. S., Youn, H. Y., Seong, J. K., et al. (2010). Effects of electroacupuncture at Zusanli and Baihui on brain-derived neurotrophic factor and cyclic AMP response element-binding protein in the hippocampal dentate gyrus. *J. Vet. Med. Sci.* 72, 1431–1436. doi: 10.1292/jvms.09-0527

Hyun, S.-H., Im, J.-W., Jung, W.-S., Cho, K.-H., Kim, Y.-S., Ko, C.-N., et al. (2014). Effect of ST36 Acupuncture on hyperventilation-induced CO_2 reactivity of the basilar and middle cerebral arteries and heart rate variability in normal subjects. *Evid. Based Complement. Altern. Med.* 2014:574986. doi: 10.1155/2014/574986

Im, J.-W., Moon, S.-K., Jung, W.-S., Cho, K.-H., Kim, Y.-S., Park, T.-H., et al. (2014). Effects of acupuncture at GB20 on CO_2 reactivity in the basilar and middle cerebral arteries during hypocapnia in healthy participants. *J. Altern. Complement. Med.* 20, 764–770. doi: 10.1089/acm.2013.0240

Jung, W.-S., Hong, J.-W., Lee, J.-W., Park, S.-U., Moon, S.-K., Ko, C.-N., et al. (2006). The clinical effect of manipulation of acupuncture to shen-men and nei-kuan on blood pressure of normal male subjects. *Korean J. Orient. Med.* 27, 57–61.

Jung, W.-S., Choi, C.-M., Hong, J.-W., Park, S.-U., Moon, S.-K., Park, J.-M., et al. (2007). The clinical effect of manipulation of acupuncture to Shen-Men and Nei-Kuan on autonomic nervous function of healthy subjects. *Korean J. Orient. Med.* 28, 69–73.

Jung, W.-S., Park, S.-U., Park, J.-M., Kim, D.-Y., Hong, I.-K., An, Y.-S., et al. (2011). Changes in SPECT cerebral blood flow following Japanese style, superficial acupuncture at LI-4 and LI-11 in healthy volunteers. *J. Altern. Complement. Med.* 17, 357–362. doi: 10.1089/acm.2010.0331

Khavjou, O., Phelps, D., and Leib, A. (2016). *Projections of Cardiovascular Disease Prevalence and Costs: 2015–2035*. Dallas, TX: American Heart Association.

Kilander, L., Nyman, H., Boberg, M., Hansson, L., and Lithell, H. (1998). Hypertension is related to cognitive impairment: a 20-year follow-up of 999 men. *Hypertension* 31, 780–786. doi: 10.1161/01.hyp.31.3.780

Langevin, H. M., Churchill, D. L., and Cipolla, M. J. (2001). Mechanical signaling through connective tissue: a mechanism for the therapeutic effect of acupuncture. *FASEB J.* 15, 2275–2282. doi: 10.1096/fj.01-0015hyp

Lee, S. Y., Baek, Y. H., Park, S. U., Moon, S. K., Park, J. M., Kim, Y. S., et al. (2009). Intradermal acupuncture on shen-men and nei-kuan acupoints improves insomnia in stroke patients by reducing the sympathetic nervous activity: a randomized clinical trial. *Am. J. Chin. Med.* 37, 1013–1021. doi: 10.1142/s0192415x09007624

Lee, B., Sur, B.-J., Kwon, S., Jung, E., Shim, I., Lee, H., et al. (2012). Acupuncture stimulation alleviates corticosterone-induced impairments of spatial memory and cholinergic neurons in rats. *Evid. Based Complement. Altern. Med.* 2012:670536. doi: 10.1155/2012/670536

Li, H., Hou, Z., Bai, Y., and Gu, S. (2011). [Comparative study on curative effects of stroke treated with acupuncture by NIRS]. *Zhongguo Zhen Jiu* 31, 998–1002. doi: 10.13703/j.0255-2930.2011.11.028

Li, P., Tjen-A-Looi, S. C., Cheng, L., Liu, D., Painovich, J., Vinjamury, S., et al. (2015). Long-lasting reduction of blood pressure by electroacupuncture in patients with hypertension: randomized controlled trial. *Med. Acupunct.* 27, 253–266. doi: 10.1089/acu.2015.1106

Li, M., Tjen-A-Looi, S. C., Guo, Z.-L., and Longhurst, J. C. (2016). Repetitive Electroacupuncture attenuates cold-induced hypertension through enkephalin in the rostral ventral lateral medulla. *Sci. Rep.* 6:35791. doi: 10.1038/srep35791

Li, D.-Z., Zhou, Y., Yang, Y.-N., Ma, Y.-T., Li, X.-M., Yu, J., et al. (2014). Acupuncture for essential hypertension: a meta-analysis of randomized sham-controlled clinical trials. *Evid. Based Complement. Altern. Med.* 2014:279478. doi: 10.1155/2014/279478

Lin, R., Chen, J., Li, X., Mao, J., Wu, Y., Zhuo, P., et al. (2016). Electroacupuncture at the Baihui acupoint alleviates cognitive impairment and exerts neuroprotective effects by modulating the expression and processing of brain-derived neurotrophic factor in APP/PS1 transgenic mice. *Mol. Med. Rep.* 13, 1611–1617. doi: 10.3892/mmr.2015.4751

Liu, Y., Park, J.-E., Shin, K.-M., Lee, M., Jung, H. J., Kim, A.-R., et al. (2015). Acupuncture lowers blood pressure in mild hypertension patients: a randomized, controlled, assessor-blinded pilot trial. *Complement. Ther. Med.* 23, 658–665. doi: 10.1016/j.ctim.2015.06.014

Liu, C.-Z., Yu, J.-C., Zhang, X.-Z., Fu, W.-W., Wang, T., and Han, J.-X. (2006). Acupuncture prevents cognitive deficits and oxidative stress in cerebral multi-infarction rats. *Neurosci. Lett.* 393, 45–50. doi: 10.1016/j.neulet.2005.09.049

Lo, M.-Y., Ong, M. W., Chen, W.-Y., Sun, W.-Z., and Lin, J.-G. (2015). The effects of acupuncture on cerebral and muscular microcirculation: a systematic review of near-infrared spectroscopy studies. *Evid. Based Complement. Alternat. Med.* 2015:839470. doi: 10.1155/2015/839470

McDonald, J., and Janz, S. (2017). *The Acupuncture Evidence Project: A Comparative Literature Review, Rev Ed*. Brisbane: Australian Acupuncture and Chinese Medicine Association.

Meschia, J. F., Bushnell, C., Boden-Albala, B., Braun, L. T., Bravata, D. M., Chaturvedi, S., et al. (2014). Guidelines for the primary prevention of stroke: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 45, 3754–3832. doi: 10.1161/STR.0000000000000046

Michikami, D., Kamiya, A., Kawada, T., Inagaki, M., Shishido, T., Yamamoto, K., et al. (2006). Short-term electroacupuncture at Zusanli resets the arterial baroreflex neural arc toward lower sympathetic nerve activity. *Am. J. Physiol. Heart Circ. Physiol.* 291, H318–H326. doi: 10.1152/ajpheart.00975.2005

Niu, J., Zhao, X., Hu, H., Wang, J., Liu, Y., and Lu, D. (2019). Should acupuncture, biofeedback, massage, Qi gong, relaxation therapy, device-guided breathing, yoga and tai chi be used to reduce blood pressure?: recommendations based on high-quality systematic reviews. *Complement. Ther. Med.* 42, 322–331. doi: 10.1016/j.ctim.2018.10.017

Park, J.-M., Shin, A.-S., Park, S.-U., Sohn, I.-S., Jung, W.-S., and Moon, S.-K. (2010). The acute effect of acupuncture on endothelial dysfunction in patients with hypertension: a pilot, randomized, double-blind, placebo-controlled crossover trial. *J. Altern. Complement. Med.* 16, 883–888. doi: 10.1089/acm.2009.0427

Petrilla, A. A., Benner, J. S., Battleman, D. S., Tierce, J. C., and Hazard, E. H. (2005). Evidence-based interventions to improve patient compliance with antihypertensive and lipid-lowering medications: evidence-based interventions to improve patient compliance. *Int. J. Clin. Pract.* 59, 1441–1451. doi: 10.1111/j.1368-5031.2005.00704.x

Pires, P. W., Dams Ramos, C. M., Matin, N., and Dorrance, A. M. (2013). The effects of hypertension on the cerebral circulation. *Am. J. Physiol. Heart Circ. Physiol.* 304, H1598–H1614. doi: 10.1152/ajpheart.00490.2012

Qaseem, A., Wilt, T. J., McLean, R. M., and Forciea, M. A. (2017). Noninvasive treatments for the clinical guidelines committee of the american college of physicians. *Ann. Intern. Med.* 166, 514–530. doi: 10.7326/M16-2367

Qiu, C., Winblad, B., and Fratiglioni, L. (2005). The age-dependent relation of blood pressure to cognitive function and dementia. *Lancet Neurol.* 4, 487–499. doi: 10.1016/s1474-4422(05)70141-1

Ratmansky, M., Levy, A., Messinger, A., Birg, A., Front, L., and Treger, I. (2016). The effects of acupuncture on cerebral blood flow in post-stroke patients: a randomized controlled trial. *J. Altern. Complement. Med.* 22, 33–37. doi: 10.1089/acm.2015.0066

Ruitenberg, A., dem Heijer, T., Bakker, S. L. M., van Swieten, J. C., Koudstaal, P. J., Hofman, A., et al. (2005). Cerebral hypoperfusion and clinical onset of dementia: the Rotterdam study. *Ann. Neurol.* 57, 789–794. doi: 10.1002/ana.20493

Sato, A., Sato, Y., Suzuki, A., and Uchida, S. (1993). Neural mechanisms of the reflex inhibition and excitation of gastric motility elicited by acupuncture-like stimulation in anesthetized rats. *Neurosci. Res.* 18, 53–62. doi: 10.1016/0168-0102(93)90105-y

Sharp, S. I., Aarsland, D., Day, S., Sønnesyn, H., Alzheimer's Society Vascular Dementia Systematic Review Group, and Ballard, C. (2011). Hypertension is a potential risk factor for vascular dementia: systematic review. *Int. J. Geriatr. Psychiatry* 26, 661–669. doi: 10.1002/gps.2572

Sibbald, B., and Roland, M. (1998). Understanding controlled trials: why are randomised controlled trials important? *BMJ* 316, 201–201. doi: 10.1136/bmj.316.7126.201

Sibbritt, D., Peng, W., Lauche, R., Ferguson, C., Frawley, J., and Adams, J. (2018). Efficacy of acupuncture for lifestyle risk factors for stroke: a systematic review. *PLoS One* 13:e0206288. doi: 10.1371/journal.pone.0206288

Sun, Z., Shen, W., Wang, X.-L., Zou, B.-L., and Fu, S. (2014). [Twisting acupuncture and sham acupuncture at Taichong (LR 3) in patients with essential hyper-tension: an fMRI study]. *Zhongguo Zhen Jiu* 34, 1191–1195. doi: 10.13703/j.0255-2930.2014.12.014

Tedla, Y. G., and Bautista, L. E. (2016). Drug side effect symptoms and adherence to antihypertensive medication. *Am. J. Hypertens.* 29, 772–779. doi: 10.1093/ajh/hpv185

Tian, G.-H., Sun, K., Huang, P., Zhou, C.-M., Yao, H.-J., Huo, Z.-J., et al. (2013). Long-term stimulation with electroacupuncture at DU20 and ST36 rescues hippocampal neuron through attenuating cerebral blood flow in spontaneously hypertensive rats. *Evid. Based Complement. Altern. Med.* 2013:482947. doi: 10.1155/2013/482947

Toyoshima, H., Takahashi, K., and Akera, T. (1997). The impact of side effects on hypertension management: a Japanese survey. *Clin. Ther.* 19, 1458–1469. doi: 10.1016/s0149-2918(97)80019-7

van Middelaar, T., van Vught, L. A., Moll van Charante, E. P., Eurelings, L. S. M., Ligthart, S. A., van Dalen, J. W., et al. (2017). Lower dementia risk with different classes of antihypertensive medication in older patients. *J. Hypertens.* 35, 2095–2101. doi: 10.1097/HJH.0000000000001411

Wang, Z., Liang, P., Zhao, Z., Han, Y., Song, H., Xu, J., et al. (2014). Acupuncture modulates resting state hippocampal functional connectivity in Alzheimer disease. *PLoS One* 9:e91160. doi: 10.1371/journal.pone.0091160

Wang, J., Xiong, X., and Liu, W. (2013). Acupuncture for essential hypertension. *Int. J. Cardiol.* 169, 317–326. doi: 10.1016/j.ijcard.2013.09.001

Wang, X.-R., Yang, J.-W., Ji, C.-S., Zeng, X.-H., Shi, G.-X., Fisher, M., et al. (2018). Inhibition of NADPH oxidase-dependent oxidative stress in the rostral ventrolateral medulla mediates the antihypertensive effects of acupuncture in spontaneously hypertensive rats. *Hypertension* 71, 356–365. doi: 10.1161/hypertensionaha.117.09759

Wiesmann, M., Zerbi, V., Jansen, D., Lütjohann, D., Veltien, A., Heerschap, A., et al. (2017). Hypertension, cerebrovascular impairment, and cognitive decline in aged AβPP/PS1 mice. *Theranostics* 7, 1277–1289. doi: 10.7150/thno.18509

World Health Organization. (2002). *Acupuncture: Review and Analysis Reports on Controlled Clinical Trials*. Geneva: World Health Organization.

Xu, J., Murphy, S., Kochanek, K., Bastian, B., and Arias, E. (2018). Deaths: final data for 2016. *Natl. Vital. Stat. Rep.* 67, 1–76. Available online at: https://www.cdc.gov/nchs/products/nvsr.htm

Xue, X., You, Y., Tao, J., Ye, X., Huang, J., Yang, S., et al. (2014). Electro-acupuncture at points of Zusanli and Quchi exerts anti-apoptotic effect through the modulation of PI3K/Akt signaling pathway. *Neurosci. Lett.* 558, 14–19. doi: 10.1016/j.neulet.2013.10.029

Yang, J., Chen, J., Yang, M., Yu, S., Ying, L., Liu, G. J., et al. (2018). Acupuncture for hypertension. *Cochrane Database Syst. Rev.* 11:CD008821. doi: 10.1002/14651858.CD008821.pub2

Yuan, X., Hao, X., Lai, Z., Zhao, H., and Liu, W. (1998). Effects of acupuncture at fengchi point (GB 20) on cerebral blood flow. *J. Tradit. Chin. Med.* 18, 102–105.

Zhao, X.-F., Hu, H.-T., Li, J.-S., Shang, H.-C., Zheng, H.-Z., Niu, J.-F., et al. (2015). Is acupuncture effective for hypertension? A systematic review and meta-analysis. *PLoS One* 10:e0127019. doi: 10.1371/journal.pone.0127019

Zhao, H., Li, D., Li, Y., Yang, Y., Liu, Y., Li, J., et al. (2019). Efficacy and safety of acupuncture for hypertension: an overview of systematic reviews. *Complement. Ther. Clin. Pract.* 34, 185–194. doi: 10.1016/j.ctcp.2018.12.003

Zheng, H., Han, Y., Du, Y., Shi, X., Huang, H., Yu, X., et al. (2018). Regulation of hypertension for secondary prevention of stroke: the possible 'bridging function' of acupuncture. *Complement. Med. Res.* 25, 45–51. doi: 10.1159/000475930

Zheng, Y., Zhang, J., Wang, Y., Wang, Y., Lan, Y., Qu, S., et al. (2016). Acupuncture decreases blood pressure related to hypothalamus functional connectivity with frontal lobe, cerebellum, and insula: a study of instantaneous and short-term acupuncture treatment in essential hypertension. *Evid. Based Complement. Alternat. Med.* 2016:6908710. doi: 10.1155/2016/6908710

Zhou, W., Fu, L. W., Tjen-A-Looi, S. C., Li, P., and Longhurst, J. C. (2005a). Afferent mechanisms underlying stimulation modality-related modulation of acupuncture-related cardiovascular responses. *J. Appl. Physiol.* 98, 872–880. doi: 10.1152/japplphysiol.01079.2004

Zhou, W., Tjen-A-Looi, S. C., and Longhurst, J. C. (2005b). Brain stem mechanisms underlying acupuncture modality-related modulation of cardiovascular responses in rats. *J. Appl. Physiol.* 99, 851–860. doi: 10.1152/japplphysiol.01365.2004

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 18 September 2019
doi: 10.3389/fphys.2019.01168

Effect of a Moderate-Intensity Aerobic Training on Joint Biomarkers and Functional Adaptations in Rats Subjected to Induced Knee Osteoarthritis

*Jeanne Brenda Martins[1,2], Vanessa Amaral Mendonça[1,2,3], Grazielle Cordeiro Aguiar[4], Sueli Ferreira da Fonseca[1,2], Jousielle Márcia dos Santos[1,3], Rosalina Tossige-Gomes[1,2], Dirceu de Sousa Melo[1,2], Murilo Xavier Oliveira[1,3], Hércules Ribeiro Leite[1,2], Ana Cristina Resende Camargos[5], Anderson José Ferreira[4], Cândido Celso Coimbra[4], Jacques Poortmans[6], Vinícius Cunha Oliveira[1,3], Sara Barros Silva[1,3], Talita Emanuela Domingues[1,2], Mário Bernardo-Filho[7] and Ana Cristina Rodrigues Lacerda[1,2,3]**

[1] Centro Integrado de Pós-Graduação e Pesquisa em Saúde (CIPq-Saúde), Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), Diamantina, Brazil, [2] Programa Multicêntrico de Pós-graduação em Ciências Fisiológicas, Sociedade Brasileira de Fisiologia (SBFis), Diamantina, Brazil, [3] Programa de Pós-Graduação em Reabilitação e Desempenho Funcional (PPGReab), Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), Diamantina, Brazil, [4] Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brazil, [5] Programa de Pós-Graduação em Ciências da Reabilitação, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, [6] Faculty of Motor Sciences, Université Libre de Bruxelles, Brussels, Belgium, [7] Departamento de Biofísica e Biometria, Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil

Background: Knee osteoarthritis ($_{k}$OA) is a common chronic disease that induces changes in redox status and inflammatory biomarkers, cell death, and motor impairment. Aerobic training can be a non-pharmacological alternative to prevent the progression of the disease.

Objective: To evaluate the effects of an 8 weeks moderate-intensity treadmill aerobic training program on redox status and inflammatory biomarkers and motor performance in $_{k}$OA-like changes induced by monosodium iodoacetate (MIA) in rats.

Methods: Twenty-seven rats were randomly divided into three groups: SHAM; induced $_{k}$OA (OA); and induced $_{k}$OA + aerobic training (OAE). Motor performance was evaluated by the number of falls on rotarod test, the total time of displacement and the number of failures on a 100 cm footbridge. Data for cytokines and histology were investigated locally, whereas plasma was used for redox status biomarkers.

Results: The OA group, compared to the SHAM group, increased 1.13 times the total time of displacement, 6.05 times the number of failures, 2.40 times the number of falls. There was also an increase in cytokine and in thiobarbituric acid reactive substances (TBARS) (IL1β: 5.55-fold, TNF: 2.84-fold, IL10: 1.27-fold, IL6: 1.50-fold, TBARS: 1.14-fold), and a reduction of 6.83% in the total antioxidant capacity (FRAP), and of 35% in the number of chondrocytes. The aerobic training improved the motor performance in all

OPEN ACCESS

Edited by:
Leonardo Alexandre Peyré-Tartaruga, Federal University of Rio Grande do Sul, Brazil

Reviewed by:
Júlio Cezar De Oliveira, Federal University of Mato Grosso, Brazil
Marli Maria Knorst, Federal University of Rio Grande do Sul, Brazil

***Correspondence:**
Ana Cristina Rodrigues Lacerda
lacerdaacr@gmail.com;
lacerda.acr@ufvjm.edu.br

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: 14 June 2019
Accepted: 29 August 2019
Published: 18 September 2019

Citation:
Martins JB, Mendonça VA, Aguiar GC, Fonseca SF, Santos JM, Tossige-Gomes R, Melo DS, Oliveira MX, Leite HR, Camargos ACR, Ferreira AJ, Coimbra CC, Poortmans J, Oliveira VC, Silva SB, Domingues TE, Bernardo-Filho M and Lacerda ACR (2019) Effect of a Moderate-Intensity Aerobic Training on Joint Biomarkers and Functional Adaptations in Rats Subjected to Induced Knee Osteoarthritis. Front. Physiol. 10:1168. doi: 10.3389/fphys.2019.01168

joint function tests matching to SHAM scores. Also, it reduced inflammatory biomarkers and TBARS level at values close to those of the SHAM group, with no change in FRAP level. The number of falls was explained by IL1β and TNF (58%), and the number of failures and the total time of displacement were also explained by TNF (29 and 21%, respectively).

Conclusion: All findings indicate the efficacy of moderate-intensity aerobic training to regulate inflammatory biomarkers associated with improved motor performance in induced $_{k}$OA-like changes, thus preventing the loss of chondrocytes.

Keywords: osteoarthritis, aerobic training, exercise, biomarkers, joint function

INTRODUCTION

Chondrocytes are responsible for tissue maintenance which impact on joint function and performance (Sophia Fox et al., 2009; Akkiraju and Nohe, 2015; Charlier et al., 2016). Current literature has reported exercise-induced chondroprotection in knee osteoarthritis ($_{k}$OA) (Loeser, 2010; Golightly et al., 2012; Geneen et al., 2017). A potential explanation is the mechanical signal transduction (O'Hara et al., 1990; Urban, 1994), preserving the cartilage proteoglycans, and promoting chondrocytes modulation (Little and Ghosh, 1997; Loeser et al., 2012). Low-magnitude mechanical stress seems to suppress the pathway of interleukin-1 beta (IL1β) and tumor necrosis factor (TNF) release (Roman-Blas et al., 2009; Leong et al., 2010; Li et al., 2013; Assis et al., 2016). The IL1β and TNF are inflammatory mediators involved in joint degeneration caused by $_{k}$OA (Blasioli and Kaplan, 2013; Mabey and Honsawek, 2015). The modulation of these cytokines in the joint would regulate the synthesis of proteoglycans and collagen, thereby attenuating the swelling process (Knobloch et al., 2008; Iijima et al., 2015).

The literature points out redox imbalance and the related increase on reactive oxygen species (ROS), swelling process and necrosis of chondrocytes as the pathophysiology of $_{k}$OA (Altindag et al., 2007; Rose et al., 2012; Hui et al., 2016). Moreover, redox imbalance and the increased inflammatory biomarkers may cause cartilage damage, neuroinflammatory disease progression, and joint disability (Kim et al., 2010; Watari et al., 2011; Attur et al., 2013, 2015; Reed et al., 2014).

Several interventions are used to improve motor performance in patients with $_{k}$**OA** (Golightly et al., 2012; Geneen et al., 2017), and a low-cost aerobic training may be an alternative (Hunter and Eckstein, 2009; Semanik et al., 2012). Aerobic training improves blood soluble TNF receptors level and brain-derived neurotrophic factor (BDNF) plasma level in people with $_{k}$OA (Gomes et al., 2012; Simão et al., 2014). Moreover, BDNF seems to impact on chondrocyte differentiation, changing it from proliferative to differentiation program (Hutchison, 2012).

Beyond insufficient information about the modulatory effect of the aerobic training in the knee joint degeneration parameters and possible relationships between joint parameters and motor performance, the literature still presents gaps regarding the effect of aerobic training in the joint preservation on $_{k}$OA-like changes. Many studies detail mechanotransduction mechanisms but still remain questions about joint function. Moreover, few studies address joint function tests to establish a new therapeutic approach in clinical practice. The current study aimed to investigate the effects of moderate-intensity aerobic training in the inflammatory and redox biomarkers modulation of $_{k}$OA-like changes, and its possible link to motor performance in rats. Therefore, because experimental $_{k}$OA induces joint swelling process, we hypothesized that moderate-intensity aerobic training would attenuate the swelling process, favoring the redox balance and preserving chondrocytes in rats with $_{k}$OA -like changes. Improved modulation in joint biomarkers levels might explain an effect on motor performance.

MATERIALS AND METHODS

Male Wistar rats were used in the current study that was part of a Masters in Physiology at the Universidade Federal dos Vales do Jequitinhonha e Mucuri (Martins, 2017). The project was approved by the local Ethics Committee (protocol 005/2015).

Twenty-seven rats were randomized into three groups: sham group (SHAM), n = 9; induced $_{k}$OA group (OA), n = 9; and induced $_{k}$OA + aerobic training (OAE), n = 9. The rats had available water and food (i.e., Nuvilab CR1, Nuvital Nutrientes S/A, Brazil) as they desire, and their environment was controlled (i.e., humidity of 60% and temperature of 22°C).

Induced Osteoarthritis

Twelve-week-old male Wistar rats were anesthetized with ketamine (80 mg/kg, i.p.) and xylazine (15 mg/kg, i.p.). Then, $_{k}$OA was induced on the right knee joint at 90° flexion by direct infiltration of monosodium iodoacetate (MIA) (1.2 mg diluted in 50 μL saline solution). We used a 29G X 1/2 BD Ultra-FineTM insulin needle (Guzman et al., 2003; Cifuentes et al., 2010; Takahashi et al., 2018). The SHAM group received an infiltration containing 50 μL of saline solution (0.9% NaCl).

Aerobic Training

To perform the aerobic training, a motorized treadmill (Insight®, SP, Ribeirão Preto, Brazil) with six individual lanes and with no inclination was used in the study. OAE group started the familiarization to the treadmill (10 min/day for 5 days) 24 h after the $_{k}$OA-induction procedure. Then, 24 h after the familiarization period, initiated the training program, which consisted of treadmill running at the velocity of 16 m/min,

3 days per week during 8 weeks (Cifuentes et al., 2010), and the duration of running sessions was increased from 30 to 50 min at the fourth week. The workload of all groups was analyzed at the end of the training program during a treadmill incremental test (initial speed of 10 m min^{-1}, 1% slope, with no electrical stimulus, an increase of 2 m min^{-1} every 3 min) (Balthazar et al., 2009; Primola-Gomes et al., 2009). The workload (W; J) was calculated as: W = body weight (kg) × total time to fatigue (min) × treadmill speed (m min^{-1}) × sin θ (treadmill inclination) × 10 (Lacerda et al., 2006).

Evaluation of Motor Performance – Forced Locomotion (Rotarod Test)

In the rotarod test (Scienlabor, Brazil), the rats are stimulated to walk around a circle drum surface. The rotarod test measures balance, coordination, physical performance, and motor-planning by calculating the number of falls during determined speed. The time that a given rat stays on this circle rod represents the joint function. We used a protocol adapted from Piel et al. (2014) to quantify the number of falls of rats during 3-min period keeping a fixed speed of 8 revolutions per minute (rpm) (Piel et al., 2014).

Locomotion Test on a Footbridge

Rats locomoted on a footbridge to investigate joint function when moving a short distance. The footbridge had a length of 100 cm delimited by 3 mm thick aluminum filets. Image and time to complete a single pass during animals' locomotion were recorded. Records were later analyzed by a blinded investigator to quantify the total time of displacement and the number of times each rat stepped out of the space between the filets (number of failures).

Euthanasia

Animals were euthanized individually by decapitation and their right knee joints were analyzed. Approximately 12 mL of blood was collected in tubes containing ethylenediaminetetraacetic acid (EDTA), then centrifuged at 500 × *g* for 10 min and the serum aliquoted and frozen in a −80°C freezer for further analysis.

Joint Lavage

For later analysis, the joint lavage (JAL) supernatants were stored at −80°C. Immediately after recovering JAL, we removed the joint capsule and stored it in a freezer at −80°C. Moreover, we homogenized the sample in phosphate buffer and also frozen it at −80°C for future analysis. IL1β, TNF, and interleukin-10 (IL10) knee joint biomarkers were analyzed according to the manufacturer's instructions by ELISA kits (DuoSet, R&D Systems, United States).

Macerated Joint Capsule

The capsule was placed into a beaker with 750 μL of cytokine extraction solution, and a tissue homogenizer (Tecnal, TE-103) was used to obtain the macerate. Then, the capsule macerate was processed at 8 rpm speed for 2 min, the volume centrifuged at 3500 xg at 4°C for 10 min and stored in a freezer. BDNF and interleukin-6 (IL6) levels were analyzed according to the manufacturer's instructions by ELISA.

Measurements of Redox Status

The reaction of the thiobarbituric acid with malondialdehyde (MDA) was used to determine lipid peroxidation by thiobarbituric acid reactive substances (TBARS) plasma levels (Ohkawa et al., 1979). The ferric reducing ability of plasma (FRAP), i.e., the reduction of ferric-tripyridyltriazine [Fe(III)-TPTZ] complex to ferrous-tripyridyltriazine [Fe(II)-TPTZ] (Benzie and Strain, 1996) was used to determine the total antioxidant capacity. The Bradford method using bovine serum albumin was used as a standard to determine the samples protein levels (Bradford, 1976).

Histology

The right knee joints were placed in 4% neutral-buffered formalin for 24 h. After that, tissues were placed in 10% EDTA at pH 7.4 for decalcification (Jimson et al., 2014). Sagittal sections were prepared from knee joints. Two slides of the femur compromised by MIA induction or SHAM were prepared for histological analyses. Three cuts in each slide. Tissues were placed in formalin, dehydrated in a graded series of ethanol and xylol, embedded in paraffin, cut into 6 μm serial sections, and stained with hematoxylin-eosin.

Quantification of Chondrocytes

To investigate the number of active cells, the middle third of the joint was used. For cell counting, the nuclei stained by hematoxylin present in the superficial, intermediate and transitional areas were considered. The analysis was performed on the articular facet of the right femur. The image was captured by a microscope with a 40 × magnification. The software Image J was used for cell counting. Two micrographs were taken in series and a blinded investigator analyzed them on different days (ICC = 0.99). For statistical analysis, the average number of cells was established and used.

Statistical Analysis

We used the SPSS statistical package, version 22.0 (Inc., United States) and Graph Pad Prism, version 5.0 (Inc., United States). Data are expressed as mean ± standard error (S.E.M). Normality of data was assessed using the Shapiro–Wilk test. For comparisons, we used the one-way ANOVA with Tukey's *post hoc* tests for parametric data (Body mass, IL6 and number of chondrocytes) and Kruskal-Wallis with Dunn's *post hoc* test for non-parametric data (IL1β, TNF, IL10, TBARS, FRAP, number of falls, total time of displacement, and number of failures). Effect Size (d) was checked in G*Power 3.1.9.2 program. Effect size conventions for test family (*F* tests) and one-way ANOVA: $d = 0.10$ (small); $d = 0.25$ (medium); $d = 0.40$ (large). The Spearman correlation investigated associations between two intra-articular biomarkers of joint damage (IL1β, TNF) and joint function tests: number of falls, total time of displacement and number of failures.

To determine intra-examiner reliability for the evaluated outcomes, the intraclass correlation coefficient (ICC) adopting a 95% confidence interval was determined. Multiple linear stepwise regression models confirmed the association between

selected biomarkers and joint function, adjusting by Bonferroni at $\alpha = 0.017$. Graphs were built using the GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, United States).

RESULTS

Twenty-seven animals were available for this study, recovered uneventfully from surgery and exercise procedure. By the end of the study, the rats were about 6 months old. No significant differences were observed in body weight between the three groups at the surgery and at the 8th-week post-surgery (**Figure 1**).

Aerobic training increased the total workload of OAE rats by 64% as compared to sham and OA groups (SHAM: 23.8 ± 8.0 J, OA: 23.7 ± 11.0 J, OAE: 69.2 ± 15.9 J, $p = 0.0002$, $d = 0.85$). Levels of IL1β, TNF, IL10 in the joint washed and the level of IL6 in the joint capsule increased respectively by 5.55, 2.84, 1.27, and 1.50-fold in the OA group. In OAE group compared with OA group, the aerobic training modulated levels of these joint cytokines close to those in the SHAM group (IL1β: $p < 0.0001$, $d = 1.18$; TNF: $p = 0.0001$, $d = 1.00$; IL10: $p < 0.0001$, $d = 1.32$; IL6: $p = 0.0001$, $d = 1.68$) (**Figure 2**).

The BDNF level in the OAE group was 41% higher than in the OA group ($p = 0.05$; $d = 0.86$). The induction of $_k$OA-like changes increased by 1.14 times the TBARS plasma level and reduced the FRAP plasma level by 6.83% ($p = 0.02$) in the OA group compared with SHAM group. In OAE group compared with OA group, the aerobic training returned the plasma level of TBARS close to those in the SHAM group, without changing the total antioxidant capacity (TBARS: $p = 0.001$; $d = 0.86$; $P = 0.95$; FRAP: $p = 0.05$; $d = 0.62$) (**Figure 3**).

The analysis of the joint function tests showed that the $_k$OA-induction (OA group) increased by 1.13 times the total time of displacement on a 100 cm footbridge and increased by 6.05 times the number of failures in the course when compared with SHAM group. The number of falls during the rotarod test increased 2.40 times in the OA group compared with SHAM. Therefore, in OAE group compared with OA group, the aerobic training had a positive effect on the disease, since it improved the performance in the three parameters evaluated, matching the SHAM score (Number of falls: $p = 0.002$; $d = 1.05$; Number of failures: $p = 0.0002$ $d = 0.26$; Total time of displacement: $p = 0.005$; $d = 0.72$) (**Figure 4**).

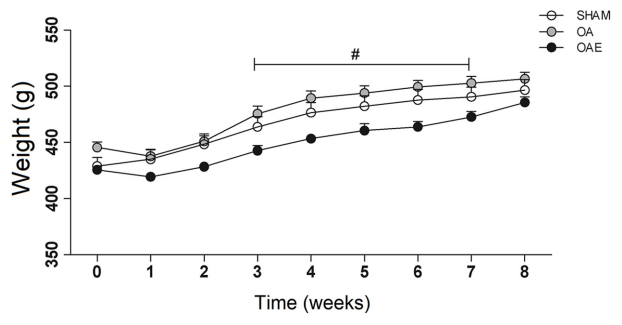


FIGURE 1 | Body mass over the 8-week interventions. (White circle) SHAM group; (Gray circle) OA: knee osteoarthritis group; (Black circle) OAE: knee osteoarthritis plus aerobic training group. $N = 9$ per group. Data are reported as Mean ± S.E.M. #Difference between OA vs. OAE.

Number of falls ($rs = 0.69$; $p = 0.0003$) and number of failures ($rs = 0.66$; $p = 0.0002$) were associated with the IL1β. Functional performance measures: number of falls in the rotarod test ($rs = 0.73$; $p < 0.0001$); total time of displacement ($rs = 0.63$; $p = 0.0005$); and number of failures ($rs = 0.52$; $p = 0.005$) were associated with the TNF. Multiple linear stepwise regression models of IL1β and TNF explained 58% of the variability in the number of falls (IL1 β: $p = 0.001$; TNF: $p = 0.002$). TNF only explained 29% of the variability of the total time of displacement ($p = 0.02$) and 21% of the variability of the number of failures ($p = 0.02$). The increase of 1 pg/mL in intra-articular IL1β level leads to an increase of 0.40 points in the number of falls. The increase of 1 pg/mL in the intra-articular TNF level leads to an increase of 0.51 points in the number of falls; 0.57 s in the total time of displacement; and 0.49 points in the number of failures (**Table 1**).

Quantification of hematoxylin-stained nuclei showed that the induction of $_k$OA reduced the number of cells (chondrocytes) alive by approximately 35% compared with the SHAM group. Aerobic training (OAE group) prevented chondrocyte cell death since the number of active cells in the trained group was 51.35% higher than in the OA group ($p < 0.0001$) (**Figure 5**).

DISCUSSION

Overall, the current study showed an association between worse joint function and high levels of joint degeneration biomarkers, as indicated by high levels of TNF and IL1β. Moreover, aerobic training could reverse local inflammatory biomarkers and decrease systemic MDA level with an improvement in gait tasks, motor, and physical performance. Histological data of the femoral joint also confirmed the beneficial effect of the proposed exercise to the $_k$OA-induced.

Scientific evidence suggests the involvement of inflammatory biomarkers and redox status parameters for the advancement and progression of $_k$OA (Regan et al., 2005; Koike et al., 2015). In patients with $_k$OA, chondrocytes and synovial cells stimulate the production of inflammatory cytokines, i.e., IL1β and TNF (Altindag et al., 2007; Kim et al., 2010; Wojdasiewicz et al., 2014; Kunisch et al., 2016). Kim et al. (2010) evidenced that IL1β and TNF are responsible for mitochondrial DNA damage in $_k$OA, promoting the development of ROS and chondrocyte death (Kim et al., 2010).

The compression forces of low magnitude during physical exercise seem to promote physiological control. Such control modulates the synthesis of collagen and proteoglycans possibly inhibited in swelling joints (Quinn et al., 1998; Fehrenbacher et al., 2003; Park et al., 2004). This could indicate the greater maintenance of proteoglycan in the joint cartilage of rats exposed to moderate exercise (Galois et al., 2003; Cifuentes et al., 2010; Li et al., 2013; Mohammadi et al., 2013). Thus, the authors suggest that the positive effects are linked to the decrease of chondrocyte necrosis in the experimental group, lowering metabolites of cell

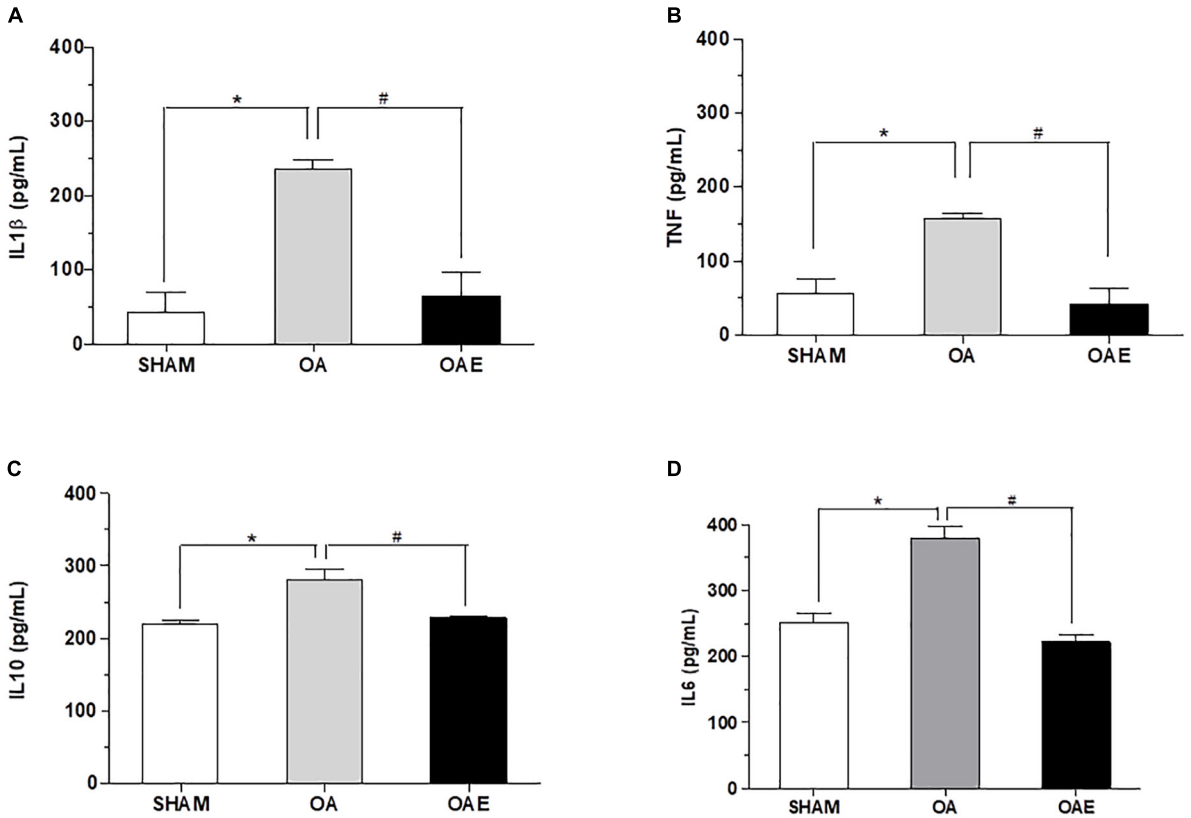


FIGURE 2 | Effect of aerobic training on inflammatory biomarkers. **(A)** Interleukin-1beta (IL1β) measured in joint wash fluid; **(B)** Tumor necrose factor (TNF) measured in joint washed; **(C)** Interleukin-10 (IL10) measured in joint washed, and **(D)** Interleukin-6 (IL6) measured in the joint capsule for SHAM group; OA: knee osteoarthritis group; OAE: knee osteoarthritis plus aerobic training group. *N* = 9 per group. Data are reported as Mean ± S.E.M. #Difference between OA vs. OAE. *Difference between OA vs. SHAM.

death and induction of inflammatory factors expression (Galois et al., 2003). The high level of ROS would act on the expression of cytokines, making worse the swelling. The ROS may initiate the cartilage degeneration and advancement of lipid peroxidation in chondrocytes (Yudoh et al., 2005; Ostalowska et al., 2006; Kim et al., 2010; Watari et al., 2011).

In $_{k}$OA, inflammatory cytokines alter the joint biochemical balance inducing chondrocyte necrosis. The production and secretion of catabolic cytokines augment ROS production inducing a redox imbalance. Free radicals interacting with chondrocyte DNA acts directly by altering cellular components, i.e., proteoglycans, collagens, and protein oxidation, favoring cartilage degeneration, which may compromise the thickness of the synovial fluid, and the synthesis of other components (Henrotin et al., 2003; Rose et al., 2012; Reed et al., 2014). Physical and functional damages occur due to tissue structural change. Catabolic cytokines (IL1β and TNF), and regulatory cytokine (IL6) are probably the main factors in this process, facilitating paths of degeneration by activating paths such as matrix metalloproteases (Rojas-Ortega et al., 2015; Assis et al., 2016). The augmented inflammatory profile marks the loss of extracellular matrix integrity, developing an oxidative injury, and, lastly, the chondrocytes death. The death of these chondrocytes seems related to the compromised joint function since this cellular type is responsible for the mobilization of essential components that assure the main functions of load distribution and reduction of friction during static or dynamic exercises that guarantee the joint function. Mohammadi et al. (2013) assessed histological data of depth ratio of lesions demonstrating that 4 weeks of moderate exercise almost treated $_{k}$OA symptoms in rats (Mohammadi et al., 2013). Moreover, the level of MDA increased in induced $_{k}$OA dogs. This intensification revealed degeneration of the type II collagen (Goranov, 2007), implying a relationship between redox imbalance and cartilage degeneration. Oxidative injury can result in cell death, triggering particles and oxidized molecules release, cellular degeneration, and increased inflammation.

$_{k}$OA subjects have greater ROS plasma level and lower antioxidant supplies (Abruzzo et al., 2013; Germanou et al., 2013). The redox imbalance can play a critical role in the cartilage degeneration (Henrotin et al., 2003; Reed et al., 2014). Thus, in both human and animal, $_{k}$OA transporters have a high level of systemic biomarkers which means cellular damage led by ROS. As a consequence, cell-matrix may be compromised. Inhibition of this course can successfully avoid degeneration of articular cartilage and neo-formation of type II collagen (Poole et al., 2002).

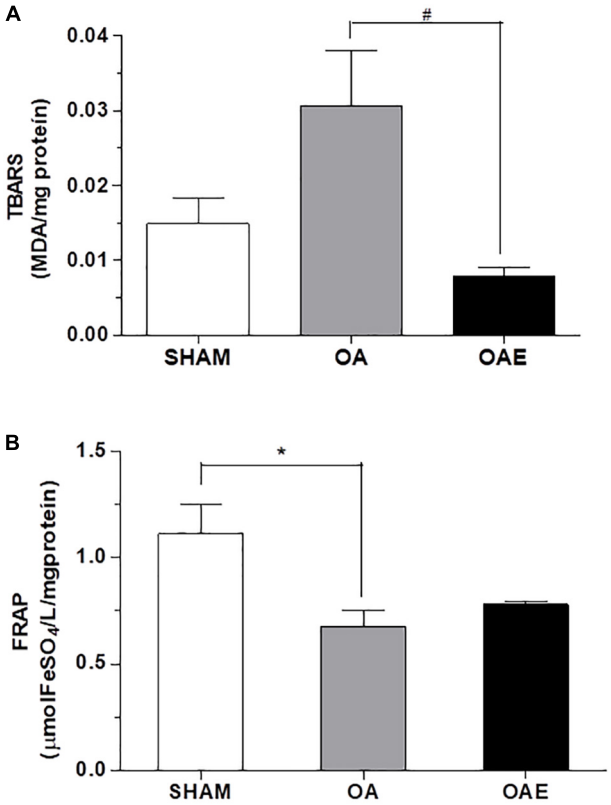


FIGURE 3 | Effect of aerobic training on status redox biomarkers. **(A)** Thiobarbituric acid-reactive substance (TBARS) measured in plasma; **(B)** The ferric reducing ability of plasma (FRAP) measured in plasma for SHAM group, OA: knee osteoarthritis group; OAE: knee osteoarthritis plus aerobic training group. *N* = 9 per group. Data are reported as Mean ± S.E.M. #Difference between OA vs. OAE. *Difference between OA vs. SHAM.

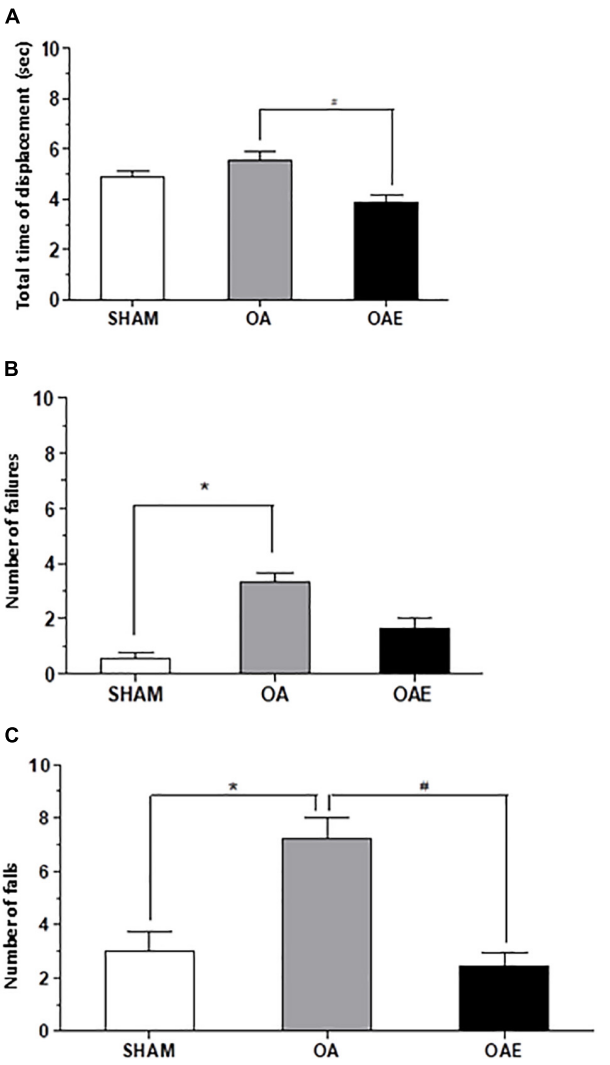


FIGURE 4 | Effect of treadmill aerobic training on joint function tests. **(A)** Total time of displacement; **(B)** Number of failures, and **(C)** Number of falls in SHAM group, OA: knee osteoarthritis group; OAE: knee osteoarthritis plus aerobic training. *N* = 9 per group. Data are reported as Mean ± S.E.M. #Difference between OA vs OAE; *Difference between OA vs. SHAM.

The transduction of mechanical signals of dynamic pressure in chondrocytes may favor the pathways that counteract tissue catabolism. Li et al. (2013) assessed anabolic responses on bovine cartilage *in vitro* cells culture inducing matrix biosynthesis with different compression ranges (10, 20, and 30%) and demonstrated that moderate dynamic compression can exert an "anti-catabolic" effect, and suppress the expression of TNF, IL6, and soluble IL6 receptors (Li et al., 2013). The catabolism control appears to relate to a range of compression frequency, amplitude, and to the low-to-moderate intensity load, showing the importance of a voltage amplitude threshold for the regulation of inflammatory paths and cell survival (Cifuentes et al., 2010; Beckett et al., 2012; Rojas-Ortega et al., 2015; Rios et al., 2018). Moreover, the exercise load influences BDNF production and release

TABLE 1 | Multiple linear stepwise regression analysis.

Joint damage biomarkers	Number of falls			Total time of displacement			Number of failures		
	B	p	R^2	β	P	R^2	B	p	R^2
			0.58			0.29			0.21
IL1β (pg/mL)	0.40	0.001*		−0.003	0.99		0.32	0.10	
TNF (pg/mL)	0.51	0.002*		0.57	0.02*		0.49	0.02*	

β, Standardized regression coefficient; R^2, Adjusted R square. IL1β, Interleukin-1 beta; TNF, Tumor necrosis factor.

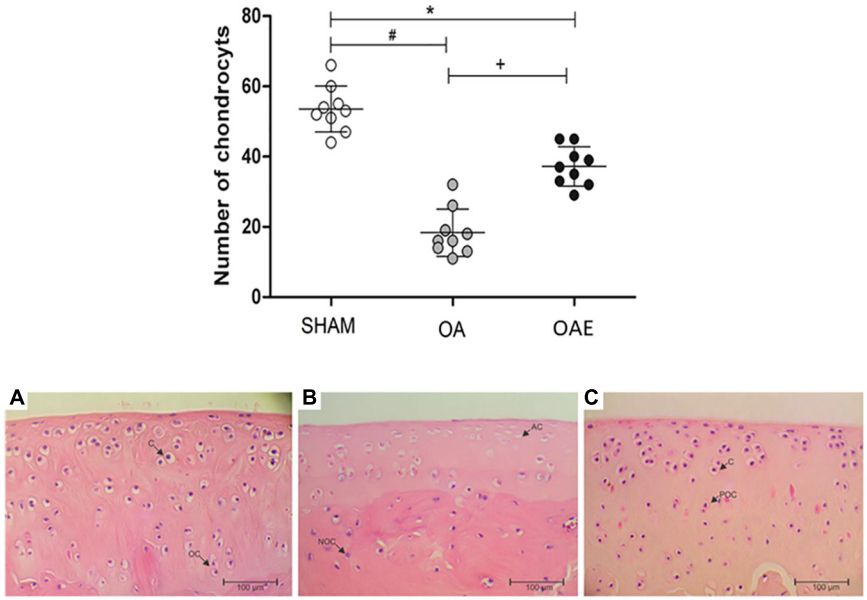


FIGURE 5 | Effect of moderate-intensity aerobic training on the number of chondrocytes, and representation of the histological cell number (chondrocytes). SHAM group (SHAM): white circles and **A**; knee osteoarthritis group (OA): gray circles and **B**; knee osteoarthritis plus moderate-intensity aerobic training group (OAE): black circles and **C**. *N* = 9 per group. Data are reported as Mean ± S.E.M. $^{+}$Difference between OA vs. OAE; *Difference between OAE vs. SHAM. $^{\#}$Difference between OA vs. SHAM. **C:** chondrocytes; OC: organized cells; AC: the absence of cells; NOC: no organized cells; POC: partially organized cells.

(Nofuji et al., 2008; Yarrow et al., 2010; Szuhany et al., 2015). BDNF is an important growth factor expressed in joint chondrocytes and in epiphyseal plaques of $_{k}$OA subjects. BDNF causes the growth and, mainly, differentiation (Hutchison, 2012) of chondrocytes, inducing the proliferation pathway that can act as a restoration mediator. Furthermore, BDNF level has been also systemically increased in patients with $_{k}$OA (Simão et al., 2014).

Because mild- to moderate-intensity exercises seem to play an anti-inflammatory role, we decided to perform a moderate-intensity aerobic training on a treadmill to promote mechanical biostimulation caused by joint compression (Galois et al., 2003; Cifuentes et al., 2010; Li et al., 2013; Assis et al., 2016; Rios et al., 2018). In the current study, we decided to use an aerobic training similar to the study of Cifuentes et al. (2010) that investigated the effects of impact exercise on the $_{k}$OA-induced cartilage aspects in rats. This protocol was chosen because the data of that investigation demonstrated that aerobic training contributed to the preservation of some joint cartilage parameters in experimental $_{k}$OA. Moreover, the overload to the intensity and inclination of exercise sessions were not applied, once it could directly affect the dynamic compression of knee joint cartilage cells, leading to misinterpretations of our results. Thus, active mechanotransduction induces changes in oxygen tension and subsequent positive effects on matrix synthesis and cell growth (Urban, 1994; Park et al., 2004). These effects are explained by the displacement of growth factors or cellular cytokines by shifting cellular metabolism (Tilwani et al., 2017). Finally, our results showed that the proposed training offered an inflammatory control, confirmed by the modulation effect in biomarkers levels (IL1β and TNF in the joint wash; IL10 and IL6 in the knee joint).

The positive effects of aerobic training are attributed to the ability of the suppression of signals transduction paths of inflammatory and catabolic mediators together with the stimulation of anabolic paths. *In vitro* studies have verified that mild to moderate mechanical stress inhibits swelling by suppression of IL1β, TNF and the transcription of various joint degeneration inflammatory biomarkers (Fehrenbacher et al., 2003; Rose et al., 2012; Yamabe et al., 2013). Experimental $_{k}$OA studies showed a positive effect of aerobic training, whereas high-intensity training had a deleterious effect (Beckett et al., 2012; Ni et al., 2012; Rojas-Ortega et al., 2015; Li et al., 2017). These data seem to determine the role of aerobic exercise appropriate dose (intensity, frequency, and duration) in modulating chondrocyte response (Ni et al., 2013; Na et al., 2014; Hill et al., 2017). In rats without previous $_{k}$OA induction, high-intensity exercises in many treadmill angles were not able to induce knee damage (Beckett et al., 2012; Rios et al., 2018). Thus, biochemical responses appear to be sensitive to the force only in the injury. Our data once again is in accordance to this premise since the proposed protocol was effective in dropping the joint IL1β and TNF in the trained group, as well as in reducing the TBARS systemic level, augmenting knee joint function and physical performance. In the present study, we identified an increased chondrocytes number in the OAE group. It is already known that chondrocytes are responsible for tissue maintenance which impact on joint function and motor performance (Sophia Fox et al., 2009; Akkiraju and Nohe, 2015). Thus, we speculate that the largest number of chondrocytes and the lower joint

degradation cytokines level (IL1β and TNF) (Kim et al., 2010; Li et al., 2013, 2015; Rojas-Ortega et al., 2015) might have preserved the proteoglycans and collagen joint which lead to better motor performance of the OAE group compared to the OA group. Based on the results, exercise prevented the increase of inflammatory biomarkers, and, consequently, prevented the loss of chondrocytes (the only marker evaluated). We cannot extrapolate our results to the whole joint, because the number of chondrocytes was the only analysis. This theory needs to be better clarified ahead. Our study is innovative as it points out the benefits of a therapeutic approach to an experimental OA model. Assessing the effect on physical performance of the animals it was demonstrated that the dose-controlled impact is achieved by ways not fully elucidated in this study. The modulation of inflammation, due to the known effects of aerobic training, has affected the redox status balance, lowering oxidative damage, improving motor performance in functional tasks.

A limitation of this study was that we did not determine $_k$OA-like changes according to the OARSI score. However, it was not possible because we used a single hematoxylin and eosin (H&E) staining for analysis. Thus, immunohistochemistry assessments on cartilage tissue would be useful in future studies.

CONCLUSION

To conclude, a moderate-intensity aerobic treadmill training appears to modulate chondrocytes via activation of anabolic paths, swelling control by IL1β and TNF levels modulation, systemic TBARS level lowering and positive regulation in joint BDNF level, resulting in physical and motor performance improvements. As a perspective, the efficacy of the training protocol used here should be investigated in older animals.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This animal study was reviewed and approved by the Commission on Ethics in Animal Use of the Universidade Federal dos Vales do Jequitinhonha e Mucuri (protocol 005/2015). Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

JM, VM, MO, HL, AC, CC, and AL conceived and designed the study. JM, VM, GA, SF, JS, RT-G, DS, MO, HL, AC, AF, CC, JP, and AL contributed to analysis and interpretation of the data. JM, VM, SF, SS, JS, RT-G, DS, TD, and AL drafted the article. JM, VM, SF, MO, HL, AC, AF, CC, JP, MB-F, and AL critically revised the article for important intellectual content. JM, VM, GA, SF, JS, RT-G, DS, MO, HL, AC, AF, CC, JP, VO, MB-F, and AL approved the final article. JM, SF, MO, HL, AC, JP, and AL statistically expertised the study. VM, MO, HL, AC, AF, CC, and AL provisioned the study materials. VM, GA, RT-G, DS, MO, AF, CC, and AL contributed to administrative, technical, or logistic support.

FUNDING

This study was supported in part by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Numbers 408721/2016-2 and 303746/2018-1), the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) (Numbers APQ-02503-16, APQ-01239-14, and APQ-03119-18), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (finance code 001).

ACKNOWLEDGMENTS

We acknowledge Martins (2017) who provided data from her master's degree conducted in the Universidade Federal dos Vales do Jequitinhonha e Mucuri. Besides, the assistance of the Brazilian Society of Physiology is gratefully acknowledged.

REFERENCES

Abruzzo, P., Esposito, F., Marchionni, C., Di Tullio, S., Belia, S., Fulle, S., et al. (2013). Moderate exercise training induces ROS-related adaptations to skeletal muscles. *Int. J. Sports Med.* 34, 676–687. doi: 10.1055/s-0032-1323782

Akkiraju, H., and Nohe, A. (2015). Role of chondrocytes in cartilage formation, progression of osteoarthritis and cartilage regeneration. *J. Dev. Biol.* 3, 177–192. doi: 10.3390/jdb3040177

Altindag, O., Erel, O., Aksoy, N., Selek, S., Celik, H., and Karaoglanoglu, M. (2007). Increased oxidative stress and its relation with collagen metabolism in knee osteoarthritis. *Rheumatol. Int.* 27, 339–344. doi: 10.1007/s00296-006-0247-8

Assis, L., Milares, L., Almeida, T., Tim, C., Magri, A., Fernandes, K., et al. (2016). Aerobic exercise training and low-level laser therapy modulate inflammatory response and degenerative process in an experimental model of knee osteoarthritis in rats. *Osteoarthr. Cartil.* 24, 169–177. doi: 10.1016/j.joca.2015.07.020

Attur, M., Krasnokutsky, S., Statnikov, A., Samuels, J., Li, Z., Friese, O., et al. (2015). Low-grade inflammation in symptomatic knee osteoarthritis: prognostic value of inflammatory plasma lipids and peripheral blood leukocyte biomarkers. *Arthritis Rheumatol.* 67, 2905–2915. doi: 10.1002/art.39279

Attur, M., Krasnokutsky-Samuels, S., Samuels, J., and Abramson, S. B. (2013). Prognostic biomarkers in osteoarthritis. *Curr. Opin. Rheumatol.* 25:136. doi: 10.1097/BOR.0b013e32835a9381

Balthazar, C. H., Leite, L. H., Rodrigues, A. G., and Coimbra, C. C. (2009). Performance-enhancing and thermoregulatory effects of intracerebroventricular dopamine in running rats. *Pharmacol. Biochem. Behav.* 93, 465–469. doi: 10.1016/j.pbb.2009.06.009

Beckett, J., Jin, W., Schultz, M., Chen, A., Tolbert, D., Moed, B. R., et al. (2012). Excessive running induces cartilage degeneration in knee joints and alters gait of rats. *J. Orthop. Res.* 30, 1604–1610. doi: 10.1002/jor.22124

Benzie, I. F., and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* 239, 70–76. doi: 10.1006/abio.1996.0292

Blasioli, D. J., and Kaplan, D. L. (2013). The roles of catabolic factors in the development of osteoarthritis. *Tissue Eng. Part B Rev.* 20, 355–363. doi: 10.1089/ten.teb.2013.0377

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254. doi: 10.1016/0003-2697(76)90527-3

Charlier, E., Relic, B., Deroyer, C., Malaise, O., Neuville, S., Collée, J., et al. (2016). Insights on molecular mechanisms of chondrocytes death in osteoarthritis. *Int. J. Mol. Sci.* 17:2146. doi: 10.3390/ijms17122146

Cifuentes, D., Rocha, L., Silva, L., Brito, A., Rueff-Barroso, C., Porto, L., et al. (2010). Decrease in oxidative stress and histological changes induced by physical exercise calibrated in rats with osteoarthritis induced by monosodium iodoacetate. *Osteoarthr. Cartil.* 18, 1088–1095. doi: 10.1016/j.joca.2010.04.004

Fehrenbacher, A., Steck, E., Rickert, M., Roth, W., and Richter, W. (2003). Rapid regulation of collagen but not metalloproteinase 1, 3, 13, 14 and tissue inhibitor of metalloproteinase 1, 2, 3 expression in response to mechanical loading of cartilage explants in vitro. *Arch. Biochem. Biophys.* 410, 39–47. doi: 10.1016/s0003-9861(02)00658-6

Galois, L., Etienne, S., Grossin, L., Cournil, C., Pinzano, A., Netter, P., et al. (2003). Moderate-impact exercise is associated with decreased severity of experimental osteoarthritis in rats. *Rheumatology* 42, 692–693. doi: 10.1093/rheumatology/keg094

Geneen, L. J., Moore, R. A., Clarke, C., Martin, D., Colvin, L. A., and Smith, B. H. (2017). Physical activity and exercise for chronic pain in adults: an overview of cochrane reviews. *Cochrane Database Syst. Rev.* 1:CD011279.

Germanou, E. I., Chatzinikolaou, A., Malliou, P., Beneka, A., Jamurtas, A. Z., Bikos, C., et al. (2013). Oxidative stress and inflammatory responses following an acute bout of isokinetic exercise in obese women with knee osteoarthritis. *Knee* 20, 581–590. doi: 10.1016/j.knee.2012.10.020

Golightly, Y. M., Allen, K. D., and Caine, D. J. (2012). A comprehensive review of the effectiveness of different exercise programs for patients with osteoarthritis. *Phys. Sports Med.* 40, 52–65. doi: 10.3810/psm.2012.11.1988

Gomes, W. F., Lacerda, A. C. R., Mendonça, V. A., Arrieiro, A. N., Fonseca, S. F., Amorim, M. R., et al. (2012). Effect of aerobic training on plasma cytokines and soluble receptors in elderly women with knee osteoarthritis, in response to acute exercise. *Clin. Rheumatol.* 31, 759–766. doi: 10.1007/s10067-011-1927-7

Goranov, N. V. (2007). Serum markers of lipid peroxidation, antioxidant enzymatic defense, and collagen degradation in an experimental (Pond-Nuki) canine model of osteoarthritis. *Vet. Clin. Pathol.* 36, 192–195. doi: 10.1111/j.1939-165x.2007.tb00208.x

Guzman, R. E., Evans, M. G., Bove, S., Morenko, B., and Kilgore, K. (2003). Mono-iodoacetate-induced histologic changes in subchondral bone and articular cartilage of rat femorotibial joints: an animal model of osteoarthritis. *Toxicol. Pathol.* 31, 619–624. doi: 10.1080/714044694

Henrotin, Y., Bruckner, P., and Pujol, J.-P. (2003). The role of reactive oxygen species in homeostasis and degradation of cartilage. *Osteoarthr. Cartil.* 11, 747–755. doi: 10.1016/s1063-4584(03)00150-x

Hill, R. J., Mason, H. M., Yeip, G., Merchant, S. S., Olsen, A. L., Stott, R. D., et al. (2017). The influence of oblique angle forced exercise in surgically destabilized stifle joints is synergistic with bone, but antagonistic with cartilage in an ovine model of osteoarthritis. *Arthritis* 2017:7481619. doi: 10.1155/2017/7481619

Hui, W., Young, D. A., Rowan, A. D., Xu, X., Cawston, T. E., and Proctor, C. J. (2016). Oxidative changes and signalling pathways are pivotal in initiating age-related changes in articular cartilage. *Ann. Rheum. Dis.* 75, 449–458. doi: 10.1136/annrheumdis-2014-206295

Hunter, D. J., and Eckstein, F. (2009). Exercise and osteoarthritis. *J. Anat.* 214, 197–207.

Hutchison, M. R. (2012). BDNF alters ERK/p38 MAPK activity ratios to promote differentiation in growth plate chondrocytes. *Mol. Endocrinol.* 26, 1406–1416. doi: 10.1210/me.2012-1063

Iijima, H., Aoyama, T., Ito, A., Yamaguchi, S., Nagai, M., Tajino, J., et al. (2015). Effects of short-term gentle treadmill walking on subchondral bone in a rat model of instability-induced osteoarthritis. *Osteoarthr. Cartil.* 23, 1563–1574. doi: 10.1016/j.joca.2015.04.015

Jimson, S., Balachander, N., Masthan, K., and Elumalai, R. (2014). A comparative study in bone decalcification using different decalcifying agents. *Int. J. Sci. Res.* 3, 1226–1229.

Kim, J., Xu, M., Xo, R., Mates, A., Wilson, G., Pearsall, A., et al. (2010). Mitochondrial DNA damage is involved in apoptosis caused by pro-inflammatory cytokines in human OA chondrocytes. *Osteoarthr. Cartil.* 18, 424–432. doi: 10.1016/j.joca.2009.09.008

Knobloch, T. J., Madhavan, S., Nam, J., Agarwal, S. Jr., and Agarwal, S. (2008). Regulation of chondrocytic gene expression by biomechanical signals. *Crit. Rev. Eukaryot. Gene Expr.* 18:139. doi: 10.1615/critreveukargeneexpr.v18.i2.30

Koike, M., Nojiri, H., Ozawa, Y., Watanabe, K., Muramatsu, Y., Kaneko, H., et al. (2015). Mechanical overloading causes mitochondrial superoxide and SOD2 imbalance in chondrocytes resulting in cartilage degeneration. *Sci. Rep.* 5:11722. doi: 10.1038/srep11722

Kunisch, E., Kinne, R. W., Alsalameh, R. J., and Alsalameh, S. (2016). Pro-inflammatory IL-1beta and/or TNF-alpha up-regulate matrix metalloproteases-1 and-3 m RNA in chondrocyte subpopulations potentially pathogenic in osteoarthritis: in situ hybridization studies on a single cell level. *Int. J. Rheum. Dis.* 19, 557–566. doi: 10.1111/1756-185X.12431

Lacerda, A. C. R., Marubayashi, U., Balthazar, C. H., Leite, L. H., and Coimbra, C. C. (2006). Central nitric oxide inhibition modifies metabolic adjustments induced by exercise in rats. *Neurosci. Lett.* 410, 152–156. doi: 10.1016/j.neulet.2006.09.067

Leong, D. J., Gu, X. I., Li, Y., Lee, J. Y., Laudier, D. M., Majeska, R. J., et al. (2010). Matrix metalloproteinase-3 in articular cartilage is upregulated by joint immobilization and suppressed by passive joint motion. *Matrix Biol.* 29, 420–426. doi: 10.1016/j.matbio.2010.02.004

Li, Y., Frank, E. H., Wang, Y., Chubinskaya, S., Huang, H.-H., and Grodzinsky, A. J. (2013). Moderate dynamic compression inhibits pro-catabolic response of cartilage to mechanical injury, TNF-α and IL-6, but accentuates degradation above a strain threshold. *Osteoarthr. Cartil.* 21, 933–941.

Li, Z., Liu, S.-Y., Xu, L., Xu, S.-Y., and Ni, G.-X. (2017). Effects of treadmill running with different intensity on rat subchondral bone. *Sci. Rep.* 7:1977. doi: 10.1038/s41598-017-02126-z

Li, Z.-C., Han, N., Li, X., Li, G., Liu, Y.-Z., Sun, G.-X., et al. (2015). Decreased expression of microRNA-130a correlates with TNF-α in the development of osteoarthritis. *Int. J. Clin. Exp. Pathol.* 8:2555.

Little, C. B., and Ghosh, P. (1997). Variation in proteoglycan metabolism by articular chondrocytes in different joint regions is determined by post-natal mechanical loading. *Osteoarthr. Cartil.* 5, 49–62. doi: 10.1016/s1063-4584(97)80031-3

Loeser, R. F. (2010). Age-related changes in the musculoskeletal system and the development of osteoarthritis. *Clin. Geriatr. Med.* 26, 371–386. doi: 10.1016/j.cger.2010.03.002

Loeser, R. F., Goldring, S. R., Scanzello, C. R., and Goldring, M. B. (2012). Osteoarthritis: a disease of the joint as an organ. *Arthritis Rheum.* 64, 1697–1707. doi: 10.1002/art.34453

Mabey, T., and Honsawek, S. (2015). Cytokines as biochemical markers for knee osteoarthritis. *World J. Orthop.* 6:95. doi: 10.5312/wjo.v6.i1.95

Martins, J. B. (2017). *Treinamento Físico Moderado Em Esteira Modula Biomarcadores Articulares E Melhora O Desempenho Funcional Em Ratos Com Osteoartrite De Joelho Induzida*. Ph.D. thesis, Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, MG.

Mohammadi, M. F., Moghaddam, A. H., and Mirkarimpur, H. (2013). The effects of a moderate exercise program on knee osteoarthritis in male wistar rats. *Ir. J. Basic Med. Sci.* 16:683.

Na, S. S., Kim, S. G., Yong, M. S., and Hwangbo, G. (2014). Study of treadmill exercise effect on rats with osteoarthritis using proteomic analysis. *J. Phys. Ther. Sci.* 26, 487–490. doi: 10.1589/jpts.26.487

Ni, G.-X., Lei, L., and Zhou, Y.-Z. (2012). Intensity-dependent effect of treadmill running on lubricin metabolism of rat articular cartilage. *Arthritis Res. Ther.* 14:R256. doi: 10.1186/ar4101

Ni, G.-X., Liu, S.-Y., Lei, L., Li, Z., Zhou, Y.-Z., and Zhan, L.-Q. (2013). Intensity-dependent effect of treadmill running on knee articular cartilage in a rat model. *BioMed. Res. Int.* 2013:172392. doi: 10.1155/2013/172392

Nofuji, Y., Suwa, M., Moriyama, Y., Nakano, H., Ichimiya, A., Nishichi, R., et al. (2008). Decreased serum brain-derived neurotrophic factor in trained men. *Neurosci. Lett.* 437, 29–32. doi: 10.1016/j.neulet.2008.03.057

O'Hara, B., Urban, J., and Maroudas, A. (1990). Influence of cyclic loading on the nutrition of articular cartilage. *Ann. Rheum. Dis.* 49, 536–539. doi: 10.1136/ard.49.7.536

Ohkawa, H., Ohishi, N., and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95, 351–358. doi: 10.1016/0003-2697(79)90738-3

Ostalowska, A., Birkner, E., Wiecha, M., Kasperczyk, S., Kasperczyk, A., Kapolka, D., et al. (2006). Lipid peroxidation and antioxidant enzymes in synovial fluid of patients with primary and secondary osteoarthritis of the knee joint. *Osteoarthr. Cartil.* 14, 139–145. doi: 10.1016/j.joca.2005.08.009

Park, S., Hung, C., and Ateshian, G. (2004). Mechanical response of bovine articular cartilage under dynamic unconfined compression loading at physiological stress levels. *Osteoarthr. Cartil.* 12, 65–73. doi: 10.1016/j.joca.2003.08.005

Piel, M. J., Kroin, J. S., van Wijnen, A. J., Kc, R., and Im, H.-J. (2014). Pain assessment in animal models of osteoarthritis. *Gene* 537, 184–188. doi: 10.1016/j.gene.2013.11.091

Poole, A., Kobayashi, M., Yasuda, T., Laverty, S., Mwale, F., Kojima, T., et al. (2002). Type II collagen degradation and its regulation in articular cartilage in osteoarthritis. *Ann. Rheum. Dis.* 61(Suppl. 2), ii78–ii81.

Primola-Gomes, T. N., Campos, L. A., Lauton-Santos, S., Balthazar, C. H., Guatimosim, S., Capettini, L. S., et al. (2009). Exercise capacity is related to calcium transients in ventricular cardiomyocytes. *J. Appl. Physiol.* 107, 593–598. doi: 10.1152/japplphysiol.91218.2008

Quinn, T. M., Grodzinsky, A. J., Buschmann, M. D., Kim, Y.-J., and Hunziker, E. B. (1998). Mechanical compression alters proteoglycan deposition and matrix deformation around individual cells in cartilage explants. *J. Cell Sci.* 111, 573–583.

Reed, K. N., Wilson, G., Pearsall, A., and Grishko, V. I. (2014). The role of mitochondrial reactive oxygen species in cartilage matrix destruction. *Mol. Cell. Biochem.* 397, 195–201. doi: 10.1007/s11010-014-2187-z

Regan, E., Flannelly, J., Bowler, R., Tran, K., Nicks, M., Carbone, B. D., et al. (2005). Extracellular superoxide dismutase and oxidant damage in osteoarthritis. *Arthritis Rheum.* 52, 3479–3491. doi: 10.1002/art.21387

Rios, J. L., Boldt, K. R., Mather, J. W., Seerattan, R. A., Hart, D. A., and Herzog, W. (2018). Quantifying the effects of different treadmill training speeds and durations on the health of rat knee joints. *Sports Med. Open* 4:15. doi: 10.1186/s40798-018-0127-2

Rojas-Ortega, M., Cruz, R., Vega-López, M. A., Cabrera-González, M., Hernández-Hernández, J. M., Lavalle-Montalvo, C., et al. (2015). Exercise modulates the expression of IL-1β and IL-10 in the articular cartilage of normal and osteoarthritis-induced rats. *Pathol. Res. Pract.* 211, 435–443. doi: 10.1016/j.prp.2015.01.008

Roman-Blas, J. A., Contreras-Blasco, M. A., Largo, R., Álvarez-Soria, M. A., Castañeda, S., and Herrero-Beaumont, G. (2009). Differential effects of the antioxidant N-acetylcysteine on the production of catabolic mediators in IL-1β-stimulated human osteoarthritic synoviocytes and chondrocytes. *Eur. J. Pharmacol.* 623, 125–131. doi: 10.1016/j.ejphar.2009.09.016

Rose, J., Söder, S., Skhirtladze, C., Schmitz, N., Gebhard, P., Sesselmann, S., et al. (2012). DNA damage, discoordinated gene expression and cellular senescence in osteoarthritic chondrocytes. *Osteoarthr. Cartil.* 20, 1020–1028. doi: 10.1016/j.joca.2012.05.009

Semanik, P. A., Chang, R. W., and Dunlop, D. D. (2012). Aerobic activity in prevention and symptom control of osteoarthritis. *PM R* 4, S37–S44. doi: 10.1016/j.pmrj.2012.02.015

Simão, A. P., Mendonça, V. A., de Oliveira Almeida, T. M., Santos, S. A., Gomes, W. F., Coimbra, C. C., et al. (2014). Involvement of BDNF in knee osteoarthritis: the relationship with inflammation and clinical parameters. *Rheumatol. Int.* 34, 1153–1157. doi: 10.1007/s00296-013-2943-5

Sophia Fox, A. J., Bedi, A., and Rodeo, S. A. (2009). The basic science of articular cartilage: structure, composition, and function. *Sports Health* 1, 461–468. doi: 10.1177/1941738109350438

Szuhany, K. L., Bugatti, M., and Otto, M. W. (2015). A meta-analytic review of the effects of exercise on brain-derived neurotrophic factor. *J. Psychiatr. Res.* 60, 56–64. doi: 10.1016/j.jpsychires.2014.10.003

Takahashi, I., Matsuzaki, T., Kuroki, H., and Hoso, M. (2018). Induction of osteoarthritis by injecting monosodium iodoacetate into the patellofemoral joint of an experimental rat model. *PLoS One* 13:e0196625. doi: 10.1371/journal.pone.0196625

Tilwani, R., Vessillier, S., Pingguan-Murphy, B., Lee, D., Bader, D., and Chowdhury, T. (2017). Oxygen tension modulates the effects of TNFα in compressed chondrocytes. *Inflamm. Res.* 66, 49–58. doi: 10.1007/s00011-016-0991-5

Urban, J. (1994). The chondrocyte: a cell under pressure. *Rheumatology* 33, 901–908. doi: 10.1093/rheumatology/33.10.901

Watari, T., Naito, K., Sakamoto, K., Kurosawa, H., Nagaoka, I., and Kaneko, K. (2011). Evaluation of the effect of oxidative stress on articular cartilage in spontaneously osteoarthritic STR/OrtCrlj mice by measuring the biomarkers for oxidative stress and type II collagen degradation/synthesis. *Exp. Ther. Med.* 2, 245–250. doi: 10.3892/etm.2011.196

Wojdasiewicz, P., Poniatowski, ŁA., and Szukiewicz, D. (2014). The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of osteoarthritis. *Mediators Inflamm.* 2014:561459. doi: 10.1155/2014/561459

Yamabe, S., Hirose, J., Uehara, Y., Okada, T., Okamoto, N., Oka, K., et al. (2013). Intracellular accumulation of advanced glycation end products induces apoptosis via endoplasmic reticulum stress in chondrocytes. *FEBS J.* 280, 1617–1629. doi: 10.1111/febs.12170

Yarrow, J. F., White, L. J., McCoy, S. C., and Borst, S. E. (2010). Training augments resistance exercise induced elevation of circulating brain derived neurotrophic factor (BDNF). *Neurosci. Lett.* 479, 161–165. doi: 10.1016/j.neulet.2010.05.058

Yudoh, K., van Trieu, N., Nakamura, H., Hongo-Masuko, K., Kato, T., and Nishioka, K. (2005). Potential involvement of oxidative stress in cartilage senescence and development of osteoarthritis: oxidative stress induces chondrocyte telomere instability and downregulation of chondrocyte function. *Arthritis Res. Ther.* 7:R380.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

STUDY PROTOCOL
published: 22 October 2019
doi: 10.3389/fmed.2019.00236

A Randomized Clinical Trial Comparing Three Different Exercise Strategies for Optimizing Aerobic Capacity and Skeletal Muscle Performance in Older Adults: Protocol for the DART Study

Dallin Tavoian[1], David W. Russ[1,2], Timothy D. Law[1], Janet E. Simon[3], Paul J. Chase[4], Emily Hill Guseman[5,6] and Brian C. Clark[1,7]**

[1] Ohio Musculoskeletal and Neurological Institute, Ohio University, Athens, OH, United States, [2] Laboratory for Integrative Muscle Biology, Division of Physical Therapy, Ohio University, Athens, OH, United States, [3] Division of Athletic Training, School of Applied Health Sciences and Wellness, Ohio University, Athens, OH, United States, [4] Division of Exercise Physiology, School of Applied Health Sciences and Wellness, Ohio University, Athens, OH, United States, [5] Diabetes Institute, Ohio University, Athens, OH, United States, [6] Department of Family Medicine, Heritage College of Osteopathic Medicine, Ohio University, Athens, OH, United States, [7] Department of Biomedical Sciences, Ohio University, Athens, OH, United States

OPEN ACCESS

Edited by:
Emanuele Marzetti, Agostino Gemelli University Polyclinic, Italy

Reviewed by:
Renato Gorga Bandeira De Mello, Federal University of Rio Grande Do Sul, Brazil
Hélio José Coelho Júnior, Campinas State University, Brazil

***Correspondence:**
Dallin Tavoian
dt114412@ohio.edu
Brian C. Clark
clarkb2@ohio.edu

Specialty section:
This article was submitted to Geriatric Medicine, a section of the journal Frontiers in Medicine

Received: *08 July 2019*
Accepted: *07 October 2019*
Published: *22 October 2019*

Citation:
Tavoian D, Russ DW, Law TD, Simon JE, Chase PJ, Guseman EH and Clark BC (2019) A Randomized Clinical Trial Comparing Three Different Exercise Strategies for Optimizing Aerobic Capacity and Skeletal Muscle Performance in Older Adults: Protocol for the DART Study. Front. Med. 6:236. doi: 10.3389/fmed.2019.00236

Background: Age-related declines in physical function lead to decreased independence and higher healthcare costs. Individuals who meet the endurance and resistance exercise recommendations can improve their physical function and overall fitness, even into their ninth decade. However, most older adults do not exercise regularly, and the majority of those who do only perform one type of exercise, and in doing so are not getting the benefits of endurance or resistance exercise. Herein we present the study protocol for a randomized clinical trial that will investigate the potential for high-intensity interval training (HIIT) to improve maximal oxygen consumption, muscular power, and muscle volume (primary outcomes), as well as body composition, 6-min walk distance, and muscular strength and endurance (secondary outcomes).

Methods and Analysis: This is a single-site, single-blinded, randomized clinical trial. A minimum of 24 and maximum of 30 subjects aged 60–75 that are generally healthy but insufficiently active will be randomized. After completion of baseline assessments, participants will be randomized in a 1:1:1 ratio to participate in one of three 12-week exercise programs: stationary bicycle HIIT, stationary bicycle moderate-intensity continuous training (MICT), or resistance training. Repeat assessments will be taken immediately post intervention.

Discussion: This study will examine the potential for stationary bicycle HIIT to result in both cardiorespiratory and muscular adaptations in older adults. The results will provide important insights into the effectiveness of interval training, and potentially support a shift from volume-driven to intensity-driven exercise strategies for older adults.

Clinical Trial Registration: This trial is registered with ClinicalTrials.gov (registration number: NCT03978572, date of registration June 7, 2019).

Keywords: aging, exercise, intervals, resistance, aerobic, power, VO_2, muscle

INTRODUCTION

Nearly half of US adults over age 60 report difficulty performing one or more activities of daily living essential to maintaining independence, a fraction that has remained stable over the last 20 years despite an overall increase in the mean age of the US population (1). Increasing numbers of older adults with disabilities will continue to drive up healthcare costs, making maintenance of health and independence a top priority for both middle-aged and older adults (2). Contributors to this functional decline include poor cardiorespiratory fitness and skeletal muscle impairments (3–5), which can partially be attributed to reduced physical activity with age (6–8). Exercise is effective at maintaining function in older adults, as evidenced by data indicating those who take part in supervised exercise programs demonstrate improvements in functional outcomes (9–11). The fact that adaptations have been seen in the oldest adults (90+ years) is particularly encouraging (12–14).

Unfortunately, <12% of older adults meet the exercise recommendations provided by the American College of Sports Medicine (ACSM) (1, 15). These recommendations include weekly accumulation of at least 150 min of moderate intensity aerobic activity or 75 min of vigorous aerobic (endurance) activity, and at least 2 days of resistance exercise to improve muscular endurance, power, and strength (15). In 2014, 36.5% of adults over the age of 65 met the aerobic exercise recommendations, while only 16.5% met the resistance exercise recommendations; only 11.7% met both aerobic and resistance recommendations simultaneously (1). Consequently, the majority of older adults who do exercise are either not getting the muscular adaptations necessary for maintaining independence, or they are not getting the necessary cardiorespiratory adaptations. However, certain types of exercise may be able to induce both cardiorespiratory and muscular adaptations in older adults.

Resistance exercise appears to have minor effects on cardiovascular disease (CVD) risk factors, though its benefits include the prevention of musculoskeletal injuries, muscle wasting, and impairments in physical function (16). On the other hand, aerobic exercise is effective at reducing CVD risk by improving heart, lung, and metabolic function, though it appears to have little effect on muscular properties (17). However, recent work indicates that the absence of muscular adaptations in response to aerobic training may be related to exercise intensity and mode of exercise (18, 19). The most popular form of physical activity in older adults is walking, and recommendations for older adults promote walking as the primary means to increase physical activity levels (15, 20, 21). While walking may challenge older adults and result in cardiovascular improvements, the relatively low intensity of muscular contractions would be unlikely to elicit hypertrophy or functional adaptations of the leg muscles (22). Even higher intensity running does not seem to result in increased muscle size or strength (19, 23), and running may blunt the muscular benefits of resistance training when performed concurrently (24). In contrast, stationary bicycle training can increase muscular strength and size (25–27), and does not seem to interfere with the muscular adaptations to resistance training (24). Bicycle high-intensity interval training (HIIT) in particular may be the ideal form of aerobic exercise able to elicit muscular adaptations. Young adults performing bicycle HIIT demonstrate improved muscle strength (28) and power (29, 30), along with enhanced cardiorespiratory function (31).

HIIT has gained popularity in recent years, with most research in middle-aged and older cardiac rehabilitation patients (32, 33). Compared to traditional aerobic training, HIIT typically has a greater effect on VO_2max (9.1% greater increase on average) and other CVD risk factors (e.g., cholesterol, blood pressure) (33). While its success in rehabilitation is encouraging, the effectiveness of HIIT as a general exercise strategy for older adults has not been adequately investigated, with few studies reporting physiological adaptations to HIIT in older adults (33–44). Furthermore, limitations make it difficult to generalize these studies to a healthy aging population. Specifically, these studies have had one or more of the following methodological confounds:

1. They lacked an active control group that followed established exercise recommendations.
2. They were short-term interventions.
3. The target population had overt diseases/health conditions, which may limit generalizability to the large number of older adults that are generally healthy.

The lack of investigations that directly compare unique exercise strategies against established strategies represents a critical barrier to progress in the field of healthy aging. With this in mind, the DART Study (Dual-benefits of Aerobic and Resistance Training) is a phase 1B proof-of-concept, proof-of mechanism clinical trial that seeks to determine if bicycle HIIT is a more efficient standalone strategy to improve cardiovascular and lower extremity muscular function than established resistance or aerobic exercise training programs. We will test this "dual-benefits hypothesis" in generally healthy older adults that have yet to develop mobility limitations, but who, without intervention, could very likely develop mobility limitations in the future due to inactivity and normal time course of aging.

METHODS AND ANALYSIS

Design

This is a single-blinded (outcomes assessor), single-site, randomized control trial. It is a three (group) by two (time)

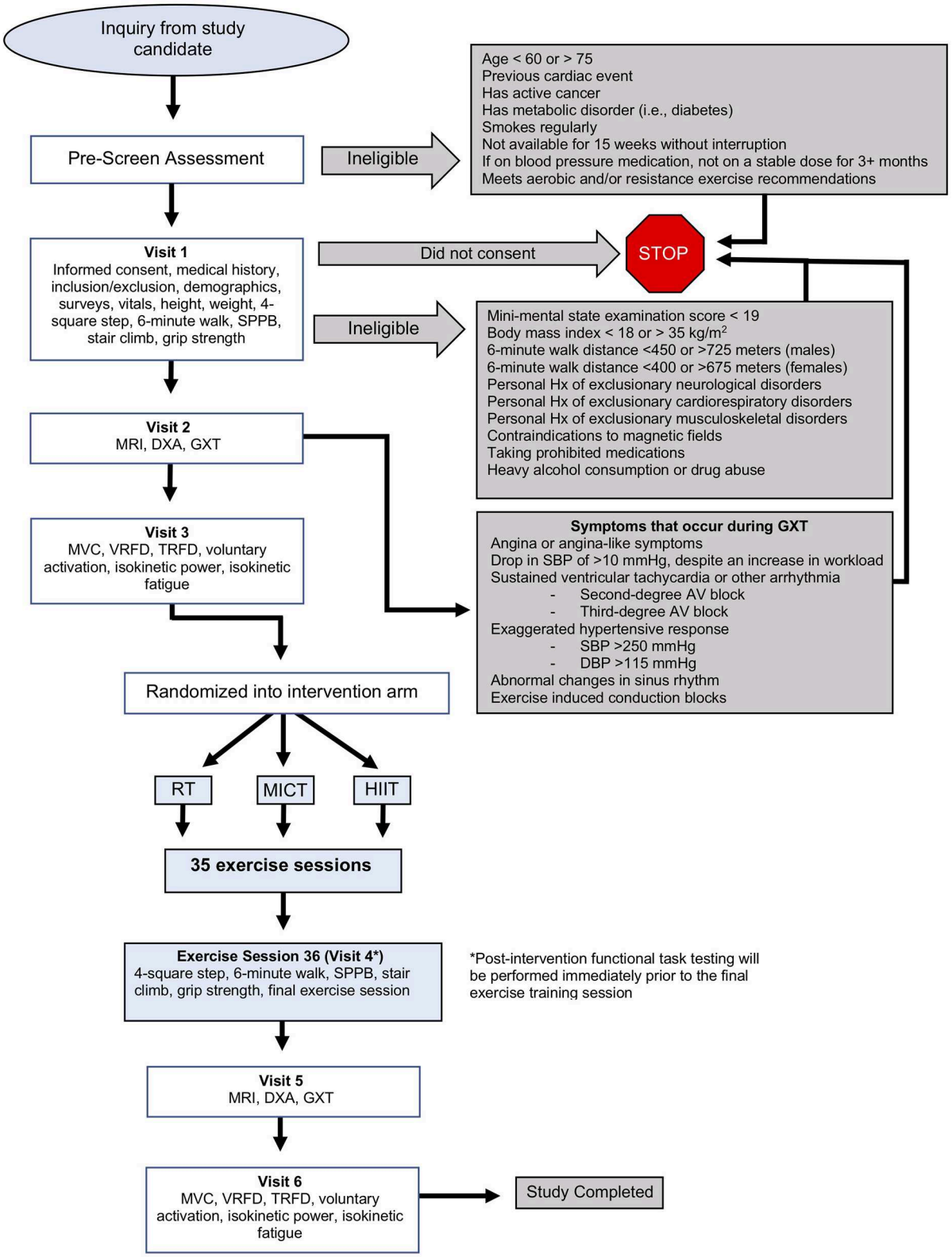


FIGURE 1 | Detailed overview of DART Study protocol. DBP, diastolic blood pressure; DXA, dual-energy X-ray absorptiometry; GXT, graded exercise test; HIIT, high-intensity interval training; Hx, history; MICT, moderate-intensity continuous training; MRI, magnetic resonance imaging; RT, resistance training; SBP, systolic blood pressure; SPPB, short physical performance battery; TRFD, twitch-evoked rate of force development; VRFD, voluntary rate of force development.

repeated measures factorial design. The overall study is illustrated in **Figure 1**.

Study Subjects

A minimum of 24 (and up to 30) subjects aged 60–75 years of age that are generally healthy but sedentary will be recruited, enrolled, and randomized in this study (n = 8–10 per group). Potential subjects will be recruited from the local community by way of flyers, community events, or emails to individuals who have previously participated in studies with the Ohio Musculoskeletal and Neurological Institute at Ohio University in Athens, Ohio, USA. All interested individuals will complete a pre-screening phone interview, and all that are not ruled ineligible will be invited for in-person screening. Written informed consent will be obtained from each subject in accordance with the Declaration of Helsinki. Ethical Approval for this study has been obtained from the Ohio University Institutional Review Board (protocol number 18-F-55).

Eligible subjects will be insufficiently active. We define "insufficiently active" as not meeting either the endurance or resistance exercise recommendations for older adults, as set forth by the American College of Sports Medicine (ACSM), for three consecutive months. The current ACSM recommendations for endurance training are 150–300 min per week of moderate-intensity activity (perceived exertion of 5–6 on 0–10 scale), or 75–150 min per week of vigorous-intensity activities (perceived exertion of 7–8 on 0–10 scale) (15). The recommendations for resistance training are at least 2 days per week of progressive weight training activities that use the major muscle groups (perceived exertion of 5–8 on 0–10 scale) (15). Additionally, the subjects must not be highly active outside of a structured exercise program (i.e., consistent hard physical labor). They must also have a 6-min walk distance within a normal range for adults aged 60–75. We calculated a normal 6-min walk range by averaging 6-min walk values from multiple studies, plus or minus one standard deviation (45–48). The calculated 6-min walk range for females was 400–675 meters, and for males was 450–725 meters. **Table 1** describes inclusion and exclusion criteria in detail. These criteria are designed to recruit a generally healthy, but insufficiently active population, and exclude individuals with poor health and physical function where there could be concerns about a subject's ability to appropriately perform the testing and exercise prescription.

Randomization

Subjects will be randomized in a 1:1:1 ratio to receive a 12-week exercise program consisting of either resistance training (RT), moderate-intensity continuous training (MICT) on a stationary bicycle, or HIIT on a stationary bicycle. Due to the small sample size in each arm, permuted-block randomization will be used via computer-generated random numbers to ensure equal sample size. Specifically, we will create blocks of three with each treatment permuted within each block. The study subjects will be enrolled and assigned to their respective interventions by an unblinded project manager.

Implementation

The allocation sequence will be generated by a biostatistician. Subjects will be enrolled in the study and assigned to their intervention group by the project manager.

Blinding

Due to the nature of the intervention neither subjects nor staff can be blinded to allocation. The outcomes assessor and data analyst will be blinded after study completion by having the subjects' demographic and intervention group information coded.

Sample Size

In this proof-of-concept, proof-of-mechanism trial, we report sample size estimates based on previous recommended literature (49). A sample size of ∼8 subjects per group will detect a moderate effect. Sample size calculation was based on expected effect sizes for the HIIT cycle, MICT cycle, and RT groups for the primary outcomes of $V0_2$max and thigh muscle CSA. Consistent with our "dual benefits hypothesis" we assumed, based on the literature, an 8% increase in thigh CSA for both the HIIT and RT groups (50) and 2% increase for the MICT group (51) and common SD across all groups of 4%. With the assumption of a 2-sided test and alpha level of significance equal to 0.05 an n = 7/group yields power of 0.83. With respect to VO_2max we assumed a 32% increase in the HIIT group, a 15% increase in the MICT group, and a 10% increase in the RT group and common SD across all groups of 14% (52). With the assumption of a 2-sided test and alpha level of significance equal to 0.05 an n = 8/group yields power of 0.80. In line with other proof-of-concept, proof-of-mechanism trials, no statistical control for type-I error from multiple comparisons will be considered, and p-values will be interpreted with care, as descriptive weights of evidence rather than as confirmatory claims. Lastly, this proof of concept, proof of mechanism trial is needed to test the complex interventions proposed, and the effect sizes calculated from this trial could be used to estimate sample size for a future large-scale clinical trial. Accordingly, we plan to enroll an n = 8–10 subjects per group.

Study Timeline

This study will have a screening/baseline assessment period of 21 days (maximum) with three sessions spaced at least 48 h apart, a 12-week exercise training period, and a post-intervention assessment period of 10 days (maximum) with two sessions spaced at least 48 h apart. Subjects will visit Ohio University's Clinical and Translational Research Unit facilities prior to the intervention for baseline assessments. During Visit 1 we will obtain informed consent and conduct a full medical history screening and a short physical performance battery (SPPB) (53) to determine if candidates meet the inclusion/exclusion criteria. Subjects who meet the criteria will be enrolled in the study and complete a series of clinical and physiological outcome measures over the three baseline visits. Upon completion of baseline assessments, subjects will be randomized into one of the

TABLE 1 | Inclusion and exclusion criteria.

Inclusion Criteria:
- Age 60–75 years with no significant health issues or conditions that, in the investigators' opinion, would limit the subject's ability to complete the study per protocol or that would impact the capability to get an accurate measurement of study endpoints.
- Body mass index between 18 and 40 kg/m^2.
- Willingness to maintain current diet and adhere to the intervention programs described for the study and willing to undergo all testing procedures.
- Able to read, understand, and complete study-related questionnaires.
- Able to read and understand, and willing to sign the informed consent form (ICF).
- Six-min walk distance of 450–725 meters for men and 400–675 meters for women.

Exclusion Criteria:
- Short physical performance batter (SPPB) score < 8.
- Any activities of daily living disability (difficulty feeding, dressing, continence, bathing, toileting, and transferring).
- Lives in a nursing home or assisted living facility.
- Known neuromuscular or neurological conditions affecting somatosensory or motor function or control (e.g., hemiplegia, multiple sclerosis, peripheral neuropathy, Parkinson's disease, Myasthenia Gravis, Ataxia, Apraxia, post-polio syndrome, mitochondrial myopathy, etc.).
- Unable to communicate because of severe hearing loss or speech disorder.
- Severe visual impairment, which would preclude completion of the assessments.
- Cancer requiring treatment currently or in the past 2 years (except primary non-melanoma skin cancer or *in situ* cervical cancer).
- Hospitalization (medical confinement for 24 h), or immobilization, or major surgical procedure requiring general anesthesia within 12 weeks prior to screening, or any planned surgical procedures during the study period.
- Chronic or relapsing/remitting gastrointestinal disorders such as inflammatory bowel disease and irritable bowel syndrome.
- Known history of human immunodeficiency virus (HIV) antibody at screening.
- Use of systemic glucocorticoids.
- Any history of angina pectoris.
- Any history of heart failure.
- Any history of myocardial infarction.
- Any coronary artery bypass graft or percutaneous coronary intervention.
- Heart disease that limits exercise (valvular, congenital, ischemic, and hypertrophic cardiomyopathy).
- Complex ventricular arrhythmias or heart block.
- Chronic obstructive pulmonary disease, cerebrovascular disease, or peripheral vascular disease.
- Diabetes mellitus
- Severe neuropathy.
- Mini-mental state exam score below 19.
- Psychiatric conditions that warrant acute or chronic therapeutic intervention (e.g., major depressive disorder, bipolar disorder, panic disorder, schizophrenia) that in the investigators' opinion may interfere with the conduct of study procedures.
- Unable to undergo magnetic resonance imaging (MRI) (e. g., body containing any metallic medical devices or equipment, including heart pacemakers, metal prostheses, implants or surgical clips, any prior injury from shrapnel or grinding metal, exposure to metallic dusts, metallic shavings or having tattoos containing metallic dyes).
- Unable to reliably undergo exercise or strength tests described for this study.
- Participation in progressive resistance exercise 2 or more days/week for most weeks over the 24 weeks prior to screening, OR 150+ min of accumulated aerobic exercise each week for most weeks over the 24 weeks prior to screening.
- Current self-reported activity level that, in the investigators' opinion, is considered highly active for older adults.
- Participation in any clinical trial within 12 weeks prior to screening.
- Limb amputation (except for toes).
- Bone fracture within 24 weeks prior to screening.
- Any disorder that will not allow completion of the motions required for resistance or aerobic exercise.
- Conditions (such as myasthenia gravis, myositis, muscular dystrophy, or myopathy, including drug-induced myopathy) leading to muscle loss, muscle weakness, muscle cramps, or myalgia.
- Acute viral or bacterial upper or lower respiratory infection at screening.
- Abnormal or uncontrolled blood pressure (BP) at the screening visit defined as BP $>$ 170/100 mmHg. If taking anti-hypertensive medication, have to be on stable doses of medication for more than 3 months.
- Current or recent history (within 1 year of screen) of heavy alcohol consumption or drug abuse that in the investigators' opinion may interfere with the conduct of study procedures.
- Reports being pregnant, lactating, or that they anticipate becoming pregnant in the next 3-months. If a woman becomes pregnant while on study protocol, they will be withdrawn from the study.

Prohibited Medications: Medications that, in the PIs opinion, would confound study integrity by interacting with study outcomes. For instance:
- Anti-obesity drugs, nutraceuticals, and dietary supplements that may affect body mass and body composition.
- Any drug or supplement known to influence muscle mass or performance including but not limited to anabolic steroids, insulin-like growth factor 1, growth hormone, replacement androgen therapy, anti-androgen therapy.

three exercise groups for the 12-week exercise intervention. All exercises will be performed on site and supervised by an exercise professional 3 days per week. All baseline assessments will be repeated upon completion of the exercise intervention. A table of events for the study is illustrated in **Table 2**.

Outcome Measures

Primary Outcomes

Knee extensor isokinetic power

Maximal isokinetic power will be measured from the knee extensors. Peak torque will be recorded from the non-dominant

TABLE 2 | Schedule of events for all groups.

	Baseline period			Exercise intervention		Follow-up	
	Visit 1[c]	**Visit 2[c]**	**Visit 3[c]**	**Sessions 1–35[a]**	**Session 36 (visit 4)[b]**	**Visit 5[d]**	**Visit 6[d]**
Day (window)	−21 to −5	−19 to −3	−17 to −1	1 to 84	80 to 83	84 to 92	86 to 94
SCREENING/BASELINE:							
Informed consent	X						
Medical history	X						
Inclusion/exclusion	X						
Demographics	X						
Vitals	X						
Height and weight	X						
SURVEYS:							
PASE	X						
MMSE	X						
SEE	X						
EXERCISE SESSIONS:							
Exercise				X	X		
FUNCTIONAL TASKS:							
4SST	X				X		
Six-min walk	X				X		
SPPB	X				X		
Stair climb	X				X		
Grip strength	X				X		
MEDICAL IMAGING:							
MRI		X				X	
DXA		X				X	
CARDIORESPIRATORY:							
GXT		X				X	
MUSCULAR TESTING:							
MVC			X				X
Ballistic			X				X
Twitch force			X				X
VA			X				X
Isokinetic power			X				X
Isokinetic fatigue			X				X
RANDOMIZATION			X				

[a] *Exercise training sessions will be performed three times per week with at least 1 day between sessions, and no more than two exercise sessions on consecutive days in the same week.*
[b] *Post-intervention functional task testing will be performed immediately prior to the final exercise training session (session 36).*
[c] *Baseline testing will be completed within 21 days of Visit 1, with at least 2 days between testing sessions.*
[d] *Post-intervention testing will be completed within 10 days of the final exercise session, with at least 2 days between testing sessions. 4SST, 4-square step test; DXA, dual-energy X-ray Absorptiometry; GXT, graded exercise test; MMSE, mini-mental state examination; MRI, magnetic resonance imaging; MVC, maximal voluntary contraction; PASE, physical activity scale for the elderly; SEE, self-efficacy for exercise scale; SPPB, short physical performance battery; VA, voluntary activation.*

leg using a Biodex System 4 Dynamometer (Biodex Medical Systems, Inc., Shirley, NY). The subject's leg will be immobilized against the lever arm with the distal end of the lever arm secured three inches superior to the medial malleolus. The axis of the lever arm will be centered at the lateral knee joint and knee range of motion will be obtained by having the subject extend their knee as far as possible against the lever arm. The maximal knee extension angle will be recorded, and the knee extension limit will be set 10° less than the maximal knee extension angle. The subject will then relax to allow the leg to return to neutral position and the knee flexion limit will be set at 80° of knee flexion. The speed of the lever arm will be set at 60°/s for both extension and flexion. The subject will then extend the knee with maximal effort until they reach the knee extension limit, and then immediately flex the knee with maximal effort until they reach the knee flexion limit. The time-series torque signal will be collected at 500 Hz by a Biopac MP150 system (Biopac Systems Inc., Santa Barbara, CA, USA). The subject will complete six isokinetic trials, with 30 s rest between trials. The average of the three highest peak torque values for both extension and flexion will be recorded at baseline

and post-intervention, and percent change from baseline will be used for analysis.

Maximal oxygen uptake (VO_2max)

VO_2max will be obtained with a ParvoMedics TrueOne 2400 metabolic measuring system with a Hans Rudolf 3813 (Shawnee Mission, KS, USA) pneumotachometer to measure ventilation. The TrueOne 2400 is a mixing chamber system that uses a paramagnetic oxygen analyzer (range 0–25%) and an infrared, single beam, single wavelength carbon dioxide analyzer (range 0–10%). Prior to each test the system will be allowed to heat up for 30 min, and then will be calibrated according to the manufacturer's recommendations. This consists of a room air auto-calibration and a gas calibration with a single gas tank (16.00% O_2, 4.008% CO_2). Additionally, the flow meter will be calibrated with a 3.000-liter Hans Rudolf 5530 series syringe, with a 5-stroke calibration using different flow rates for each stroke. Ten ECG electrodes will be placed on the subject's body according to Mason-Likar procedures (54, 55). Prior to placement, the areas will be shaved with a disposable razor, wiped with an alcohol pad, and then lightly abraded with fine-grit sandpaper. The subject will be fitted with a Hans Rudolf Oro-Nasal reusable facemask (Shawnee Mission, KS, USA) with a 2-way non-rebreathing valve connected to the metabolic cart with large-bore, low-resistance tubing. The testing protocol will be explained to the subject, as well as how to communicate with investigators while wearing the facemask (i.e., hand signals). Resting VO_2 values will be collected from the subject after 3 min of rest in a seated position on the cycle ergometer. Resting blood pressure will be taken immediately after resting VO_2 is obtained. The subject will begin cycling on a magnetically braked cycle Lode Corival CPET ergometer (Lode B.V., Groningen, NL) at 60–80 RPMs (depending on subject comfort level) with a starting power output of 15 watts (W) for 1 min, collecting VO_2 values every 20 s. Power output will be increased by 15 W every minute until subject can no longer continue the test or criteria have been met (**Table 3**). With 20 s remaining in each stage, the subject will indicate their RPE on a 6–20 Borg scale (56). With 10 s left in each stage, heart rate will be recorded from ECG readouts. Blood pressure will be taken at the beginning of even numbered stages. Subjects will be verbally encouraged throughout the test, and airflow will be provided through the use of a rotating fan. After test termination, the subject will perform a 5-min cycling cool down at 30 W. Blood pressure will be taken every 5 min for 20 min after the completion of the graded exercise test (GXT). Maximal heart rate will be determined from ECG readings during the final stage of the GXT for all subjects. Absolute VO_2max measured in L/min will be recorded at baseline and post-intervention, and change from baseline will be used for analysis. Additionally, VO_2max relative to body mass measured in mL/kg/min will be recorded at baseline and post-intervention, and change from baseline will be used for analysis.

Quadriceps muscle volume

Quadriceps muscle volume will be obtained via magnetic resonance imaging (MRI) scans performed with a 0.25-Tesla Musculoskeletal MRI system (Esaote G-Scan Brio, Genoa, Italy) to acquire contiguous transverse T-1 weighted spin echo image slices in the thigh region with a slice thickness of 10 mm and an inter-slice distance of 10 mm. The isocenter will be positioned at mid-thigh, midway between the patella and the inguinal crease, and the subjects will be supine. Images will be transferred to a computer for calculation of quadriceps anatomical cross-sectional area (CSA). Beginning with the slide with the first discernable visual of the rectus femoris and including the subsequent four proximal slides, quadriceps muscle area will be traced using a polygon tool, excluding bone, as well as fat tissue surrounding the muscles (MIPAV version 7.3.0). Intramuscular fat will then be subtracted by applying a shading correction to each slide, determining average voxel density and standard deviation voxel density from a sample of the lightest area of fat tissue, computing a cutoff value at three standard deviations darker than the sample voxel density, and excluding all pixels with a voxel density at or below the computed value. Pre- and post-intervention slides will be displayed simultaneously, and slides will be visually compared to ensure that tracing patterns are identical and that the same structures are excluded (i.e., neurovascular bundle, intermuscular fat) on both slides before CSA values are recorded. This process will be completed for each analyzed slide, resulting in five measures of quadriceps CSA with intermuscular and intramuscular fat excluded for both pre and post time points. Muscle volume will then be calculated using the Cavalieri method [$MV = T\ (A_1 + A_2 + A_3 + A_4 + A_5)$], where MV = muscle volume, T = the known distance between slices, and A = area (57). Quadriceps muscle volume will be recorded at baseline and post-intervention, and percent change from baseline will be used for analysis.

Secondary Outcomes

Knee extensor isometric strength

Maximal isometric force production will be measured via three maximal voluntary contractions (MVCs) of the knee extensors while the subject is positioned in the Biodex Dynamometer as described above, with the lever arm immobilized at 90° of knee flexion. The subject will be instructed to gradually increase force for the first second, and then exert maximal effort for ∼3–4 s. The subject will perform three MVCs with a 30–60 s rest period between contractions. Verbal encouragement will be provided during each trial. The time-series torque signal will be collected at 500 Hz by a Biopac MP150 system (Biopac Systems Inc., Santa Barbara, CA, USA). The trial with the highest value will be recorded at baseline and post-intervention, and percent change from baseline will be used for analysis.

Knee extensor isokinetic fatigue

Fatigue resistance of the knee extensors will be measured with the subject positioned in the Biodex dynamometer as described above. Subjects will be asked to perform a series of isokinetic leg extensions at 120°/s (the flexion component will be passive at a speed of 240°/s). First, study subjects will perform three isokinetic extensions recorded as pre-fatigue peak torque values. Next, study subjects will be given 3 min of rest before beginning the fatigue portion of the test. For the fatigue test, subjects will perform 120 consecutive maximal isokinetic leg extension

TABLE 3 | Criteria for test termination during graded exercise testing.

Criteria for maximal effort-related test termination	Criteria for health concern-related test termination
• Subject requests to stop • Physical or verbal manifestations of severe fatigue • Failure of heart rate to increase with increased exercise intensity • Unable to maintain a cycle frequency of 50 RPMs for >5 s Or 2 of the 4 following criteria: • RPE of 17 or greater (Borg 6–20 scale) • Peak heart rate that is 85% of age-predicted maximal heart rate (220-age) • Plateau of VO2 • Respiratory exchange ratio (RER) of 1.1 or higher	• Angina or angina-like symptoms (subjective score of 2 or greater on ACSM angina scale) • Shortness of breath, wheezing, leg cramps, or claudication (subjective score of 3 or greater on ACSM claudication scale) • Signs of poor perfusion: light-headedness, confusion, ataxia, pallor, cyanosis, nausea, or cold and clammy skin • Drop in systolic blood pressure of >10 mm Hg, despite an increase in workload • Central nervous system symptoms (e.g., ataxia, dizziness, or near syncope) • Sustained ventricular tachycardia or other arrhythmia, including second- or third-degree atrioventricular block, that interferes with normal maintenance of cardiac output during exercise • Exaggerated hypertensive response (systolic blood pressure >250 mm Hg or diastolic blood pressure >115 mm Hg) • Abnormal changes in sinus rhythm • Exercise induced conduction blocks

ACSM, American College of Sports Medicine; RPE, rating of perceived exertion; RPM, rotations per minute; VO2, volume of oxygen consumption.

contractions (test time ∼4-min). Peak force for each of the 120 contractions will be summed to calculate total work output and will be recorded at baseline and post-intervention, and change from baseline will be used for analysis. The subject will be verbally encouraged throughout the test. Lastly, post-fatigue peak torque will be assed at 2, 5, and 10 min after the completion of the fatigue test. For all fatigue measurement, the time-series torque signal will be collected at 500 Hz by a Biopac MP150 system (Biopac Systems Inc., Santa Barbara, CA, USA). Average torque of three contractions at each of the post-fatigue timepoints will be recorded and expressed relative to the pre-fatigue torque at baseline and post-intervention, and percent change from baseline will be used for analysis.

Six-minute walk distance

A 6-min walk distance test will be performed in a 30-meter hallway marked off with cones at either end, and distance marked every three meters. The subject will start at the end of the hallway (starting cone) and be instructed to walk as quickly as they can for 6 min. They will walk toward the end of the hallway, around the second cone, and then back toward the starting cone. The subject will complete as many laps as possible within 6 min, rounding the cones each lap. Subjects will be given feedback on elapsed/remaining time every 30 s, and encouraged to continue walking as quickly as possible. Distance covered in 6 min will be recorded to the nearest meter at baseline and post-intervention, and change from baseline will be used for analysis.

Body composition

Total body fat mass will be obtained via whole-body dual-energy X-ray absorptiometry (DXA) scans (Hologic Discovery QDR model Series, Waltham, MA, USA) using the system's software package (Hologic APEX, Version 4.0.2). Subjects will be scanned at the same time of day pre- and post-intervention and will be encouraged to maintain a similar sleeping and eating schedule for both scans. Subjects will be advised to report to the laboratory in a hydrated state and will be given scrubs to wear during the scan. They will also be given the opportunity to use the restroom prior to the scan. Care will be taken to follow The International Society for Clinical Densitometry guidelines for positioning during the scan (58). Total body fat mass will be recorded at baseline and post-intervention, and percent change from baseline will be used for analysis.

Other Outcomes

Knee extensor voluntary rate of force development (VRFD)

VRFD of the knee extensors will be measured with the subject positioned in the Biodex Dynamometer as described above with the lever arm set to 90° of knee flexion. The subject will extend the knee into the immobilized lever arm with maximal effort over ∼500 ms, then immediately relax. The process will be explained to the subject so they understand that they are to produce as much force as possible, as quickly as possible, and then to relax (e.g., pretend you are kicking a tire as hard as you can). The time-series torque signal will be collected at 500 Hz by a Biopac MP150 system (Biopac Systems Inc., Santa Barbara, CA, USA). The trial with the highest VRFD (slope of force tracing between 10 and 90% of maximal force) will be calculated at baseline and post-intervention, and change from baseline will be used for analysis.

Twitch-evoked rate of force development (TRFD) and voluntary activation

TRFD and voluntary activation of the knee extensors will be obtained with the subject positioned in the Biodex dynamometer as described above with the lever arm set to at 90° of knee flexion. A surface stimulating electrode will be placed on the distal motor point of the vastus medialis and another on the proximal motor point of the vastus lateralis. Single pulses of incrementally increasing current will be delivered via a Digitimer DS7AH constant current stimulator (Digitimer Ltd., Hertfordshire, UK) until twitch force plateaus. The time-series torque signal will be collected at 500 Hz by a Biopac MP150 system (Biopac Systems Inc., Santa Barbara, CA, USA). TRFD (slope of the twitch response between 10 and 90% of maximal force) at maximal

stimulation intensity will be calculated at baseline and post-intervention, and change from baseline will be used for analysis. Subjects will then perform two MVCs separated by 2 min, and during the MVC a doublet will be delivered at maximal intensity (intensity at which twitch force plateaus), followed by a second doublet delivered to resting muscle. Voluntary activation is expressed as [1–(doublet force during MVC/doublet force during relaxation)] * 100. The time-series torque signal will be collected at 500 Hz by a Biopac MP150 system (Biopac Systems Inc., Santa Barbara, CA, USA). The subject will perform two voluntary activation trials, and the average of the two measures will be recorded at baseline and post-intervention, and change from baseline will be used for analysis.

4-square step test (4SST)

The 4SST is a test that challenges motor planning and initiation as well as motor sequencing and recall (59, 60). A four-foot by four-foot square will be marked with athletic tape and split into quadrants (**Figure 2**). The subjects will start in square 1, facing square 2. The subject then steps forward into square 2, laterally to square 3, backwards to square 4, laterally to square 1, laterally to square 4, forwards to square 3, laterally to square 2, and backwards to square 1, facing the same direction throughout the entire sequence. Subjects must place both feet in the specified quadrant before they can move into the next square, and must have at least one foot on the ground at all times. The test will be timed with a stop watch to the nearest 0.01 s. When the subject is ready the investigator will say "ready, set, go." The timer will begin at "Go" and stopped when the subject has placed both feet back in square 1 after completing a clockwise and counter-clockwise cycle. The subject will perform two trials with 30 s rest between trials, and the fastest will be recorded at baseline and post-intervention. Change from baseline will be used for analysis.

Stair climb power

Stair climb power will be calculated as [power = force x velocity] where force = body mass in kilograms x acceleration due to gravity, and velocity = cumulative stair height/stair climb time. The subject will be weighed to the nearest pound, and free weights equivalent to 20% of their body weight will be distributed evenly into two canvas bags. The bags will be set aside for later weighted stair climb testing. The subject will stand at the bottom of a flight of eight stairs (each stair 7″ in height) with feet together. The subject will then be instructed to safely climb the stairs as quickly as possible without skipping any stairs, maintaining at least one foot on a stair at all times. The subject will also be instructed that they may use the handrails if necessary for safety purposes. The test will be timed with a stop watch to the nearest 0.01 s. When the subject is ready the investigator will say "ready, set, go." The timer will begin at "Go" and stopped when the subject's first foot makes contact with the eighth stair. The subject will perform two trials, and the fastest will be recorded at baseline and post-intervention. Stair climb power will be calculated as described above, and change from baseline will be used for analysis. The subject will repeat the test while carrying the weighted bags equivalent to 20% of their body weight, one bag in each hand. The subject will perform two trials and the fastest will be recorded at baseline and post-intervention. Stair climb power will be calculated as described above, and change from baseline will be used for analysis. 30–60 s rest will be allowed between each trial.

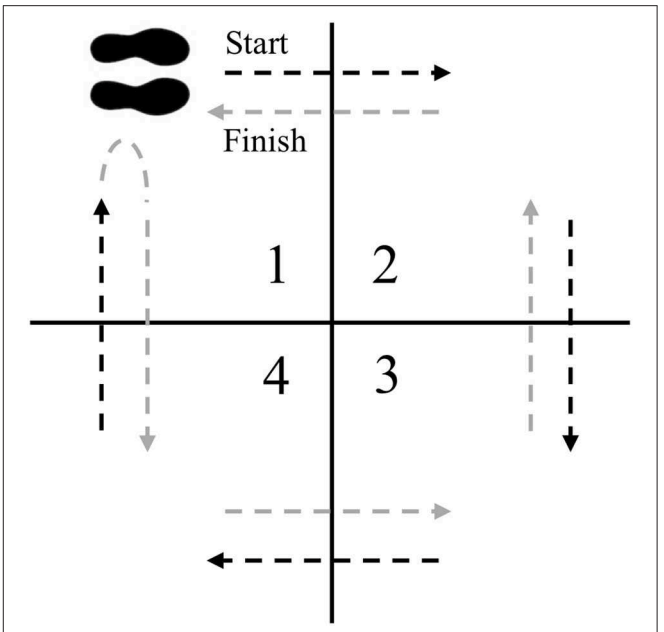


FIGURE 2 | 4SST setup. The subject starts in square 1 facing square 2. The subject faces the same direction as they step into squares 2, 3, 4, 1, 4, 3, 2, and then 1.

Grip strength

Maximal grip strength will be obtained with a Jamar hydraulic grip strength dynamometer (Performance Health, LLC, Akron, OH USA). Handle position of the dynamometer will be standardized at position II (61, 62). Hand dominance will be determined by asking the subject if they were right-handed or left-handed. The dominant hand will be tested first. The subject will be seated with the shoulder in neutral position and the elbow unsupported and flexed to 90° with the forearm and wrist in neutral position (61). The subject will squeeze the dynamometer handle as hard as possible for 3 s. Maximal force will be recorded in kg. The subject will perform three trials with each hand, and 15 s rest will be allowed between trials. Average force of the three trials for each hand will be recorded at baseline and post-intervention, and change from baseline will be used for analysis.

Exercise Intervention

Each subject will perform their prescribed exercise three times per week for 12 weeks. Time between visits will generally be 48–72 h, but may range from 24 to 96 h to meet the demands of the subject's schedule. Subjects may be allowed to exercise on consecutive days one time per week, but must have at least 48 h before a third exercise session is completed (for example, Monday, Tuesday, Thursday is acceptable, but Monday, Tuesday, Wednesday is not). Prior to each exercise session an exercise supervisor will perform a brief medical safety check. If the

subject reports changes in health status that are concerning, has abnormal vital signs (blood pressure readings that exceed 170 mmHg for systolic blood pressure or 100 mmHg for diastolic blood pressure), or has changed medications/dosage, they will not be allowed to continue exercising until the study physician has reviewed the changes and clears the subject. After the medical safety check, the subject will perform a 5-min warm-up on the stationary bike at a low intensity (i.e., output 50% or less of their maximal output during the GXT). After the subject has completed the prescribed exercise for the day they will perform a 5-min cooldown at a low intensity on the stationary bicycle. For the RT and MICT exercise protocols we will employ a pragmatic trial design that follow the recommendations set forth by the ACSM, but that are not necessarily matched by time. There are currently no recommendations for HIIT in older adults, and the exercise sessions are shorter in duration due to the high-intensity nature.

Exercise Interventions

RT

Subjects will perform 10 resistance exercises for the major muscle groups (**Table 4**). The training program is lower extremity-focused, with 70% of exercises isolating the lower extremities. For the first 2 weeks subjects will perform 1–2 sets of 15 repetitions for each of the ten exercises, using a weight that elicits a rating of perceived exertion (RPE) at the end of the final repetitions of the respective sets of 5–6 (0–10 scale) as reported by the subject. Rest between sets/exercises will range from 30 to 60 s. Weeks 3–4 subjects will perform 2–3 sets of 12–20 repetitions at an RPE of 5–8 with 60–90 s rest between sets. Weeks 5–8 subjects will perform 3–4 sets of 10–20 repetitions at an RPE of 6–8 with 60–90 s rest between sets. Weeks 9–12 subjects will perform 3–5 sets of 6–20 repetitions at an RPE of 7–8 with 60–90 s rest between sets. Contraction velocity for each exercise will be moderate (180–240°/sec), with duration lasting ∼2 s for concentric actions and 2 s for eccentric actions (63). This protocol meets the ACSM resistance training recommendations for older adults (15), and progresses from entry-level to a more demanding protocol. Duration is expected to last ∼45–75 min. Subjects will occasionally be asked their perceived effort level on 1–10 scale after individual exercise sets to ensure that they are exercising at the prescribed intensity.

TABLE 4 | Resistance training group exercises.

Daily exercises	Rotating exercises
Leg press	Lunges
Knee extensions	Step-ups (weighted or unweighted)
Leg curls	Hip abduction
Calf raises	Hip bridge (single- or double-leg)
Chest press	Box squat
	Sumo squat
	Planks (knee and elbows or knees and toes)
	Biceps curls
	Push-ups (incline or flat)
	Seated cable pull-down
	Seated cable row
	Triceps extensions
	Shoulder overhead press
	Lateral arm raises

The five Daily Exercises are performed during each exercise session. The remaining five exercises each session will be chosen by the exercise supervisor from the list of Rotating Exercises.

MICT

The MICT will be performed on a stationary bicycle (Peloton Interactive, Inc. New York City, NY, USA) interfaced with a computer monitor that plays selected pre-recorded "spin classes." Heart rate and power output are displayed in real time. The goal of MICT is to maintain an output that elicits the prescribed heart rate throughout the exercise session. Heart rate reserve (HRR) will be calculated by subtracting the subject's resting heart rate (obtained during medical history check at visit 1) from their maximal heart rate (obtained during the GXT). The progression of the 12-week program will go as follows. For the first week subjects will cycle for 20–30 min at 50–60% of their HRR. Week 2 subjects will cycle for 20–30 min at 55–65% of their HRR. Weeks 3–4 subjects will cycle for 30 min at 60–70% of their HRR. Weeks 5–8 subjects will cycle for 30–45 min at 65–75% HRR, and in weeks 9–12 subjects will cycle for 45 min at 70–75% HRR. These ranges are based on recommendations from a meta-analysis describing a dose-response relationship between exercise intensity and VO_2max adaptations in older adults (64), An exercise supervisor will oversee each exercise session. The target output (in watts) will be determined during the first exercise session by having the subject cycle for 5 min at a cadence of 75 RPMs with a resistance that produces 50 W. Once a consistent heart rate is established (using a chest-strap heart rate monitor), we will increase or decrease the resistance until the target heart rate is maintained. The target output for each subsequent session will be the highest average output from the previous week. If there was no increase from the previous week, target average output will be manually increased by 3%. If the subject is maintaining the target output but not achieving the target heart rate, output will be increased incrementally by 2–5 W until the heart rate is maintained in the target range. During each exercise session the subject will follow the cadence recommendations of the spin class instructor (ranging 50–100 RPMs) while the in-person exercise supervisor modifies the resistance to ensure the output is maintained within the target range. At times the subject may be cycling at the target output but have a heart rate that exceeds the target. In these cases heart rate range takes precedence over output range, and output will be decreased until the target heart rate is maintained. This may occur when cycling at higher cadences (e.g., 90–100 RPMs). At the end of each exercise session subjects will be asked their perceived effort level for the entire session on a 1–10 scale and a Borg 6–20 scale. See **Table 5** for exercise duration of each session.

HIIT

Subjects in the HIIT group will use the same stationary bicycle setup as in the MICT group. The progression of the 12-week program will go as follows. Subjects will cycle continuously for 20–30 min at 50–60% of their HRR for the first week, and 20–30 min at 55–65% of HRR for the second week, similar to the

TABLE 5 | MICT and HIIT exercise groups cycling duration.

Session #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
	Week 1			**Week 2**			**Week 3**			**Week 4**		
MICT	20	20	30	30	20	30	30	30	30	30	30	30
HIIT	20	20	30	30	20	30	15	15	15	15	15	15
Session #	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**29**	**21**	**22**	**23**	**24**
	Week 5			**Week 6**			**Week 7**			**Week 8**		
MICT	30	30	30	30	45	30	30	45	30	45	30	45
HIIT	15	20	15	15	20	15	20	15	20	20	15	20
Session #	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
	Week 9			**Week 10**			**Week 11**			**Week 12**		
MICT	45	45	45	45	45	45	45	45	45	45	45	45
HIIT	20	20	20	20	30	20	20	30	20	20	30	20

Duration (in minutes) of cycling for the moderate-intensity continuous training (MICT) and the high-intensity interval training (HIIT) groups for exercise sessions 1–36.

MICT group. During the subsequent weeks, subjects will perform bouts of higher intensity cycling (target intensity of 80–100% of their HRR) interspersed with low- intensity rest periods (target intensity of 40–60% of their HRR). Here, the duration of the bout (or interval) will range from as little as 15 s and upwards of 1 min, and rest periods will be matched in a work/rest ratio of 2:1, 1:1, or 1:2 (1:1 on average). During weeks 3–4 the intensity will be at 80–95% of their HRR and the overall duration will last 15–20 min. Weeks 5–8 the intensity will be at 80–100% of their HRR with a duration of 15–30 min. During weeks 9–12 intensity will be at 85–100% of their HRR and duration will last 20–30 min. When the subject begins week three, the target average output will be determined by multiplying the subject's best average output from the first 2 weeks by 1.2. The subject will cycle at the target average output for the first 2–3 min of each session, at 150–300% of their target average output during the high-intensity intervals, and at 25–50% of their target average output during their low-intensity intervals in order to achieve the target heart rate ranges. The target average output for each subsequent week of exercise sessions will be the highest average output from the previous week. If there was no increase from the previous week, target average output will be manually increased by 3%. Subjects will complete 15, 20, or 30-min sessions throughout the study (**Table 5**) and will begin each session by cycling at the target average output for 2–4 min, followed by several high- and low-intensity intervals. Subjects will cycle at a high intensity for ∼6 total min during 15 min sessions, ∼8 total min during 20 min sessions, and ∼10 total min during 30 min sessions, maintaining an average work/rest ratio of 1:1. The subject will follow the cadence recommendations (ranging 50–100 RPMs) of the spin class instructor while the in-person exercise supervisor modifies the resistance to ensure the output is maintained within the target ranges. It is unlikely that the subject's heart rate will reach 40–60% of HRR during the rest intervals, however, an output that is 25–50% of the target average output should elicit a heart rate that is 40–60% of HRR under normal conditions. Therefore, rest intervals will be long enough that the subject's heart rate will decrease by 5–20 beats per minute prior to the start of the next high-intensity interval. At the end of each exercise session subjects will be asked their perceived effort level for the entire session on a 1–10 scale and a Borg 6–20 scale. See **Table 5** for exercise duration of each session.

Intervention Discontinuation

Participation in the study will be discontinued if the subject fails to follow the study requirements, experiences serious side effects (e.g., heart attack, exercise related injury), changes the dosage of anti-hypertensive medication or starts taking an exclusionary medication during the course of the study (**Table 1**), or requests to be removed from the study.

Concomitant Exercise and Diet

It is possible that some subjects may be participating in some low-volume physical activity prior to study enrollment and still be eligible for the study (e.g., yoga, yard work). To control for this, all subjects will be encouraged to continue normal activity outside of the study throughout their enrollment in the study. Similarly, study subjects will be asked to maintain their normal diet throughout the study.

Adherence

Successful adherence will be defined as a study subject who achieves at least an 80% adherence rate (i.e., attends 29 of 36 exercise sessions).

Data Management

Data will be entered into the database immediately after each measure is performed by the investigator collecting the measure, and then again at the end of the study by the outcomes assessor. Any incongruous measures will be re-analyzed separately by the outcomes assessor and another blinded investigator. Demographic and intervention group data will be coded and the code will be stored in a locked cabinet unavailable to the outcomes assessor.

Statistical Methods

The three intervention arms (HIIT, MICT, and RT) will be compared against each other for all primary analyses. For total fat mass and the primary and secondary knee extensor-related outcomes (isokinetic power, muscle volume, MVC, and isokinetic fatigue), we will compute a percentage change from baseline to 12 weeks. For absolute and relative VO_2max and 6-min walk distance we will compute absolute change from baseline to 12 weeks. We will test differences in group means using one-way ANOVA with Sidak option in SPSS v. 25.0. Tukey *post hoc* tests will be performed if significant differences exist, alpha level set at 0.05. Additionally, as this is a proof-concept, proof of mechanism trial we will calculate effect sizes between groups using the corrected effect size Hedge's g with 95% confidence intervals for small sample sizes (65).

Harms

In our study, adverse events (AEs) will be defined as an unexpected medical problem that happens during the course of the study related or unrelated to the intervention or assessments.

All AEs occurring after informed consent is signed and until study completion will be recorded. An AE that meets the criteria for a serious adverse event (SAE) will be reported to the Ohio University IRB as a SAE. A SAE is defined as an unexpected medical problem that is believed by the investigators to be causally related to the study intervention or assessments and results in any of the following: a life-threatening condition, severe or permanent disability, prolonged hospitalization, or death. Prior to each exercise session the exercise supervisor will perform a brief medical safety check and will ask the subject if they experienced any health issues since the last exercise session. Any issues will be recorded and reviewed by the project manager. The issue will then be recorded as an AE, and will be noted if the project manager believes the issue is related to the intervention (e.g., muscle soreness).

ANTICIPATED RESULTS

The Dual-Benefits Hypothesis

The HIIT group will see improvements in endurance measures (VO_2max, 6-min walk distance, isokinetic fatigue) equal to the MICT group and greater than the RT group, and improvements in muscular characteristics (isokinetic power, MVC, thigh muscle volume, VRFD, TRFD, voluntary activation) equal to the RT group and greater than the MICT group.

Other Hypotheses

All groups will exhibit similar changes in the 4-square step test and stair climb.

The RT group will see greater improvements in grip strength than either MICT or HIIT groups.

The MICT group will see greater reductions in fat mass than either HIIT or RT groups.

DISCUSSION

High-intensity interval training has become a popular exercise strategy for young adults for the equivalent or greater health benefits to traditional aerobic training with only a fraction of the time commitment (30). HIIT may also be a highly beneficial exercise strategy for older adults with potential to elicit both muscular and cardiorespiratory adaptations. However, there have been few studies assessing the effects of HIIT in older adults, with the majority being performed in individuals with overt health conditions (33, 35). Although a preliminary study, this is, to our knowledge, the first randomized controlled trial to compare muscular and cardiorespiratory adaptations of bicycle HIIT to adaptations seen in response to traditional bicycle aerobic training or resistance training in generally health older adults. Schjerve et al. (52) compared the effects of HIIT, MICT, and RT in middle-aged, obese adults, reporting large improvements in VO_2max but no strength gains in the HIIT group. However, this study used a walking protocol for both MICT and HIIT groups, and we postulate the lack of effect on strength was due to the lower levels of muscular activity and force associated with this protocol [e.g., there is evidence that >60% of maximal muscle force must be produced during training to see increases in muscle size and/or function (22)]. HIIT has already been demonstrated to improve cardiorespiratory fitness equal to, or greater than, MICT in older adults (33, 38). Therefore, we propose in this study to determine if HIIT can produce muscular adaptations similar to those seen in response to resistance training. We will be using a cycling protocol for the HIIT group, as improvements in strength and power have been demonstrated in response to HIIT cycling protocols in young adults (28, 30).

The largest potential problem that could arise throughout the course of the study relates to the possibility of AEs. The risk of cardiovascular events in adults participating in HIIT programs is low, as reported by Rognmo et al. (66), who found that of the 4,800+ cardiac rehabilitation patients participating in either HIIT or MICT at a cardiac rehabilitation center, three suffered from cardiac arrest during training (one fatal during MICT, two non-fatal during HIIT). The rate of complications to the number of patient exercise hours was 1 per 23,182 h for HIIT (66). The target population for the current study will include insufficiently active older adults, but those with certain known heart conditions will be excluded from the study, and any subjects who display specific irregular cardiac responses during the baseline GXT (**Table 3**) will also be excluded. As such, risk of cardiac events is expected to be lower for our population than has been reported in cardiac rehabilitation patients. The American College of Sports Medicine recognizes the risks associated with exercise, but states that the health benefits of exercise outweigh the risks (15).

This study will assess the effectiveness of a high-intensity interval cycling exercise protocol in older adults to produce both cardiorespiratory and muscular adaptations. If shown to be effective it will pave the way for a paradigm shift from volume- to intensity-driven exercise recommendations for older adults, with the ultimate goal of improving health and reducing age-related physical dysfunction.

ETHICS STATEMENT

Research Ethics Approval

This study has been approved by the Ohio University Institutional Review Board (approval number: 18-F-55).

Protocol Amendments

Any modifications to the protocol which may impact the conduct of the study, potential benefits to the subject, or affect subjects safety (including changes to study objectives, study design, patient population, sample sizes, study procedures, or significant administrative changes) will require a formal amendment to the protocol. Such amendments will be agreed upon by the principal investigators, and approved by the Ohio University Institutional Review Board prior to implementation.

Consent

Informed consent will be obtained from each subject after the study has been fully described to the subject, and the consenting investigator believes that the subject fully understands the study requirements and can make an informed decision.

Confidentiality

Each subject will be assigned a number and experimental data will be recorded using that number. Any identifiable personal information will be kept in a locked cabinet in OMNI, and only the PI will have key access to the information.

Access to Data

The principal investigators will have direct access to all data sets. Data dispersed to other project team members will be blinded of any identifying subject information.

Ancillary and Post-trial Care

There are no provisions for ancillary or post-trial care.

Dissemination Policy

The investigators will provide the subjects with estimates of when the trial will end and when data will be published. The American Heart Association Predoctoral Fellowship requires that all journal articles be made freely available in PubMed Central within 12 months of publication. Eligibility for authorship of manuscripts resulting from this study include (1) substantial contributions to the conception or design of the project, or the acquisition, analysis, or interpretation of the data, AND (2) drafting or revising the manuscript, AND (3) final approval of the manuscript. There is no intention to use professional writers.

AUTHOR CONTRIBUTIONS

DT, BC, and DR conceived of the study and initiated the study design. PC and EG helped with implementation. TL provided medical oversight. JS provided statistical expertise. All authors contributed to refinement of the study protocol and approved the final manuscript.

FUNDING

This work was supported by American Heart Association Grant #19PRE34380496/Dallin Tavoian/2019 (DT), the Osteopathic Heritage Foundations Graduate Fellowship Program at Ohio University Heritage College of Osteopathic Medicine (DT), and the John J. Kopchick Molecular and Cellular Biology/Translational Biomedical Sciences Research Fellowship Award (DT).

REFERENCES

1. FIFoA-R. *Federal Interagency Forum on Aging-Related Statistics. Older Americans 2016: Key Indicators of Well Being*. Washington, DC: US Government Printing Office (2016).
2. NORC. *Perceptions of Aging During Each Decade of Life After 30*. Chicago, IL (2017).
3. Anton SD, Woods AJ, Ashizawa T, Barb D, Buford TW, Carter CS, et al. Successful aging: advancing the science of physical independence in older adults. *Ageing Res Rev.* (2015) 24:304–27. doi: 10.1016/j.arr.2015.09.005
4. Lee D, Artero EG, Xuemei S, Blair SN. Review: mortality trends in the general population: the importance of cardiorespiratory fitness. *J Psychopharmacol.* (2010) 24:27–35. doi: 10.1177/1359786810382057
5. Visser M, Goodpaster BH, Kritchevsky SB, Newman AB, Nevitt M, Rubin SM, et al. Muscle mass, muscle strength, and muscle fat infiltration as predictors of incident mobility limitations in well-functioning older persons. *J Gerontol A Biol Sci Med Sci.* (2005) 60:324–33. doi: 10.1093/gerona/60.3.324
6. DiPietro L. Physical activity in aging: changes in patterns and their relationship to health and function. *J Gerontol A Biol Sci Med Sci.* (2001) 56:13–22. doi: 10.1093/gerona/56.suppl_2.13
7. Keadle SK, McKinnon R, Graubard BI, Troiano RP. Prevalence and trends in physical activity among older adults in the United States: a comparison across three national surveys. *Prev Med.* (2016) 89:37–43. doi: 10.1016/j.ypmed.2016.05.009
8. Sun F, Norman IJ, While AE. Physical activity in older people: a systematic review. *BMC Public Health.* (2013) 13:449. doi: 10.1186/1471-2458-13-449
9. Davidson L, Hudson R, Kilpatrick K, Kuk J, McMillan K, Janiszewski P, et al. Effects of exercise modality on insulin resistance and functional limitation in older adults: a randomized controlled trial. *Arch Intern Med.* (2009) 169:122–31. doi: 10.1001/archinternmed.2008.558
10. Mangione KK, Miller AH, Naughton IV. Cochrane review: improving physical function and performance with progressive resistance strength training in older adults. *Phys Ther.* (2010) 90:1711–5. doi: 10.2522/ptj.20100270
11. Solberg PA, Kvamme NH, Raastad T, Ommundsen Y, Tomten SE, Halvari H, et al. Effects of different types of exercise on muscle mass, strength, function and well-being in elderly. *Eur J Sport Sci.* (2013) 13:112–25. doi: 10.1080/17461391.2011.617391
12. Billat V, Dhonneur G, Mille-Hamard L, Le Moyec L, Momken I, Launay T, et al. Case studies in physiology: maximal oxygen consumption and performance in a centenarian cyclist. *J Appl Physiol.* (2017) 122:430–4. doi: 10.1152/japplphysiol.00569.2016
13. Cadore EL, Casas-Herrero A, Zambom-Ferraresi F, Idoate F, Millor N, Gómez M, et al. Multicomponent exercises including muscle power training enhance muscle mass, power output, and functional outcomes in institutionalized frail nonagenarians. *Age.* (2014) 36:773–85. doi: 10.1007/s11357-013-9586-z
14. Serra-Rexach JA, Bustamante-Ara N, Hierro Villarán M, González Gil P, Sanz Ibáñez MJ, Blanco Sanz N, et al. Short-term, light- to moderate-intensity exercise training improves leg muscle strength in the oldest old: a randomized controlled trial. *J Am Geriatr Soc.* (2011) 59:594–602. doi: 10.1111/j.1532-5415.2011.03356.x
15. Chodzko-Zajko WJ, Proctor DN, Fiatarone Singh MA, Minson CT, Nigg CR, Salem GJ, et al. Exercise and physical activity for older adults. *Med Sci Sports Exerc.* (2009) 41:1510–30. doi: 10.1249/MSS.0b013e3181a0c95c
16. Braith RW. Resistance exercise training: its role in the prevention of cardiovascular disease. *Circulation.* (2006) 113:2642–50. doi: 10.1161/CIRCULATIONAHA.105.584060
17. Wewege MA, Thom JM, Rye K-A, Parmenter BJ. Aerobic, resistance or combined training: a systematic review and meta-analysis of exercise to reduce cardiovascular risk in adults with metabolic syndrome. *Atherosclerosis.* (2018) 274:162–71. doi: 10.1016/j.atherosclerosis.2018.05.002
18. Hudelmaier M, Wirth W, Himmer M, Ring-Dimitriou S, Sänger A, Eckstein F. Effect of exercise intervention on thigh muscle volume and anatomical cross-sectional areas-quantitative assessment using MRI. *Magn Reson Med.* (2010) 64:1713–20. doi: 10.1002/mrm.22550
19. Ozaki H, Loenneke J, Thiebaud R, Stager J, Abe T. Possibility of leg muscle hypertrophy by ambulation in older adults: a brief review. *Clin Interv Aging.* (2013) 8:369–75. doi: 10.2147/CIA.S43837
20. DHHS. *A Report of the Surgeon General. Physical Activity and Health: Older Adults*. U.S. Department of Health and Human Services. US Department of Health and Human Services (1999).
21. Garber CE, Blissmer B, Deschenes MR, Franklin BA, Lamonte MJ, Lee I-M, et al. Quantity and quality of exercise for developing and maintaining cardiorespiratory, musculoskeletal, and neuromotor fitness in apparently healthy adults: guidance for prescribing exercise. *Med Sci Sports Exerc.* (2011) 43:1334–59. doi: 10.1249/MSS.0b013e318213fefb
22. Wernbom M, Augustsson J, Thomee R. The influence of frequency, intensity, volume and mode of strength training on whole muscle

cross-sectional area in humans. *Sports Med.* (2007) 37:225–64. doi: 10.2165/00007256-200737030-00004
23. Nybo L, Sundstrup E, Jakobsen MD, Mohr M, Hornstrup T, Simonsen L, et al. High-intensity training versus traditional exercise interventions for promoting health: *Med. Sci Sports Exerc.* (2010) 42:1951–8. doi: 10.1249/MSS.0b013e3181d99203
24. Wilson JM, Marin PJ, Rhea MR, Wilson SMC, Loenneke JP, Anderson JC. Concurrent training: a meta-analysis examining interference of aerobic and resistance exercises. *J Strength Cond Res.* (2012) 26:2293–307. doi: 10.1519/JSC.0b013e31823a3e2d
25. Harber MP, Konopka AR, Undem MK, Hinkley JM, Minchev K, Kaminsky LA, et al. Aerobic exercise training induces skeletal muscle hypertrophy and age-dependent adaptations in myofiber function in young and older men. *J Appl Physiol.* (2012) 113:1495–504. doi: 10.1152/japplphysiol.00786.2012
26. Konopka AR, Douglass MD, Kaminsky LA, Jemiolo B, Trappe TA, Trappe S, et al. Molecular adaptations to aerobic exercise training in skeletal muscle of older women. *J Gerontol A Biol Sci Med Sci.* (2010) 65A:1201–7. doi: 10.1093/gerona/glq109
27. Lovell DI, Cuneo R, Gass GC. Can aerobic training improve muscle strength and power in older men? *J Aging Phys Act.* (2010) 18:14–26. doi: 10.1123/japa.18.1.14
28. Martinez-Valdes E, Falla D, Negro F, Mayer F, Farina D. Differential motor unit changes after endurance or high-intensity interval training. *Med Sci Sports Exerc.* (2017) 49:1126–36. doi: 10.1249/MSS.0000000000001209
29. Siahkouhian M, Khodadadi D, Shahmoradi K. Effects of high-intensity interval training on aerobic and anaerobic indices: comparison of physically active and inactive men. *Sci Sports.* (2013) 28:e119–25. doi: 10.1016/j.scispo.2012.11.006
30. Weston M, Taylor KL, Batterham AM, Hopkins WG. Effects of low-volume high-intensity interval training (HIT) on fitness in adults: a meta-analysis of controlled and non-controlled trials. *Sports Med.* (2014) 44:1005–17. doi: 10.1007/s40279-014-0180-z
31. Burgomaster KA, Howarth KR, Phillips SM, Rakobowchuk M, MacDonald MJ, McGee SL, et al. Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans: metabolic adaptations to sprint or endurance training in humans. *J Physiol.* (2008) 586:151–60. doi: 10.1113/jphysiol.2007.142109
32. Pattyn N, Coeckelberghs E, Buys R, Cornelissen VA, Vanhees L. Aerobic interval training vs. moderate continuous training in coronary artery disease patients: a systematic review and meta-analysis. *Sports Med.* (2014) 44:687–700. doi: 10.1007/s40279-014-0158-x
33. Weston KS, Wisløff U, Coombes JS. High-intensity interval training in patients with lifestyle-induced cardiometabolic disease: a systematic review and meta-analysis. *Br J Sports Med.* (2014) 48:1227–34. doi: 10.1136/bjsports-2013-092576
34. Ahmaidi S, Masse-Biron J, Adam B, Choquet D, Freville M, Libert J-P, et al. Effects of interval training at the ventilatory threshold on clinical and cardiorespiratory responses in elderly humans. *Eur J Appl Physiol.* (1998) 78:170–6. doi: 10.1007/s004210050403
35. Beauchamp MK, Nonoyama M, Goldstein RS, Hill K, Dolmage TE, Mathur S, et al. Interval versus continuous training in individuals with chronic obstructive pulmonary disease- a systematic review. *Thorax.* (2010) 65:157–64. doi: 10.1136/thx.2009.123000
36. Grace F, Herbert P, Elliott AD, Richards J, Beaumont A, Sculthorpe NF. High intensity interval training (HIIT) improves resting blood pressure, metabolic (MET) capacity and heart rate reserve without compromising cardiac function in sedentary aging men. *Exp Gerontol.* (2017) 109:75–81. doi: 10.1016/j.exger.2017.05.010
37. Huang S-C, Wong M-K, Lin P-J, Tsai F-C, Fu T, Wen M-S, et al. Modified high-intensity interval training increases peak cardiac power output in patients with heart failure. *Eur J Appl Physiol.* (2014) 114:1853–62. doi: 10.1007/s00421-014-2913-y
38. Hwang C-L, Yoo J-K, Kim H-K, Hwang M-H, Handberg EM, Petersen JW, et al. Novel all-extremity high-intensity interval training improves aerobic fitness, cardiac function and insulin resistance in healthy older adults. *Exp Gerontol.* (2016) 82:112–9. doi: 10.1016/j.exger.2016.06.009
39. Jabbour G, Iancu H-D, Mauriège P, Joanisse DR, Martin LJ. High-intensity interval training improves performance in young and older individuals by increasing mechanical efficiency. *Physiol Rep.* (2017) 5:e13232. doi: 10.14814/phy2.13232
40. Knowles A-M, Herbert P, Easton C, Sculthorpe N, Grace FM. Impact of low-volume, high-intensity interval training on maximal aerobic capacity, health-related quality of life and motivation to exercise in ageing men. *Age.* (2015) 37:25. doi: 10.1007/s11357-015-9763-3
41. Makrides L, Heigenhauser GJ, Jones NL. High-intensity endurance training in 20- to 30- and 60- to 70-yr-old healthy men. *J Appl Physiol.* (1990) 69:1792–8. doi: 10.1152/jappl.1990.69.5.1792
42. Morris N, Gass G, Thompson M, Bennett G, Basic D, Morton H. Rate and amplitude of adaptation to intermittent and continuous exercise in older men: *Med. Sci Sports Exerc.* (2002) 34:471–7. doi: 10.1097/00005768-200203000-00014
43. Sculthorpe NF, Herbert P, Grace F. One session of high-intensity interval training (HIIT) every 5 days, improves muscle power but not static balance in lifelong sedentary ageing men: a randomized controlled trial. *Medicine.* (2017) 96:e6040. doi: 10.1097/MD.0000000000006040
44. Støren Ø, Helgerud J, Sæbø M, Støa EM, Bratland-Sanda S, Unhjem RJ, et al. The effect of age on the VO2max response to high-intensity interval training. *Med Sci Sports Exerc.* (2017) 49:78–85. doi: 10.1249/MSS.0000000000001070
45. Camarri B, Eastwood PR, Cecins NM, Thompson PJ, Jenkins S. Six minute walk distance in healthy subjects aged 55–75 years. *Respir Med.* (2006) 100:658–65. doi: 10.1016/j.rmed.2005.08.003
46. Lusardi MM, Pellecchia GL, Schulman M. Functional performance in community living older adults. *J Geriatr Phys Ther.* (2003) 26:14–22. doi: 10.1519/00139143-200312000-00003
47. Steffen TM, Hacker TA, Mollinger L. Age- and gender-related test performance in community-dwelling elderly people: six-minute walk test, Berg balance scale, timed up & go test, and gait speeds. *Phys Ther.* (2002) 82:128–37. doi: 10.1093/ptj/82.2.128
48. Troosters T, Gosselink R, Decramer M. Six minute walking distance in healthy elderly subjects. *Eur Respir J.* (1999) 14:270–4. doi: 10.1034/j.1399-3003.1999.14b06.x
49. Khan I, Sarker S-J, Hackshaw A. Smaller sample sizes for phase II trials based on exact tests with actual error rates by trading-off their nominal levels of significance and power. *Br J Cancer.* (2012) 107:1801–9. doi: 10.1038/bjc.2012.444
50. Osawa Y, Tabata S, Katsukawa F, Ishida H, Oguma Y, Kawai T, et al. Effects of 16-week high-intensity interval training using upper and lower body ergometers on aerobic fitness and morphological changes in healthy men: a preliminary study. *Open Access J Sports Med.* (2014) 5:257–65. doi: 10.2147/OAJSM.S68932
51. Abe T, Fujita S, Nakajima T, Sakamaki M, Ozaki H, Oga R. Effects of low-intensity cycle training with restricted leg blood flow on thigh muscle volume and VO2max in young men. *J Sports Sci Med.* (2010) 9:452–8. Available online at: https://www.jssm.org/hf.php?id=jssm-09-452.xml
52. Schjerve IE, Tyldum GA, Tjønna AE, Stølen T, Loennechen JP, Hansen HEM, et al. Both aerobic endurance and strength training programmes improve cardiovascular health in obese adults. *Clin Sci.* (2008) 115:283–93. doi: 10.1042/CS20070332
53. Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, et al. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. *J Gerontol.* (1994) 49:M85–94. doi: 10.1093/geronj/49.2.M85
54. Kligfield P, Gettes LS, Bailey JJ, Childers R, Deal BJ, Hancock EW, et al. Recommendations for the standardization and interpretation of the electrocardiogram: part I: the electrocardiogram and its technology: a scientific statement From the American Heart Association Electrocardiography and Arrhythmias Committee, Council on Clinical Cardiology; the American College of Cardiology Foundation; and the Heart Rhythm Society: endorsed by the International Society for Computerized Electrocardiology. *Circulation.* (2007) 115:1306–24. doi: 10.1161/CIRCULATIONAHA.106.180200
55. Mason RE, Likar I. A new system of multiple-lead exercise electrocardiography. *Am Heart J.* (1966) 71:196–205. doi: 10.1016/0002-8703(66)90182-7

56. Borg G. Perceived exertion as an indicator of somatic stress. *J Rehabil Med.* (1970) 2:92–8. doi: 10.1037/t58166-000
57. Lund H, Christensen L, Savnik A, Danneskiold-Samsøe B, Bliddal H. Volume estimation of extensor muscles of the lower leg based on MR imaging. *Eur Radiol.* (2002) 12:1982–7. doi: 10.1007/s00330-002-1334-1
58. Hangartner TN, Warner S, Braillon P, Jankowski L, Shepherd J. The official positions of the International Society for Clinical Densitometry: acquisition of dual-energy X-ray absorptiometry body composition and considerations regarding analysis and repeatability of measures. *J Clin Densitom.* (2013) 16:520–36. doi: 10.1016/j.jocd.2013.08.007
59. Dite W, Temple VA. A clinical test of stepping and change of direction to identify multiple falling older adults. *Arch Phys Med Rehabil.* (2002) 83:1566–71. doi: 10.1053/apmr.2002.35469
60. Moore M, Barker K. The validity and reliability of the four square step test in different adult populations: a systematic review. *Syst Rev.* (2017) 6:187. doi: 10.1186/s13643-017-0577-5
61. Mathiowetz V, Weber K, Volland G, Kashman N. Reliability and validity of grip and pinch strength evaluations. *J Hand Surg.* (1984) 9:222–6. doi: 10.1016/S0363-5023(84)80146-X
62. Shiratori AP, Iop Rda R, Borges Júnior NG, Domenech SC, Gevaerd Mda S. Evaluation protocols of hand grip strength in individuals with rheumatoid arthritis: a systematic review. *Rev Bras Reumatol Engl Ed.* (2014) 54:140–7. doi: 10.1016/j.rbr.2014.03.009
63. Ratamess N, Alvar B, Evetoch T, Housh T, Kibler W, Kraemer W, et al. Progression models in resistance training for healthy adults: *Med. Sci Sports Exerc.* (2009) 41:687–708. doi: 10.1249/MSS.0b013e31819 15670
64. Huang G, Wang R, Chen P, Huang SC, Donnelly JE, Mehlferber JP. Dose–response relationship of cardiorespiratory fitness adaptation to controlled endurance training in sedentary older adults. *Eur J Prev Cardiol.* (2016) 23:518–29. doi: 10.1177/2047487315582322
65. Hedges LV. Distribution theory for glass's estimator of effect size and related estimators. *J Educ Stat.* (1981) 6:107–28. doi: 10.3102/10769986006002107
66. Rognmo O, Moholdt T, Bakken H, Hole T, Molstad P, Myhr NE, et al. Cardiovascular risk of high- versus moderate-intensity aerobic exercise in coronary heart disease patients. *Circulation.* (2012) 126:1436–40. doi: 10.1161/CIRCULATIONAHA.112.123117

Conflict of Interest: DT has received research funding from the American Heart Association, the Osteopathic Heritage Foundations, and the John J. Kopchick Fellowship Award from Ohio University. In the past 5-years BC has received research funding from the NIH, Regeneron Pharmaceuticals, Astellas Pharma Global Development, Inc., RTI Health Solutions, Biophytis, and the Osteopathic Heritage Foundations. In the past 5-years BC has received consulting fees from Regeneron Pharmaceuticals, Abbott Laboratories, and the Gerson Lehrman Group. Additionally, BC is co-founder with equity, and serves as the Chief of Aging Research, of AEIOU Scientific, LLC.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

SYSTEMATIC REVIEW
published: 24 October 2019
doi: 10.3389/fnagi.2019.00291

Emerging Cognitive Intervention Technologies to Meet the Needs of an Aging Population: A Systematic Review

Fady Alnajjar[1,2†], Sumayya Khalid[1], Alistair A. Vogan[1], Shingo Shimoda[2], Rui Nouchi[3] and Ryuta Kawashima[3]*

[1] College of Information Technology, United Arab Emirates University, Al Ain, United Arab Emirates, [2] Intelligent Behavior Control Unit, CBS-TOYOTA Collaboration Center, RIKEN, Nagoya, Japan, [3] Institute of Development, Aging and Cancer (IDAC), Tohoku University, Sendai, Japan

Background: Cognitive training helps to promote healthy aging and ease activities of daily living for older adults. Recently, experiments have been conducted using robots to perform this cognitive training.

Methods: A review was conducted to examine the effects of computer-based cognitive interventions for older adults who were either healthy or experiencing mild cognitive impairment (MCI). A second study also examined the evolution of socially assistive robots (SAR) and their effectiveness at administering cognitive training for older adults.

Results: Eighty-one studies published between 2009 and 2019 were identified for review, 56 of which focused on computerized cognitive training (CCT) while 25 examined the use of robotics. Twenty-four of the 56 CCT studies met the inclusion criteria. These were further classified into two groups: studies which used self-designed programs, and studies using commercially available ones. Of the 25 studies examining the use of robotics in cognitive intervention 7 met the inclusion criteria. Review shows that CCT improves cognitive function but that robots are more effective tools for improving cognition.

Conclusion: It can be concluded that CCT is beneficial for older adults and though there are drawbacks to this approach they are overcome by the introduction of robots into the training process. Culture, language, and socio-economic considerations vis-a-vis robot design and training methodology should be included in future research.

Keywords: cognitive impairment, cognitive training, computerized cognitive training, socially assistive robots, robotics for elderly

OPEN ACCESS

Edited by:
Trentham Furness, Australian Catholic University, Australia

Reviewed by:
Lakshmi Rajagopal, Northwestern University, United States
Corey Joseph, Monash Health, Australia

***Correspondence:**
Fady Alnajjar
fady.alnajjar@uaeu.ac.ae;
fady@riken.jp

†ORCID:
Fady Alnajjar
orcid.org/0000-0001-6102-3765

Received: *09 July 2019*
Accepted: *09 October 2019*
Published: *24 October 2019*

Citation:
Alnajjar F, Khalid S, Vogan AA, Shimoda S, Nouchi R and Kawashima R (2019) Emerging Cognitive Intervention Technologies to Meet the Needs of an Aging Population: A Systematic Review. Front. Aging Neurosci. 11:291. doi: 10.3389/fnagi.2019.00291

INTRODUCTION

In recent decades, the world has witnessed measures of poverty drop while, on average, those of education, income, quality of life, and life expectation have risen significantly (Pinker, 2018). Indeed, on many measures there is reason for optimism. However, a quick study of the demographics indicates a rapidly aging global population[1]. It is during this period that many begin

[1]United Nations: World Population Prospects The 2015 Revisions (2015). Available online at: http://esa.un.org/unpd/wpp/Publications/Files/Key_Findings_WPP_2015.pdf (accessed September 2015).

to experience the challenges of performing simple daily self-care and other independent living activities (McColl et al., 2013). This is because, in the later years, cognitive functions such as working memory have been found to diminish, while the prevalence of various diseases and disorders, including age-related dementia and Alzheimer's Disease, grows (Bozoki et al., 2013). In fact, dementia is one of the main reasons for the increased dependency of older people since it results in the deterioration of those specific cognitive functions needed in daily life (McColl et al., 2013). This deterioration is manifested in symptoms such as loss of memory, problems of orientation, depression, behavioral changes, and impaired communication skills. According to recent findings, each year over 9.9 million new cases of dementia are identified worldwide; this suggests a new case emerging every 3.2 s[2] At this rate, by 2050, those experiencing dementia will have reached an alarming 131.5 million[2]. The financial impact of this has the potential to be overwhelming. While the present economic worldwide cost of dementia is approximately 818 billion US dollars, in only a few years it is expected to climb to a trillion-dollar challenge[2]. This, too, applies acutely to the United Arab Emirates (UAE). At present, the UAE possesses a relatively young population; however, in the coming years this is projected to change. According to the United Nations World Economic Situation and Prospects 2010 Report, by 2050 there will be a substantial rise in the population of those aged 60 or over, from 2.4% to an alarming 27%[1]. For healthcare systems to fully-prepare themselves to meet this new reality significant innovations will need to be explored. Since no effective treatment or cure for dementia exists, an increased effort is being made to establish the efficacy of non-pharmaceutical strategies. One of these strategies is targeted cognitive training for older adults which may lead to prevention of dementia or delay of its onset (Brinke et al., 2018).

Cognitive training has been shown to maintain, or even improve, cognitive function for elderly (Kueider et al., 2012). In the past two decades this form of training has gained popularity. Studies have demonstrated its effectiveness in improving memory, attention and cognitive skills (Willis et al., 2006; Mowszowski et al., 2010; Kelly et al., 2014; Rebok et al., 2014). One randomized trial using cognitive training found diminishment in the decline of instrumental activities of daily living (IADL), thus leading to prevention and reduction of further risk of developing functional decline in elderly (Rebok et al., 2014).

Cognitive training involves a well-structured practice of complex mental exercises. Training can be given in multiple ways; it can be process-based, comprising repetitive training on specific tasks, or more strategic, individualized intervention based on memory formation strategies (Walton et al., 2015). However, there are hurdles to its widespread implementation. The traditional method of cognitive training requires a trained instructor, for example. This necessitates face-to-face interaction, which entails a meeting location, the coordination of schedules and travel time. Additionally, training can be very expensive since trainers usually charge by the hour; there is also the added cost of equipment and materials. Furthermore, not all elderly individuals are comfortable in traveling regularly to a meeting location. In fact, some older adults may be home-bound, live in an assisted living or nursing home facility, or may simply not be able to easily access transportation (Kueider et al., 2012). As a result, it becomes difficult to take part in these programs with regularity.

Recent informational technological advancements which potentially alleviate this problem have made their way into healthcare, and now play a significant role in cognitive training. Computer-based cognitive training (CCT) has been found to be easier to implement since it is cost-effective, can be accessed from anywhere and at any time, and can be performed from the comfort of the user's home (Kueider et al., 2012). Also, it can be customized according to specific needs of individuals. Moreover, CCT provides real-time performance assessment and feedback, and allows for the adjustment of application difficulty level accordingly. There are three approaches to impart CCT: (1) brain-training programs, (2) working memory training programs, and (3) video game training programs (Boot and Kramer, 2014). Computer and video games are designed to be fun and exciting. This serves to motivate users to maintain engagement throughout the training program. However, while there are many cognitive training products in the market, there is still a lack of evidence supporting their effectiveness at imparting cognitive training with significant improvement in cognitive function (Kueider et al., 2012). One study, a meta analytical review concluded that a commercially available computer-based training program for working memory skills was only able to have short-term specific training effects and did not generalize to "real world" cognitive skills, which raises question regarding the methodological approach or theoretical support for the current available training mechanisms (Melby-Lervåg et al., 2016).

To provide effective care or to improve the efficacy of the training program, it is important that the methods used should be comfortable for users. For example, the elderly may feel more at ease when training is conducted in their native tongue and when it is developed with an orientation to their individual culture. The consequence of this will be that training is more impactful and enjoyable since the user will be more greatly motivated to engage within each session. Furthermore, with increasing research being conducted on interventions for age-related cognitive impairment, it is important to understand and distinguish the effectiveness of various methodologies used. The effectiveness of any individual methodology may be determined, for practical purposes, by the extent to which a transfer effect is produced. The transfer effect, in this case, would refer to the effect that the knowledge or abilities acquired in one area might have on the knowledge acquisition in other areas. From this understanding, the transfer effect can be bifurcated into "near" and "far" transfer effect, with the further distinction that the production of a range of "near" and "far" transfer effects might identify a quality methodology (Nouchi and Kawashima, 2014). While short-term cognitive training has been demonstrated to produce a limited and temporary effect, training conducted regularly and with vigor over an extended period of time can have a sustained meaningful impact (Tapus and Vieru, 2013).

[2]Dementia Statistics, Alzeihmer's Disease International. Available online at: https://www.alz.co.uk/research/statistics.

Unfortunately, the healthcare facilities are already under pressure with a shortage of staff and space. It is very challenging to provide a customized setup for each individual, according to their specific requirements (Tapus and Vieru, 2013).

Intelligent robotic systems have been designed for human-robot interaction (HRI). HRI is a field of study dedicated to understanding, designing and evaluating robotic systems for use by or with humans. It's a communication link between human and robots[3] As per Wikipedia the purpose of HRI is to model human expectations, regarding robotic interaction, to aid in robot design and algorithm development, which can allow more natural and effective interaction between human and robots.

Newly developed robotic systems have evolved considerably and can now be effectively used to provide that individualized care to the elderly, and from the comfort of the user's home (McColl et al., 2013). Additionally, socially interactive robot may have a tremendous impact on overall cognitive and social well-being. Developing a more human-like social robot with natural gestures and speech can engage a user and more fully support them as they carry out their exercises (Tapus and Vieru, 2013). Robots such as these could be programmed to help users choose from a variety of exercises and could motivate them along the way by giving applause, praises, or encouraging feedback during the training. Additionally, social assistive robots (SAR) have been shown to provide a companionship which improves user engagement in activities. This plays a powerful role in cognitive health (Tapus and Vieru, 2013). Furthermore, the specific needs of the user can be met through a customization of the social robot appearance; in particular, they have been designed to resemble pets, such as dogs or seals, producing positive benefits. Robots like Paro, ICat, Albo, and Pearl have been studied for their effect on the elderly, and a positive psychological and social impact has been demonstrated, such as improvement to both the mood and well-being of the users (Broekens et al., 2000). Unfortunately, most interventions have been limited to nursing homes or health facility. Few studies exist which examine the impact of assistive robots on the basic daily activities of the elderly in their own homes.

It seems however, a future socially assistive humanoid robot could help not only with the daily activities of the elderly and by providing company, but also by performing cognitive training with regularity and accuracy. When doing this it would be able to maintain users' scores and learn and adapt continuously to these individuals over time as cognition improved. This adaptive, user-friendly, reliable robot would provide an engaging and motivating customized therapy to users, establishing a life-enriching human-robot relationship.

To make this possible, some system requirements that can be identified are two-way communication, safety, services and assistive functions, therapy and smart situation awareness (Gross et al., 2011). Furthermore, to ensure a good human robot relationship and interaction, we need to make the robot as human-like as possible. The main requirements would be for it to have more appealing, human-like interaction capabilities, demonstrate appropriate social behavior and be able to focus user attention in order to help achieve specific goals (Tapus et al., 2007).

There are a few areas of focus that need to be addressed when designing a humanoid robot: (1) physical appearance, (2) personality, (3) empathy, (4) engagement, (5) adaptation, and (6) transfer (Tapus et al., 2007).

A vast amount of literature exists which explores the effects of cognitive training (both computerized and non-computerized) on the elderly.

This review focuses on the effect of CCT on samples (healthy older adults and healthy older adults with MCI), to delay or prevent onset of dementia. For the purpose of this review, related articles published over the last 10 years were researched to answer the following questions: (1) To what degree has CCT been impactful as a tool for cognitive training of individuals experiencing age-related cognitive decline? (2) Can CCT delay or prevent the onset of dementia and which programs have been found to be most effective? (3) How have robots been used for cognitive training? (4) What challenges have been identified in robot development as they relate to cognitive training of the elderly?

METHODS

To carry out this review, a methodological approach was followed.

Search Strategy

Databases were searched systematically to identify possible studies for inclusion. The databases were searched using the following keywords: *aging, smart aging, elderly, old, adults, computerized, cognitive, computerized cognitive, training, interactive gaming, cognition, cognitive abilities, video games, trainings, robots, socially assistive robots*. Databases used were *PubMed, Psych Info, SCOPUS, Google Scholar, MEDLINE, and CINAHIL*. We also dug deep into references of some these studies to find out more relevant studies.

Inclusion and Exclusion Criteria

Studies met the inclusion criteria if they: (a) were published in the last 10 years, (b) were in English, (c) were randomized control trials, (d) had a sample of healthy older adults and healthy older adults with mild cognitive impairment, (e) Patients more than 55 years of age [as 3 relevant studies (Ballesteros et al., 2014; Marusic et al., 2016; Zhang et al., 2019) used older adults aged from 55 years old] (f) used only CCTs as intervention (either commercially available or video games). Studies that did not use computer-based trainings were excluded. All studies in which participants had dementia or Alzheimer's disease were excluded. Studies which focused on computerized cognitive training were sorted into two groups, one which used training programs that were specifically designed for the study, the other which used commercially available cognitive training programs, **Figure 1**. Each study was reviewed and key information (participants, age,

[3]*Introduction: Human – Robot Interaction.* Available online at: https://humanrobotinteraction.org/1-introduction/.

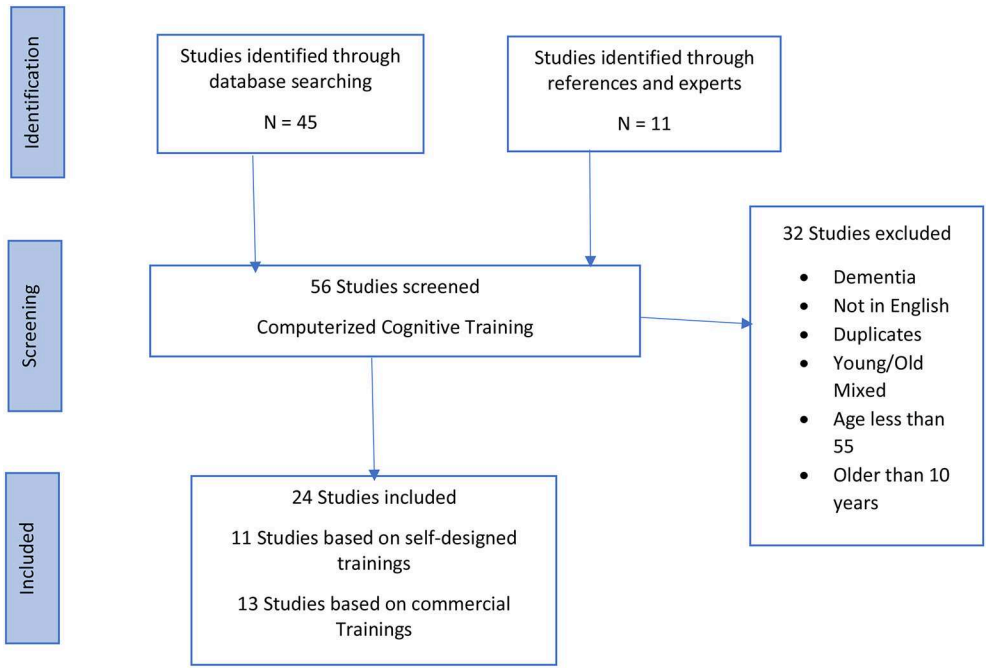


FIGURE 1 | Computerized Cognitive Training (CCT)—flow diagram of search results.

type of intervention, cognitive status, and cognitive outcomes) pertaining to study design was extracted.

In addition, analyzed separately were a few studies, shown in **Figure 2**, that focused on elderly care using robots. Among these, two types of studies were identified. The first set of studies were based on socially assistive robots, or service type robots, that assisted the elderly in independent living activities. The second set of studies focused on robots which resembled pets, or companion robots, meant to keep users company to mitigate loneliness and depression. Few studies existed which examined the use of robots to impart cognitive training to the elderly. Consequently, studies that were considered eligible for review were those that met a purpose to serve the elderly in any manner. Studies with elderly people with dementia were also included. Excluded, however, were those meant only to gauge the acceptance of robot presence by the elderly, or studies using surgically assistive robots. However, since research examining the effectiveness of using robots for cognitive training is still in its infancy, a time range was not defined for including studies relevant to usage of robotics in elderly care, and the studies possessing participants with dementia were also included for this section.

Quality Assessment Methodology

Each study was assessed for quality using a modified Delphi list (Verhagen et al., 1998). To improve the assessment quality, the following elements were considered: age range, sample size, and intervention duration. The latter two were considered with the understanding that a smaller sample size and a shorter intervention duration might negatively impact generalizability and reproducibility. Additional considerations were given to a study's limit or extensiveness of the cognitive functions under examination, whether the interventions were conducted at home or at a center, and finally, whether they were supervised or non-supervised. The choice of elements included in the quality assessment was guided by a previously published systematic review conducted by Nouchi et al. (Nouchi and Kawashima, 2014).

RESULTS

Fifty-six RCTs based on CCT were identified for this review published in the last 10 years. Each study was reviewed and information pertaining to the study design, sample characteristics (e.g., age, cognitive status), cognitive outcomes were extracted. Based on the above-mentioned inclusion and exclusion criteria 24 of the 56 publications related to computerized cognitive training, were eligible for current review. Common reasons for exclusion were: samples having dementia or with participants younger than 55 years of age, duplicate studies, studies not published in English. The 24 studies used in this review focused on different cognitive measures. **Table 1** shows the methodological quality of the included studies. **Tables 2**, **3** summarize the findings of each individual study. Twelve (Barnes et al., 2009; Herrera et al., 2012; Rose et al., 2012; Bozoki et al., 2013; McAvinue et al., 2013; Corbett et al., 2015; Gooding et al., 2015; Marusic et al., 2016; Nouchi et al., 2016; Yeo et al., 2018; Requena and Rebok, 2019; Zhang et al., 2019) studies designed their own CT programs. Twelve (Finn and McDonald, 2011; Peretz et al., 2011; Miller et al., 2013; Strenziok et al., 2013; Ballesteros et al., 2014; Hughes et al., 2014; Hyer et al., 2015; Styliadis et al., 2015; Walton et al., 2015; Lin et al., 2016; Toril

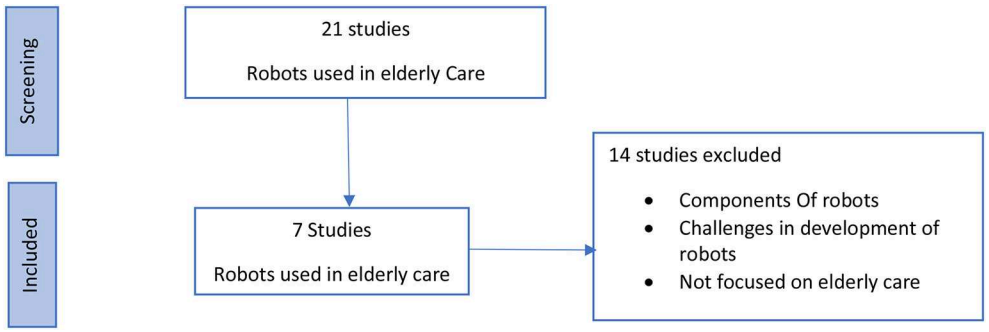


FIGURE 2 | Robots used in elderly care—flow diagram of search results.

et al., 2016; Simon et al., 2018) used a commercially available cognitive training (CT) program based on video game trainings, such as Lumosity, Cog Med, Brain Age etc.

Quality Assessment

Table 1 presents an assessment of the methodological quality of the included studies. The quality assessment ranged from 3 to 7, with an average of 5.25 out of 9. All included studies have a "good" methodology. A study by Corbett et al. (2015) had the highest methodological quality. The score of item 1 was low among the included studies because most of the studies had a small sample size; below 100. All studies have clearly mentioned the age range or mean age group of their sample, except a study by Šabanović et al. (2013) which identifies their sample only as "older adults." While all studies had intervention duration longer than 3 weeks, one study by Walton et al. (2015) was identified as having a very short duration at just 28 days.

Participants, Sample Size, and Duration of Intervention

Among the 24 studies, all the participants were older adults. However, 15 studies had healthy older adults (Verhagen et al., 1998; Tapus et al., 2007; Finn and McDonald, 2011; Gross et al., 2011; Herrera et al., 2012; McAvinue et al., 2013; Strenziok et al., 2013; Gooding et al., 2015; Hyer et al., 2015; Walton et al., 2015; Marusic et al., 2016; Nouchi et al., 2016; Toril et al., 2016; Yeo et al., 2018)[3], 8 studies (Peretz et al., 2011; Rose et al., 2012; Ballesteros et al., 2014; Corbett et al., 2015; Styliadis et al., 2015; Marusic et al., 2016; Simon et al., 2018; Requena and Rebok, 2019) used participants with MCI and 1 study (Zhang et al., 2019) had participants with subclinical cognitive decline. The age range of these studies varied between 55 and 90, with only 1 study (Finn and McDonald, 2011) with an age range not provided, though they did identify the participants as "older adults." The duration of interventions was between 14 days and 6 months, with 1–3 sessions per week on an average.

Cognitive Functions Measured

Among the 24 studies, a variety of foci were employed examining the impact on cognitive function. Some studies focused on a single function, whereas others explored two or more. The following are areas where quantitative data were gathered: processing speed, memory attention, and reasoning. Processing speed is defined as the ability to quickly process information. A total of 8 studies (Barnes et al., 2009; Finn and McDonald, 2011; Bozoki et al., 2013; Ballesteros et al., 2014; Walton et al., 2015; Lin et al., 2016; Marusic et al., 2016; Nouchi et al., 2016) measured processing speed. Results of 5 studies (Ballesteros et al., 2014; Walton et al., 2015; Lin et al., 2016; Marusic et al., 2016; Nouchi et al., 2016) showed there was improvement. Memory is the ability to retain, store, and recall information (Kueider et al., 2012). There are many different types of memory (e.g., recall, recognition, episodic, verbal, visual, and working). While most studies only examined memory as an overall cognitive function, some divided it into subcategories. Eleven studies (Barnes et al., 2009; Bozoki et al., 2013; McAvinue et al., 2013; Strenziok et al., 2013; Ballesteros et al., 2014; Hyer et al., 2015; Walton et al., 2015; Nouchi et al., 2016; Toril et al., 2016; Simon et al., 2018; Yeo et al., 2018) focused on working memory; 2 studies (Hyer et al., 2015; Simon et al., 2018) out of these had working memory as the sole cognitive domain for the study. Both (Hyer et al., 2015; Simon et al., 2018) showed significant improvement in the area of working memory; 1 study (Hyer et al., 2015) had participants with mild cognitive impairment (MCI) while the other (Simon et al., 2018) had healthy older adults. Both studies used commercially available CogMed video games in their training. The study with healthy older adults demonstrated transfer effects resulting in improvement of processing speed as well. Out of the remaining 9 studies (Barnes et al., 2009; Bozoki et al., 2013; McAvinue et al., 2013; Strenziok et al., 2013; Ballesteros et al., 2014; Walton et al., 2015; Nouchi et al., 2016; Toril et al., 2016; Yeo et al., 2018) which focused on multiple domains in addition to working memory, 3 studies (Barnes et al., 2009; Walton et al., 2015; Toril et al., 2016) showed improvement, whereas 6 studies (Bozoki et al., 2013; McAvinue et al., 2013; Strenziok et al., 2013; Ballesteros et al., 2014; Nouchi et al., 2016; Yeo et al., 2018) showed no significant change in working memory. Other categories of memory, like short-term memory and episodic memory, showed improvement after interventions. Some studies which considered memory as a single domain also showed levels of improvement. However, there was one study that showed no improvement at all (Gross et al., 2011). Attention can be understood as the process by which an individual directs or focuses on specific auditory

TABLE 1 | Scores of methodological qualities.

References	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Total Score (max = 9)
McAvinue et al. (2013)	N	Y	Y	N	N	N	Y	?	Y	4
Yeo et al. (2018)	Y	Y	Y	Y	Y	?	Y	N	Y	7
Bozoki et al. (2013)	N	Y	Y	Y	N	N	Y	Y	Y	6
Corbett et al. (2015)	Y	Y	Y	Y	N	N	Y	Y	Y	7
Rose et al. (2015)	N	Y	Y	N	Y	Y	?	?	Y	5
Nouchi et al. (2016)	N	Y	Y	Y	N	N	N	Y	Y	5
Requena and Rebok (2019)	N	Y	Y	Y	Y,	Y	N	?	Y	6
Zhang et al. (2019)	N	Y	Y	Y	Y	Y	?	?	Y	6
Barnes et al. (2009)	N	Y	Y	Y	N	N	Y	?	Y	5
Marusic et al. (2016)	N	Y	Y	Y	Y	Y	Y	?	Y	7
Herrera et al. (2012)	N	Y	Y	Y	Y	Y	N	N	Y	6
Gooding et al. (2015)	N	Y	Y	Y	Y	?	N	?	Y	5
Hyer et al. (2015)	N	Y	Y	Y	Y	N	Y	?	Y	6
Walton et al. (2015)	N	Y	Y	Y	N	N	Y	Y	Y	6
Toril et al. (2016)	N	Y	Y	Y	Y	Y	Y	?	Y	7
Simon et al. (2018)	N	Y	Y	N	N	N	Y	?	Y	4
Strenziok et al. (2013)	N	N	Y	Y	N	N	N	?	Y	3
Peretz et al. (2011)	Y	Y	Y	Y	N	N	N	Y	Y	6
Finn and McDonald (2011)	N	Y	Y	Y	N	N	Y	?	Y	5
Ballesteros et al. (2014)	N	Y	Y	Y	Y	Y	Y	?	Y	7
Styliadis et al. (2015)	N	Y	Y	Y	?	?	N	N	Y	4
Miller et al. (2013)	N	Y	Y	Y	?	?	Y	?	Y	5
Lin et al. (2016)	N	Y	Y	N	N	N	N	?	Y	3
Hughes et al. (2014)	N	Y	Y	?	N	N	N	?	Y	3

Q1. Sample size > 199; Q2. Age mentioned; Q3. Duration > 3 weeks; Q4. Cognitive domains > 2; Q5. Supervised intervention; Q6. Carried out at center; Q7. Subjects similar at baseline; Q8. Patients blinded to trial; Q9. Statistically significant.
Y, Yes—The study met the criteria. N, No—The study did not meet the criteria. ?, No information.

or visual stimuli in the environment. There were 10 studies (Finn and McDonald, 2011; Herrera et al., 2012; Ballesteros et al., 2014; Corbett et al., 2015; Gooding et al., 2015; Lin et al., 2016; Marusic et al., 2016; Yeo et al., 2018; Requena and Rebok, 2019; Zhang et al., 2019) which included attention as one of the focused domains. Attention was seen to improve significantly in 8 studies (Finn and McDonald, 2011; Herrera et al., 2012; Ballesteros et al., 2014; Corbett et al., 2015; Gooding et al., 2015; Lin et al., 2016; Marusic et al., 2016; Requena and Rebok, 2019) while 2 studies (Yeo et al., 2018; Zhang et al., 2019) found no improvement. Reasoning is the action of thinking about something in a logical, sensible way. Five studies (Bozoki et al., 2013; Miller et al., 2013; Corbett et al., 2015; Nouchi et al., 2016; Zhang et al., 2019) focused on reasoning as one of the cognitive domains. While 2 studies (Bozoki et al., 2013; Nouchi et al., 2016; Zhang et al., 2019) did not find evidence of impact after from intervention, the 3 remaining studies (Miller et al., 2013; Corbett et al., 2015) found significant improvements.

Robots Used in Elderly Care

Out of another set of 25 studies, 7 (Tanaka et al., 2012; Kim et al., 2015; Moyle et al., 2015, 2017; Soler et al., 2015; Jøranson et al., 2016; Thodberg et al., 2016) were shortlisted that implied usage of robots in elderly care (**Table 4**). Of these, 2 studies (Kim et al., 2015; Soler et al., 2015) examined the effect of using a robot for cognitive training with the elderly. Most of the studies were based on the usage of robots as a companion (Jøranson et al., 2016; Moyle et al., 2017) or explored their role in affective therapy (Tanaka et al., 2012; Moyle et al., 2015; Thodberg et al., 2016). However, all robotic studies yielded a positive outcome, thus making it one of the most suitable methods to impart cognitive training at home since it can be supervised by the robot. Also, the difficulty level of the program can be controlled through the robot; by maintaining and analyzing the data, and then adjusting the difficulty level of the training accordingly.

DISCUSSION

This review first summarizes the types of CCT programs that have been employed for improving cognitive function or attenuating cognitive decline in both healthy older adults and older adults with MCI. It also examines the impact of these programs. Based on this review, CCT appears very promising as a tool to improve the cognitive abilities of healthy older

TABLE 2 | Self-designed cognitive trainings.

References	Type of intervention	No of subjects and trial period	Cognitive domain focused	Findings	Limitations
McAvinue et al. (2013)	• Computerized training task • Control group	36 healthy older subjects Age range: 64–79 years old 5-week training period + a 6-month follow up	• Short-term memory • Working memory	• Improvement in short-term memory, together with transfer of training gains to long-term episode memory tasks • No significant improvement in working memory	• A small sample size • Lack of inclusion of a measure of visuo-spatial short-term or working memory • Non-adaptive version of the training program for control group
Yeo et al. (2018)	• Cognitive training system, BRAINMEM	240 healthy participants Age range: 60–80 years old 24 sessions over 8 weeks and three-monthly booster sessions	• Attention • Working memory • Delayed recall	• No significant differences in overall cognitive performance post-intervention between subjects	• Lack of a sham control • Unbiased testing of effect sustainability of the training not done • Lack of generalizability
Bozoki et al. (2013)	• Online games designed for the program • Active group only	60 Healthy older subjects Age range: 60–80 years old 6 weeks	• Visual attention • Working memory • Processing Speed • Reasoning	• No effects, only improvements on games	• A small sample size; a short-term trial • No control group • Low program intensity
Corbett et al. (2015)	• Problem-solving cognitive training (ReaCT) • General Cognitive Training (GCT) • A control treatment • Group	2,192 healthy older subjects; Age mean: 65 years old 6 months	• Reasoning • Problem solving • Attention • Memory • Visuospatial ability	• Improved cognition, particularly the reasoning skills, evident from week 6	• Only people who could access computer were included into the trial • Only people with higher levels of education; retention strategies need to be improved
Rose et al. (2012)	• Virtual Week Training • Program • Active Control • Group (ACG)	59 healthy older subjects Age mean: 67.4 years old 1 month 12 sessions, each 1 h long	• Prospective memory	• Improved prospective memory • Transfer to real-world settings, reflected in participants' daily activities	• A small sample size • A short-term trial period • A lack of effective strategies used by participants
Nouchi et al. (2016)	• Processing Speed Training Game (PSTG) • Knowledge and Quiz Training Game (KQTG) • Active control group	72 healthy older adults Age range: 60 years old or more 4 weeks	• Processing speed • Reasoning • Short term memory • Working memory • Episodic memory	• PSTG had a small improvement in processing speed, inhibition and depressive mood • No improved performance in reasoning, shifting, short term/working memory, and episodic memory	• Short-term training period • No follow-up assessment • A small effect size
Requena and Rebok (2019)	• Experimental control group • G1—Training with Lumosity • G2—Training with paper and pencil	54 healthy older adults • Age range: 65 years and older 32 sessions held weekly during the months of October to May during the years 2015–2017	• Attention • Memory • Psychological well-being	• No differences in the psychological well-being in either groups • Significant difference in attention, everyday memory and brain activity • CCT outperformed paper-and-pencil training	• Difference in age and educational level
Zhang et al. (2019)	• Multi-domain cognitive training via tablet	27 older adults with MCI Age range: 55 years and above Twice a week/12 weeks	• Reasoning • Memory • Visuospatial skills • Language • Calculation • Attention	• Improvement in immediate memory and visuospatial memory abilities • No significant difference in neuropsychological test scores observed from baseline	• A small sample size • Inadequate training duration • Lack of control group

(Continued)

TABLE 2 | Continued

References	Type of intervention	No of subjects and trial period	Cognitive domain focused	Findings	Limitations
Barnes et al. (2009)	• Computer-based cognitive training (CCT) program developed by Posit Science Corporation (San Francisco, CA)	47 subjects with mild cognitive impairment Age mean: 74 years old 100 min/day, 5 days/week for 6 weeks	• Processing speed • Accuracy • Primary Memory • Working auditory memory	• Primary outcome of global cognitive function between the intervention and control groups not statistically significant • Effect sizes for measures of verbal learning and memory consistently favored the intervention	• Small sample size • Stimulating cognitive and physical lifestyle activities outside of intervention not controlled
Marusic et al. (2016)	• Computerized spatial navigation training (CSNT) protocol • Experiment-control groups	16 healthy men Age range: 55–65 years old 14 days training, 28-day recovery program	• Executive function • Attention • Processing speed	• Improved spatial navigation • Improved performance (fidelity), but visible also across other cognitive domains known to be associated with brain areas sub served by those that involve spatial navigation	• A small sample size • A short duration
Herrera et al. (2012)	• Programmed training exercises (visual recognition task) • Attention training task	22 older adults with amnestic MCI Age range: 65–90 years old 12 weeks	• Memory • Attention	• Improved episodic memory • Transfer effect between recognition vs. recall • Attention training with the visual focused attentional tasks improved information processing	• Parameters very frequently manipulated so that training tasks would continue to challenge each patient's abilities throughout training
Gooding et al. (2015)	• Randomized clinical trial • Computerized Cognitive Training (CCT) • Cognitive Vitality Training (CVT) • An Active Control Group (ACG)	96 male participants Age mean: ~76 years old 30 h of training/16-weeks	• Memory • Attention • Executive Function	• CVT showed significant improvement relative to ACG • No significant difference between participants of CCT and CVT	• Restricted demographics sample • Did not include measures to assess everyday functioning

adults and adults with MCI who have a higher risk of acquiring dementia or Alzheimer's disease. Timely training may prolong the onset of dementia and Alzheimer's disease; still, there are a few concerns to be discussed in this section. This review next presents, how robots have been used in elderly care to ease their living.

In our shortlisted studies, the cognitive training can be categorized as self-designed cognitive training, custom-made for the program (Barnes et al., 2009; Herrera et al., 2012; Rose et al., 2012; Bozoki et al., 2013; McAvinue et al., 2013; Corbett et al., 2015; Gooding et al., 2015; Marusic et al., 2016; Nouchi et al., 2016; Yeo et al., 2018; Requena and Rebok, 2019; Zhang et al., 2019), and as commercially-available training programs and video games (Finn and McDonald, 2011; Peretz et al., 2011; Miller et al., 2013; Strenziok et al., 2013; Ballesteros et al., 2014; Hughes et al., 2014; Hyer et al., 2015; Styliadis et al., 2015; Walton et al., 2015; Lin et al., 2016; Toril et al., 2016; Simon et al., 2018). Based on this review, self-designed cognitive training interventions demonstrated an improvement in processing speed, working memory, executive function, visual spatial ability, and attention. For example, in one of the studies; Corbett et al. (2015), an online 6-month randomized 3-arm controlled trial was conducted. The study compared general cognitive training (GCT), evidence-based reasoning and problem-solving cognitive training (ReaCT) and a control group. ReaCT focused on 3 reasoning and 3 problem solving tasks and GCT involved cognitive tasks covering mathematics, attention, memory and visuospatial ability. Participants were asked to undertake these training for 10 min daily. As participants improved the task difficulty increased to maintain the challenge and improve performance. The control group performed equivalent internet-based tasks involving a game in which people were asked to put a series of statements in correct numerical order. This trial showed that there was considerable improvement in all the cognitive domains mentioned above.

Commercially available trainings like Cogmed and Lumosity improved visuo-spatial functions, episodic memory, working memory and attention as these commercially available programs have designed games in a manner which helps in improving the above-mentioned cognitive domains. For example, Hyer et al. (2015) used Cogmed for their study, which had participants with MCI, the study focused on improvement of working memory. Twenty-five sessions were conducted in 5–7 weeks for 40 min per day, where the participants were given exercises, that involved the temporary storage and manipulation of sequential visuospatial and/or verbal information. Each participant had a coach who ensured the proper completion of tasks in a timely manner. Improvement in working memory of participants was seen after the training (Hyer et al., 2015). Video game-based training had a significant impact on measures of reaction time and processing speed but were not very impactful on executive function or memory (Ballesteros et al., 2014). For example, Ballesteros et al. (2014) in their study used non-action video games for training, 50 healthy older adults for 20–1 h sessions for 12 weeks. They observed enhancements in controlled processing and attention but no significant improvement in working memory and executive functions. Consequently, interventions

TABLE 3 | Commercially available programs and video games.

References	Type of intervention	No of subjects and trial period	Cognitive domain focused	Findings	Limitations
Hyer et al. (2015)	• Cognitive training program Cog Med for the intervention group and Sham for the active control group	68 older subjects with Mild Cognitive Impairment (MCI) Age range: 65 years and above 7 weeks	• Working memory	• Improved working memory of both groups • Cog Med group had higher satisfaction ratings	• A small sample size • A short-term period of the trial; a lack of the program intensity
Walton et al. (2015)	• Internet-based commercially available program- Brain trainer • Active-control group	28 healthy older subjects Age mean: 64.18 years old 28 days	• Processing speed • Memory execution function • Visuospatial memory and ability • Working memory	• Improved reaction time for both groups • Significant improvement in accuracy and spatial working memory for treatment group	• A lack of the follow up assessment • A small sample size • A short-term period
Toril et al. (2016)	• Commercially available video games Lumosity used for training • Experimental control group	39 healthy older adults Age mean: 69.95 years old (experimental group) & 73.20 years old (control group) 7–8 weeks	• Visuospatial working memory • Episodic memory • Short-term memory	• Improved visuospatial and working memory performance • Effects maintained over a 3-month no-contact follow-up period in short term memory and episodic memory	• A small sample size • Study did not evaluate the effects of training older adults with video games on everyday life tasks • A passive control group
Simon et al. (2018)	• CogMed for training participants • An active control group	82 healthy older adults from 2 countries Age range: 65 and above 5 times a week/5 weeks	• Working memory	• Improved working memory and processing speed	• Not able to conclude if cultural differences between sites affect cognitive measures • A moderate sample size • The transfer effect observed on only one cognitive task • Lacked a baseline assessment
Strenziok et al. (2013)	• Brain Fitness (BF-auditory perception) • Space Fortress (SF-visuomotor/working memory) • Rise of Nations (RON strategic reasoning)	42 healthy 'older adults' Age not specified 6-week training session	• Auditory perception • Visuomotor working memory • Strategic reasoning	• BF training: improvement in everyday problem-solving and reasoning • SF: improvement in untrained everyday problem-solving • RON: no effect on everyday problem-solving or reasoning and reduced working memory performance	• Benefits common to all three tasks were less detectable
Peretz et al. (2011)	• CogniFit Personal Coach, • Computer games group	155 healthy older adults Age range: 61–75 years old 3 sessions/week/3-month period	• 17 cognitive abilities	• Both approaches generated cognitive benefits • Conclusion: Regular mental stimulation will result in improved cognitive ability	• The ceiling effects in the measurement instrument • The lack of health and quality-of-life endpoints • The absence of a strict follow-up to monitor additional aspects of adherence • The lack of a postintervention follow-up
Finn and McDonald (2011)	• Lumosity	25 participants with MCI Age range: 60 years and above 6–8 weeks	• Attention • Processing speed • Visual memory • Cognitive control.	• Improved performance on the trained tasks over time • Improvement on a measure of visual sustained attention in treatment group • No significant changes noted on other primary outcome measures • No generalization to self-reported memory functioning or perceptions of control over memory	• A small sample size • No control group • Training at home, using own computers

(Continued)

TABLE 3 | Continued

References	Type of intervention	No of subjects and trial period	Cognitive domain focused	Findings	Limitations
Ballesteros et al. (2014)	• Lumosity	40 healthy older adults Age range: 57–80 years old 20 1 h non-action video game training sessions/10–12 weeks	• Processing speed • Attention • Executive control • Spatial working memory • Episodic memory • Subjective well-being	• Trained group showed enhancements in controlled processing, attention, immediate and delayed • Recall memory • Affection and assertiveness. • Trained participants neither showed transfer to executive control nor to spatial WM • Reduced distractibility in trainees by improving alertness and attention filtering • Marginal improvement observed in affection and assertiveness (two dimensions of subjective well-being) • No significant impact of video game training on executive functions	• A small sample size • Generalizability to everyday life tasks not examined • Additional time spent, and development of rapport/relationship, with researchers, may have effected motivation of experimental group—complicating conclusions regarding neuroplasticity
Styliadis et al. (2015)	• Posit training • Divided into five groups: three experimental groups: cognitive and/or physical training; two control groups: active and passive	70 right-handed MCI older adults Aged 60 years old and above 8 weeks	• Verbal memory • Executive functions • Independent living	• Combined interventions, occurring either sequentially or simultaneously, show promise in maintaining or improving cognitive functions • Combined training can improve general cognitive performance and subjective measures of functional status as compared to a no-treatment control • The other experimental (CT, PT) and control groups (AC) did not show significant alterations in their cortical activity after training	• Not a blind study • A small sample size
Miller et al. (2013)	• Brain Fitness • Intervention-control group	84 healthy older adults Age mean: 81.8 years old 5 days a week/20–25 min each day/8 weeks	• Short and long-term memory • Language • Visual spatial processing • Reasoning/problem solving • Calculation skills	• Improved delayed memory scores • Improved cognitive performance over extended period, including memory and language	• A small sample size • A comparatively short follow-up period of 6 months • Most participants were well-educated and Caucasian • Wide variability in number of sessions for each group • Participants not screened for MCI
Lin et al. (2016)	• INSIGHT online training program (Posit Science) • Active control group	21 older adults with MCI Age range: 60 years or above 6 weeks VSOP training	• Processing speed • Attention	• VSOP lead to improvement in trained and untrained domains like working memory	• A small sample size • The training effects not specified
Hughes et al. (2014)	• Group-based Wii interactive video gaming	20 older adults with MCI Age mean: 77.4 years old 90 min sessions/24 weeks	• Not specifically mentioned	• Older adults with MCI are capable of engaging in interactive video gaming over a period of 6 months • Community-dwelling older adults with MCI are capable of, enjoy, and are stimulated by, interactive video games	• A small sample size

TABLE 4 | Robots used in elderly care.

References	Type of robot and role of robot	No of subjects and trial duration	Place of study	Intervention	Outcome
Kim et al. (2015)	• Role: assisted in cognitive training • Robots—Silbot and Mero	85 participants Age range: 60 and above 12-week study	N/A	• Participants randomized into 3 groups: • Traditional cognitive training • Robot-assisted cognitive training • No intervention group	• Conventional cognitive training group showed less cortical thinning • Robot-assisted group showed greater results
Tanaka et al. (2012)	• Role: therapeutic • Robot–Nodding Kabochan communication robot	34 healthy female adults Age range: 66–84 years old 8 weeks study	Home	• Participants randomized into 2 groups: • Group with communicative Kabochan robot which communicated with users • Group with a control robot looked like Kabochan but did not communicate	• The experimental group slept better • Had decreased levels of saliva cortisol • Showed improved cognitive function, (executive and verbal memory function)
Jøranson et al. (2016)	• Role: companion • Robot—PARO	53 older adults with MCI or dementia Age range: 65 and above 12 weeks study	Nursing home	Participants randomized into two groups: • The intervention group with PARO—the harp seal robot • The control group which carried out treatment as before the study	• The intervention group showed improved quality of life levels but only for patients with severe dementia • No significant difference seen in quality of life levels in mild-to-moderate dementia in the intervention group as compared to control group
Thodberg et al. (2016)	• Role: affective therapy • Robot—PARO	100 participants Age mean: 85.5 years old 6 weeks	Nursing home	• Supervised interaction with a dog, PARO or a toy cat	• The dog and the robot gained more interaction than the toy cat • Over time robot interaction deceased as compared to interaction with dog • Depression scores improved over the study
Moyle et al. (2017)	• Role: companion therapy • Robot PARO	415 participants with dementia Age mean: 85 years old 10 weeks	Long-term care facilities	• One-on-one interaction with PARO • Switched on and with PARO switched off and a control group	• Participants in the PARO switched on group were more engaged verbally and visually than compared to the users in PARO switched off group • PARO switched on group had improved pleasure and reduced agitation levels
Moyle et al. (2015)	• Role: therapeutic • Robot—CuDDler	5 female participants with dementia Age mean: 84 years old 5 weeks	Nursing home	• One–to–one interaction with the CuDDler	• Agitation levels increased among the 5 participants
Soler et al. (2015)	• Role: cognitive and physical therapy • Robots—NAO & PARO	Phase 1—101 participants with dementia Age mean: 84.7 years old 3 months Phase 2—110 participants with dementia Age mean: 84.7 years old 3 months	Nursing Home	• Phase 1–supervised cognitive, musical, and physical group therapy with NAO • Phase 2–supervised cognitive, musical, and physical group therapy with PARO	Phase 1: • Decreased apathy in both the groups • Increased delusion in the NAO group • Increased irritability in both groups Phase 2: • Increased hallucinations and irritability in both the groups

based solely on video games may not be, strictly speaking, of significant benefit to improve cognitive function.

In addition, most studies used a small sample size, and duration of training was short. Many did not carry out follow-up checks after training to ascertain the existence of long-term effects. One of the studies (Lampit et al., 2014) indicated that CCT should be done for a minimum of 30 min because synaptic plasticity is only possible after 30–60 min of stimulation. It also noted that training sessions should not exceed three sessions per week, otherwise the training appeared to produce the opposite of the intervention objective (Lampit et al., 2014). This observation could be utilized for designing a program with methodology that produces the best outcome, Researchers also claim that computer-based cognitive training has moderate effects in improving cognitive functioning in healthy older individuals, but the training's effectiveness varies across cognitive domains and is determined by design choices.

A very important element regarding the efficacy of cognitive training is the question of transfer effect, which is explained in the introduction section above. Even though the above statements raise concerns regarding the efficacy of these trainings, research is still in progress to develop CCT programs for elderly as clinical studies show that these trainings may generate meaningful transfer effects (Bozoki et al., 2013). The reviewed studies show that the transfer effect of cognitive training to untrained tasks is mixed; some trainings have achieved far transfer and some have not. This is not to say that targeted training did not improve specific cognitive measurements. It was seen that some tasks improve cognitive performance, but without transfer to untrained tasks.

Another issue which may produce hindrance for cognitive benefits is allowing participants the freedom to choose which games they play and the option to set the levels of difficulty according to their personal preference (Bozoki et al., 2013). Importantly, the findings indicate that participants tend to select options with the least challenge. This produces diminished cognitive benefits since the resulting focus of training is often limited to fewer cognitive domains. While some CCT can be conducted from home, which is more convenient for those home-bound, and allows users to work at their own pace and to focus more on the areas that need improvement, there are a few points to consider. For CCT to produce long-term benefits it needs to be performed for a significant duration; additionally, it should be rigorous, repetitive, and consistently challenging (Klimova, 2016). The training to be effective at home must be supervised with a specialized trainer/care giver, if executed in an impromptu manner it may not yield the sought-after results, as stated above. Thus, even after certain benefits, the need of a trainer persists.

There are additional issues concerning the development of CCT programs for the elderly. Older individuals, for example, may not possess the required interest to use computer programs—though most do not require the user to be tech-savvy, and basic instructions are given prior to the start of the program. There may also be the need to develop age-specific (Wolfson and Kraiger, 2014) and culture-specific computer-based training programs and formats. Interestingly, little is known about the impact cultural background can have on this form of training. Since culture is a way of social life for people, it influences lifestyle, personal identity and one's relationship with others (Bruno et al., 2017). A cultural innovation can trigger changes in general cognitive capabilities (Bender, 2019). It has its share of effect on cognitive skills and information processing, because of this, a consideration of user culture when designing a robot would ensure older users are more comfortable and motivated. The use of language is one of the key abilities that contributes to our existence, it facilitates cooperation and allows us to agree on values and norms, making the foundation of our communities (Bender, 2019). If a program is culture specific and is designed in the native language of the user, they may relate more, especially elder people; as everyone may not be familiar with the standard English language and would understand the instructions better in their native language. Also, if the program is designed based on their local culture values and traditions, they may connect more to the exercise. This, in turn would help them complete the tasks assigned, without leaving the study mid-session. Another concern, possibly related, is that many users may not find it sufficiently engaging or motivating to carry out the trainings via a PC or a tablet, whereas the presence of a physical entity in the form of a social robot may more strongly compel users to complete the trainings since the robot can appraise, remind and encourage users to carry out the trainings.

While most studies suggest cognitive training works best when done for a longer duration, and computer-based cognitive training makes it easier for participants to receive this training since it is from the comfort of their homes, there is still the requirement of a specialized trainer/care-giver to monitor improvement and adherence to learner best practice. Also, specialized therapists need to be present to guide users through their execution, to continuously challenge them as they master a particular level of a program and to provide feedback during the task. Specialized therapists, additionally, need to keep track of performance to later draw a conclusion and maintain a progress report over a period of time (Broekens et al., 2000).

Most of the participants in our studies were above 60 (total 3,270 participants; 7.7% with MCI), except for the patients in three studies (Ballesteros et al., 2014; Marusic et al., 2016; Zhang et al., 2019), where participants were 55 and above years of age (total 83 participants, 32.5% with MCI). If the sample age is divided into two sections as young older adults (below 75 years) and old-older adults (above 75 years), only 3 studies (Miller et al., 2013; Gooding et al., 2015; Lin et al., 2016) have specified that the sample was above 75 (total of 200 participants, 10% with MCI). However, on analyzing the outcomes, there were no specific effects seen on CCT because of age difference. It can be said that CCT has a generalized positive effect for elderly (aged above 55 years) and is not age dependent.

It can be summarized that the CCT is mostly beneficial and shows improvement in older adults. Issues related to training location need to be addressed; either it is performed at a facility, or at home. When done at the former, the participant is required to attend regularly. This may be a demotivating factor for some since all participants may not have easy access to transportation and the commute may be a challenge. This challenge may be further compounded by, the additional cost of public or

private paid transports. When performed at home it is usually unsupervised and, as a consequence, the participant carries out the training in a sequence according to their own choice of activity. This may result ineffective engagement, since it is highly plausible participants will choose exercises that require less than optimal effort, and so limit the potential cognitive benefits.

Robots, however, have come a long way and are now available widely to assist in numerous ways. Human-robot interaction (HRI) has improved meaningfully over the past decade and is a technology being used in the healthcare industry in the form of socially assistive robots (SAR). SARs have proven to have immense potential in elderly care, promising to reform its delivery. In this review the existing studies have been extensively searched and the following roles of SAR can be outlined broadly as: (1) Companion Robots—the function of these robots is to provide companionship and alleviate anxiety and loneliness. They are commonly designed in animal-like forms providing companionship as a pet would, without the overhead of animal care. (2) Care Robots—these are designed to assist the elderly in their daily activities, for example reminding them to take their medicine on time, connecting them with their loved ones through voice or video calls, detecting falls, and notifying the appropriate authority in the case of an emergency. Some are even designed to fetch things from other rooms etc. They are very suitable, in fact, for elderly people living alone or people who have difficulty performing certain movements. (3) Therapy Robots—they are designed to carry out therapy sessions, whether physical exercises, or cognitive training. With this objective, they monitor the improvements of its users, intelligently adjusting the level of difficulty of exercises when needed to place them specifically within the range of proximal development, and to assess user mood, interest, and level of engagement with a variety of sensors and programs which a simple computerized cognitive training program is unable to achieve. The additional information gathered by the robot creates a qualitatively superior, and hence, more meaningful interaction for the user. Furthermore, the appearance of a robot can be altered to produce a pet robot or humanoid; likewise, a robot can be programmed to mimic enthusiasm and other emotions, to possess an appealing voice or the preferred gender. These elements enhance communication considerably. Because of this, in contrast to the effectiveness of tablets and CCT programs, where robotic intervention excels is in its pronounced capacity to engage and therefore motivate users to complete exercises and succeed in cognitive training. Research has shown that users are able to develop an emotional connection and a virtual relationship with their robots, helping to produce the superior social, emotional and cognitive benefits of the intervention, and raise quality of life scores (Gazzola et al., 2007; Šabanović et al., 2013; Liang et al., 2017; Bender, 2019).

Still, there are, in fact, few studies that use robotics exclusively as a medium for cognitive training. There is ample opportunity to do extensive research in the development of assistive robots for cognitive training, in particular, developing culturally appropriate robots for maximum benefit to the user. In contrast to conventional CCT programs, we believe the use of robots for cognitive training of elderly individuals experiencing age-related cognitive decline may produce significantly greater positive impact. For example, Kim et al. (2015) used both cognitive training and robot-assisted training in their study, they found the robot assisted training to be more effective.

There are a few limitations that require further research. Firstly, future trials should aim for a larger sample size and a longer intervention duration; additionally, they must directly compare the different alternatives of training to identify the most effective. Secondly, studies focusing on multiple cognitive domains should have greater organization when carrying out activities on specific domains. This would make it easier to conclude which domains have benefited the most. Thirdly, hardly any studies are available which show culturally appropriate cognitive training approaches. Future research which consider this may produce improved cognitive function. Fourthly, many studies have inconsistent research designs making it difficult to extract a clear and crisp conclusion. This should be considered. Also, only studies published in English were included, other language studies may have more insights, but were excluded as it would be difficult to understand and interpret them correctly for this review; which could have resulted in a biased outcome. A final limitation seen is the absence of research which addresses the needs of a minimally educated elderly population. We should aim to develop programs that are able to cater to people with basic levels of education, or no education at all. Ultimately, this would allow these programs to have a wider reach, since many countries around the world may have an elderly population that is not highly educated or literate.

CONCLUSION

Based on this review, we can conclude that computerized cognitive training targeting healthy older adults and adults with MCI is moderately beneficial in improving various cognitive functions. Various methods of available trainings have yielded improved working memory, attention, processing speed, episodic memory, visuo-spatial functions, executive functions; however, there are a few issues with CCT which can be overcome by the introduction of SARs in this field.

Future studies will need to focus more closely on the key psychological, cultural and socio-economic factors of its participants. Researchers should double their efforts to identify the key-mechanisms for improving the cognitive and everyday functions of elderly. Large sample, longer-duration experiments are needed with a control group preferably to get a more generalized outcome. Furthermore, cultural considerations, as mentioned, need to be made by developers to strengthen acceptance and engagement by users. Considerations, such as language and lifestyle, would help reach a more wide-ranging population. Finally, most of the present examples of SARs are designed to provide companionship or assistance to the elderly in their daily activities. Future research should aim to make a SAR, not just culturally oriented in its disposition, but able to carry out the much needed cognitive trainings with the elderly in a highly organized manner targeting multiple domains, while maintaining the results and scores and adapting the program to make it constantly challenging and fun for its user. All of this

would result in greater success and produce opportunities for smart aging.

AUTHOR CONTRIBUTIONS

FA, SK, AV, SS, RN, and RK contributed conception and design of the study. FA, SS, AV, and RN prepared the materials. FA, SK, and AV wrote the manuscript. SS, RN, and RK reviewed the manuscript and gave comments. All authors contributed to the final manuscript revision, read and approved the submitted version.

FUNDING

The work was funded by the Zayed Center for Health Sciences-United Arab Emirates University (Grant No. 31R178).

REFERENCES

Ballesteros, S., Prieto, A., Mayas, J., Toril, P., and Pita, C. (2014). Brain training with non-action video games enhances aspects of cognition in older adults: a randomized controlled trial. *Front. Aging Neurosci.* 6:277. doi: 10.3389/fnagi.2014.00277

Barnes, D. E., Yaffe, K., Belfor, N., Jagust, W. J., DeCarli, C., Reed, B. R., et al. (2009). Computer-based cognitive training for mild cognitive impairment. *Alzheimer Dis. Assoc. Disord.* 23, 205–210. doi: 10.1097/WAD.0b013e31819c6137

Bender, A. (2019). The role of culture and evolution for human cognition. *Top. Cogn. Sci.* doi: 10.1111/tops.12449. [Epub ahead of print].

Boot, W. R., and Kramer, A. F. (2014). *The Brain-Games Conundrum: Does Cognitive Training Really Sharpen the Mind?* Available online at: http://dana.org/Cerebrum/2014/The_BrainGames_Conundrum__Does_Cognitive_Training_Really_Sharpen_the_Mind_/ (accessed March 29, 2016).

Bozoki, A., Radovanovic, M., Winn, B., Heeter, C., and Anthony, J. C. (2013). Effects of a computer-based cognitive exercise program on age-related cognitive decline. *Arch. Gerontol. Geriatr.* 57, 1–7. doi: 10.1016/j.archger.2013.02.009

Brinke, T., Best, J. R., Crockett, R. A., and Liu-Ambrose, T. (2018). The effects of an 8-week computerized cognitive training program in older adults: a study protocol for a randomized controlled trial. *BMC Geriatr.* 18:31. doi: 10.1186/s12877-018-0730-6

Broekens, J., Heerink, M., and Rosendal, H. (2000). Assistive social robots in elderly care: a review. *Gerontechnology* 8, 94–103. doi: 10.4017/gt.2009.08.02.002.00

Bruno, B., Chong, N., Kamide, H., Kanoria, S., Lee, J., Lim, Y., et al. (2017). "The CARESSES EU-Japan project: making assistive robots culturally competent," in *Conference: Ambient Assisted Living* (Genova).

Corbett, A., Owen, A., Hampshire, A., Grahn, J., Stenton, R., Dajani, S., et al. (2015). The effect of an online cognitive training package in healthy older adults: an online randomized controlled trial. *J. Am. Med. Dir. Assoc.* 16, 990–997. doi: 10.1016/j.jamda.2015.06.014

Finn, M., and McDonald, S. (2011). Computerized cognitive training for older persons with mild cognitive impairment: a pilot study using a randomised controlled trial design. *Brain Impairm.* 12, 187–199. doi: 10.1375/brim.12.3.187

Gazzola, V., Rizzolatti, G., Wicker, B., and Keysers, C. (2007). The anthropomorphic brain: the mirror neuron system responds to human and robotic actions. *NeuroImage* 35, 1674–1684. doi: 10.1016/j.neuroimage.2007.02.003

Gooding, A. L., Choi, J., Fiszdon, J. M., Wilkins, K., Kirwin, P. D., van Dyck, C. H., et al. (2015). Comparing three methods of computerised cognitive training for older adults with subclinical cognitive decline. *Neuropsychol. Rehabil.* 26:810821. doi: 10.1080/09602011.2015.1118389

Gross, H. M., Schroeter, C., Mueller, S., Volkhardt, M., Einhorn, E., Bley, A., et al. (2011). "Progress in developing a socially assistive mobile home robot companion for the elderly with mild cognitive impairment," in *2011 IEEE/RSJ International Conference on Intelligent Robots and Systems (IROS)* (San Francisco, CA), 2430–2437. doi: 10.1109/IROS.2011.6094770

Herrera, C., Chambon, C., Michel, B. F., Paban, V., and Alescio-Lautier, B. (2012). Positive effects of computer-based cognitive training in adults with mild cognitive impairment. *Neuropsychologia* 50, 1871–1881. doi: 10.1016/j.neuropsychologia.2012.04.012

Hughes, T. F., Flatt, J. D., Fu, B., Butters, M. A., Chang, C.-C. H., and Ganguli, M. (2014). Interactive video gaming compared with health education in older adults with mild cognitive impairment: a feasibility study. *Int. J. Geriatr. Psychiatry* 29, 890–898. doi: 10.1002/gps.4075

Hyer, L., Scott, C., Atkinson, M. M., Mullen, C. M., Lee, A., Johnson, A., et al. (2015). Cognitive training program to improve working memory in older adults with MCI. *Clin. Gerontol.* 39, 410–427. doi: 10.1080/07317115.2015.1120257

Jøranson, N., Pedersen, I., Rokstad, A. M. M., and Ihlebaek, C. (2016). Change in quality of life in older people with dementia participating in Paro-activity: a cluster-randomized controlled trial. *J. Adv. Nurs.* 72, 3020–3033. doi: 10.1111/jan.13076

Kelly, M. E., Loughrey, D., Lawlor, B. A., Robertson, I. H., Walsh, C., and Brennan, S. (2014). The impact of cognitive training and mental stimulation on cognitive and everyday functioning of healthy older adults: asystematic review and meta-analysis. *Ageing Res. Rev.* 15, 28–43. doi: 10.1016/j.arr.2014.02.004

Kim, G. H., Jeon, S., Im, K., Kwon, H., Lee, B. H., Kim, G. Y., et al. (2015). Structural brain changes after traditional and robot-assisted multi-domain cognitive training in community-dwelling healthy elderly. *PLoS ONE* 10:e0123251. doi: 10.1371/journal.pone.0123251

Klimova, B. (2016). Computer-based cognitive training in aging. *Front. Aging Neurosci.* 8:313. doi: 10.3389/fnagi.2016.00313

Kueider, A. M., Parisi, J. M., Gross, A. L., and Rebok, G. W. (2012). Computerized cognitive training with older adults: a systematic review. *PLoS ONE* 7:e40588. doi: 10.1371/journal.pone.0040588

Lampit, A., Hallock, H., and Valenzuela, M. (2014). Computerized cognitive training in cognitively healthy older adults: a systematic review and meta-analysis of effect modifiers. *PLoS Med.* 11:e1001756. doi: 10.1371/journal.pmed.1001756

Liang, A., Piroth, I., Robinson, H., Macdonald, B., Fisher, M., Nater, U., et al. (2017). A pilot randomized trial of a companion robot for people with dementia living in the community. *J. Am. Med. Dir. Assoc.* 18, 871–878. doi: 10.1016/j.jamda.2017.05.019

Lin, F., Heffner, K. L., Ren, P., Tivarus, M. E., Brasch, J., Chen, D.-G., et al. (2016). Cognitive and neural effects of vision-based speed-of-processing training in older adults with amnestic mild cognitive impairment: a pilot study. *J. Am. Geriatr. Soc.* 64, 1293–1298. doi: 10.1111/jgs.14132

Marusic, U., Giordani, B., Moffat, S. D., Petrič, M., Dolenc, P., Pišot, R., et al. (2016). Computerized cognitive training during physical inactivity improves executive functioning in older adults. *Aging Neuropsychol. Cogn.* 25, 49–69. doi: 10.1080/13825585.2016.1263724

McAvinue, L. P., Golemme, M., Castorina, M., Tatti, E., Pigni, F. M., and Salomone, S. (2013). An evaluation of a working memory training scheme in older adults. *Front. Aging Neurosci.* 5:20. doi: 10.3389/fnagi.2013.00020

McColl, D., Louie, W. G., and Nejat, W. G. (2013). Brian 2.1: a socially assistive robot for the elderly and cognitively impaired. *IEEE Robot. Autom. Mag.* 20, 74–83. doi: 10.1109/MRA.2012.2229939

Melby-Lervåg, M., Redick, T., and Hulme, C. (2016). Working memory training does not improve performance on measures of intelligence or other measures of "far transfer": evidence from a meta-analytic review. *Perspect. Psychol. Sci.* 11, 512–534. doi: 10.1177/1745691616635612

Miller, K. J., Dye, R. V., Kim, J., Jennings, J. L., O'Toole, E., Wong, J., et al. (2013). Effect of a computerized brain exercise program on cognitive performance in older adults. *Am. J. Geriatr. Psychiatry* 21, 655–663. doi: 10.1016/j.jagp.2013.01.077

Mowszowski, L., Batchelor, J., and Naismith, S. L. (2010). Early intervention for cognitive decline: can cognitive training be used as a selective prevention technique? *Int. Psychogeriatr.* 22, 537–548. doi: 10.1017/S1041610209991748

Moyle, W., Jones, C., Sung, B., Bramble, M., O'Dwyer, S., Blumenstein, M., et al. (2015). What effect does an animal robot called CuDDler have on the

engagement and emotional response of older people with dementia? A pilot feasibility study. *Int. J. Soc. Robot.* 8, 145–156. doi: 10.1007/s12369-015-0326-7

Moyle, W., Jones, C. J., Murfield, J. E., Thalib, L., Beattie, E. R. A., Shum, D. K. H., et al. (2017). Use of a robotic seal as a therapeutic tool to improve dementia symptoms: a cluster-randomized controlled trial. *J. Am. Med. Dir. Assoc.* 18, 766–773. doi: 10.1016/j.jamda.2017.03.018

Nouchi, R., and Kawashima, R. (2014). Improving cognitive function from children to old age: a systematic review of recent smartageing intervention studies. *Adv. Neurosci.* 2014, 1–15. doi: 10.1155/2014/235479

Nouchi, R., Saito, T., Nouchi, H., and Kawashima, R. (2016). Small acute benefits of 4 weeks processing speed training games on processing speed and inhibition performance and depressive mood in the healthy elderly people: evidence from a randomized control trial. *Front. Aging Neurosci.* 8:302. doi: 10.3389/fnagi.2016.00302

Peretz, C., Korczyn, A., Shatil, E., Aharonson, V., Birnboim, S., and Giladi, N. (2011). Computer-based, personalized cognitive training versus classical computer games: a randomized double-blind prospective trial of cognitive stimulation. *Neuroepidemiology* 36, 91–99. doi: 10.1159/000323950

Pinker, S. (2018). *Enlightenment Now: The Case for Science, Reason, Humanism, and Progress*. New York, NY: Viking.

Rebok, G. W., Ball, K., Guey, L. T., Jones, R. N., Kim, H.-Y., and King, J. W. (2014). Ten-year effects of the advanced cognitive training for independent and vital elderly cognitive training trial on cognition and everyday functioning in older adults. *J. Am. Geriatr. Soc.* 62, 16–24. doi: 10.1111/jgs.12607

Requena, C., and Rebok, G. (2019). Evaluating successful aging in older people who participated in computerized or paper-and-pencil memory training: the memoria mejor program. *Int. J. Environ. Res. Publ. Health* 16:191. doi: 10.3390/ijerph16020191

Rose, N. S., Craik, F. I. M., Kliegel, M., Hering, A., and Rendell, P. G. (2012). "Cognitive and neural plasticity in older adults' prospective memory following training with the virtual week computer game," in *Poster presented at the Annual Meeting of the Psychonomic Society* (Minneapolis, MN). doi: 10.1037/e502412013-662

Rose, N. S., Rendell, P. G., Hering, A., Kliegel, M., Bidelman, G. M., and Craik, F. I. M. (2015). Cognitive and neural plasticity in older adults' prospective memory following training with the virtual week computer game. *Front. Hum. Neurosci.* 9:592. doi: 10.3389/fnhum.2015.00592

Šabanović, S., Bennett, C. C., Chang, W. L., and Huber, L. (2013). "PARO robot affects diverse interaction modalities in group sensory therapy for older adults with dementia," in *IEEE International Conference on Rehabilitation Robotics (ICRR)* (Seattle, WA), 1–6. doi: 10.1109/ICORR.2013.6650427

Simon, S. S., Tusch, E. S., Feng, N. C., Håkansson, K., Mohammed, A., and Daffner, K. R. (2018). Is computerized working memory training effective in healthy older adults? Evidence from a multi-site, randomized controlled trial. *J. Alzheimer's Dis.* 65, 931–949. doi: 10.3233/JAD-180455

Soler, V. M., Agüera-Ortiz, L., Rodríguez, J. O., Rebolledo, C. M., Muñoz, P., Pérez, A. R., et al. (2015). Social robots in advanced dementia. *Front. Aging Neurosci.* 7:133. doi: 10.3389/fnagi.2015.00133

Strenziok, M., Parasuraman, R., Clarke, E., Cisler, D., Thompson, J., and Greenwood, P. M. (2013). Neurocognitive enhancement in older adults: comparison of three cognitive training tasks to test a hypothesis of training transfer in brain connectivity. *NeuroImage* 85(Pt 3), 1027–1039. doi: 10.1016/j.neuroimage.2013.07.069

Styliadis, C., Kartsidis, P., Paraskevopoulos, E., Ioannides, A. A., and Bamidis, P. D. (2015). Neuroplastic effects of combined computerized physical and cognitive training in elderly individuals at risk for dementia: an eLORETA controlled study on resting states. *Neural Plast.* 2015:172192. doi: 10.1155/2015/172192

Tanaka, M., Ishii, A., Yamano, E., Ogikubo, H., Okazaki, M., Kamimura, K., et al. (2012). Effect of a human-type communication robot on cognitive function in elderly women living alone. *Med. Sci. Monit.* 18, CR550–CR557. doi: 10.12659/MSM.883350

Tapus, A., Mataric, M., and Scassellati, B. (2007). Socially assistive robotics [Grand Challenges of Robotics]. *IEEE Robot. Autom. Mag.* 14, 35–42. doi: 10.1109/MRA.2007.339605

Tapus, A., and Vieru, A. M. (2013). "Robot cognitive stimulation for the elderly," in *Natural and Artificial Models in Computation and Biology: 5th International Work-Conference on the Interplay Between Natural and Artificial* (Mallorca). doi: 10.1007/978-3-642-38637-4_10

Thodberg, K., Sørensen, L. U., Videbech, P. B., Poulsen, P. H., Houbak, B., and Damgaard, V. (2016). Behavioral responses of nursing home residents to visits from a person with a dog, a robot seal or a toy cat. *Anthrozoös* 29, 107–121. doi: 10.1080/08927936.2015.1089011

Toril, P., Reales, J. M., Mayas, J., and Ballesteros, S. (2016). Video game training enhances visuospatial working memory and episodic memory in older adults. *Front. Hum. Neurosci.* 10:206. doi: 10.3389/fnhum.2016.00206

Verhagen, A., De Vet, H., Bie, R., Kessels, A., Boers, M., Bouter, L., et al. (1998). The Delphi list. *J. Clin. Epidemiol.* 51, 1235–1241. doi: 10.1016/S0895-4356(98)00131-0

Walton, C. C., Kavanagh, A., Downey, L. A., Lomas, J., Camfield, D. A., and Stough, C. (2015). Online cognitive training in healthy older adults: a preliminary study on the effects of single versus multi-domain training. *Transl. Neurosci.* 6, 13–19. doi: 10.1515/tnsci-2015-0003

Willis, S. L., Tennstedt, S. L., Marsiske, M., Ball, K., Elias, J., Koepke, K. M., et al. (2006). Long-term effects of cognitive training on everyday functional outcomes in older adults. *JAMA* 296, 2805–2814. doi: 10.1001/jama.296.23.2805

Wolfson, N. E., and Kraiger, K. (2014). Cognitive aging and training: the role of instructional coherence and advance organizers. *Exp. Aging Res.* 40, 164–186. doi: 10.1080/0361073X.2014.882206

Yeo, S. N., Lee, T. S., Sng, W. T., Heo, M. Q., Bautista, D., Cheung, Y. B., et al. (2018). Effectiveness of a personalized brain-computer interface system for cognitive training in healthy elderly: a randomized controlled trial. *J. Alzheimer's Dis.* 66, 127–138. doi: 10.3233/JAD-180450

Zhang, H., Wang, Z., Wang, J., Lyu, X., Wang, X., Liu, Y., et al. (2019). Computerized multi-domain cognitive training reduces brain atrophy in patients with amnestic mild cognitive impairment. *Transl. Psychiatry* 9:48. doi: 10.1038/s41398-019-0385-x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

MINI REVIEW
published: 15 November 2019
doi: 10.3389/fnagi.2019.00318

The Effect of Balance and Coordination Exercises on Quality of Life in Older Adults: A Mini-Review

*Ayelet Dunsky**

School of Human Movement and Sport Sciences, The Academic College at Wingate, Wingate Institute, Netanya, Israel

The ability to control balance during activities of daily living (ADL) is impaired in older adults as a result of deterioration in the sensory systems (i.e., vestibular, visual, somatosensory), the cognitive system (central nervous system), and the musculoskeletal system. Consequently, many older adults face a risk of falling during their ADL. In most cases, falls and related injuries impair the quality of life and result in physical limitations, anxiety, loss of confidence, and fear of falling. Among a variety of fall prevention interventions, adapted physical activity programs have been suggested for improving balance control during ADL. These programs challenge the sensory, cognitive, and musculoskeletal systems while addressing balance constraints such as orientation in space, changes in direction, and the speed or height of the center of mass during static and dynamic situations resembling ADL. The above-mentioned elements can be dealt with through a combination of balance and coordination exercises that challenge the postural control systems in multiple dimensions—including vertical and horizontal changes of the center of mass, standing on unstable surfaces with a reduced base of support, and changing body directions. Consequently, such exercises require environmental information-processing. The combination of dual-task, function-oriented challenges while controlling balance stimulates the sensory and neuromuscular control mechanisms. Among older adults, these programs have been found to improve static and dynamic stability, as well as a number of aspects in the quality of life. Recently, they have also been found to improve cognitive functions such as memory and spatial cognition.

Keywords: balance, coordination, exercise, older adults, quality of life

OPEN ACCESS

Edited by:
Trentham Furness, Australian Catholic University, Australia

Reviewed by:
Wendy Gilleard, Southern Cross University, Australia
Bianca Share, Australian Catholic University, Australia

***Correspondence:**
Ayelet Dunsky
ayelet@wincol.ac.il

Received: *28 June 2019*
Accepted: *01 November 2019*
Published: *15 November 2019*

Citation:
Dunsky A (2019) The Effect of Balance and Coordination Exercises on Quality of Life in Older Adults: A Mini-Review. Front. Aging Neurosci. 11:318. doi: 10.3389/fnagi.2019.00318

INTRODUCTION

Aging is subjected to longitudinal processes as a consequence of physiological changes, such as a higher level of stress, mitochondrial dysfunction, abnormality of inflammatory processes, decreased hormone production, and decreased metabolic rate which can lead to catabolism and degeneration of organs (Cesari et al., 2013; Sieber, 2017). These processes lead to a progressive loss of nerve extensions, bone mass, skeletal muscle mass, and strength (Chang and Lin, 2015; Sieber, 2017; Nascimento et al., 2019). Consequently, frailty and sarcopenia may be present in approximately 10% of people over the age of 65 and 25–50% of those aged over 85 (World Health Organization, 2007). These are phenomena known to impair the ability to perform activities of daily living (ADL), reduce the quality of life, and increase the risk of falling (Chang and Lin, 2015; Landi et al., 2015; Sieber, 2017; Nascimento et al., 2019).

The increased risk of falling, shown to be a consequence of the above-mentioned processes, is mainly due to the difficulties of older adults in maintaining postural control while performing ADL (Rubenstein, 2006; Kojima, 2015). Postural control is based on the ability to synchronize several systems in an ongoing cycle: the sensory systems (i.e., vestibular, visual, somatosensory), the cognitive system (central nervous system), and the musculoskeletal system. During normal aging, physiological changes occur in one's visual, vestibular, and somatosensory inputs, as well as in the central processing and muscular effectors (Horak, 2006; Rubenstein, 2006). In the visual system, a reduced ability to detect low contrast hazards, judge distances, and perceive spatial relationships appears to be the significant impairment associated with falls among older adults (Lord, 2006). Changes in the vestibular system are expressed in a reduced number of hair cells in the semicircular canals, in the maculae of the saccule and the utricle, as well as in the primary and secondary vestibular neurons (Ishiyama, 2009). The reduced capacity to detect position and direction of movement, together with reduced lower-limb strength and sensation, are considered to be significant predictors of fall risk among older adults (Lord et al., 1996). Moreover, inter-joint coordination and the appropriate timing of muscle action during ADL, such as in walking, is also affected; thus, the ability of older people to use the fall avoidance strategies practiced by young people is reduced (Rubenstein, 2006).

Several therapeutic approaches have been shown to be effective for fall prevention among older adults, and there is a large body of literature documenting the favorable effects of physical activity training programs. Evidence suggests that programs based on aerobic and resistance exercise can be used to restore or maintain functional independence in older adults, and may also potentially prevent, delay, or reverse frailty (de Vries et al., 2012; de Labra et al., 2015). Aerobic training enhances cardiovascular function, as it was shown to preserve motor units and mitochondrial function. Additionally, it was found to prevent muscle atrophy and to improve the health-related quality of life (Navas-Enamorado et al., 2017). Resistance training was shown to increase protein synthesis and muscle mass, as well as to improve neural recruitment and muscle strength, explained by neural and morphological adaptations (Guizelini et al., 2018). The combination of these training modes has yielded beneficial effects in body composition and physical function, as well as in cognitive and emotional function, among frail patients (de Labra et al., 2015; Tarazona-Santabalbina et al., 2016). It is important to note that in most cases aerobic and strength exercises require the participants to be highly mobile. Older adults with low mobility might have difficulty in benefiting from these exercises because of their limited locomotive ability. Therefore, exercise with reduced locomotion requirements, such as balance or coordination exercise, may provide similar benefits to older adults with a variety of mobility abilities (Kwok et al., 2011).

The possibility of improving the quality of life of older people through simple exercises can be a solution for people with a wide range of functional abilities. Thus, the current mini-review has two purposes: (a) to present studies examining the effect of performing balance and coordination exercises on various aspects of the quality of life of older people; and (b) to present suggestions for practical applications.

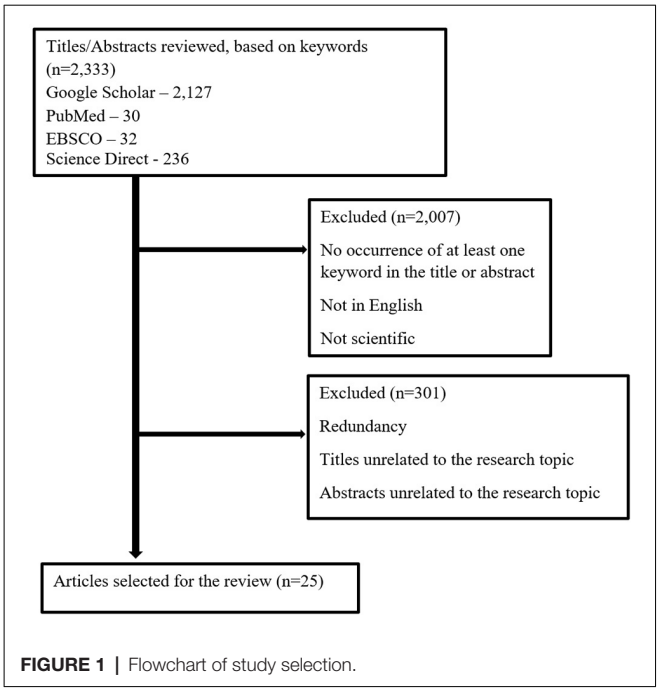


FIGURE 1 | Flowchart of study selection.

METHODS

The author performed a literature review of available studies on the research topic dealing with different effects of balance and/or coordination exercise programs on various aspects of older adults' lives. Research studies were selected on the basis of research topics that included the following keywords: balance exercise, coordination exercise, postural control, risk of falling, frailty, and quality of life, found in the world's acknowledged databases: PubMed, EBSCO (SPORTDiscus, Academic Search Complete, EDS), Google Scholar, and Science Direct. The search was not limited by any period of time. From the database/journal searches, 2,333 titles/abstracts were retrieved. The majority of the studies were detected in the Google Scholar database. The titles and abstracts of some identified articles were then checked for relevance. Subsequently, the search was performed again, focusing on the occurrence of at least one keyword in the title or abstract of articles written in English and representing scientific study, thereby significantly narrowing down the selection. Altogether, 326 studies were found. After removing duplicates and titles/abstracts unrelated to the research topic, 25 studies were found to be relevant to the research topic (see **Figure 1** for the flowchart of study selection). The information found in the selected studies on balance and coordination exercise and its effect on postural control, cognitive function, and the quality of life of older adults was evaluated, and it is summarized in **Table 1** and described and discussed in the following sections.

TABLE 1 | Description of studies that included balance and/or coordination and changes in postural control, cognitive performance, or quality of life among adults.

Study	Participants	Intervention/ Assessments	Outcome measures	Major findings
BALANCE AND POSTURAL CONTROL				
Bisson et al. (2007)	Older adults *n* = 24, Age: 74 Study groups: 1. Virtual reality (VR; *n* = 12) 2. Biofeedback (*n* = 12)	Training program: 20 sessions over 10 weeks of dynamic balance exercises using VR ("juggling" a virtual ball while standing), or dynamic balance exercises with visual biofeedback (moving a curser that represents Center of Pressure (COP) while standing on force plate)	Static balance—sways of COP in different postures; Simple reaction time task—while maintaining balance; Functional balance and mobility—the Community Balance and Mobility Scale (CB&M)	After a 10-week intervention, both groups significantly improved their functional balance and mobility, as well as their reaction time during standing.
de Vries et al. (2018)	Older adults *n* = 30, Age: 70 Young adults *n* = 30, Age: 22 Study conditions: 1. FLOS task 2. VR—Kinski 3. VR—Wiiski	One session of VR game: steer an avatar skiing down a slalom track	Time to complete the game; Maximum displacement of Center of Mass (COM); Peak COM speed	COM displacement during the Kinski was significantly larger in all directions compared to the Wiiski. Peak COM speed was significantly higher in the Kinski.
Dunsky et al. (2017b)	Older adults *n* = 112, Age: 75 Study groups: 1. women *n* = 862. men *n* = 36	Associations between static balance and dynamic balance	TUG; FR; COP length of sway; COP sway intensities	In general: low correlations were found between static and dynamic balance measures, for both women and men
Nagy et al. (2007)	Older adults *n* = 19, Age: 79 Young adults *n* = 11, Age: 22 Study groups: 1. training (*n* = 9) 2. control (*n* = 10) 3. young adults (*n* = 11)	Training program: 16 sessions over 8-weeks, static and dynamic balance, strength, flexibility and aerobic Control: routine	COP path and frequencies (AP, ML) during standing with eyes open and eyes closed. TUG test	Training group: Improved postural control in the ML direction. Improved performance on TUG test
Rendon et al. (2012)	Older adults *n* = 40, Age: 60–95 Study groups: 1. virtual reality (VR) 2. control	Training program: 18 session over 6 weeks of VR balance games Control: routine	Dynamic balance – 8 feet up and go test; Balance confidence—Activities–specific Balance Confidence Scale (ABC); Depression—Geriatric Depression Scale	After 6 weeks of intervention, the VR group showed significant improvement in the dynamic balance test and in the ABC score compared with the control group.
BALANCE AND COGNITIVE PERFORMANCE				
Mouthon and Taube (2019)	Young adults *n* = 26, Age: 24 Study groups: 1. training (*n* = 13) 2. control (*n* = 13)	Training program: six sessions, over 2-weeks, balancing on a movable platform. Control: routine physical activity	Time kept in horizontal position on a movable platform; EMG of tibialis anterior and soleus muscles; Short-interval intracortical inhibition (SICI)	The balance training led to an increased intracortical inhibition during balance tasks, as well as improved balance performance, with reduced EMG activities during unstable conditions.
Netz et al. (2018)	Older adults *n* = 112, Age: 77 Study groups: 1. young women (*n* = 41, age: 69) 2. old women (*n* = 38, age: 79) 3. men (*n* = 33, age: 77)	Associations between static and dynamic balance and attention inhibition	TUG; FR; COP length of sway; COP sway intensities; Computerized go/no-go test	Attention inhibition was significantly correlated to static balance for young women, to dynamic balance for men, and not correlated to balance for older women.

(Continued)

TABLE 1 | (Continued)

Study	Participants	Intervention/ Assessments	Outcome measures	Major findings
Rogge et al. (2017)	Adults *n* = 40, Age: 18–65 Study groups: 1. balance (*n* = 19) 2. relaxation (*n* = 21)	Training program: 24 sessions over 12-weeks, Balance circuit training in eight stations. Or Relaxation training using progressive muscle relaxation and autogenic training.	Dynamic balance—platform time at horizontal position; BESS; COP sway velocity; Cardiorespiratory fitness; Memory—auditory verbal paired associated learning task; Spatial cognition—Orienting and Perspective Taking test; Figure orientation; Mirror Images; Stroop test	The balance training improved participants' dynamic balance. Significantly higher memory scores for the balance training group. No changes were found in the BESS or the cardiorespiratory fitness.
Rogge et al. (2018)	Adults *n* = 37, Age: 19–65 Study groups: 1. balance (*n* = 19) 2. relaxation (*n* = 18)	Training program: 24 sessions over 12-weeks, Balance circuit training in eight stations. Or: Relaxion training using progressive muscle relaxation and autogenic training.	Dynamic balance—platform time at horizontal position; MRI Cortical thickness; Subcortical gray matter volume	The balance training group had significantly improved balance performance, and showed significantly higher cortical thickness increases following the intervention.
BALANCE TRAINING AND QUALITY OF LIFE				
Gouveia et al. (2018)	Older adults *n* = 52, Age: 65–85 Study groups: 1. intervention (*n* = 26) 2. control (*n* = 26)	Training program: 24 sessions over 12-weeks, gait, balance, functional training, strengthening, flexibility and 3D training	SF-36 questionnaire	Significant improved quality of life for the intervention group.
Halvarsson et al. (2011)	Older adults *n* = 59, Age: 67–93 Study groups: 1. intervention (*n* = 38) 2. control (*n* = 21)	Training program: 36 sessions over 12-weeks, progressive and specific balance exercises for ADL Control: regular life during the study period.	Falls Efficacy Scale International (FES-I); Reaction time of step execution; Gait—spatio-temporal variables	Intervention group showed significant improvements in FES-I, in reaction time parameters and several gait parameters. In general—it led to decreased fear of falling.
Halvarsson et al. (2013)	Older adults *n* = 59, Age: 67–93 Study groups: 1. intervention (*n* = 38) 2. control (*n* = 21)	Training program: 36 sessions over 12-weeks, progressive and specific balance exercises for ADL Control: regular life during the study period.	Gait speed; Step execution; Fear of falling; likelihood of depression	Gait speed, step execution and fear of falling were still improved in the intervention group at 9-months follow-up. At 15-months follow-up, only fear of falling was significantly improved. Other parameters were significantly better compared to the control group.
Halvarsson et al. (2015a)	Older adults Age: 66–89	Training program: 36 sessions over 12-weeks, balance demanding exercises at three levels of progression	FES-I; Fear of falling; Gait speed with and without cognitive task; Balance performance; physical function	All intervention groups had significantly better scores in FES-I, walking speed with dual-task, balance performance and lower extremities' function, and reduced fear of falling compared to the control group.
Halvarsson et al. (2015b)	Older adults with osteoporosis *n* = 96, Age: 66–87 Study groups: 1. training (*n* = 34)	Training program: 36 sessions over 12-weeks, progressive	FES-I; Fear of falling; Gait speed with and without cognitive task;	Both intervention groups had significantly better scores in FES-I, walking speed with dual-task, balance

(Continued)

TABLE 1 | (Continued)

Study	Participants	Intervention/ Assessments	Outcome measures	Major findings
	2. training + physical activity (*n* = 31) 3. control (*n* = 31)	balance with dual and multi-task, and physical activity	Balance performance; physical function	performance and lower extremities' function, compared to the control group.
Taguchi et al. (2010)	Older adults *n* = 65, Age: 74–96 Study groups: 1. intervention (*n* = 31) 2. control (*n* = 34)	Training program: one session per week for 12-months of various exercise related to flexibility, strength, aerobic and balance	Lower limb strength; Sit-and-reach test; Grip strength; 6-min walking; Falls Efficacy Scale; MMSE; IADL	After 12 months of intervention, the intervention group had significant improvement in lower-limb strength, sit-and-reach test, as well as in the Falls Efficacy Scale, representing improvement in quality of life.
COORDINATION TRAINING AND BALANCE PERFORMANCE				
Lelard et al. (2010)	Older adults *n* = 28, Age: 70–85 Study groups: 1. Tai-Chi (*n* = 14) 2. balance training (*n* = 14)	Training program: 24 sessions over 12-weeks, of 10 Tai-chi forms adapted for older adults, Or balance exercises that involved shifting the body part (or COM) in different positions.	Static postural control: COP sways with eyes open and closed; Walking speed over 10-m course.	After a 12-week intervention no significant differences were found in walking speed or postural control for both groups.
Wong et al. (2001)	Older adults *n* = 39, Age: 66–76 Study groups: 1. Tai-Chi (*n* = 25) 2. control (*n* = 14)	The Thai-Chi group practiced Thai-Chi for 2–35 years	Static postural stability; Dynamic balance test	The Thai-Chi group had significantly better results in complication static conditions (eyes closed, sway surface), as well as in one of the dynamic balance tests, compared to the control.
COORDINATION AND COGNITIVE FUNCTION				
Gao et al. (1996)	Adults *n* = 6	Performance of four different tasks: passive and active cutaneous discrimination tasks, active grasp objects task with two hands and coordinated finger movements	Dentate nuclei activation using MRI	The highest cerebellar activity was find during the coordinative activity. Dentate activation was greatly enhanced when sensory discrimination was paired with finger movements.
Kwok et al. (2011)	Older adults *n* = 40, Age: 66–90 Study groups: 1. coordination group (*n* = 20) 2. towel exercises group (*n* = 20)	Training program: 8 sessions over 8-week of simple coordination exercises, Or stretching exercises using a towel, mainly training upper limbs.	Two cognitive function assessments: Chinese Mini-Mental State Examination; Chinese Dementia Rating Scale (CDRS); TUG	After an 8-week intervention, the CDRS scores of the coordination group improved significantly.
Niemann et al. (2014)	Older adults *n* = 49, Age: 62–79 Study groups: 1. cardiovascular training (*n* = 17) 2. coordination training (*n* = 19) 3. control (*n* = 13)	Training program: three sessions per week for 12 months of Nordic Walking program (for the cardiovascular group), Or: eye-hand and leg-arm coordination, spatial orientation and reaction to moving objects exercises (for the	After 12-months of intervention cardiovascular fitness significantly improved in the cardiovascular group; action speed performance and hippocampal volume significantly improved in both	

(Continued)

TABLE 1 | (Continued)

Study	Participants	Intervention/ Assessments	Outcome measures	Major findings
		coordination group), Or: stretching and relaxation training (for the control group).	cardiovascular and the coordination groups.	
Voelcker-Rehage et al. (2011)	Older adults *n* = 44, Age: 62–79 Study groups: 1. cardiovascular training (*n* = 17) 2. coordination training (*n* = 19) 3. control (*n* = 13)	Training program: three sessions per week for 12 months of Nordic Walking program (for the cardiovascular group), Or: eye-hand and leg-arm coordination, spatial orientation and reaction to moving objects exercises (for the coordination group), Or: stretching and relaxation training (for the control group).	Functional MRI—changes in brain activation patterns; Executive function; Perceptual speed	After 12-months of intervention both cardiovascular and coordination groups improved in executive function and perceptual speed. In addition, brain activity patterns changed, indicating more efficient information processing.
THE COMBINATION OF BALANCE AND COORDINATION EXERCISE				
Dizdar et al. (2018)	Older adults, postmenopaus-al females with osteoporosis *n* = 68, Age: 50–75 Study groups: 1. balance and coordination (*n* = 25) 2. strengthening (*n* = 25) 3. aerobic (*n* = 25)	Training program: 36 sessions over 12 weeks, of balance and coordination exercises, Or strengthening exercises on abdominals and back muscles and upper and lower extremities, Or aerobic training by walking on treadmill.	Static balance (COP sways); Dynamic balance: TUG and Berg Balance Scale; Pain assessment; Life quality assessment	After 12 weeks of intervention the balance and coordination group significantly improved in static and dynamic balance performances. Both balance and coordination and strengthening groups showed improvement in general health, however, the strengthening group had significant improvement in terms of mental function compared to the other groups.
Dunsky et al. (2017a)	Older adults *n* = 36, Age: 72 Study groups: 1. step aerobics (SA; *n* = 14) 2. stability ball (SB; *n* = 13) 3. control (*n* = 9)	Training program: 16 sessions over 8 weeks, of strength, balance and coordination exercises using aerobic steps, or stability ball. Control: ceramic sculpture class	Balance assessments: TUG, One-Leg Stand test, FR, The Tinetti Performance-Oriented Mobility Assessment (POMA); Quality of life assessment—The Short Form-36 Health Survey questionnaire (SF-36)	After an 8-week intervention the SA group significantly improved their TUG and POMA performances; General health perception improved significantly among both SA and SB groups compared to the control.
Segev et al. (2019)	Older adults with cardio-vascular diseases *n* = 26, Age: 74 Study groups: 1. intervention (*n* = 13) 2. control (*n* = 13)	Training program: 24 sessions over 12-weeks, balance and coordination exercises within 20 min of warm-up. As part of 80 min physical activity for cardiac rehabilitation. Control: Traditional warm-up	TUG test FR test BESS FTSST	Significant improvement in TUG, BESS and FTSST only in the intervention group
Taylor-Piliae et al. (2006)	Older adults with cardio-vascular diseases *n* = 39, Age: 66	Training program: 36 session over 12 weeks of Tai Chi 24 postures	Mood state (POMS); The Perceived Stress Scale test; Tai-Chi exercise self-efficacy	Significant improvements in all measures of psychosocial status were found following the intervention, as well as increased Tai-Chi self-efficacy.

BESS, Balance Error Scoring System; COM, Center of Mass; COP, Center of Pressure; FLOS, Functional Limits of Stability; FR, Functional Reach; FTSST, Five Time Sit to Stand Test; TUG, Time Up and Go; VR, Virtual Reality; POMA, The Tinetti Performance-Oriented Mobility Assessment.

BALANCE AND POSTURAL CONTROL

During ADL people are susceptible to changes in both dynamic as well as static balance. The ability to control those changes represents a complex challenge for the neuromuscular control system, which must cope with rapid environmental changes and is based upon the ability to proact or react to these changes for successful locomotion and fall prevention. This challenge can be met if there is proper function of the visual, vestibular, proprioceptive, and tactile senses for correct sensory input, and if they work together with the neuromuscular system to control body alignment with the correct subsequent motor output (Hayes, 1982; Horak, 2006; Dunsky et al., 2017b). The proactive balance strategy activates postural adjustments prior to the occurrence of destabilizing forces upon the body. The reactive balance strategy activates postural adjustments after an external disturbance is encountered, thus assuring balance recovery (Hayes, 1982; Wong et al., 2001). As people age, their ability to use these strategies—and in particular the reactive balance strategy—is impaired, as a consequence of the physiological and cognitive changes mentioned above. Thus, specific training programs that are based on postural control exercises are suggested for older adults (Arampatzis et al., 2011). If the purpose of training is to evoke improvements in reactive balance, then the training program may be associated with all postural control systems, including the musculoskeletal system, the cognitive system, as well as the somatosensory feedback system, while challenging the body in different environmental situations and unexpected perturbations (Tinetti et al., 1986; Robbins et al., 1989; Arampatzis et al., 2011). During balance training, participants perform exercises that include static vs. dynamic stability postures, reducing the base of support (bipedal vs. tandem vs. one-leg stance), changes in the height of the center of gravity, changes in the standing surface (such as floor, wobble boards, wobble cushions, foam, or perturbation platforms), and reducing the source of visual information, while attempting to simulate perturbations leading to falls during daily activities (e.g., eyes open vs. closed; Zech et al., 2010; Sibley and Salbach, 2015; Rogge et al., 2018). Nagy et al. (2007) found that an 8-week program that included static and dynamic balance exercises combined with strength, flexibility, and aerobic exercises, improved the postural control of older adult participants, especially in the more challenging direction (i.e., mediolateral), with and without visual control. The authors suggested that improvement was a consequence of the establishment of a new postural control strategy that was developed by the participants as a result of the specific balance intervention. de Vries et al. (2018) suggested using virtual reality training for postural control improvement, as it may challenge all aspects of the balance control system. Virtual reality can incorporate anticipatory postural adjustments, postural responses, muscle loading, cognitive challenges, transitioning into different poses, and taking steps. The authors examined two types of virtual reality training of skiing and found that the Kinski game elicited a larger center of mass displacements than the Wiiski game, and thus suggested the Kinski as a better method of balance training for older adults. In another study, an interventional program of 20 sessions of virtual reality was shown to improve functional balance and mobility among older adults, with results similar to biofeedback training (Bisson et al., 2007). Additionally, a training program of 18 sessions of virtual reality was shown to improve dynamic balance and balance confidence, in comparison to the control group (routine activities) older adults (Rendon et al., 2012).

The use of balance training for postural control is only one aspect of quality of life improvement. It has also been suggested as beneficial for cognitive function beneficial for cognitive function improvement.

BALANCE AND COGNITIVE PERFORMANCE

Physical activity, and in particular aerobic exercise, has been discussed as a promising means for increasing neurogenesis and plasticity of the brain, in order to improve cognitive functions as well as to protect against the age-related decline in the ability of the brain to adapt to environmental demands (Stimpson et al., 2018). As balance training provides a stimulus to the vestibular, neuromuscular, and proprioceptive systems, which then send signals to specific areas in the brain that make connections between vestibular nuclei and the cerebellum, hippocampus, as well as prefrontal and parietal cortices, it may affect cognitive functions such as spatial functions, navigation, and memory (Taube et al., 2007; Smith and Zheng, 2013). It has been speculated that increased stimulation of the vestibular system during balance exercise may be a mediator between physical exercise and cognitive functioning (Smith et al., 2010). In a cross-sectional study, Netz et al. (2018) found that attention inhibition was correlated to static balance among older women, while in older men it was associated with dynamic balance. Based on their results, the authors recommended that men include static balance exercises in their exercise routine and that women include dynamic exercise.

Rogge et al. (2017) found that 12 weeks of balance training improved memory and spatial cognition among healthy adults. In addition, they found increased cortical thickness in specific regions of the brain (i.e., the superior temporal cortex, visual association cortices, the posterior cingulate cortex, the superior frontal sulcus, and in the precentral gyri), among the same group. These changes were found to be correlated with improved balance performances (Rogge et al., 2018). The authors suggested that the brain's regions that show changes play a role in spatial orienting and memory, stimulating visual-vestibular pathways during self-motion, and thus they may mediate the beneficial effects of balance exercise on cognition. Mouthon and Taube (2019) found that 2 weeks of balance training on an unstable platform improved postural control that was correlated with improvements in intracortical inhibition. The authors suggested that these changes may demonstrate the occurrence of cortical plasticity and adaptation of inhibitory behavior

for the acquisition of a balance task following the balance training intervention.

BALANCE TRAINING AND QUALITY OF LIFE

Gouveia et al. (2018) found that 12 weeks of a rehabilitation program that included gait, balance, functional training, strengthening, and flexibility training in community-dwelling older adults can lead to significant improvements in multiple aspects of quality of life (assessed by the Short Form Health Survey—SF-36). The authors suggested that this improvement was based on the enhanced balance performance, as well as on the educational aspect of the program that was used in their study, since quality of life is strongly associated with both physical and mental age-related factors. Taguchi et al. (2010) found that a 12-months program of various exercises related to flexibility, strength, aerobic and balance significantly improved lower-limb strength as well as fall efficacy scale, but not other measures of quality of life. Halvarsson et al. (2011) found that individually adjusted, progressive, and specific balance group training for 3 months positively affected the fear of falling of community-dwelling elderly people. The authors mentioned that exercise in groups provides a social belonging which may contribute to an increased attendance rate, and thus can also influence the domain participation and improve the quality of life through increased activity in daily life (Lelard and Ahmaidi, 2015). In several continuous studies, Halvarsson et al. (2013, 2015a,b) found that this same balance progressive program improved gait speed and lower-limb strength reduced the likelihood of depression and improved balance confidence among older adults.

COORDINATION TRAINING AND COGNITIVE FUNCTION

Coordination exercise with low velocity, low impact, and a high-interest level, which also provides a good training effect, is preferred for most older persons (Wong et al., 2001). These exercises were associated with high activation in visual-spatial networks in the brain of older adults (Voelcker-Rehage et al., 2011; Niemann et al., 2014). Coordinative exercise is known to involve an activation of the cerebellum (Gao et al., 1996), which is responsible for motor control and motor learning (Manto et al., 2012), and was also found to influence a variety of higher cognitive functions, including divided attention and working memory (Gottwald et al., 2003), and verbal learning and memory (Tomlinson et al., 2014). In addition, bimanual coordination movements have been shown to lead to activation in the pre-frontal cortex, specifically the medial frontal region, which is also involved in attention to demanding cognitive tasks, spatial memory, self-initiated movement, and conflict resolution (Spinella et al., 2004).

An 8-week coordination training program based on 11 movements, including coordination of fingers, hands, eyes, and legs while the participant is sitting (which is a simplified version of Tai Chi), significantly improved cognitive function (as assessed by the Chinese Dementia Rating Scale) of older adults (Kwok et al., 2011). Voelcker-Rehage et al. (2011) and Niemann et al. (2014) found that 12 months of coordination training was associated with increased activation in the visual-spatial network, and led to changes in the total hippocampal volume of older adults. The authors suggested that this result was a consequence of the fact that to a high degree coordinative exercises rely on and practice spatial orientation, and the hippocampus is known to be involved in spatial memory processes.

THE COMBINATION OF BALANCE AND COORDINATION EXERCISE

As coordination exercise was suggested to improve cognitive functions, and balance exercise was shown to improve balance, cognitive functions, as well as quality of life, it was suggested that the combination of balance and coordination exercise may result in greater improvement in the quality of life among the elderly. One example of such a combination is suggested by Tai Chi exercises, which are based on a series of movements linked together in a continuous sequence (i.e., coordination) while the body is constantly shifting from foot to foot, with the knees and hips held in flexion (i.e., balance). During each movement, different parts of the body take turns playing the role of stabilizer and mover, allowing smooth movements to be executed without compromising balance and stability. Older adults who performed this form of exercise were found to have significantly better postural control and stability in conditions with simultaneous disturbance of vision and proprioception, compared to active nonpractitioners (Wong et al., 2001). This form of exercise was also examined in a 12-week Tai Chi exercise program and has been found effective in reducing perceived stress and improving mood state, as well as increasing perceived social support (Taylor-Piliae et al., 2006). On the contrary, 24 sessions of Tai Chi exercises during a 12-week intervention did not improve static postural control or walking speed among older adults (Lelard et al., 2010).

Another example of the combination of balance and coordination exercises, together with resistance and aerobic training, was suggested by Dizdar et al. (2018) for fall prevention among women with osteoporosis. The part of balance and coordination in the exercises included: single leg stances with eyes open and closed, tandem stance, toe walking, heel walking, tandem gait, reciprocal lower extremity movement, half-squatting, bridging, modified Romberg exercise on hard and soft ground with eyes closed, edge walking, walking on a balance board, reciprocal leg movements, slowly sitting down and standing up from a chair, and going up and down the stairs. The authors found that 12 weeks of this combined program improved the participants' quality of life (as assessed by the Quality of Life Questionnaire of the European Foundation for Osteoporosis). The authors suggested that this improvement may be the result of performing exercises as a group, which enhanced the social aspect of the participant (Dizdar et al., 2018). This combination was

also studied by Dunsky et al. (2017a), who found that 8 weeks of balance training combined with coordination by the means of dual-task exercises had a significant positive effect on the quality of life (as assessed by the Short Form Health Survey—SF-36) of community-dwelling older adults. In this study, two balance programs were conducted: step aerobics—while using the step as an obstacle (moving near and around it, stepping on it, adding music while walking, and adding dual-task exercises and resistance exercises while walking around and on the step); and the stability ball—while using the ball as an unstable surface (sitting or lying on the ball while performing resistance exercises, and adding music and adding dual-task exercises while having postural control of the body on the ball). The authors suggested that the improved quality of life was probably the consequence of high compliance to the intervention in both groups, which led their participants to feel more comfortable in performing ADL and about other aspects of life, thus leading to a higher general health perception at the end of the study. Recently, Segev et al. (2019) studied the effect of balance and coordination exercises that are incorporated within a traditional cardiac-rehabilitation program for older adults with cardiovascular diseases. Following a 12-week program in which they trained twice a week, the participants of the intervention group improved their static and dynamic balance, as well as their functional strength. These skills are considered major components in ADL, and thus their improvement may imply a better quality of life.

DISCUSSION AND CONCLUSION

The current mini-review points to the advantages of a combination of balance and coordination exercise on behavioral and neurophysiological outcomes among older adults. Based on the above literature, it is suggested that older adults be exposed to a program that includes such a combination for 2–3 sessions each week, for periods of at least 8 weeks, as a tool for quality of life improvement. Instructors or clinicians who wish to include such combination exercises in a program for older adults should introduce them gradually, allowing for the proper adjustment of the trainees while ensuring their safety. The program should incorporate exercises that include static vs. dynamic stability postures, changes in the base of support, variations in the height of the center of gravity, and different standing surfaces. Additionally, it should progressively reach higher levels of challenges in the form of more complex exercises involving both motor and cognitive tasks (dual- and multi-task activities). When planning each training session, it is important to keep in mind the participants' adaptation process to the exercises and to determine the session's optimum duration, allowing for gradual and safe exposure to new equipment or a new exercise.

AUTHOR CONTRIBUTIONS

AD performed the drafting, analyses, and final version of the entire manuscript.

REFERENCES

Arampatzis, A., Peper, A., and Bierbaum, S. (2011). Exercise of mechanisms for dynamic stability control increases stability performance in the elderly. *J. Biomech* 44, 52–58. doi: 10.1016/j.jbiomech.2010.08.023

Bisson, E., Contant, B., Sveistrup, H., and Lajoie, Y. (2007). Functional balance and dual-task reaction times in older adults are improved by virtual reality and biofeedback training. *Cyberpsychol. Behav.* 10, 16–23. doi: 10.1089/cpb.2006.9997

Cesari, M., Vellas, B., and Gambassi, G. (2013). The stress of aging. *Exp. Gerontol.* 48, 451–456. doi: 10.1016/j.exger.2012.10.004

Chang, S. F., and Lin, P. L. (2015). Frail phenotype and mortality prediction: a systematic review and meta-analysis of prospective cohort studies. *Int. J. Nurs. Stud.* 52, 1362–1374. doi: 10.1016/j.ijnurstu.2015.04.005

de Labra, C., Guimaraes-Pinheiro, C., Maseda, A., Lorenzo, T., and Millán-Calenti, J. C. (2015). Effects of physical exercise interventions in frail older adults: a systematic review of randomized controlled trials. *BMC Geriatr.* 15:154. doi: 10.1186/s12877-015-0155-4

de Vries, A. W., Faber, G., Jonkers, I., Van Dieen, J. H., and Verschueren, S. M. P. (2018). Virtual reality balance training for elderly: similar skiing games elicit different challenges in balance training. *Gait Posture* 59, 111–116. doi: 10.1016/j.gaitpost.2017.10.006

de Vries, N. M., van Ravensberg, C. D., Hobbelen, J. S., Olde Rikkert, M. G., Staal, J. B., and Nijhuis-van der Sanden, M. W. (2012). Effects of physical exercise therapy on mobility, physical functioning, physical activity and quality of life in community-dwelling older adults with impaired mobility, physical disability and/or multi-morbidity: a meta-analysis. *Ageing Res. Rev.* 11, 136–149. doi: 10.1016/j.arr.2011.11.002

Dizdar, M., Irdesel, J. F., Dizdar, O. S., and Topsaç, M. (2018). Effects of balance-coordination, strengthening and aerobic exercises to prevent falls in postmenopausal patients with osteoporosis: a 6-month randomized parallel prospective study. *J. Aging Phys. Act.* 26, 41–51. doi: 10.1123/japa.2016-0284

Dunsky, A., Yahalom, T., Arnon, M., and Lidor, R. (2017a). The use of step aerobics and the stability ball to improve balance and quality of life in community-dwelling older adults-a randomized exploratory study. *Arch. Gerontol. Geriatr.* 71, 66–74. doi: 10.1016/j.archger.2017.03.003

Dunsky, A., Zeev, A., and Netz, Y. (2017b). Balance performance is task specific in older adults. *Biomed. Res. Int.* 2017:6987017. doi: 10.1155/2017/6987017

Gao, J. H., Parsons, L. M., Bower, J. M., Xiong, J., Li, J., and Fox, P. T. (1996). Cerebellum implicated in sensory acquisition and discrimination rather than motor control. *Science* 272, 545–547. doi: 10.1126/science.272.5261.545

Gottwald, B., Mihajlovic, Z., Wilde, B., and Mehdorn, H. M. (2003). Does the cerebellum contribute to specific aspects of attention? *Neuropsychologia* 41, 1452–1460. doi: 10.1016/s0028-3932(03)00090-3

Gouveia, B. R., Gouveia, É. R., Ihle, A., Jardim, H. G., Martins, M. M., Freitas, D. L., et al. (2018). The effect of the ProBalance programme on health-related quality of life of community-dwelling older adults: a randomised controlled trial. *Arch. Gerontol. Geriatr.* 74, 26–31. doi: 10.1016/j.archger.2017.08.012

Guizelini, P. C., de Aguiar, R. A., Denadai, B. S., Caputo, F., and Greco, C. C. (2018). Effect of resistance training on muscle strength and rate of force development in healthy older adults: a systematic review and meta-analysis. *Exp. Gerontol.* 102, 51–58. doi: 10.1016/j.exger.2017.11.020

Halvarsson, A., Dohrn, I. M., and Ståhle, A. (2015a). Taking balance training for older adults one step further: the rationale for and a description of a proven balance training programme. *Clin. Rehabil.* 29, 417–425. doi: 10.1177/0269215514546770

Halvarsson, A., Franzén, E., and Ståhle, A. (2015b). Balance training with multi-task exercises improves fall-related self-efficacy, gait, balance performance and physical function in older adults with osteoporosis: a randomized controlled trial. *Clin. Rehabil.* 29, 365–375. doi: 10.1177/0269215514544983

Halvarsson, A., Franzén, E., Farén, E., Olsson, E., Oddsson, L., and Ståhle, A. (2013). Long-term effects of new progressive group balance training for elderly people with increased risk of falling-a randomized controlled trial. *Clin. Rehabil.* 27, 450–458. doi: 10.1177/0269215512462908

Halvarsson, A., Olsson, E., Farén, E., Pettersson, A., and Stahle, A. (2011). Effects of new, individually adjusted, progressive balance group training for elderly people with fear of falling and tend to fall: a randomized controlled trial. *Clin. Rehabil.* 25, 1021–1031. doi: 10.1177/0269215511411937

Hayes, K. C. (1982). Biomechanics of postural control. *Exerc. Sport Sci. Rev.* 10, 363–391. doi: 10.1249/00003677-198201000-00011

Horak, F. B. (2006). Postural orientation and equilibrium: what do we need to know about neural control of balance to prevent falls? *Age Ageing* 35, ii7–ii11. doi: 10.1093/ageing/afl077

Ishiyama, G. (2009). Imbalance and vertigo: the aging human vestibular periphery. *Semin. Neurol.* 29, 491–499. doi: 10.1055/s-0029-1241039

Kojima, G. (2015). Frailty as a predictor of future falls among community-dwelling older people: a systematic review and meta-analysis. *J. Am. Med. Dir. Assoc.* 16, 1027–1033. doi: 10.1016/j.jamda.2015.06.018

Kwok, T. C., Lam, K. C., Wong, P. S., Chau, W. W., Yuen, K. S., Ting, K. T., et al. (2011). Effectiveness of coordination exercise in improving cognitive function in older adults: a prospective study. *Clin. Interv. Aging* 6, 261–267. doi: 10.2147/CIA.s19883

Landi, F., Calvani, R., Cesari, M., Tosato, M., Martone, A. M., Bernabei, R., et al. (2015). Sarcopenia as the biological substrate of physical frailty. *Clin. Geriatr. Med.* 31, 367–374. doi: 10.1016/j.cger.2015.04.005

Lelard, T., and Ahmaidi, S. (2015). Effects of physical training on age-related balance and postural control. *Neurophysiol. Clin.* 45, 357–369. doi: 10.1016/j.neucli.2015.09.008

Lelard, T., Doutrellot, P. L., David, P., and Ahmaidi, S. (2010). Effects of a 12-week Tai Chi Chuan program versus a balance training program on postural control and walking ability in older people. *Arch. Phys. Med. Rehabil.* 91, 9–14. doi: 10.1016/j.apmr.2009.09.014

Lord, S. R. (2006). Visual risk factors for falls in older people. *Age Ageing* 35, ii42–ii45. doi: 10.1093/ageing/afl085

Lord, S. R., Lloyd, D. G., and Keung Li, S. E. K. (1996). Sensori-motor function, gait patterns and falls in community-dwelling women. *Age Ageing* 25, 292–299. doi: 10.1093/ageing/25.4.292

Manto, M., Bower, J. M., Conforto, A. B., Delgado-García, J. M., da Guarda, S. N., Gerwig, M., et al. (2012). Consensus paper: roles of the cerebellum in motor control-the diversity of ideas on cerebellar involvement in movement. *Cerebellum* 11, 457–487. doi: 10.1007/s12311-011-0331-9

Mouthon, A., and Taube, W. (2019). Intracortical inhibition increases during postural task execution in response to balance training. *Neuroscience* 401, 35–42. doi: 10.1016/j.neuroscience.2019.01.007

Nagy, E., Feher-Kiss, A., Barnai, M., Domján-Preszner, A., Angyan, L., and Horvath, G. (2007). Postural control in elderly subjects participating in balance training. *Eur. J. Appl. Physiol.* 100, 97–104. doi: 10.1007/s00421-007-0407-x

Nascimento, C. M., Ingles, M., Salvador-Pascual, A., Cominetti, M. R., Gomez-Cabrera, M. C., and Viña, J. (2019). Sarcopenia, frailty and their prevention by exercise. *Free Radic. Biol. Med.* 132, 42–49. doi: 10.1016/j.freeradbiomed.2018.08.035

Navas-Enamorado, I., Bernier, M., Brea-Calvo, G., and de Cabo, R. (2017). Influence of anaerobic and aerobic exercise on age-related pathways in skeletal muscle. *Ageing Res. Rev.* 37, 39–52. doi: 10.1016/j.arr.2017.04.005

Netz, Y., Zeev, A., and Dunsky, A. (2018). Postural control and posture-unrelated attention control in advanced age—An exploratory study. *Maturitas* 116, 130–136. doi: 10.1016/j.maturitas.2018.08.003

Niemann, C., Godde, B., and Voelcker-Rehage, C. (2014). Not only cardiovascular, but also coordinative exercise increases hippocampal volume in older adults. *Front. Aging Neurosci.* 6:170. doi: 10.3389/fnagi.2014.00170

Rendon, A. A., Lohman, E. B., Thorpe, D., Johnson, E. G., Medina, E., and Bradley, B. (2012). The effect of virtual reality gaming on dynamic balance in older adults. *Age Ageing* 41, 549–552. doi: 10.1093/ageing/afs053

Robbins, A. S., Rubenstein, L. Z., Josephson, K. R., and Schulman, B. L. (1989). Predictors of falls among elderly people: results of two population based studies. *Arch. Intern. Med.* 149, 1628–1633. doi: 10.1001/archinte.149.7.1628

Rogge, A. K., Röder, B., Zech, A., and Hötting, K. (2018). Exercise-induced neuroplasticity: balance training increases cortical thickness in visual and vestibular cortical regions. *Neuroimage* 1, 471–479. doi: 10.1016/j.neuroimage.2018.06.065

Rogge, A. K., Röder, B., Zech, A., Nagel, V., Hollander, K., Braumann, K. M., et al. (2017). Balance training improves memory and spatial cognition in healthy adults. *Sci. Rep.* 7:5661. doi: 10.1038/s41598-017-06071-9

Rubenstein, L. Z. (2006). Falls in older people: epidemiology, risk factors and strategies for prevention. *Age Ageing* 35, ii37–ii41. doi: 10.1093/ageing/afl084

Segev, D., Hellerstein, D., Carasso, R., and Dunsky, A. (2019). The effect of a stability and coordination training programme on balance in older adults with cardiovascular disease: a randomised exploratory study. *Eur. J. Cardiovasc. Nurs.* doi: 10.1177/1474515119864201 [Epub ahead of print].

Sibley, K. M., and Salbach, N. M. (2015). Applying knowledge translation theory to physical therapy research and practice in balance and gait assessment: case report. *Phys. Ther.* 95, 579–587. doi: 10.2522/ptj.20130486

Sieber, C. C. (2017). Frailty-from concept to clinical practice. *Exp. Gerontol.* 87, 160–167. doi: 10.1016/j.exger.2016.05.004

Smith, P. F., Darlington, C. L., and Zheng, Y. (2010). Move it or lose it-is stimulation of the vestibular system necessary for normal spatial memory? *Hippocampus* 20, 36–43. doi: 10.1002/hipo.20588

Smith, P. F., and Zheng, Y. (2013). From ear to uncertainty: vestibular contributions to cognitive function. *Front. Integr. Neurosci.* 7:84. doi: 10.3389/fnint.2013.00084

Spinella, M., Yang, B., and Lester, D. (2004). Prefrontal system dysfunction and credit card debt. *Int. J. Neurosci.* 114, 1323–1332. doi: 10.1080/00207450490476011

Stimpson, N. J., Davison, G., and Javadi, A. H. (2018). Joggin' the noggin: towards a physiological understanding of exercise-induced cognitive benefits. *Neurosci. Biobehav. Rev.* 88, 177–186. doi: 10.1016/j.neubiorev.2018.03.018

Taguchi, N., Higaki, Y., Inoue, S., Kimura, H., and Tanaka, K. (2010). Effects of a 12-month multicomponent exercise program on physical performance, daily physical activity and quality of life in very elderly people with minor disabilities: an intervention study. *J. Epidemiol.* 20, 21–29. doi: 10.2188/jea.je20081033

Tarazona-Santabalbina, F. J., Gómez-Cabrera, M. C., Pérez-Ros, P., Martínez-Arnau, F. M., Cabo, H., Tsaparas, K., et al. (2016). A multicomponent exercise intervention that reverses frailty and improves cognition, emotion and social networking in the community-dwelling frail elderly: a randomized clinical trial. *J. Am. Med. Dir. Assoc.* 17, 426–433. doi: 10.1016/j.jamda.2016.01.019

Taube, W., Gruber, M., Beck, S., Faist, M., Gollhofer, A., and Schubert, M. (2007). Cortical and spinal adaptations induced by balance training: correlation between stance stability and corticospinal activation. *Acta Physiol.* 189, 347–358. doi: 10.1111/j.1748-1716.2007.01679_1.x

Taylor-Piliae, R. E., Haskell, W. L., Waters, C. M., and Froelicher, E. S. (2006). Change in perceived psychosocial status following a 12-week Tai Chi exercise programme. *J. Adv. Nurs.* 54, 313–329. doi: 10.1111/j.1365-2648.2006.03809.x

Tinetti, M. E., Williams, T. F., and Mayewski, R. (1986). Fall risk index for elderly patients based on chronic disabilities. *Am. J. Med.* 80, 429–434. doi: 10.1016/0002-9343(86)90717-5

Tomlinson, S. P., Davis, N. J., Morgan, H. M., and Bracewell, R. M. (2014). Cerebellar contributions to verbal working memory. *Cerebellum* 13, 354–361. doi: 10.1007/s12311-013-0542-3

Voelcker-Rehage, C., Godde, B., and Staudinger, U. M. (2011). Cardiovascular and coordination training differentially improve cognitive performance and neural processing in older adults. *Front. Hum. Neurosci.* 5:26. doi: 10.3389/fnhum.2011.00026

Wong, A. M., Lin, Y. C., Chou, S. W., Tang, F. T., and Wong, P. Y. (2001). Coordination exercise and postural stability in elderly people: effect of Tai Chi Chuan. *Arch. Phys. Med. Rehabil.* 82, 608–612. doi: 10.1053/apmr.2001.22615

World Health Organization. (2007). *WHO Global Report on Falls Prevention in Older Age*. Geneva: WHO Press, World Health Organization.

Zech, A., Hübscher, M., Vogt, L., Banzer, W., Hänsel, F., and Pfeifer, K. (2010). Balance training for neuromuscular control and performance enhancement: a systematic review. *J. Athl. Train* 45, 392–403. doi: 10.4085/1062-6050-45.4.392

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

ORIGINAL RESEARCH
published: 22 November 2019
doi: 10.3389/fnagi.2019.00321

A Delayed Advantage: Multi-Session Training at Evening Hours Leads to Better Long-Term Retention of Motor Skill in the Elderly

Carmit Gal[1,2], Ella Gabitov[3], Rinatia Maaravi-Hesseg[1,2], Avi Karni[1,2†] and Maria Korman[1†]*

[1] The Edmond J. Safra Brain Research Center for the Study of Learning Disabilities, University of Haifa, Haifa, Israel, [2] Laboratory for Human Brain and Learning, Sagol Department of Neurobiology, Faculty of Natural Sciences, University of Haifa, Haifa, Israel, [3] McConnell Brain Imaging Center, Montreal Neurological Institute, McGill University, Montreal, QC, Canada

The acquisition and retention of motor skills is necessary for everyday functioning in the elderly and may be critical in the context of motor rehabilitation. Recent studies indicate that motor training closely followed by sleep may result in better engagement of procedural ("how to") memory consolidation processes in the elderly. Nevertheless, elderly individuals are mostly morning oriented and a common practice is to time rehabilitation programs to morning hours. Here, we tested whether the time-of-day wherein training is afforded (morning, 8–10:30 a.m., or evening, 6–9 p.m.) affects the long-term outcome of a multi-session motor practice program (10 sessions across 3–4 weeks) in healthy elderly participants. Twenty-nine (15 women) older adults (60–75 years) practiced an explicitly instructed five-element key-press sequence by repeatedly generating the sequence "as fast and accurately as possible." The groups did not differ in terms of sleep habits and quality (1-week long actigraphy); all were morning-oriented individuals. All participants gained robustly from the intervention, shortening sequence tapping duration and retaining the gains (> 90%) at 1-month post-intervention, irrespective of the time-of-day of training. However, retesting at 7-months post-intervention showed that the attrition of the training induced gains was more pronounced in the morning trained group compared to the evening group (76 and 56.5% loss in sequence tapping time; 7/14 and 3/14 participants showed a > 5% decline in accuracy relative to end of training, respectively). Altogether, the results show that morning-oriented older adults effectively acquired skill in the performance of a sequence of finger movements, in both morning and evening practice sessions. However, evening training leads to a significant advantage, over morning training, in the long-term retention of the skill. Evening training should be considered an appropriate time window for motor skill learning in older adults, even in individuals with morning chronotype. The results are in line with the notion that motor training preceding a sleep interval may be better consolidated into long-term memory in the elderly, and thus result in lower forgetting rates.

Keywords: elderly, motor skill, multi-session training, retention, morning, evening, chronotype, actigraphy

OPEN ACCESS

Edited by:
Michael George Bemben, The University of Oklahoma, United States

Reviewed by:
Talitha Best, Central Queensland University, Australia
Carla Silva-Batista, University of São Paulo, Brazil
Mavis Akosua Asantewaa Tenkorang-Impraim, University of Nebraska Medical Center, United States

****Correspondence:***
Carmit Gal
carmitgal21@hotmail.com

† These authors have contributed equally to this work

Received: *30 July 2019*
Accepted: *05 November 2019*
Published: *22 November 2019*

Citation:
Gal C, Gabitov E, Maaravi-Hesseg R, Karni A and Korman M (2019) A Delayed Advantage: Multi-Session Training at Evening Hours Leads to Better Long-Term Retention of Motor Skill in the Elderly. Front. Aging Neurosci. 11:321. doi: 10.3389/fnagi.2019.00321

INTRODUCTION

Motor functioning and, specifically, the ability of older adults to acquire new fine motor skills and generate effective long-term procedural ("how to") memory are often reduced compared to young adults (Voelcker-Rehage, 2008). The search for interventions that can attenuate the age-related decrements in the performance of the existing repertoire of motor skills, as well as in the ability to master newly acquired skills is of importance (King et al., 2013). The decline in motor learning abilities was suggested to reflect a general decrease in neuroplasticity with aging (King et al., 2013), or stricter control ("gating") of the brain's plasticity mechanisms subserving procedural long-term memory (Korman et al., 2015), or both. The latter notion implies that in specific bio-behavioral conditions, devised to meet the age-related constraints on plasticity, the potential of older adults to master motor skills may be better expressed.

One critical constraining or "gating" aspect of skill learning in the elderly is related to the circadian correlates of biological aging—the changes in activity-rest rhythms toward morning chronotype and the decrease in sleep quality and duration (Duffy et al., 2015). Aging is characterized by a blunted circadian rhythmicity in the core body temperature, cortisol, and melatonin, suggesting that changes in sleep architecture may be linked to weakened circadian regulation (Hood and Amir, 2017). Due to the morning-oriented preferred activity times, early awakening hours, and high day-time fatigue (Goldman et al., 2008), it is generally accepted that the assessment of cognitive functioning may be confounded by a decrease in alertness in the late afternoon or evening in the elderly (Hood and Amir, 2017); thus, scheduling training to evening hours is considered sub-optimal. Indeed, morning type older adults, accounting for more than 60% of the elderly population (Roenneberg et al., 2007; Fischer et al., 2017), often show prominent deterioration in cognitive performance over the day (May et al., 2005; Veneman et al., 2013; Tsokanaki et al., 2016). Nevertheless, recent studies suggest that time-of-day effects may differ across cognitive domains (Schmidt et al., 2015); implicit memory retrieval may, in fact, be better at off-peak than at peak alertness hours in both young and elderly (May et al., 2005).

The acquisition of new motor skills has been extensively studied using different versions of the finger tapping task. This task is viewed as an ecologically relevant model for the acquisition of complex manual skills, from writing/typing to playing a musical instrument, that involves the explicitly guided concatenation of single movements into sequences (Friedman and Korman, 2012, 2016). Along the course of learning, sequential performance becomes progressively faster and smoother, without compromising, and sometimes, improving accuracy (Hikosaka et al., 2002; Sosnik et al., 2004). Mastering a novel motor sequence is a multi-session process, whereby each training session elicits both immediate (online) and delayed (offline) changes in movement speed and accuracy (Karni and Sagi, 1993; Karni et al., 1995, 1998; Korman et al., 2003). An important outcome of multi-session training in young adults is that the gains are both sequence and effector specific, and thus are only partially generalizable to the performance of new sequences (Karni et al., 1995; Korman et al., 2003).

Although there are age-related declines in baseline motor performance, the ability to learn within-session is well preserved in the elderly (Durkin et al., 1995; Howard et al., 2004; Yan et al., 2010; Ehsani et al., 2015; Korman et al., 2015) [a possible exception may be learning under conditions of high task complexity (Rieckmann and Bäckman, 2009)]. However, offline learning, expressed as delayed between-session gains in performance, is consistently reported to be impaired in older adults (Brown et al., 2009; King et al., 2013; Korman et al., 2015). These delayed gains are considered to be a behavioral hallmark of memory consolidation processes and are time-dependent and, specifically in relation to movement sequence learning, time-in-sleep dependent (Karni et al., 1998; Korman et al., 2003). Thus, the relative deficits in the generation of delayed, consolidation-phase, gains following a single training session scheduled to the morning or day hours (Korman et al., 2015) may accumulate over multi-session practice and manifest as an overall slower rate of learning in older adults (Spencer et al., 2007; Wilson et al., 2012).

In young adults, both the magnitude of the delayed gains in explicitly instructed motor sequence practice and the time-course of the evolution of these gains are dependent on post-training sleep, either night-time or day-time, or both (Walker et al., 2003; Korman et al., 2007; Doyon et al., 2009; Walker and Stickgold, 2010). The role of sleep is conceptualized both as protecting from behavioral interference by subsequent motor experience and as promoting memory stabilization and enhancement. Because changes in sleep, often negative, occur in advanced age (Phillips and Ancoli-Israel, 2001; Huang et al., 2002), these changes were linked to the decline in cognitive functioning in the elderly (Mander et al., 2017; Dzierzewski et al., 2018) and, specifically, to the decrease in motor learning abilities (King et al., 2013). A large number of studies indicate that sleep-dependent procedural memory consolidation phase gains in performance are particularly weakened in older adults (Spencer et al., 2007; Wilson et al., 2012; Albouy et al., 2013; King et al., 2013; Terpening et al., 2013; Backhaus et al., 2016). Nevertheless, when sleep is allowed immediately after an evening training session (Mantua et al., 2016) or napping is afforded after training at late morning hours (Korman et al., 2015), healthy elderly were shown to generate significant overnight delayed consolidation phase gains in performance. It may be the case that evening training with proximity to a sleep period may promote better memory consolidation and minimize unspecific interference from everyday motor activity (Korman et al., 2015).

A critical aspect in evaluating the effectiveness and utility of any training intervention beyond the robustness of the acquired gains in performance is the durability of the skill. In the serial reaction time task single-session learning, both general (faster reaction times) and sequence-specific knowledge were retained, though not fully, over a 1-year period by older adults (Romano et al., 2010). Robust retention was reported in mirror-tracing skills in healthy older adults who practiced for 3 separate days and were retested 5 years later (Rodrigue et al., 2005). However, other evidence suggests that aging is related to faster forgetting rates (Malone et al., 2016; Griffin et al., 2017).

Given that, on the one hand, training in the morning hours may meet the circadian preference of older adults, but, on the other hand, that morning training may be less beneficial to the mastering of motor skills as a long time interval separates the practice experience from the interval of sleep at night; a direct comparison of the effects of affording a program of motor training at peak, and off-peak times, in the elderly, is warranted from both theoretical and practical perspectives. Here, using the well-established paradigm of the finger tapping sequence learning (FTSL) task (Karni et al., 1995, 1998), we investigated whether the time-of-day wherein training is afforded (morning or evening) is a significant factor in motor skill acquisition, the ability to generalize the gains in performance, and importantly, the retention of the skill, in healthy elderly participants. The effects of an extensive, 10-session training intervention were assessed in terms of sequence completion time and between key-press transition times [model task of motor sequence learning (Friedman and Korman, 2012)]. Accuracy levels, as markers of a possible speed-accuracy trade-off, were assessed in terms of the percentage of correct transitions from the total number of transitions (= 59 per block).

MATERIALS AND METHODS

Participants

The study was approved by the University of Haifa Human Experimentation Ethics Committee. All participants gave written informed consent of participation in the study after being provided with explanations of its purpose. Twenty-nine active, community-dwelling elderly participants (60–75 years old; mean age = 67.3 ± 4.23, 16 women) took part in the current study. In the evening group: 9/15 = 60% were women and in the morning group 7/14 = 50% were women. The group size of the current exploratory study was based on the effect sizes of the offline memory consolidation effects in terms of sequence duration, observed in the same task in Korman et al. (2015) (with healthy elderly participants): the group that had a nap immediately after training improved by 17%, whereas the group that did not nap improved by 4%, when re-tested 24 after the training. The Eve group was expected to show better overnight memory consolidation, with similar differences in performance improvement following training afforded during morning hours compared to evening hours, due to proximity to the sleep interval of the latter. Based on a standard deviation in each group of 11.5%, and a power of 0.95, 15 subjects in each group were required, based on a mixed-design ANOVA. No previous data were available to perform power analysis for the effects of multi-session training; the differences in the long-term representation of the skill after a single training session were expected to accumulate over the course of multi-session training.

The participants were recruited through public advertisements and a "snow-ball" approach from a single kibbutz in northern Israel, if meeting the basic requirements of good health and right-handedness. All participants were retired from their permanent work, however, engaged in variable jobs in the kibbutz, for at least 4 hours a day, during the study period. Individuals with neurological, psychiatric, or musculoskeletal system disorders, unstable cardiovascular status, users of psychotropic medication, those who have been diagnosed with diabetes, sleep disorders/insomnia, or impaired thyroid function, as well as overweight individuals were excluded. Since there is no BMI cut-off score that is universally accepted in older adults (Yan et al., 2004), the inclusion criterion in the current study was BMI < 27 (upper limit of normal BMI in young adults is 24.9). People designating themselves as heavy smokers and heavy alcohol and caffeine consumers (> 3 drinks per day on average); professional musicians or/and professional typists; as well as shift-workers, were excluded from the experiment. Participants reporting frequent, habitual, day-time napping were also excluded.

All participants were first evaluated using a structured telephone interview. Inclusion criteria were thoroughly verified at the first meeting, using standard questionnaires for general health and sleep-activity habits. All participants were right-handed according to the Edinburgh handedness inventory (scored > 50 points) (Oldfield, 1971). Emotional health was evaluated using the Beck's anxiety and depression inventory (Steer et al., 1986). Given the recognized effects of sleep on learning, the sleep–wake parameters of participants were assessed using the Pittsburg Sleep Quality Index (PSQI) (Buysse et al., 1989), Daytime Sleepiness Questionnaire (Epworth: Johns, 1991), and the Morningness–Eveningness Questionnaire (MEQ) for assessment of the circadian type (Horne and Ostberg, 1976).

Participants meeting the inclusion criteria were randomly assigned to one of the study groups, using the block randomization method: to be trained either in the morning (8–10:30 a.m.), or in the evening (6–9 p.m.) hours. No differences were found between the groups in terms of age and level of education, and in scores of the screening questionnaires (**Table 1**).

The Setup and the FTSL Task

All participants were trained and tested in their private home, by the same experimenter. Participants were seated in a quiet room that included a table and a chair; this location was used throughout the study. A 17-in screen laptop was positioned about 50 cm from the participant and the response box. The left hand of the participant was placed on the four numeric keys, arranged in an ergonomic position, of a response box (Expert gaming Keypad-Razer Nostromo), with key-to-number assignment from right to left; the little finger designated 4 (**Figure 1A**).

The task practiced in the current study was a computerized version of the finger opposition sequence learning task, initially developed by Karni et al. (1995) (**Figure 1A**) implemented as the FTSL task; i.e., with key presses substituting the opposition movements. All participants had no prior experience with the task. The task consisted of repeating (tapping), as quickly and accurately as possible, a sequence of five finger movements using the left, non-dominant hand. Two mirror-reversed sequences of equal length and complexity (41234 or 42314, **Figure 1A**) were used; each participant

TABLE 1 | Mean scores for demographic data and screening questionnaires, by group.

	Variable	Morning *N* = 14	Evening *N* = 15	Interpretation
		Mean + *SD*	Mean + *SD*	
	Age	62.7 ± 5.86	63.5 ± 5.09	
	Education (years)	11.64 ± 1.33	11.14 ± 1.02	
	Handedness score	89.19 ± 18.53	100	
Emotional health	Beck anxiety inventory	2.53 ± 4.37	3.64 ± 3.91	> 9 minimal anxiety
	Beck depression inventory	2.66 ± 4.12	2.94 ± 3.11	> 9 minimal depression
Sleep and wake	Epworth	5.92 ±4.92	5.0 ±3.08	> 10 excessive sleepiness
	PSQI	4.69 ±1.88	5.3 ±2.32	> 5 sleep disturbances
	MEQ	60 ± 8.7	63.15 ± 5.33	> = 59 morning type

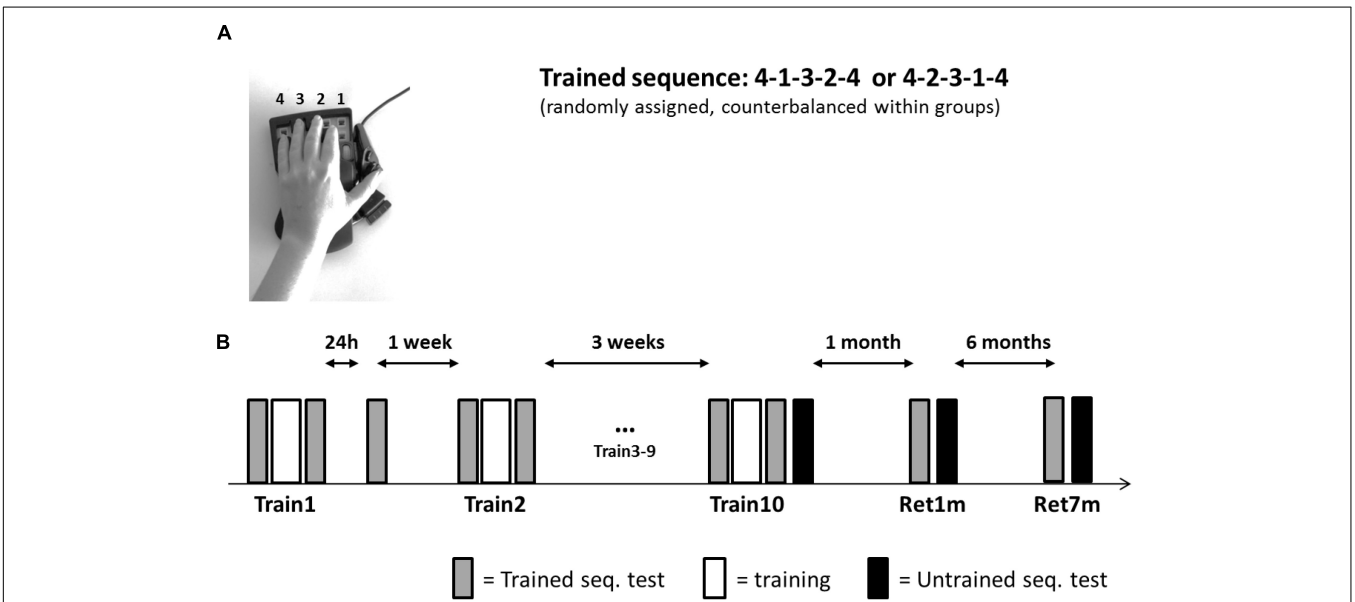


FIGURE 1 | Setup, task, and training protocol. **(A)** The Finger Tapping Sequence Learning (FTSL) task. **(B)** The training protocol. One of the two sequences (4-1-3-2-4 or 4-2-3-1-4) was used as the trained sequence; its mirror reversed counterpart sequence was used in transfer tests (untrained sequence). Participants in both groups (evening and morning) were initially trained in a single training session (Train1), retested at 24 h post-training, and a week later continued in the multi-session training protocol—nine additional training sessions (Train2–Train10) spaced 2–3 days apart. Altogether, the training period spanned 3–4 weeks. Performance of the untrained (transfer) sequence was tested immediately after the 10th session (Train10) and the trained and untrained sequences were retested at 1 month (Ret1m) and 7 months (Ret7m) after the Train10 session. Each session included 14 blocks; gray bars—the four initial and the final four blocks of the session were used as tests of the trained sequence; white bars—six blocks of training; black bars—four test blocks on the untrained sequence.

was randomly assigned one sequence for training (trained sequence) and the second sequence served for the transfer tests (untrained sequence).

Training Protocol and Performance Assessments

The experiment involved 10 training (intervention) sessions and two long-term retention tests (**Figure 1B**). Following the first training session, a 24-h performance re-test was performed (data not reported here). A week later, sessions 2–10 were afforded, on separate days spaced 2–3 days apart; thus, the training phase spanned 3–4 weeks in total. Retention was tested at 1 and 7 months after the completion of the 10th session of the intervention.

The training times were the same as the testing times in both groups (± 30 mins) throughout the study: participants of the morning group were trained at 8–10:30 a.m. and the participants of the evening group were trained at 6:30–9:00 p.m. Each training session lasted approximately 30 min. Tests for the ability to transfer the gains acquired in practice to a novel sequence, the performance of the untrained, mirror-reversed sequence, were performed at the end of the 10th session (Train10) and at the end of the 1-month and 7-months' retention tests (Ret1m and Ret7m, respectively). Performance of the trained sequence was always tested first.

Full explicit knowledge and a demonstration of the required movement sequence were provided prior to each training session by verbal instruction and a presentation, on the computer screen, of the numbered keys comprising the to be trained or

tested sequence, by the experimenter. The participants interacted with the same experimenter throughout the study. The training session began only after three consecutive, correct iterations of the target sequence were executed by the participant, indicating that the participant understood the required sequence.

All training sessions were identical in structure. Each training session consisted of 14 blocks, each block comprised of 12 repetitions of the assigned sequence (i.e., each block consisted of a total of 60 key presses). A 30-s rest period was afforded between consecutive blocks. The average of the four initial and four final blocks were used in the analysis as the measures of performance at the beginning (pre-test) and end (post-test) of each training session, respectively (Doyon et al., 2009). Each of the retention tests, at 1 and 7 months' post-intervention, also included four consecutive blocks of the performance of the trained sequence; four consecutive blocks were also used to assess performance in the transfer condition, the performance of the untrained sequence. Participants were instructed not to practice the experimental task between sessions. All participants confirmed at the beginning of each meeting they followed this instruction.

Before the beginning of each block of trials, the participants were instructed/reminded to continuously tap the sequence "as fast and accurately as possible," using their left non-dominant hand; starting immediately after the onset of the "go" signal on screen ("green cross"). Participants were then instructed that at the end of each block, a "red cross"("stop" cue) will appear, as the cue to stop tapping the sequence. The screen background remained black throughout the session. No feedback on performance was provided online or offline. Participants were instructed that occasional errors should not be corrected, and were required to continue the task without a pause even in the case of an error.

One day prior to the first session of training, each participant was asked to wear an actigraph (ActiGraph wGT3X, ActiGraph, LLC) in order to record the participant's sleep-activity cycles. The participants were instructed to wear the actigraph on their non-dominant wrist (left), continuously for a 7-day period, with the exception of time spent during water activities (shower, etc.). In addition, participants were asked to keep a sleep diary with entries for each day of the week they wore the actigraph. At the conclusion of the 7 days' period, actigraph devices were collected and the data were retrieved using Actilife6 software. Participants were instructed to maintain their usual sleep habits and avoid day-time napping throughout the whole experiment.

Data Extraction and Statistical Analyses

The timing and corresponding number of each key-press were recorded. The motor sequence performance measures were derived from the time differences between two consecutive key presses within each correctly performed and completed sequence, as well as between sequences (the final key-press of a sequence and the first key-press of the next sequence) in each task block using a custom MATLAB script (The Mathworks, Inc., Natick, MA, United States, version:2007). The pairs of movements (transitions) for analysis were four *within-sequence transitions*—transition from finger 4 to 1, 1 to 3, 3 to 2, and 2 to 4 for participants training on sequence 4-1-3-2-4 or the corresponding four transitions for sequence 4-2-3-1-4 in participants assigned the latter sequence. In addition, *between-sequence transition* times, the transition time from end of one sequence (finger 4) to the first movement of the next iteration (finger 4), was computed for each block. The main behavioral measures of performance were: (*i*) speed, calculated as the mean correct sequence duration – sum of within-sequence transitions in a given trial. The sequence duration analysis included only the correctly performed and completed sequences during each block; (*ii*) accuracy, calculated as the percentage correct transitions from the total number of transitions (= 59 per block).

Data were analyzed using the Statistical Package for the Social Sciences (SPSS Statistics for Windows, Version 24; IBM Corp., Armonk, NY, United States). A Kolmogorov–Smirnov test for normality was used to test the distribution of the data on the primary outcome measures. The magnitude of learning the trained sequence was assessed using mixed repeated measures general linear models (GLMs) [with two groups (morning/evening) × 10 sessions (Train1–Train10) × two time-points for each session (pre-test, post-test)], carried out separately for each performance measure. Retention of skill in the execution of the trained sequence across the intervals of 1-month and 7-months post-intervention compared to the last training was assessed using repeated measures GLMs (Rm-GLMs) [two groups (morning/evening) × three time-points (post-test of Train10, Ret1m, Ret7m)], carried out separately for each performance measure. To test for the degree of specificity of the acquired motor sequence knowledge, performance of the trained sequence was compared to the performance of a novel, untrained, sequence using paired *t*-tests at Train10, Ret1m, and Ret7m. Because the error percentage (accuracy) was not normally distributed, the non-parametric Chi-square test was used for assessing changes in accuracy.

The raw activity scores from the actigraphy were translated to sleep–wake scores based on standard software (scoring algorithm) (Actilife6). Mean total sleep time (TST), sleep efficiency, sleep latency, and wake time after sleep onset (WASO), number of awakening, and average of awakening duration. To assess possible difference in sleep quality and duration between the intervention groups, the means of the groups for each sleep parameter were compared using independent sample uncorrected *t*-tests and Mann–Whitney test for the non-parametric sleep parameters. The significance level was at $p \leq 0.05$.

RESULTS

Effects of Multi-Session Practice Across the Intervention Interval

Overall, both groups showed significant gains in mean correct sequence duration with no loss in accuracy (no speed-accuracy trade-off) (**Figure 2A**). Mixed Rm-GLM analyses comparing the performance across the 10 training sessions (time-point, between-sessions) for the initial and final four blocks of each session (within-session) in the two groups (Eve and Morn,

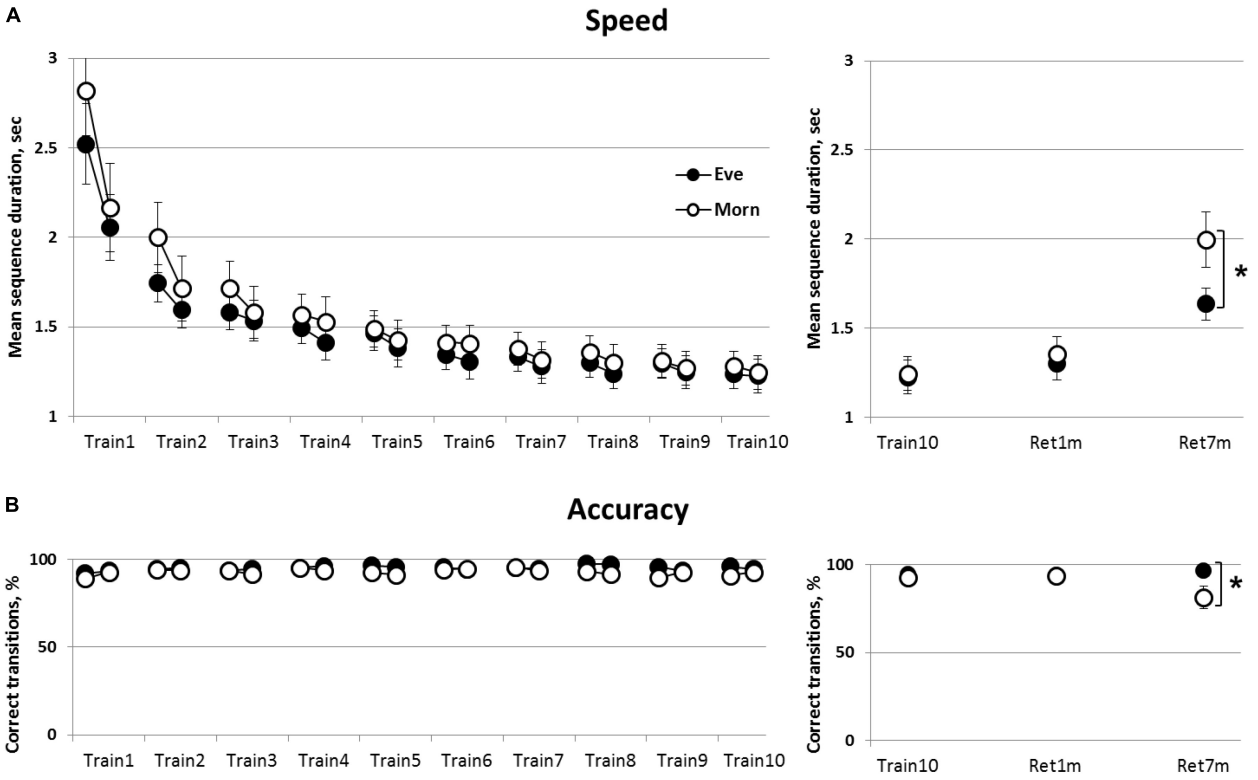


FIGURE 2 | The time-course of learning and retention of a key-press sequence; evening (Eve) and morning (Morn) trained groups. Performance at the initial and the final practice blocks at each of the 10 practice sessions is shown (averages of first four and last four performance blocks, respectively) (left panels).
(A) Speed—mean duration for correct sequences. **(B)** Accuracy—percent of correct transitions from the total number of key transitions (= 59 per block). The right panels show retention of the trained sequence at 1 (Ret1m) and 7 months (Ret7m) compared to the end of training (Train10). Open circles—morning group, filled circles—evening group. Bars—standard error of the mean (SEM), *—significant difference between the performance levels of the Eve and Morn groups, $p \leq 0.05$.

between-subjects factor) showed that overall, there was a significant decrease in mean sequence duration, time-points [$F(9,243) = 74.46$, $p < 0.001$, $\eta^2 = 0.73$]. There was, however, no significant main effect of group [$F(9,243) = 0.65$, $p = 0.75$, $\eta^2 = 0.024$] and also no significant group × time-points interaction [$F(1,27) = 0.58$, $p = 0.45$, $\eta = 0.021$] (**Figure 2A**).

There was also a significant within-session effect [$F(1,27) = 30.56$, $p < 0.001$, $\eta^2 = 0.53$] and a significant time-point × within-session effect [$F(9, 243) = 23.40$, $p < 0.001$, $\eta^2 = 0.46$]; indicating that gains in performance speed (reduction of sequence duration) occurred within the initial sessions (sessions 1–3, 4; Train1–3,4 in **Figure 2A**).

However, there was no significant group × within-session or group × time-point × within-session interaction [$F(1,27) = 0.58$, $p = 0.45$, $\eta^2 = 0.021$, $F(9,243) = 1.33$, $p = 0.22$, $\eta^2 = 0.05$, respectively] indicating that the time-course of learning, within-sessions and across sessions, was independent of the time-of-day wherein the training sessions were afforded.

Accuracy throughout the training programs was high; with the number of correct sequences > 90% throughout the intervention sessions in both groups (**Figure 2B**). Non-parametric repeated measures Friedman tests showed that there was a trend toward a (small) increase in accuracy in the Eve trained group [$\chi 2(19) = 28.65$, $p = 0.07$] and no significant change in the accuracy of performance in the Morn group [$\chi 2(19) = 18.07$, $p = 0.52$] (**Figure 2B**). The gains in speed, therefore, evolved with no costs of accuracy, i.e., there was no speed-accuracy tradeoff.

The results, therefore, indicate that training at morning hours did not confer an advantage; both training protocols were effective in inducing learning across the 10 training sessions, without apparent differences in the magnitude of the learning gains or the time-course of within or between sessions acquisition of skill.

Long-Term Maintenance of Skill

The retention of the gains in the performance of the practiced key-tapping sequence was tested at 1 month post-training and again by 7 months post-training (**Figure 2**, right panels). To assess retention, performance was compared across three time-points: the final training session, 1-month post-training and 7 months post-training (Train10, Ret1m, and Ret7m, respectively) (**Figure 2**). There was a significant deterioration in speed with sequence duration increasing significantly [$F(2,52) = 27.57$, $p < 0.001$, $\eta^2 = 0.52$]. Nevertheless, although there was no significant group effect [$F(1,26) = 1.2$, $p = 0.29$, $\eta^2 = 0.04$], there was a trend for a time-point × group interaction [$F(2,52) = 2.97$, $p = 0.06$, $\eta^2 = 0.10$]. This trend reflected a greater deterioration in sequence duration from

Train10 to Ret7m in the Morn group (from 1.28 to 1.99 s) than in the Eve group (from 1.25 to 1.63 s) (**Figure 2A**, upper right panel).

Participants practicing in the morning hours, the Morn group, tended also to show a deterioration in accuracy across the 7 months' retention interval (**Figure 2**, lower right panel). Accuracy, in the Morn group, was reduced from 92 to 81% on average, but no losses were apparent in the Eve group (from 94 to 96%). A non-parametric $\chi 2$ (chi square) test was used to assess the proportion of participants in the two groups who experienced a loss of accuracy across the 7 months' retention interval. To this end, we designated the participants, in the two groups, that experienced losses in accuracy on the order of 5% or more [comparing the last training session (Train10) to the retention test 7 months' post-training]. The results showed that, in the Morn group, 7/14 participants showed a loss in accuracy; a proportion not statistically different from a 50:50 chance of either gaining and retaining or showing losses in accuracy [$\chi 2(1) = 0.00$, $p = 1.0$]. The proportion of participants showing such losses in accuracy during the retention interval, in the Eve group, was only 3/14; a proportion significantly different from a 50:50 chance of either improving or retaining the gains to showing losses [$\chi 2(1) = 4.57$, $p < 0.05$]. A direct comparison of the proportion of participants showing losses in accuracy (above 5%) over the 7 months' retention interval showed a significant advantage for the Eve group [$\chi 2(1) = 4.57$, $p < 0.05$].

The between-group differences, in sequence duration and accuracy, at each time-point assessed using *post hoc t*-tests and Mann–Whitney *U*-tests comparisons are shown in **Table 2**.

Altogether, the results showed that by 7 months after the end of the intervention, there was a substantial forgetting of the skill, reflected in both sequence duration and accuracy, in the Morn group; however, the losses in speed were somewhat smaller in the Eve trained group, while accuracy was well maintained across the 7 months' interval.

Transfer

To test the generalizability of the acquired knowledge, we compared the performance of the trained sequence to the performance of a newly introduced, untrained sequence, at the end of the intervention (Train10). The mean duration of completing a correct trained (Lt_T) and an untrained sequence (Lt_U) composed of the same component movements ordered in reverse to their order in the Lt_T sequence are presented in **Figures 3A,B**. There was a significant advantage for the Lt_T in both groups (Eve and Morn) in terms of mean sequence duration [$F(1,27) = 62.80$, $p < 0.001$, $\eta^2 = 0.7$], with no significant sequence × group interaction [$F(1,27) = 1.26$, $p = 0.272$, $\eta^2 = 0.04$] or group [$F(1,27) = 0.75$, $p = 0.4$, $\eta^2 = 0.03$] effect. For accuracy, a non-parametric Friedman test of differences showed that in both groups, there was a significant difference in favor of the trained sequence [$\chi 2(1) = 4.57$, $p < 0.05$, $\chi 2(1) = 7.14$, $p < 0.005$; Eve and Morn, respectively]. Thus, in both groups, full transfer (generalization) of the acquired gains to the untrained condition did not occur.

We directly compared sequence duration for the trained sequence to the untrained sequence at 1 and 7 months' post-training in the two groups, with time-point and sequence as within-subject's effects. A mixed Rm-GLM analysis showed that there was a significant time-point effect [$F(1,26) = 10.09$, $p < 0.005$, $\eta^2 = 0.28$] with no significant group × time-point interaction [$F(1,26) = 2.28$, $p = 0.14$, $\eta^2 = 0.08$] or group effect [$F(1,26) = 2.06$, $p = 0.16$, $\eta^2 = 0.07$]. There was a significant sequence effect [$F(1,26) = 35.37$, $p < 0.001$, $\eta^2 = 0.58$] but no group × sequence interaction [$F(1,26) = 0.04$, $p = 0.85$, $\eta^2 = 0.01$].

A non-parametric comparison for accuracy showed that there was no significant difference between the two sequences in the Eve group [$\chi 2(1) = 1.33$, $p = 0.25$] or in the Morn group [$\chi 2(1) = 3.77$, $p = 0.12$] at Train10. This was also the case at 7 months [$\chi 2(1) = 0.0$, $p = 1.0$; $\chi 2(1) = 0.29$, $p = 0.59$, Eve and Morn groups, respectively]. Thus, overall there was no significant difference between the two group in terms of transfer abilities.

Sleep and Chronotype

Independent samples Mann–Whitney *U*-test showed no significant differences between the participants of the Morn and Eve groups in respect to chronotype as assessed by MEQ scores [$U(28) = 73.0$, $p = 0.25$, $d' = 1.14$]. Parsing of the continuous MEQ scores into chronotype categories (Horne and Ostberg, 1976; Caci et al., 2009) showed that the participants of the study were mostly moderately morning chronotypes (**Table 3**).

In the PSQI questionnaires, both the Eve (mean of 5.3 ± 2.32) and Morn (4.69 ± 1.88) groups' scores were within the normal range (a score > 5 indicates poor sleep quality). Independent samples Mann–Whitney *U*-tests showed no significant differences between the two groups [$U(26) = 73.0$, $p = 0.55$, $d' = 0.29$]. In the ESS, assessing the level of daytime sleepiness, there were also no significant differences

TABLE 2 | Between-group comparisons of the mean sequence duration and accuracy at three time-points: Train10, Ret1m, and Ret7m (group mean ± SD).

	Train10	Ret1m	Ret7m
Sequence duration, seconds	Eve: 1.22 ± 0.36, Morn:1.24 ± 0.35 $t(27) = -0.15$, $p = 0.884$, $d' = 0.06$	Eve: 1.30 ± 0.36, Morn: 1.36 ± 0.36 $t(27) = -0.39$, $p = 0.7$, $d' = 0.17$	Eve: 1.63 ± 0.35, Morn: 1.99 ± 0.58 $t(26) = -1.98$, $p = 0.05$, $d' = 0.75$
Accuracy, %	Eve: 94.43 ± 10.98, Morn: 92.70 ± 9.55 $U(29) = 95.5$, $p = 0.66$, $d' = 0.17$	Eve: 93.95 ± 14.35, Morn: 93.79 ± 7.63 $U(29) = 79.5$, $p = 0.25$, $d' = 0.01$	Eve: 96.4 ± 3.08, Morn: 81.3 ± 24.05 $U(28) = 55.0$, $p = 0.049$, $d' = 0.88$

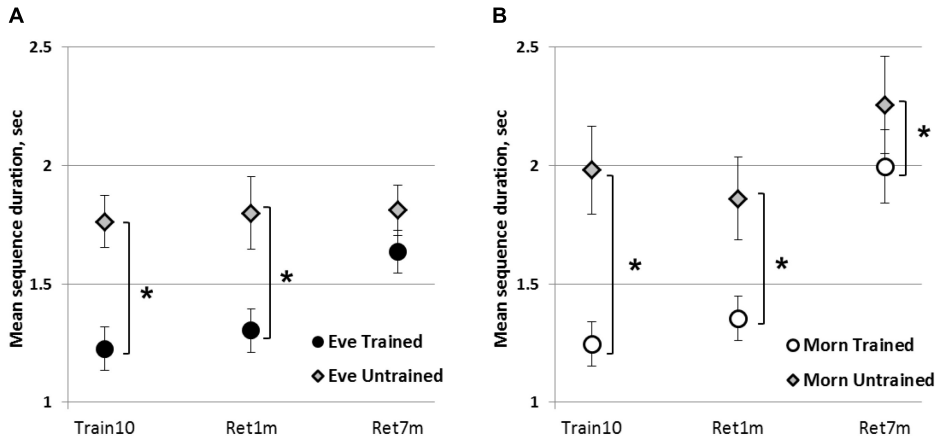


FIGURE 3 | Generalizability of the acquired knowledge to the performance of the untrained sequence. **(A)** Eve group. **(B)** Morn group. Each data point represents the averaged performance of four test blocks in terms of mean duration for correct sequences. Train10—end of the last training session; Ret1m—1 month after the termination of training; Ret7m—7 months after the termination of training; error bars—SEM, *—significant difference between the performance levels of the trained and the untrained sequence, $p \leq 0.05$.

TABLE 3 | Chronotypes based on the MEQ scores in the two groups (Eve and Morn).

Chronotype	Morn group	Eve group
MEQ mean ±SD	60 ±8.7	63.15 ±5.33
Definitely morning type	1	1
Moderately morning type	11	14
Intermediate type	2	–
Moderately evening type	–	–
Definitely evening type	–	–

The MEQ scores were parsed into categorical chronotypes using standard cut-off criteria (Horne and Ostberg, 1976; Caci et al., 2009).

TABLE 4 | Actigraphy parameters of the two groups (Morn, Eve), averaged across 7 days of recording during the first week of the training intervention.

Sleep parameter	Morn group	Eve group	Significance
Latency	5.29 ±4.93	10.82 ±11.42	*T*-test, ns
Efficiency	82.77 ±7.63	82.96 ±6.39	*U*-test, ns
TTB	409.91 ±43.53	428.52 ±51.71	*U*-test ns
TST	339.89 ±58.83	353.43 ±53.87	*T*-test, ns
WASO	64.63 ±28.44	64.23 ±24.38	*U*-test, ns
Nu_awake	14.86 ±2.73	12.56 ±31.86	*U*-test, ns
Avg_awake	4.37 ±1.71	5.23 ±1.72	*T*-test, ns

A two-tailed independent samples t-test or a Mann–Whitney U-test was used to compare between the groups; ns, not significant; $p > 0.05$.

between the two groups with no or only moderate levels of day time sleepiness (5 ± 3.08, 5.92 ± 4.92; the Eve and the Morn groups, respectively). The actigraphy-based sleep measures of the two groups were compared by averaging the sleep measures across seven nights, starting from the night of the first training session. The average scores of actigraphy-derived sleep parameters, including sleep efficiency, sleep onset latency, TST, total time in bed (TTB), wake after sleep onset (WASO), as well as the number of awakenings (Nu_awake) and average awakening time are shown in **Table 4**. There were no significant differences between the two groups (Eve, Morn) in any of these sleep parameters, suggesting that overall, the differences in the performance measures of the two groups may not be ascribed to differences in sleep habits, quality, and duration.

DISCUSSION

In the current study, we tested whether the time-of-day wherein training was afforded (morning or evening groups) during a multi-session motor training program affects the acquisition and mastering of a movement sequence or the long-term outcome of the training program in healthy morning-oriented elderly participants. The results of the current study show that the multi-session intervention program, a total of 10 training sessions, resulted in robust gains in performance (mean sequence tapping duration and accuracy) irrespective of whether participants engaged in training on the task at morning or at evening hours. Thus, although evening hours are considered a non-preferred, "off peak," circadian phase in most elderly, and in participants who were moderate to definite morning chronotype, the evening trained group was able to acquire experience-dependent gains in performance across the intervention period as robustly as their peers training at morning hours. Moreover, at 1 month after the end of intervention, both groups retained more than 90% of the gains attained by the end of the multi-session training program; but when re-tested at 7 months post-training, the Eve trained group was found to have a clear mnemonic advantage, showing significantly less attritions of the gains attained in training compared to the Morn trained group. Importantly, the mnemonic differences between the groups, in the current study, cannot be ascribed to differences in sleep parameters; no differences were found in the sleep parameters, subjective or objective, of the two

experimental groups. The sleep assessments showed that all participants were moderately morning chronotype, and had age-adequate sleep.

Altogether, the current results show that a motor skill was effectively acquired by morning-oriented older adults; irrespective of whether training was afforded at morning or at evening. However, the current results also show that successful acquisition of skill across a multi-session training program may not suffice to secure the long-term retention of the skill; there was a significant deterioration in the performance of the task after an interval of a few months (a time interval wherein the task was not executed). Nevertheless, the time-of-day in which the training was afforded was a critical factor in determining retention; retention was better in the evening group compared to the morning group in terms of retaining the gains in speed and accuracy attained in the performance of the task during the practice program.

Multi-session training was found beneficial for the generation of skill, and its retention, in the elderly (Spencer et al., 2007; Fraser et al., 2009; Nemeth et al., 2010; Romano et al., 2010; Wilson et al., 2012), with gains in performance surpassing the gains attained in a single session. Previous studies suggest that the consolidation of motor memories following motor sequence training may be either impaired or less readily mobilized (i.e., selectively engaged) in older adults (Spencer et al., 2007; Harand et al., 2012; Wilson et al., 2012; Korman et al., 2015).

In line with this notion, are the results of studies showing that older adults may need more practice in order to attain a level of mastery in a given task compared to younger trainees (e.g., Rodrigue et al., 2005). However, beyond the quantitative aspects of the training experience (such as the number of sessions or the number of task iterations afforded) other, in a sense, orthogonal, conditions may contribute to the successful learning process in the elderly. Specifically, the affordance of sleep was suggested as a critical modifier of the long-term outcomes of motor skill training, in particular, the ability to express delayed, offline, gains in motor performance in both young (Walker et al., 2003; Korman et al., 2007) and older (e.g., Korman et al., 2015) adults though not in children, i.e., before puberty (e.g., Ashtamker and Karni, 2013).

Both the magnitude of delayed gains and the time-course of the evolution of these gains were shown to be dependent on post-training sleep, either night-time or day-time in young (Walker et al., 2003; Korman et al., 2007; Doyon et al., 2009; Walker and Stickgold, 2010) and older adults (Korman et al., 2015; Mantua et al., 2016). For example, the study by Korman et al. (2015) suggested that while morning training by itself may not constitute a sufficient condition for the subsequent expression of delayed gains in the performance of a newly learned and practiced movement sequence, an interval of day-time sleep afforded shortly after the practice session can make possible the expression of robust delayed gains in the elderly. The authors proposed that the temporal proximity of the practice to a post-training sleep interval is critical, in the elderly; perhaps because opportunities for the interference of subsequent motor experiences in procedural memory consolidation processes (triggered by the practice experience) are reduced. It was conjectured that an increase in the susceptibility to interference by subsequent everyday experience may be one mechanism whereby stricter selectivity about what is or is not prioritized for maintenance in long-term memory is imposed in the elderly, an additional constraint on mnemonic processes (but not learning or the acquisition of within-session, online, gains *per se*) compared to younger adults (Korman et al., 2015).

The results of the current study are in line with the notion that motor training in temporal proximity to a sleep interval may be beneficial to the ability of older adults to retain "how to" motor knowledge. However, the advantage of training at evening hours was not apparent in the overall achievements of the evening trained participants (compared to their peers trained at morning) or the rate of improvement within and between sessions during the multi-session training protocol; the advantage of training at evening was apparent only after more than a month after the termination of practice, i.e., in terms of long-term retention.

Nevertheless, the very fact that in the current study older adults, with a morning orientation in terms of diurnal preferences, managed to acquire a motor skill when trained at evening, at a rate not different from the rate of learning in a morning trained group, raises the need for re-conceptualizing the notion that training at morning is universally the preferable time-of-day for elderly (Roenneberg et al., 2007; Fischer et al., 2017; Hood and Amir, 2017).

Only a few studies have directly addressed the effects of training interventions on the retention of skills, in the elderly. The focus of most studies was on the ability of elderly individual to express within-session, online, gains, or the gains attained by the end of multi-session training programs in comparison to the comparative gains attained by younger adults in similar training regimes (Durkin et al., 1995; Howard et al., 2004; Yan et al., 2010; Ehsani et al., 2015; Korman et al., 2015). A number of studies, however, addressed the retention of practice related gains in task performance in older adults in comparison to younger adults (Shea et al., 2006; Fraser et al., 2009; Nemeth et al., 2010; Wilson et al., 2012). While younger adults were able to retain training-related gains for long periods of time (across time intervals with no additional training), the passage of time (with no additional training afforded) was found to adversely affect the performance gains in older adults (Malone et al., 2016; Griffin et al., 2017). It was concluded that long intervals of no training may lead to forgetting and skill attrition, the loss of gains previously acquired, in the elderly (Malone et al., 2016; Griffin et al., 2017). The current results suggest that perhaps as a default, the motor system of older adults may treat motor "how to" knowledge that is not in continuous use as less prioritized for long-term retention. Given that the long-term maintenance of motor skill in motor cortex is an active and biologically/metabolically "expensive" process and that long-term memory-related plasticity is selectively maintained (Xu et al., 2009; Yu and Zuo, 2011; Yang and Gan, 2012), this may reflect a stricter selectivity in what is to be retained in long-term memory as one ages (see, e.g., Korman et al., 2015).

It is important to stress that the current results were obtained in the socially active and healthy older adult sample,

including only participants who did not take frequent day-time naps. However, frequent day-time napping is a common habit among elderly, affecting night-time sleep, cognitive, and physical fitness (Mantua and Spencer, 2017). This may limit the transfer of these findings to a potential clinical setting. Additional factors, such as preventative health medications and some health challenges common to elderly adults that may be related to circadian rhythms (e.g., fatigue, decrements in executive functioning, low physical and social activity) and thus, interfere with the training outcomes, or capacity to engage, should be addressed in the future.

The participants were not asked during the follow-up about their experience with other similar tasks, because finger-tapping tasks are omnipresent, such as in computer and cell phone use. Although the participants were instructed not to practice the experimental task between sessions, the absence of knowledge about the extent of similar experiences between experimental sessions is an additional limitation of the present study. Nevertheless, we note that the participants did not practice the specific sequence of finger movements used in the study task, were not proficient in typing (did not use all fingers for computer work), and had no experimental response box at their disposal (the spatial arrangement of the keys in response box is different from the computer keyboard).

Additional limitation of the current study is that both the training and the assessments were performed at specific time-of-day, only during morning (8–10:30 a.m.) or evening (6:30–9:00 p.m.) hours, for each group. Moreover, the motor task used in this study was a short sequence of fine finger movements; the current results may not generalize to other types of motor training. Responsiveness for physical training and assessment was shown in several recent studies to be dependent on the time of day, suggesting that time-of-day is an important modifier of exercise capacity and associated metabolic pathways (Brito et al., 2019; Ezagouri et al., 2019; Youngstedt et al., 2019).

Thus, our results can serve as a proof-of-concept justifying future studies that will explore conditions or interventions in older adults in which the potential for motor learning, as well as for mastering other types of cognitive tasks, can be harnessed and perhaps facilitated. Specifically, future studies should directly address the possibility, previously raised by Korman et al. (2015), that motor learning and consolidation can be enhanced if elderly participants are restricted in experiencing potentially interfering tasks before the first post-learning sleep interval. The current results also raise the possibility that boost sessions may be beneficial in older adults to better maintain skill in tasks wherein everyday experience is limited or separated by long time intervals. There is evidence underscoring the importance of continuous practice on motor performance in aging; for example, boost sessions were found beneficial for professional pianists to maintain their performance skills, including motor speed, into advanced age (Krampe and Ericsson, 1996) and skilled typists were able to maintain their speed of typing up to 72 years of age, if some practice was afforded every other day (Salthouse, 1996).

We propose that the time-of-day in which practice is afforded, is an important parameter to be considered when it comes to motor skill learning and maintenance in the elderly population; this consideration may also apply in the context of rehabilitation of movement routine (e.g., Korman et al., 2018). On the one hand, morning training may be preferable because it meets the circadian preference of older adults (Roenneberg et al., 2007; Fischer et al., 2017; Hood and Amir, 2017). However, morning training may be less beneficial to the mastering of motor skills, as a long time interval separates the practice experience from the interval of sleep at night; evening training with the proximity to a sleep period may promote better memory consolidation and minimize unspecific interference from everyday motor activity (Korman et al., 2015). These considerations can be taken into account by practitioners in structuring home/rehabilitation programs for older adults, making the scheduling of the intervention sessions to morning hours less restrictive. The time-of-day of practice, specifically in the rehabilitation of function in elderly individuals who are less well-entrained to the environmental cues of the diurnal rhythms (Camargo-Sanchez et al., 2015; Duffy et al., 2015; Mattis and Sehgal, 2016; Cornelissen and Otsuka, 2017; Logan and McClung, 2019) may need to be addressed as a critical factor in the studies of long-term effects of intervention protocols.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the University of Haifa, Human Experimentation Ethics Committee of the Natural Sciences Faculty. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors read and approved the final version of the manuscript. CG, AK, and MK conceived and designed the experiments. CG collected the data. CG and EG analyzed the raw data. CG, RM-H, AK, and MK made the statistical analysis and interpretation of the data. CG, EG, AK, and MK wrote the article.

FUNDING

The E.J. Safra Brain Research Center for the Study of Learning Disabilities at the University of Haifa, ERA-NET NEURON JTC2011: COGSTROKE, Chief Scientist's Office, Ministry of Health, Israel, and Joy Ventures are gratefully acknowledged for partially funding this project.

REFERENCES

Albouy, G., King, B. R., Maquet, P., and Doyon, J. (2013). Hippocampus and striatum: dynamics and interaction during acquisition and sleep-related motor sequence memory consolidation. *Hippocampus* 23, 985–1004. doi: 10.1002/hipo.22183

Ashtamker, L., and Karni, A. (2013). Motor memory in childhood: early expression of consolidation phase gains. *Neurobiol. Learn. Mem.* 106, 26–30. doi: 10.1016/j.nlm.2013.07.003

Backhaus, W., Braass, H., Renné, T., Gerloff, C., and Hummel, F. C. (2016). Motor performance is not enhanced by daytime naps in older adults. *Front. Aging Neurosci.* 8:125. doi: 10.3389/fnagi.2016.00125

Brito, L. C., Pecanha, T., Fecchio, R. Y., Rezende, R. A., Sousa, P., and Da Silva-Júnior, N. (2019). Morning versus evening aerobic training effects on blood pressure in treated hypertension. *Med. Sci. Sports Exerc.* 51, 653–662. doi: 10.1249/MSS.0000000000001852

Brown, R. M., Robertson, E. M., and Press, D. Z. (2009). Sequence skill acquisition and off-line learning in normal aging. *PLoS One* 4:e6683. doi: 10.1371/journal.pone.0006683

Buysse, D. J., Reynolds, C. F., Monk, T. H., Berman, S. R., and Kupfer, D. J. (1989). The Pittsburgh sleep quality index: a new instrument for psychiatric practice and research. *Psychiatry Res.* 28, 193–213. doi: 10.1016/0165-1781(89)90047-4

Caci, H., Deschaux, O., Adan, A., and Natale, V. (2009). Comparing three morningness scales: age and gender effects, structure and cut-off criteria. *Sleep Med.* 10, 240–245. doi: 10.1016/j.sleep.2008.01.007

Camargo-Sanchez, A., Niño, C. L., Sánchez, L., Echeverri, S., Gutiérrez, D. P., Duque, A. F., et al. (2015). Theory of inpatient circadian care (TICC): a proposal for a middle-range theory. *Open Nurs. J.* 9, 1–9. doi: 10.2174/1874434601509010001

Cornelissen, G., and Otsuka, K. (2017). Chronobiology of aging: a mini-review. *Gerontology* 63, 118–128. doi: 10.1159/000450945

Doyon, J., Korman, M., Morin, A., Dostie, V., Tahar, A. H., Benali, H., et al. (2009). Contribution of night and day sleep vs. simple passage of time to the consolidation of motor sequence and visuomotor adaptation learning. *Exp. Brain Res.* 195, 15–26. doi: 10.1007/s00221-009-1748-y

Duffy, J. F., Zitting, K.-M., and Chinoy, E. D. (2015). Aging and circadian rhythms. *Sleep Med. Clin.* 10, 423–434. doi: 10.1016/j.jsmc.2015.08.002.Aging

Durkin, M., Prescott, L., Furchtgott, E., Cantor, J., and Powell, D. A. (1995). Performance but not acquisition is severely impaired in the elderly. *Arch. Gerontol. Geriatr.* 20, 167–183. doi: 10.1016/0167-4943(94)00594-w

Dzierzewski, J. M., Dautovich, N., and Ravyts, S. (2018). Sleep and cognition in older adults. *Sleep Med. Clin.* 13, 93–106. doi: 10.1016/j.jsmc.2017.09.009.Sleep

Ehsani, F., Abdollahi, I., Ali, M., Bandpei, M., Zahiri, N., and Jaberzadeh, S. (2015). Motor learning and movement performance: older versus younger adults. *Basic Clin. Neurosci.* 6, 231–238.

Ezagouri, S., Zwighaft, Z., Sobel, J., Baillieul, S., Doutreleau, S., Ladeuix, B., et al. (2019). Physiological and molecular dissection of daily variance in exercise capacity. *Cell Metab.* 30, 78–91. doi: 10.1016/j.cmet.2019.03.012

Fischer, D., Lombardi, D. A., Marucci-Wellman, H., and Roenneberg, T. (2017). Chronotypes in the US – influence of age and sex. *PLoS One* 12:e0178782. doi: 10.1371/journal.pone.0178782

Fraser, S. A., Li, K. Z. H., and Penhune, V. B. (2009). A comparison of motor skill learning and retention in younger and older adults. *Exp. Brain Res.* 195, 419–427. doi: 10.1007/s00221-009-1806-5

Friedman, J., and Korman, M. (2012). Kinematic strategies underlying improvement in the acquisition of a sequential finger task with self-generated vs. cued repetition training. *PLoS One* 7:e52063. doi: 10.1371/journal.pone.0052063

Friedman, J., and Korman, M. (2016). Offline optimization of the relative timing of movements in a sequence is blocked by retroactive behavioral interference. *Front. Hum. Neurosci.* 10:623. doi: 10.3389/fnhum.2016.00623

Goldman, S. E., Ancoli-Israel, S., Boudreau, R., Cauley, J. A., Hall, M., Stone, K. L., et al. (2008). Sleep problems and associated daytime fatigue in community-dwelling older individuals. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* 63, 1069–1075. doi: 10.1093/gerona/63.10.1069

Griffin, J. W., John, S., Adams, J. W., Bussell, C. A., Saurman, J. L., and Gavett, B. E. (2017). The effects of age on the learning and forgetting of primacy, middle, and recency components of a multi- trial word list. *J. Clin. Exp. Neuropsy.* 39, 900–912. doi: 10.1080/13803395.2017.1278746

Harand, C., Bertran, F., Doidy, F., Guénolé, F., Desgranges, B., Eustache, F., et al. (2012). How aging affects sleep-dependent memory consolidation? *Front. Neurol.* 3:8. doi: 10.3389/fneur.2012.00008

Hikosaka, O., Nakamura, K., Sakai, K., and Nakahara, H. (2002). Central mechanisms of motor skill learning. *Curr. Opin. Neurobiol.* 12, 217–222. doi: 10.1016/s0959-4388(02)00307-0

Hood, S., and Amir, S. (2017). The aging clock: circadian rhythms and later life Find the latest version: the aging clock: circadian rhythms and later life. *J. Clin. Invest.* 127, 437–446. doi: 10.1172/JCI90328

Horne, J. A., and Ostberg, O. (1976). A self assessment questionnaire to determine morningness eveningness in human circadian rhythms. *Int. J. Chronobiol.* 4, 97–110.

Howard, D. V., Howard, J. H. J., Japikse, K., Diyanni, C., Thompson, A., and Somberg, R. (2004). Implicit sequence learning: effects of level of structure, adult age, and extended practice. *Psychol. Aging* 19, 79–92. doi: 10.1037/0882-7974.19.1.79

Huang, Y.-L., Liu, R.-Y., Wang, Q.-S., Van Someren, E. J. W., Xu, H., and Zhou, J.-N. (2002). Age-associated difference in circadian sleep-wake and rest-activity rhythms. *Physiol. Behav.* 76, 597–603. doi: 10.1016/s0031-9384(02)00733-3

Johns, M. W. (1991). A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep* 14, 540–545. doi: 10.1093/sleep/14.6.540

Karni, A., Meyer, G., Jezzard, P., Adams, M. M., Turner, R., and Ungerleider, L. G. (1995). Functional MRI evidence for adult motor cortex plasticity during motor skill learning. *Nature* 377, 155–158. doi: 10.1038/377155a0

Karni, A., Meyer, G., Rey-Hipolito, C., Jezzard, P., Adams, M. M., Turner, R., et al. (1998). The acquisition of skilled motor performance: fast and slow experience-driven changes in primary motor cortex. *PNAS* 95, 861–868. doi: 10.1073/pnas.95.3.861

Karni, A., and Sagi, D. (1993). The time course of learning a visual skill. *Nature* 365, 250–252. doi: 10.1038/365250a0

King, B. R., Fogel, S. M., Albouy, G., and Doyon, J. (2013). Neural correlates of the age-related changes in motor sequence learning and motor adaptation in older adults. *Front. Hum. Neurosci.* 7:142. doi: 10.3389/fnhum.2013.00142

Korman, M., Dagan, Y., and Karni, A. (2015). Nap it or leave it in the elderly: a nap after practice relaxes age-related limitations in procedural memory consolidation. *Neurosci. Lett.* 606, 173–176. doi: 10.1016/j.neulet.2015.08.051

Korman, M., Doyon, J., Doljansky, J., Carrier, J., Dagan, Y., and Karni, A. (2007). Daytime sleep condenses the time course of motor memory consolidation. *Nat. Neurosci.* 10, 1206–1213. doi: 10.1038/nn1959

Korman, M., Raz, N., Flash, T., and Karni, A. (2003). Multiple shifts in the representation of a motor sequence during the acquisition of skilled performance. *PNAS* 100, 12492–12497. doi: 10.1073/pnas.2035019100

Korman, M., Shaklai, S., Cisamariu, K., Gal, C., Maaravi-Hesseg, R., Levy, I., et al. (2018). Atypical within-session motor procedural learning after traumatic brain injury but well-preserved between-session procedural memory consolidation. *Front. Hum. Neurosci.* 12:10. doi: 10.3389/fnhum.2018.00010

Krampe, R. T., and Ericsson, A. K. (1996). Maintaining excellence: deliberate practice and elite performance in young and older pianists. *J. Exp. Psychol. Gen.* 125, 331–359. doi: 10.1037/0096-3445.125.4.331

Logan, R. W., and McClung, C. A. (2019). Rhythms of life: circadian disruption and brain disorders across the lifespan. *Nat. Rev. Neurosci.* 20, 49–65. doi: 10.1038/s41583-018-0088-y

Malone, L. A., Bastian, A. J., and Maryland, B. (2016). Age-related forgetting in locomotor adaptation. *Neurobiol. Learn. Mem.* 2715, 1–6. doi: 10.1016/j.nlm.2015.11.003.Age-related

Mander, B. A., Winer, J. R., and Walker, M. P. (2017). Sleep and human aging hhs public access. *Neuron* 94, 19–36. doi: 10.1016/j.neuron.2017.02.004

Mantua, J., Baran, B., and Spencer, R. M. C. (2016). Sleep benefits consolidation of visuo-motor adaptation learning in older adults. *Exp. Brain Res.* 234, 587–595. doi: 10.1007/s00221-015-4490-7

Mantua, J., and Spencer, R. M. C. (2017). Exploring the nap paradox: are mid-day sleep bouts a friend or foe? *Sleep Med.* 37, 88–97. doi: 10.1016/j.sleep.2017.01.019

Mattis, J., and Sehgal, A. (2016). Circadian rhythms, sleep, and disorders of aging. *Trends Endocrinol. Metab.* 27, 192–203. doi: 10.1016/j.tem.2016.02.003

May, C. P., Hasher, L., and Foong, N. (2005). Implicit memory, age, and time of day. *Psychol. Sci.* 16, 96–100. doi: 10.1111/j.0956-7976.2005.00788.x

Nemeth, D., Janacsek, K., Londe, Z., Ullman, M. T., Howard, D. V., and Howard, J. H. J. (2010). Sleep has no critical role in implicit motor sequence learning in young and old adults. *Exp Brain Res.* 201, 351–358. doi: 10.1007/s00221-009-2024-x

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* 9, 97–113. doi: 10.1016/0028-3932(71)90067-4

Phillips, B., and Ancoli-Israel, S. (2001). Sleep disorders in the elderly. *Sleep Med.* 2, 99–114. doi: 10.1016/S1389-9457(00)00083-6

Rieckmann, A., and Bäckman, L. (2009). Implicit learning in aging: extant patterns and new directions. *Neuropsychol. Rev.* 19, 490–503. doi: 10.1007/s11065-009-9117-y

Rodrigue, K. M., Kennedy, K. M., and Raz, N. (2005). Aging and longitudinal change in perceptual-motor skill acquisition in healthy adults. *J. Gerontol.* 60, 174–181.

Roenneberg, T., Kuehnle, T., Juda, M., Kantermann, T., Allebrandt, K., Gordijn, M., et al. (2007). Epidemiology of the human circadian clock. *Sleep Med. Rev.* 11, 429–438. doi: 10.1016/j.smrv.2007.07.005

Romano, J. C., Howard, J. H. J., and Howard, D. V. (2010). One-year retention of general and sequence-specific skills in a probabilistic, serial reaction time task. *Memory* 18, 427–441. doi: 10.1080/09658211003742680

Salthouse, T. A. (1996). The processing-speed theory of adult age differences in cognition. *Psychol. Rev.* 103, 403–428. doi: 10.1037//0033-295x.103.3.403

Schmidt, C., Collette, F., Reichert, C. F., Micheline, M., Vandewalle, G., Peigneux, P., et al. (2015). Pushing the limits: chronotype and time of day modulate working memory-dependent cerebral activity. *Front. Neurol.* 6:199. doi: 10.3389/fneur.2015.00199

Shea, C. H., Park, J.-H., and Braden, H. W. (2006). Age-related effects in sequential motor learning. *Phys. Ther.* 86, 478–488.

Sosnik, R., Hauptmann, B., Karni, A., and Flash, T. (2004). When practice leads to co-articulation: the evolution of geometrically defined movement primitives. *Exp. Brain Res.* 156, 422–438. doi: 10.1007/s00221-003-1799-4

Spencer, R. M. C., Gouw, A. M., and Ivry, R. B. (2007). Age-related decline of sleep-dependent consolidation. *Learn. Mem.* 14, 480–484. doi: 10.1101/lm.569407

Steer, R. A., Beck, A. T., Riskind, J. H., and Brown, G. (1986). Differentiation of depressive disorders from generalized anxiety by the Beck Depression Inventory. *J. Clin. Psychol.* 42, 475–478. doi: 10.1002/1097-4679(198605)42:3<475::aid-jclp2270420311>3.0.co;2-d

Terpening, Z., Naismith, S., Melehan, K., Gittins, C., Bolitho, S., and Lewis, S. J. G. (2013). The contribution of nocturnal sleep to the consolidation of motor skill learning in healthy ageing and Parkinson's disease. *J. Sleep Res.* 22, 398–405. doi: 10.1111/jsr.12028

Tsokanaki, P., Moraitou, D., and Papantoniou, G. (2016). The combined effect of sleep and time of day on emotion decoding from dynamic visual cues in older adults. *Neuropsychiatr. Dis. Treat.* 12, 2283–2291. doi: 10.2147/NDT

Veneman, C. E., Gordon-Salant, S., Matthews, L. J., and Dubno, J. R. (2013). Age and measurement time-of-day effects on speech recognition in noise. *Ear Hear.* 34, 288–299. doi: 10.1097/AUD.0b013e31826d0b81

Voelcker-Rehage, C. (2008). Motor-skill learning in older adults-a review of studies on age-related differences. *Eur. Rev. Aging Phys. Act.* 5, 5–16. doi: 10.1007/s11556-008-0030-9

Walker, M. P., Brakefield, T., Hobson, J. A., and Stickgold, R. (2003). Dissociable stages of human memory consolidation and reconsolidation. *Nature* 425, 8–12. doi: 10.1038/nature01951.1

Walker, M. P., and Stickgold, R. (2010). Overnight alchemy: sleep-dependent memory evolution. *Natl. Rev. Neurosci.* 11, 1–5. doi: 10.1038/nrn2762-c1. Overnight

Wilson, J. K., Baran, B., Pace-Schott, E. F., Ivry, R. B., and Spencer, R. M. C. (2012). Sleep modulates word-pair learning but not motor sequence learning in healthy older adults. *Neurobiol. Aging* 33, 991–1000. doi: 10.1016/j.neurobiolaging.2011.06.029

Xu, T., Yu, X., Perlik, A. J., Tobin, W. F., Zweig, J. A., Jones, T., et al. (2009). Enduring motor memories. *Nature* 462, 915–919. doi: 10.1038/nature08389. Rapid

Yan, J. H., Abernethy, B., and Li, X. (2010). The effects of ageing and cognitive impairment on on-line and off-line motor learning. *Appl. Cogn. Psychol.* 24, 200–212. doi: 10.1002/acp.1551

Yan, L. L., Daviglus, M. L., Liu, K., Pirzada, A., Garside, D. B., Schiffer, L., et al. (2004). BMI and health-related quality of life in adults 65 years and older. *Obes. Res.* 12, 69–76. doi: 10.1038/oby.2004.10

Yang, G., and Gan, W.-B. (2012). Sleep contributes to dendritic spine formation and elimination in the developing mouse somatosensory cortex. *Dev. Neurobiol.* 72, 1391–1398. doi: 10.1038/jid.2014.371

Youngstedt, S. D., Elliott, J. A., and Kripke, D. F. (2019). Human circadian phase-response curves for exercise. *J. Physiol.* 597, 2253–2268. doi: 10.1113/JP276943

Yu, X., and Zuo, Y. (2011). Spine plasticity in the motor cortex. *Curr. Biol.* 21, 169–174. doi: 10.1016/j.micinf.2011.07.011.Innate

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Physiology

ORIGINAL RESEARCH
published: 18 February 2020
doi: 10.3389/fphys.2020.00045

The Effects of Calorie Restriction and Exercise on Age-Related Alterations in Corpus Cavernosum

Caglar Macit[1], Unsal V. Ustundag[2], Ozge C. Dagdeviren[3], Guldem Mercanoglu[4] and Goksel Sener[5]*

[1] Department of Pharmacology, School of Pharmacy, Istanbul Medipol University, Istanbul, Turkey, [2] Department of Biochemistry, Faculty of Medicine, Istanbul Medipol University, Istanbul, Turkey, [3] Faculty of Medicine, Department of Biochemistry, Adnan Menderes University, Aydın, Turkey, [4] Faculty of Pharmacy, Department of Pharmacology, University of Health Sciences, Istanbul, Turkey, [5] Faculty of Pharmacy, Department of Pharmacology, Marmara University, Istanbul, Turkey

OPEN ACCESS

Edited by:
Mario Bernardo-Filho, Rio de Janeiro State University, Brazil

Reviewed by:
Owen Llewellyn Woodman, Monash University, Australia
Edson Antunes, Campinas State University, Brazil

****Correspondence:***
Guldem Mercanoglu
guldemiko@gmail.com

Specialty section:
This article was submitted to Integrative Physiology, a section of the journal Frontiers in Physiology

Received: *11 July 2019*
Accepted: *21 January 2020*
Published: *18 February 2020*

Citation:
Macit C, Ustundag UV, Dagdeviren OC, Mercanoglu G and Sener G (2020) The Effects of Calorie Restriction and Exercise on Age-Related Alterations in Corpus Cavernosum. Front. Physiol. 11:45. doi: 10.3389/fphys.2020.00045

Background: Aging is an important risk factor for erectile dysfunction (ED). Both calorie restriction (CR) and physical exercise (PE) have been established as a non-medical method for the improvement of detrimental changes in aging. It is well documented that both CR and PE influence on sympathetic and parasympathetic systems; however, there are few studies on non-adrenergic non-cholinergic pathways. This study aims to investigate the NO-mediated mechanisms of CR and PE on corpus cavernosum in aged rats.

Materials and Methods: 3 and 15 month-old rats were divided into five experimental groups: young rats fed ad libitum (Y-C), aged rats fed ad libitum (O-S), aged rats with CR (O-CR), aged rats with PE (O-PE), and aged rats with CR and PE (O-CR-PE). CR was applied to animals as a 40% reduction of daily food intake for 6 weeks. PE was moderate swimming at 30 min at 3 days/week. The effects of CR and PE were evaluated by histologic, biologic, and in-vitro tissue bath studies.

Results: The outcomes in CR and PE groups (characterized by decreased nitrosative damage together with increased antioxidant capacity) were improved in comparison to the O-S. Apoptotic biomarkers were also lower and both endothelial and smooth muscle cell' functions were preserved too. There was no statistical difference between apoptosis, antioxidant capacity, and nitrosative damage parameters. Contractile responses to phenylephrine and relaxation responses to carbachol were: O-CR > O-PE > O-CR-PE. In these groups, NOS protein levels determined by western-blot were: eNOS: O-CR = O-CR + PE > O-PE; iNOS: O-CR = O-PE > O-CR-PE; nNOS: O-PE > O-CR-PE > O-CR.

Conclusion: In our study, both CR and PE prevented age-related changes in the corpus cavernosum of rats. Reducing nitrosative damage in the neurovascular structure was the

main mechanism. CR and exercise restored the endothelial and smooth muscle cells in corpus cavernosum by decreasing apoptosis. The mechanism of enhancing functional response in corpus cavernosum with CR was the improvement of endothelial function via eNOS activation however it involves increases in the NO-cGMP signaling pathway by an endothelium-independent mechanism with PE. This might be a direct stimulation of smooth muscle cells by NO, which released from the cavernous nerve endings via nNOS activation.

Keywords: erectil dysfunction, calorie restriction, physical exercise, aging, nitric oxide

INTRODUCTION

Erectile dysfunction (ED) is one of the most common disorders in males (Kaya et al., 2017). The prevalence of ED in the general population ranges from 30 to 65% in men aged 40–80 years (Campbell et al., 2019). The Massachusetts Male Aging study emphasized the close association between aging and ED by showing an almost 1.5 times increased prevalence of ED after the age of 70 years (Feldman et al., 1994).

The development of a penile erection can simply be summarized in two sequential steps: (1) the transportation of blood into the cavernosal sinusoids resulting in the enlargement and rigidity of the penis, and (2) the reduction of venous outflow via veno-occlusion to maintain the enlargement and rigidity of the penis (Ferrini et al., 2017). However, this two-step process depends on a complex balance and coordination of neurogenic, vascular, and humoral events (Jang et al., 2017), such as the parasympathetic, sympathetic, and nitrergic nerves, neurotransmitters, blood vessels, and cavernous muscles (Claudino et al., 2004). Nitric oxide (NO) plays a key role in this coordination, because the increase in the blood flow via dilation of arterial vessels as well as the increase in the size of corporal sinusoids via the relaxation of smooth muscles is regulated by the NO (Ferrini et al., 2017). Both the nitrergic fibers and vascular endothelial cells supply the corpus cavernosum with NO (Claudino et al., 2004). Decreased nerve and endothelium-mediated corpus cavernosum relaxation, reductions in smooth muscle cell content, and pathological remodeling of the pudendal artery were shown in age-related ED. Moreover, they were attributed largely to increased oxidative stress and endothelial dysfunction (Johnson et al., 2011). However, the underlying molecular mechanisms have not been fully identified (Johnson et al., 2011). Furthermore, conflicting results have been reported, especially in relation to NO-mediated mechanisms (Ferrini et al., 2001; Gonzalez and Rajfer, 2004; Johnson et al., 2011).

A causal connection between oxidative-stress, aging and age-related pathologies (Davalli et al., 2016), as well as the beneficial effects of calorie restriction (CR) and regular physical exercise (PE) on oxidative stress in age-related pathologies is known (Testa et al., 2014; Simioni et al., 2018). Again, the beneficial effects of CR and PE on erectile function have also been shown separately (Ferrini et al., 2001; Claudino et al., 2004; Gonzalez and Rajfer, 2004; Johnson et al., 2011; Testa et al., 2014; Davalli et al., 2016; Souza et al., 2017; Simioni et al., 2018). This study aims to investigate the NO-mediated mechanisms of CR and PE on the corpus cavernosum in aged rats.

MATERIALS AND METHODS

Animals and Study Protocol

A sample of 3-month-old young and 15-month-old aged male Wistar albino rats were divided into five groups of 15 each: young rats fed *ad libitum* (Y-C); aged rats fed *ad libitum* (O-S); aged rats with CR (O-CR); aged rats that were exercise trained (O-PE); and aged rats with CR and that were exercise trained (O-CR-PE).

The *ad libitum* groups of animals were fed with standard chow pellets (composed of 21% soybean protein, 15% sucrose, 43.7% dextrin, 10% corn oil, 0.15% a-methionine, 0.2% choline chloride, 5% salt mix, 2% vitamin mix, and 3% Solka-Floc fiber) and had free access to food and water. CR was applied to animals as a 40% reduction of daily food intake for 6 weeks (Whidden et al., 2011). Exercise training was moderate swimming for 30 min 3 days/week for 6 weeks (Leite et al., 2013). At the end of the study period, animals were then decapitated and corpus cavernosum tissues were excised. In half of the rats in each group, the corpus cavernosum was immediately prepared for the *in vitro* contractility studies. In the other half of the rats in each group, samples were stored at −70°C for the measurement of biochemical parameters. Extra tissue samples were fixed in 10% buffered formalin solution and prepared for routine paraffin embedding for histological analysis.

Animals were housed individually in polycarbonate cages with wood chip bedding and maintained in a temperature-controlled room (50–60% of humidity at 24°C) with a 12-h light-dark cycle (lights on at AM 7:00 and off at PM 7:00).

All experimental procedures were done in accordance with the Guide for the Care and the Use of Laboratory Animals published by the US National Institutes of Health. The local animal ethic committee approval was obtained for all experimental procedures.

Tissue Antioxidant Capacity and Oxidant Status

While superoxide dismutase (SOD) and glutathione (GSH) levels were measured for antioxidant activity; malondialdehyde (MDA) as a marker of lipid oxidation and 8-hydroxyguenosine (8-OHdG) as an indicator of oxidative DNA damage were measured in tissue samples homogenized in ice-cold 10% trichloroacetic

acid (TCA). MDA was determined spectropohometrically by measuring the presence of thiobarbituric acid-reactive substances (Okhawa et al., 1979). SOD enzyme activity determination was done based on the production of H_2O_2, from xanthine by xanthine oxidase, and the reduction of nitro blue tetrazolium as previously described (Aladag et al., 2003). 8-OHdG was measured by ELISA (OxiSelect Oxidative DNA Damage Elisa Kit, Cell Biolabs) according to the manufacturer's instruction. The DNA isolation from the samples was performed by a commercial kit, according to the manufacturer's instruction (PureLink® Genomic DNA Mini Kit, Life Technology).

Tissue Nitric Oxide (NO), Peroxynitrite (ONO_2^-), and Cyclic Guanylate Cyclase (cGMP) Levels

Nitric oxide was measured in the tissue supernatants as nitrite/nitrate (NOx) concentration by spectrophotometry (Roche, United States). Briefly, tissues were harvested, washed in cold 5% TCA, suspended with an ice-cold assay buffer (ready to use), homogenized on ice, and centrifuged for 2–5 min at 4°C. The supernatant was collected in a clean tube, deproteinized, and neutralized with ice cold 4 M PCA and 2 M KOH, respectively. Samples were added to wells, nitrate reductase was added to convert nitrate to nitrite, and colored with Griess Reagent. The intensity of the color was measured against the standard at 550 nm by spectrophotometer.

To assess the activation of either NO/ONO_2^- or NO/cGMP pathway, ONO_2^- and cGMP levels were measured. For the cGMP measurement, frozen samples were homogenized with 5% TCA and centrifuged. The Supernatant was extracted with water-saturated ether and dried. Reconstituted samples were measured by using an enzyme-linked immunoassay (ELISA) using a commercial kit (ADI-900-013, Enzo Life). ONO_2^- measurement was performed by enzyme-linked immunoassay (ELISA) using a commercial kit (HycultBiotech, PA, United States) according to the manufacturer's instruction. Accordingly, tissue homogenates were incubated with a biotynlated tracer antibody and transferred to a microplate precoated with nitrated-HSA and allowed to incubate. After incubation, the plate was washed to remove excess traces of antibody. Streptavidin peroxidase followed by TBM was added to the plate to facilitate color development. The reaction was stopped using oxalic acid and color intensity was measured against the standard at 450 nm by spectrophotometer. Tissue protein levels were determined using the method proposed by Folin-Lowry (Lowry et al., 1951).

Apoptotic/Anti-apoptotic Biomarkers

Western blot was used to detect the change of apoptosis-related proteins. Accordingly, the lysis of Adipose-Derived Stem Cells (ADSCs) exploited the Laemmli Sample Buffer (Bio-Rad). After centrifugation at 4°C, protein components were attained and determined by using a BCATM Protein Assay Kit (Thermo Scientific). Proteins were loaded in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Obtaining discrete proteins were then transferred to nitrocellulose membranes and incubated overnight at 4°C with primary antibodies of Bcl-xL (sc-892, Santa Cruz), Caspase-3 (sc-7148, Santa Cruz), Bax (sc-7480, Bax Antibody (B-9), Santa Cruz), and p53 (sc-6243, Santa Cruz) over 1 h at room temperature with corresponding secondary antibodies. β-actin was used as a reference protein.

Total Nitric Oxide Synthase (NOS) Activity and NOS Isoforms

Tissue NOS activity was detected by ELISA (ENOS-100, EnzyChrom™ Nitric Oxide Synthase Assay Kit, Bioassay Systems) according to the manufacturer's instruction.

The NOS isoforms, nNOS (Sc-5302, NOS1 (A-11) Antibody, Santa Cruz), iNOS (Sc-7271, NOS2 Antibody (C-11), and eNOS (Sc-654 NOS3 Antibody (C-20), Santa Cruz), were determined by Western blotting in tissue homogenates using specific antibodies as described above.

Histologic Examination

A mid-portion of each penile segment was harvested and immediately fixed in 10% paraformaldehyde for 24 h at 4°C. Specimens were cut into 5-μm sections, stained with hematoxylin-eosin, and evaluated under the light microscope (Olympus BX51).

Corpus Cavernosum Function

Contractile responses in organ bath study were used to measure the function of corpus cavernosum. The penis was removed and the excised corpus cavernosum from the rats was dissected free of the tunica albuginea and cut into 2 × 2 × 15 mm strips. Corporeal strips were bathed in Krebs–Henseleit solution (118,14 mM NaCl, 4,7 mM KCl, 2,5 mM $CaCl_2$, 25 mM $NaHCO_3$, 1,2 mM MgSO4, 1,2 mM KH_2PO_4, and 11,1 mM glucose) The strips were mounted in 10-mL organ baths containing Krebs solution at 37°C continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide (pH 7.4). The tissues were equilibrated for 45 min under a resting tension of 500 mg. Changes in the isometric force were recorded using a PowerLab 400 Data Acquisition System (Software Chart, version 4.2, AD Instruments, Colorado Springs, CO, United States).

To verify the contractile ability of the preparations, 118 mM KCl solution was added to the organ baths at the end of the equilibration period. The contractile responses of the corporeal strips to 10^{-9}–10^{-3} M phenylephrine were obtained cumulatively and expressed as the percentage of the maximal contraction induced by 118 mM KCl. After a 30-min washout period, corporeal tissues were contracted with a submaximal dose (10^{-5} M) of phenylephrine. The relaxation responses of the pre-contracted tissues were evaluated by adding increasing concentrations of carbachol (10^{-9}–10^{-3} M) (Paskaloglu et al., 2004). Potency (pEC_{50}) presented as a log of molar concentration to produce 50% of the maximal contractile response elicited by an agonist relative to KCl (118 mmol/L)-induced contraction.

Statistical Analysis

Statistical analyses were performed with SPSS 24.0 software program (Chicago, IL, United States). All variables were expressed as mean ± SEM. Differences between groups were

analyzed by ANOVA and *Post hoc* Bonferroni test and $p < 0.05$ was considered to be statistically significant.

RESULTS

Tissue Antioxidant Capacity and Oxidant Status

Both tissue SOD and GSH levels were low in the O-S compared to the Y-C ($p < 0.01$). Parallel with these findings tissue MDA and 8-OHdG levels were high in the O-S group too ($p < 0.01$).

Compared O-S, both CR, and PE trained groups were characterized with decreased oxidative damage (characterized by increased SOD and GSH) together with increased antioxidant capacity (characterized by decreased cavernosal MDA and 8-OHdG levels) ($p < 0.01$ for all comparisons). There was no statistically significant difference between the CR, PE, and CR + PE groups ($p > 0.05$ for all comparisons) (**Table 1**).

Tissue NO, ONO_2^-, and cGMP Levels

Tissue NO, ONO_2, and cGMP levels are given in **Table 2**. Compared to Y-C, in the O-S group NO, ONO_2^- levels were increased, while cGMP levels were significantly depressed ($p < 0.05$). On the other hand, both CR and PE treatments reduced the tissue NO and ONO_2^- levels significantly ($p < 0.05$) and prevented the reduction in tissue cGMP levels ($p < 0.05$). However, there was no statistically significant difference in NO, ONO_2^-, and cGMP levels between the CR, PE, and CR + PE groups ($p > 0.05$ for all comparisons).

Apoptotic Biomarkers

Apoptosis-related protein bands determined by Western blotting and protein levels that were calculated by comparing the density of the control protein, β-actin, are shown in **Figures 1**, **2**, respectively. As shown in **Figure 2**, the O-S group was characterized by increased pro-apoptotic (Bax, p53) and decreased in anti-apoptotic protein (Bcl-XL) levels ($p < 0.001$). On the contrary, compared to the O-S group, CR, and PE groups were characterized by increased anti-apoptotic (Bcl-XL) and decreased pro-apoptotic (Bax, p53) protein levels. There were no differences between the PE, CR, and CR + PE groups also ($p > 0.05$ for all comparisons).

TABLE 1 | Tissue oxidative status and the antioxidant capacity.

Group	Antioxidant capacity		Oxidative status	
	SOD (U/gr tissue)	GSH (μmole/gr tissue)	MDA (nmole/gr tissue)	8-OHdG (ng/mg DNA)
Y-C	7.46 ± 0.32	2.48 ± 0.21	8.43 ± 0.24	1.42 ± 0.14
O-S	3.06 ± 0.13*	0.96 ± 0.10*	12.53 ± 1.23*	3.47 ± 0.20*
O-CR	4.08 ± 0.25*,&	2.24 ± 0.15&	8.71 ± 0.52&	1.87 ± 0.17&
O-PE	4.38 ± 0.16*,&	2.26 ± 0.08&	8.23 ± 0.21&	1.78 ± 0.27&
O-CR-PE	4.36 ± 0.09*,&	2.19 ± 0.19&	8.32 ± 0.18&	1.63 ± 0.30&

*$p < 0.01$ *: compared to Y-C; &: compared to O-S; n = 6. Y-C: young control; O-S: old sedentary; O-CR: old calorie restricted; O-PE: old exercise treated; O-CR-PE: old calorie restricted and exercise treated.*

Total NOS Activity, NOS Isoforms, and Histologic Assessment

Histologic slices of the corpus cavernosum of the Y-C group of animals with normal morphology were characterized by regular endothelial and smooth muscle cells bordered by a layer of dense collagenous (tunica albuginea) and trabecular pattern of veins with irregular vascular channels surrounded by collagen, elastic fiber, and smooth muscle (**Figure 3**). On the contrary, the O-S group of corpus cavernosum tissue was characterized by moderate endothelial and smooth muscle cell degeneration and inflammatory cell infiltration (**Figure 3**). CR and PE treated groups of slices were almost in normal morphology of corpus cavernosum (**Figure 3**).

Compared to the Y-C animals, the O-S group of rats was characterized by increased tissue NOS levels together with decreased eNOS and nNOS and increased iNOS proteins (**Figure 4**). In the CR and PE trained groups, NOS protein levels determined by Western-blotting were eNOS: O-CR = O-CR-PE > O-PE; iNOS: O-CR = O-PE > O-CR-PE; and nNOS: O-PE > O-CR-PE > O-CR (**Figure 5**).

Corpus Cavernosum Function

In the pre-contracted (118 mM KCl) corpus cavernosum strips of Y-C, cumulatively added phenylephrine (10^{-9}–10^{-3} M) caused a concentration-dependent contraction with a pEC_{50} of 6.26 ± 0.05 (**Figure 6**). By contrast, in the O-S group, the contractile response of corpus cavernosum to phenylephrine was decreased, causing a significant effect on pEC_{50} (5.65 ± 0.02, $p < 0.05$). Both CR and PE treatment prevented the contractile response of corpus cavernosum to phenylephrine ($p < 0.05$ for all comparisons) (**Figure 6**). Contractile responses to phenylephrine calculated as pEC_{50} were: O-CR > O-PE > O-CR-PE (**Table 3**).

Cumulatively added carbachol (10^{-9}–10^{-3} M) to corporal tissues, which were pre-contracted with a submaximal dose of phenylephrine (10^{-5} M), caused relaxation in Y-C in a dose-dependent manner (6.49 ± 0.03) (**Figure 7**). Compared to the Y-C group, relaxation responses to carbachol were markedly reduced (pEC50 = 6.04 ± 0.04, $p < 0.05$) in the O-S group. In the CR and PE treated groups, however, the relaxation responses were higher (**Figure 7**). Relaxation responses to carbachol calculated as pEC_{50} were O-CR > O-PE > O-CR-PE (**Table 3**).

DISCUSSION

It is well known that aging is closely associated with ED with significantly increased prevalence after the age of 70 years (Feldman et al., 1994). Here, we also showed that erectile function in aged rats was significantly impaired compared with their young littermates, as was evident by decreased cavernosal function.

Penile erectile tissue is composed of two corporal bodies called the corpora cavernosa. These cavernosal bodies are composed of sinusoidal spaces with trabecular meshwork, which are lined

TABLE 2 | Tissue nitric oxide, peroxynitrite and cyclic guanylate cyclize levels.

Parameter	Y-C	O-S	O-CR	O-PE	O-CR-PE
NOx (nmol/mg protein)	145 ± 7.2	348 ± 22.3*	188 ± 11.6*,&	193 ± 20.3*,&	175 ± 12.4*,&
ONO_2^-(nmol/g tissue)	114 ± 3.1	208 ± 18.2*	150 ± 32.3*,&	164 ± 29.8*,&	134 ± 24.9*,&
cGMP (pmol/mg protein)	12.98 ± 0.32	8.57 ± 0.12*	11.41 ± 0.25&	11.03 ± 0.12&	12.24 ± 0.18&

*$p < 0.05$ * compared to Y-C; & compared to O-S; n = 6. NO, nitrite/nitrate, ONO_2^-, peroxynitrite, cGMP, cyclic guanylate cyclize; Y-C, young control; O-S, old sedentary; O-CR, old calorie restricted; O-PE, old exercise treated; O-CR-PE, old calorie restricted and exercise treated.*

by endothelium (Agarwal et al., 2006). A penile erection is a two-step mechanical process summarized as: (1) transportation of blood into the expanded cavernosal sinusoids, which results in the enlargement and rigidity of the penis for the initiation of the erection, and (2) maintenance of that enlargement and rigidity by cavernosal veno-occlusion (CVO) in order to prevent the leakage of the blood from the veins before the sexual act is completed (Johnson et al., 2011). In the first step, while the blood flows into the corporal sinusoids via cavernosal arteries, corporal sinusoids expand to provide a space for the pooling of the blood. In the second step, the increase in the intra-corporal pressure via pooling of the blood into the expanded sinusoids shuts the venous channels and decreases the outflow to prevent the leakage of the blood from the vein. The increase in the inflow of the blood via cavernosal arteries, located within the corporal bodies, and expansion of the corporal sinusoids is dependent on the relaxation of smooth muscles located both in the arterial system and corporal sinusoids (Lue and Tanagho, 1987). Therefore, a penile erection is a well-coordinated neuro-vascular process, which includes closed functional interaction between the penis

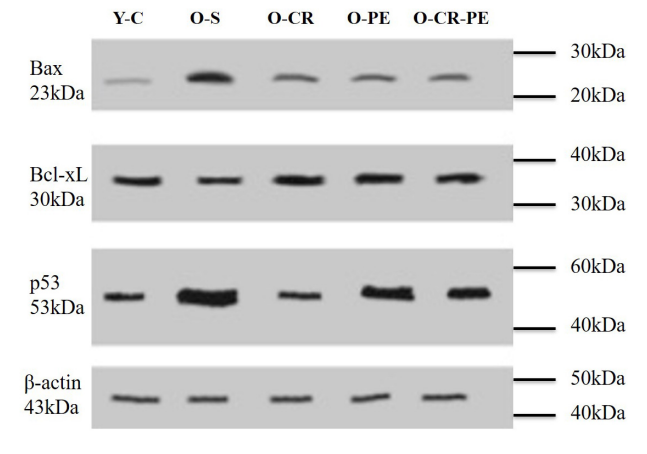


FIGURE 1 | Anti-apoptotic and apoptotic protein bands determined by Western blotting.

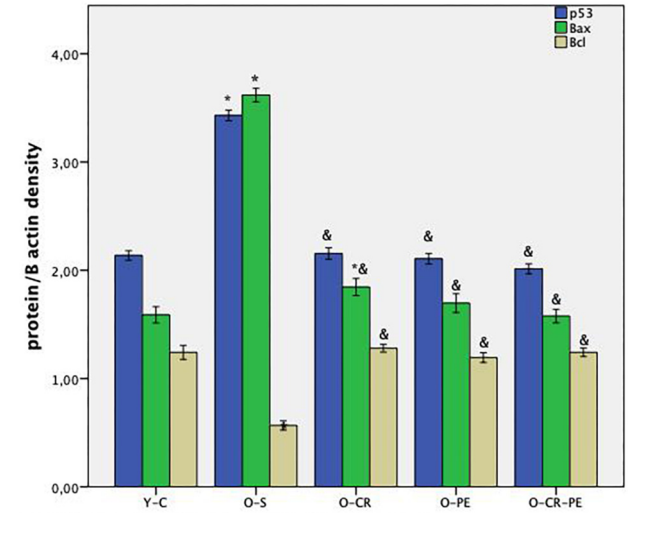


FIGURE 2 | Anti-apoptotic and apoptotic protein levels. $P < 0.05$ * compared to Y-C; & compared to O-S; $n = 6$ Y-C, young control; O-S, old sedentary; O-CR, old calorie restricted; O-PE, old exercise treated; O-CR-PE, old calorie restricted and exercise treated.

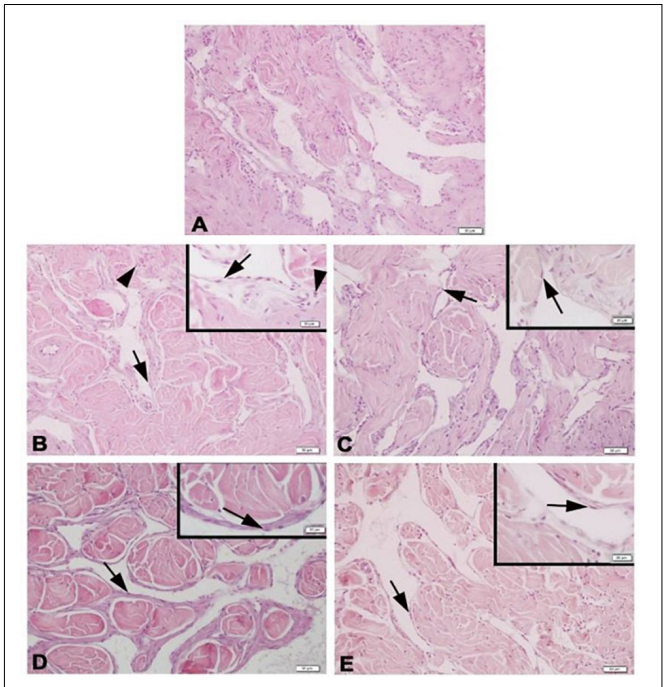


FIGURE 3 | Photomicrographs of corpus cavernosum Normal corpus cavernosum morphology in Y-C group **(A)**; Desquamous endothelial cells and inflammatory cells in O-S group **(B)**; Endothelial and smooth muscle cells of O-CR group **(C)**; Endothelial and smooth muscle cells of O-PE **(D)**; Endothelial and smooth muscle cells of O-CR-PE group **(E)**. arrow: endothelium. arrowhead: inflammatory cells.

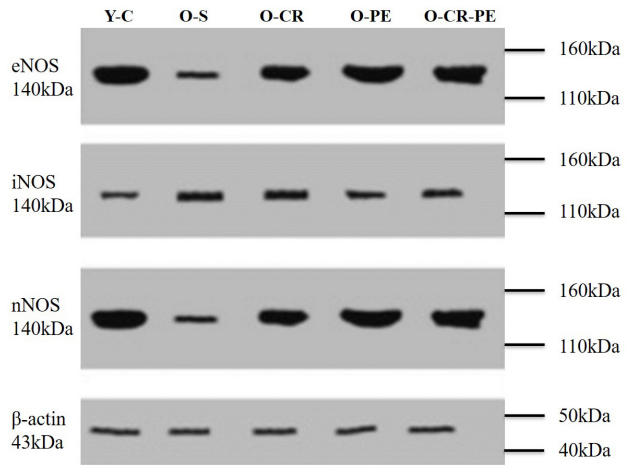


FIGURE 4 | NOS bands determined by Western blotting.

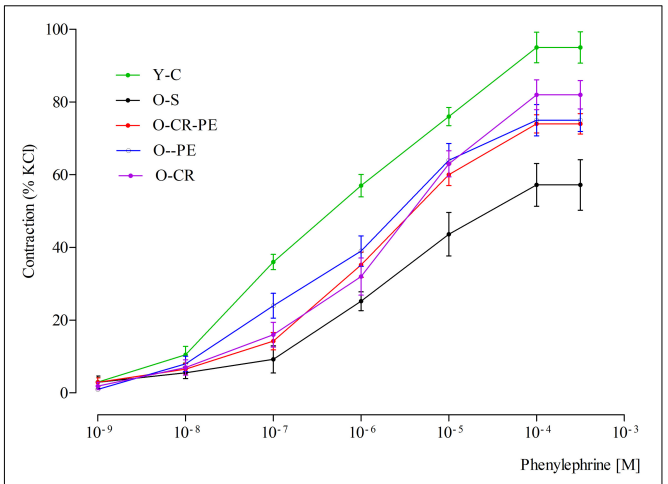


FIGURE 6 | Contractile responses to phenylephrine.

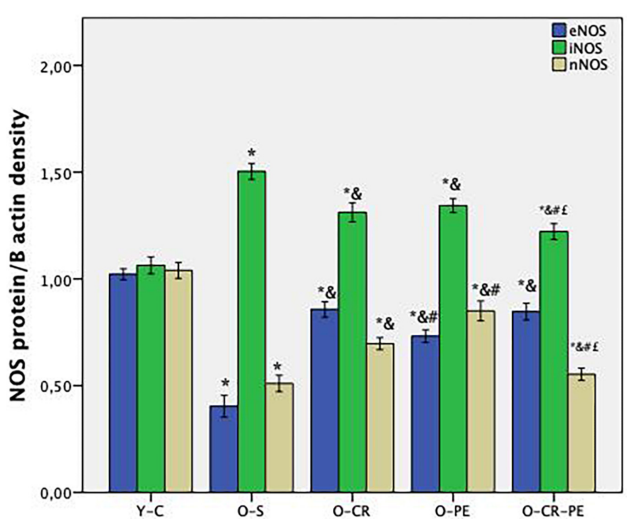


FIGURE 5 | NOS protein levels ($n = 6$) $p < 0.05$ * compared to Y-C; & compared to O-S; # compared to O-CR; £ compared to O-PE; Y-C, young control; O-S, old sedentary O-CR, old calorie restricted O-PE, old exercise trained; O-CR-PE, old calorie restricted and exercise trained.

vascular and nervous system (Andersson, 2011). NO plays an important role in this coordination via both nitrergic fibers and vascular endothelial cells (Ferrini et al., 2017; Jang et al., 2017). NO synthesized by nNOS, located in the terminal axons of the nerve innervating the corporal smooth muscle, enters the corporal smooth muscle cell and activates guanylyl cyclase to form cGMP from GTP and induces smooth muscle relaxation and initiates the erection (Burnett et al., 1992). The length of time of erection is dependent on the length of time for maintaining the corporal muscle in its relaxed state. Since the erection requires a balance between inflow and outflow of the blood within the corporal sinusoids, NO produced by eNOS plays a role both in the initiation and continuation of a penile erection (Hannan et al., 2010). NO, synthesized from the endothelial cells by eNOS, diffuses into the smooth muscle cells and causes relaxation with a mechanism similar to nNOS. Also, iNOS may originate from the smooth muscle cell, and NO produced by iNOS acts within the mitochondria. However, iNOS-mediated effects in the corpus cavernosum are quite controversial (Wang et al., 2013; Zhao et al., 2016). Although iNOS is generally considered to be expressed in an inflammatory condition, increasing evidence has demonstrated that iNOS has an important role in the protection of the penile corpus cavernosum either by combating the oxidative stress associated with the ongoing apoptotic process (Gonzalez and Rajfer, 2004; Ferrini et al., 2015) or by inhibiting the breakdown of cGMP (Ferrini et al., 2007). In our study, both three isoforms of NOS (eNOS, nNOS, and nNOS) protein levels in the Y-C group of rats were almost the same. Still, iNOS protein levels were almost doubled in the O-S group. Based on this knowledge, we can speculate that iNOS plays different roles in the penile erection depending on the level of the oxidative status of the penis.

It is known that about 15% loss of functional corporal muscle mass leads the symptomatic ED (Agarwal et al., 2006). The aging-dependent decrease in the amount of the functioning corporal smooth muscle, mainly by apoptosis, was shown (Grünewald and Beal, 1999). Again, the progressive loss of anti-apoptotic genes (Bcl-2 and Bcl-x) in the corpus cavernosum of aged rats was also shown (Yamanaka et al., 2002). In line with these studies, we also showed a decrease in the anti-apoptotic protein (Bcl-XL) levels together with the increase in pro-apoptotic protein (Bax, p53) levels in the O-S group. Again, in our study, decreased contraction and relaxation responses were observed in the functional study of the corpus cavernosum. These findings were consistent with the findings of endothelial and smooth muscle cell degeneration in the histological slices in the O-S group.

Oxidative stress is believed to be the primary trigger for apoptosis (Agarwal et al., 2006). Oxidative stress occurs when the cells are exposed to excessive levels of reactive oxygen spices (ROS) resulting from the imbalance between pro-oxidant and protective mechanisms (Zalba et al., 2000).

TABLE 3 | Potency obtained from the concentration-response curves.

	pEC_{50}				
	Y-C	**O-S**	**O-CR**	**O-PE**	**O-CR-PE**
Phenylephrine	6.26 ± 0.05	5.65 ± 0.02*	6.04 ± 0.03&	5.92 ± 0.04*,&	5.74 ± 0.02*,&,#,£
Carbachol	6.49 ± 0.03	6.04 ± 0.04*	6.76 ± 0.04*	6.52 ± 0.05#	6.26 ± 0.03*,#,£

*$p < 0.05$, * compared to Y-C; & compared to O-S; # compared to O-CR; £ compared to O-PE; n = 6. Potency (pEC_{50}) represented as log of molar concentration to produce 50% of maximal contractile response elicited by agonist relative to KCl (118 mmol/L)-induced contraction.*

Under physiological conditions, the tissue antioxidant enzymes (SOD, GSH, and catalase) prevent the continuous formation of ROS. For example, the detoxification of ROS by cavernosal endothelial cells by using these enzymes is known (Dobrina and Patriarca, 1986). Although the upregulation of these enzymes is the main compensatory mechanism, sustained high levels of ROS diminish the activity of these enzymes (Ushiyama et al., 2004; Lagoda et al., 2007). Age-related increase in ROS together with a decrease in antioxidant capacity, which is characterized by reduced levels of SOD and glutathione, have been shown in different study settings (Bivalacqua et al., 2003; Ferrini et al., 2004). Consistent with these findings, in our study, the O-S group of animals was characterized with decreased antioxidant capacity (statistically significant decrease in SOD and GSH levels) and increased oxidant status (statistically increase in MDA and 8-OHdG levels). It should be emphasized that high MDA levels are indicative of lipid changes in the lipid matrix and cell membrane, whereas high 8-OHdG levels indicate DNA damage. Thus, DNA damage and lipid peroxidation might be responsible for the damage to the cavernosal structure in our study. The observation of an increased level of pro-apoptotic protein levels together with the decreased antiapoptotic protein levels in the O-S group supports this argument.

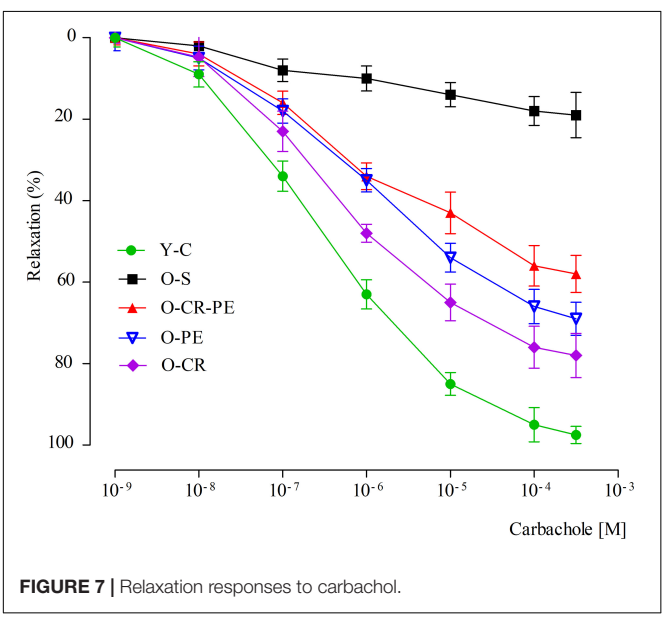


FIGURE 7 | Relaxation responses to carbachol.

It is well known that NO is a highly reactive free radical that reacts with ROS, especially with the superoxide (O_2^-), to form ONO_2^- (Beckman and Koppenol, 1996). According to the body of evidence, ONO^-_2 affects the corpus cavernosum in four different ways. Firstly, ONO^-_2 causes smooth muscle relaxation, which is less potent than the NO, resulting in ineffective relaxation in the corpus cavernosum (Khan et al., 2001). We showed decreased relaxation and increased ONO_2^- together with decreased NO/cGMP levels in the O-S group of animals. Secondly, ONO_2^- increases the incidence of apoptosis in the endothelium, which leads to denudation of endothelium and a further reduction of available NO (Yamanaka et al., 2002; Fan et al., 2012). In the present study, increased NO and ONO_2^- together with decreased cGMP levels were consistent with the increased iNOS and decreased eNOS protein levels in the O-S group. In this group, the decreased anti-apoptotic protein levels together with the increased pro-apoptotic protein levels also supported these findings. Thirdly, reduced availability of NO by ONO_2^- induced the adhesion of the platelets and leukocytes in the vascular endothelium, which caused vasoconstriction and aggravated ED (Jeremy et al., 2000). Lastly, particularly in the presence of inflammation, produced ONO_2^- from a high level of NO can lead to a cytotoxic effect on cavernosal muscle (Wink et al., 1998). In our study, the observation of inflammatory cells in the histologic slices in the O-S group supported the argument that inflammation results in oxidative stress, and that, in increased oxidative stress conditions, the source of the high level of NO is the iNOS induced by inflammation in age-related ED. As discussed earlier, during the low level of oxidative stress, NO produced by iNOS within the smooth muscle cells combats the oxidative stress associated with the ongoing apoptosis. On the contrary, during the high level of oxidative stress, NO produced by iNOS, induced by inflammation, reacts with the reactive oxygen species to form ONO_2^-. This causes endothelial dysfunction, vasoconstriction, a decrease in the availability of NO, and the inhibition of SOD; all these lead to ED.

A causal connection between oxidative stress, aging, and age-related pathologies (Davalli et al., 2016) as well as the beneficial effects CR and PE on oxidative stress in age-related pathologies is known (Testa et al., 2014; Simioni et al., 2018). Again, the beneficial effects of CR and PE on erectile function have also been shown separately (Claudino et al., 2004; Souza et al., 2017). In our study, although the contractile response to phenylephrine (an alpha agonist) was attenuated in O-S, this was prevented

both in the O-CR and O-PE groups. Moreover, when the CR and PE groups were compared, the response to phenylephrine was significantly higher in the O-CR than in the O-PE group. This might indicate that CR and PE show their beneficial effects on functional responses of corpus cavernosum through different mechanisms. Increased eNOS levels together with increased response to phenylephrine in the O-CR group support that the effect of CR is mediated by eNOS activation. Other researchers reported similar results (Whidden et al., 2011). On the contrary, lower eNOS protein levels together with mild phenylephrine response in the O-PE group shows the involvement of the NO-cGMP signaling pathway in an endothelium-independent manner. We speculate that this could be a direct stimulation of smooth muscle cells by nNOS, which is released from the cavernous nerve endings. Increased nNOS protein levels in this group supports this hypothesis. Similarly, Claudino et al. (2004) reported an eNOS-independent relaxation response in the corpus cavernosum from trained rats. Again, in the present study, alteration in the relaxation responses of corpus cavernosum to carbachol (a muscarinic agonist) also supports our hypothesis.

CONCLUSION

In the present study, CR and PE prevented age-related changes in the corpus cavernosum. Reducing nitrosative damage in the neurovascular structure was the main mechanism. CR and PE restored the endothelial and smooth muscle cells in the corpus cavernosum by decreasing apoptosis. The main mechanism of enhancing functional response in the corpus cavernosum with CR was the maintenance of endothelial function via eNOS activation (supported by increased eNOS levels together with the increased response to phenylephrine an alpha-1 agonist in O-CR group). However, it involves an increase in the NO-cGMP signaling pathway in an endothelium-independent manner with PE. This could be a direct stimulation of smooth muscle cells by nNOS, which is released from the cavernous nerve endings (supported by increased nNOS levels and mild response to carbachol a muscarinic agonist). As a result, lifestyle changes, such as CR and PE, should be considered in combination with pharmacological treatment strategies in the treatment of age-related changes in ED.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The animal study was reviewed and approved by the Yeditepe University Experimental Animals Ethics Committee.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

REFERENCES

Agarwal, A., Nandipati, K. C., Sharma, R. K., Zippe, C. D., and Raina, R. (2006). Role of oxidative stress in the pathophysiological mechanism of erectile dysfunction. *J. Androl.* 27, 335–347. doi: 10.2164/jandrol.05136

Aladag, M. A., Turkoz, Y., Sahna, E., Parlakpinar, H., and Gul, M. (2003). The attenuation of vasospasm by using a SOD mimetic after experimental subarachnoidal hemorrhage in rats. *Acta Neurochir.* 145, 673–677. doi: 10.1007/s00701-003-0052-z

Andersson, K. E. (2011). Mechanisms of penile erection and basis for pharmacological treatment of erectile dysfunction. *Pharmacol. Rev.* 63, 811–859. doi: 10.1124/pr.111.004515

Beckman, J. S., and Koppenol, W. H. (1996). Nitric oxide, superoxide, and peroxyni- trite: the good, the bad, and ugly. *Am. J. Physiol.* 271, 1424–1437.

Bivalacqua, T. J., Armstrong, J. S., Biggerstaff, J., Abdel-Mageed, A. B., Kadowitz, P. J., Hellstrom, W. J., et al. (2003). Gene transfer of extracellular sod to the penis reduces superoxide and improves erectile function in aged rats. *Am. J. Physiol. Heart Circ. Physiol.* 284, H1408–H1421.

Burnett, A. L., Lowenstein, C. J., Bredt, D. S., Chang, T. S., and Snyder, S. H. (1992). Nitric oxide: a physiologic mediator of penile erection. *Science* 257, 401–403. doi: 10.1126/science.1378650

Campbell, J. D., Trock, B. J., Oppenheim, A. R., Anusionwu, I., Gor, R. A., and Burnett, A. L. (2019). Meta-analysis of randomized controlled trials that assess the efficacy of low-intensity shockwave therapy for the treatment of erectile dysfunction. *Ther. Adv. Urol.* 11:1756287219838364.

Claudino, M. A., Priviero, F. B. M., Teixeria, C. E., Nucci, G. D., Antunes, E., and Zanesco, A. (2004). Improvemnt in relaxation response in corpus cavernosum from trained rats. *Urology* 63, 1004–1008. doi: 10.1016/j.urology.2003.11.034

Davalli, P., Mitic, T., Caporali, A., Lauriola, A., and D'Arca, D. (2016). ROS, cell senescence and novel molecular mechanisms in aging anda ge-related diseases. *Oxid. Med. Cell. Longev.* 2016:3565127. doi: 10.1155/2016/3565127

Dobrina, A., and Patriarca, P. (1986). Neutrophil-endothelial cell interaction. Evidence for and mechanisms of the self-protection of bovine microvascular endothelial cells from hydrogen peroxide-induced oxidative stress. *J. Clin. Invest.* 78, 462–471. doi: 10.1172/jci112598

Fan, M., Xu, X., He, X., Chen, L., Qian, L., Liu, J., et al. (2012). Protective effects of hydrogen- rich saline against erectile dysfunction in a streptozotocin induced diabetic rat model. *J. Urol.* 190, 350–356. doi: 10.1016/j.juro.2012.12.001

Feldman, H. A., Goldstein, I., Hatzichristou, D. G., Krane, R. J., and McKinlay, J. B. (1994). Impotence and its medical and psychosocial correlates: results of the Massachusetts Male Aging Study. *J. Urol.* 151, 54–61. doi: 10.1016/s0022-5347(17)34871-1

Ferrini, M., Magee, T. R., Vernet, D., Rajfer, J., and González-Cadavid, N. F. (2001). Aging-related expression of inducible nitric oxide synthase and markers of tissue damage in the rat penis. *Biol. Reprod.* 64, 974–982. doi: 10.1095/biolreprod64.3.974

Ferrini, M. G., Cadavid, N. F. G., and Raifer, J. (2017). Aging related erectile dysfunction—potential mechanism to halt or delay its onset. *Transl. Androl. Urol.* 6, 20–27. doi: 10.21037/tau.2016.11.18

Ferrini, M. G., Davila, H. H., Valente, E. G. A., Gonzalez-Cadavid, N. F., and Rajfer, J. (2004). Aging-related induction of inducible nitric oxide synthase is vasculo-protective to the arterial media. *Cardiovasc. Res.* 61, 796–805. doi: 10.1016/j.cardiores.2003.12.006

Ferrini, M. G., Hlaing, S. M., Chan, A., and Artaza, J. N. (2015). Treatment with a combination of ginger, L-citrulline, muira puama and *Paullinia cupana* can reverse the progression of corporal smooth muscle loss, fibrosis and veno-occlusive dysfunction in the aging rat. *Andrology* 4:132.

Ferrini, M. G., Kovanecz, I., Sanchez, S., Vernet, D., Davila, H. H., Rajfer, J., et al. (2007). Long-term continuous treatment with sildenafil ameliorates aging- related erectile dysfunction and the underlying corporal fibrosis in the rat. *Biol. Reprod.* 76, 915–923. doi: 10.1095/biolreprod.106.059642

Gonzalez, C. N. F., and Rajfer, J. (2004). Molecular pathophysiology and gene therapy of aging-related erectile dysfunction. *Exp. Gerontol.* 39, 1705–1712. doi: 10.1016/j.exger.2004.06.022

Grünewald, T., and Beal, M. F. (1999). NOS knockouts and neuroprotection. *Nat. Med.* 5, 1354–1355. doi: 10.1038/70918

Hannan, J. L., Blaser, M. C., Oldfield, L., Pang, J. J., Adams, S. M., Pang, S. C., et al. (2010). Morphological and functional evidence for the contribution of the pudendal artery in aging-induced erectile dysfunction. *J. Sex. Med.* 7, 3373–3384. doi: 10.1111/j.1743-6109.2010.01920.x

Jang, H., Bae, W. J., Kim, S. J., Cho, H. J., Yuk, S. M., Han, D. S., et al. (2017). The herbal formula KH-204 is protective against erectile dysfunction by minimizing oxidative stress and improving lipid profiles in a rat model of erectile dysfunction induced by hypercholesterolemia. *BMC Complement. Altern. Med.* 17:129. doi: 10.1186/s12906-017-1588-4

Jeremy, J. Y., Angelini, G. D., Khan, M., Mikhailidis, D. P., Morgan, R. J., Thompson, C. S., et al. (2000). Platelets, oxidant stress and erectile dysfunction: an hypothesis. *Cardiovasc. Res.* 46, 50–54. doi: 10.1016/s0008-6363(00)00009-2

Johnson, J. M., Bivalacqua, T. J., Lagoda, G. A., Burnett, A. L., and Musicki, B. (2011). eNOS-uncoupling in age-related erectile function. *Int. J. Impot. Res.* 23, 43–48. doi: 10.1038/ijir.2011.2

Kaya, E., Sikka, S. C., Kadowitz, P. J., and Gur, S. (2017). Aging and sexual health: getting to the problem. *Aging Male* 20, 65–80. doi: 10.1080/13685538.2017.1295435

Khan, M. A., Thompson, C. S., Mumtaz, F. H., Mikhailidis, D. P., Morgan, R. J., Bruckdorfer, R. K., et al. (2001). The effect of nitric oxide and peroxynitrite on rabbit cavernosal smooth muscle relaxation. *World J. Urol.* 19, 220–224. doi: 10.1007/s003450000162

Lagoda, G., Jin, L., Lehrfeld, T. J., Liu, T., and Burnett, A. L. (2007). Fk506 and sildenafil promote erectile function recovery after cavernous nerve injury through antioxidative mechanisms. *J. Sex. Med.* 4, 908–916. doi: 10.1111/j.1743-6109.2007.00519.x

Leite, N. C., Ferreira, T. R., Rickli, S., Borck, P. C., Mathias, P. C. F., Emilio, H. R. O., et al. (2013). Glycolytic and mitochondrial metabolism in pancreatic islets from MSG-treated obese rats subjected to swimming training. *Cell. Physiol. Biochem.* 31, 242–256. doi: 10.1159/000343365

Lowry, O. H., Rosebrough, N. J., Farr, A. C., and Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193, 265–266.

Lue, T. F., and Tanagho, E. A. (1987). Physiology of erection and pharmacological management of impotence. *J. Urol.* 137, 829–836. doi: 10.1016/s0022-5347(17)44267-4

Okhawa, H., Ohshi, N., and Yagi, K. (1979). Assay or lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95, 351–358. doi: 10.1016/0003-2697(79)90738-3

Paskaloglu, K., Sener, G., and Ayanğolu, D. G. (2004). Melatonin treatment protects against diabetes-induced functional and biochemical changes in rat aorta and corpus cavernosum. *Eur. J. Pharmacol.* 499, 345–354. doi: 10.1016/j.ejphar.2004.08.002

Simioni, C., Zauli, G., Martelli, A. M., Vitale, M., Sacchetti, G., Gonelli, A., et al. (2018). Oxidative stress: role of physical exercise and antioxidant nutraceutics in adulhood and aging. *Oncotarget* 9, 17181–17198. doi: 10.18632/oncotarget.24729

Souza, I. L. L., Barros, B. C., Oliveira, G. A., Queiroga, F. R., Toscano, L. T., Silva, A. S., et al. (2017). Hypercaloric diet establishes erectile dysfunction in rat: mechanisms underlying the endothelial damage. *Front. Physiol.* 8:760. doi: 10.3389/fphys.2017.00760

Testa, G., Biasi, F., Poli, G., and Chiarpotto, E. (2014). Calorie restriction and dietary restriction mimetics: a strategy for improving healthy aging and longevity. *Curr. Pharm. Des.* 20, 2950–2977. doi: 10.2174/13816128113196660699

Ushiyama, M., Morita, T., Kuramochi, T., Yagi, S., and Katayama, S. (2004). Erectile dysfunction in hypertensive rats results from impairment of the relaxation evoked by neurogenic carbon monoxide and nitric oxide. *Hypertens. Res.* 27, 253–261. doi: 10.1291/hypres.27.253

Wang, T., Li, M., Yuan, H., Zhan, Y., Xu, H., Wang, S., et al. (2013). saRNA guided iNOS up-regulation improves erectile function of diabetic rats. *J. Urol.* 190, 790–798. doi: 10.1016/j.juro.2013.03.043

Whidden, M. A., Kirichenko, N., Halici, Z., Erdos, B., Foster, T. C., and Tümer, N. (2011). Lifelong caloric restriction prevents age-induced oxidative stress in the sympathoadrenal system of Fischer 344 x Brown Norway rats. *Biochem. Biophys. Res. Commun.* 408, 454–458. doi: 10.1016/j.bbrc.2011.04.051

Wink, D. A., Feelisch, M., Fukuto, J., Chistodoulou, D., Jourd'heuil, D., Grisham, M. B., et al. (1998). The cytotoxicity of nitroxyl: possible implications for the pathophysiological role of NO. *Arch. Biochem. Biophys.* 351, 66–74. doi: 10.1006/abbi.1997.0565

Yamanaka, M., Shirai, M., Shiina, H., Shirai, M., Tanaka, Y., Fujime, M., et al. (2002). Loss of anti-apoptotic genes in aging rat crura. *J. Urol.* 168, 2296–2300. doi: 10.1097/00005392-200211000-00117

Zalba, G., Beaumont, J., San Jose, G., Fortuno, A., Fortuno, M. A., and Diez, J. (2000). Vascular oxidant stress: molecular mechanisms and pathophysiological implications. *J. Physiol. Biochem.* 56, 57–64. doi: 10.1007/bf03179777

Zhao, S., Liu, L., Kang, R., Li, F., Li, E., Zhang, T., et al. (2016). Shengjing capsule improves erectile function through regulation of nitric oxide-induced relaxation in corpus cavernosum smooth muscle in a castrated rat model. *Urology* 91, 243.e7–243.e12. doi: 10.1016/j.urology.2016.02.021

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

ORIGINAL RESEARCH
published: 06 March 2020
doi: 10.3389/fnagi.2020.00051

A Neurovisceral Integrative Study on Cognition, Heart Rate Variability, and Fitness in the Elderly

Felipe de Oliveira Matos[1]*, *Amanda Vido*[1], *William Fernando Garcia*[1], *Wendell Arthur Lopes*[1] and *Antonio Pereira*[2]

[1]Departament of Physical Education, Health Sciences Center, State University of Maringá (UEM), Maringá, Brazil, [2]Institute of Technology, Federal University of Pará (UFPA), Belém, Brazil

The association between physical fitness and cognitive performance has been widely investigated in the literature. However, the neurophysiological mechanisms underlying this relationship are not yet clear. Here, we aim to evaluate the interactions between executive function measures, heart rate variability (HRV), and physical fitness in the context of the neurovisceral integration (NVI) theory. Twenty-eight healthy elderly subjects (>60 years) were submitted to evaluation of executive performance with three computerized tests: the N-back test measured working memory capacity, the Stroop Color test evaluated inhibitory control and selective attention, and the Wisconsin Card Sorting Test (WCST) evaluated abstract reasoning and cognitive flexibility. We also used the Physical Testing Battery for the Elderly to measure aerobic capacity, dynamic balance, upper body flexibility, and handgrip strength. Our results confirm the relationship between executive function and physical fitness, particularly between working memory, cardiorespiratory fitness, and dynamic balance. We also demonstrate an association between executive performance and HRV in older people, corroborating previous results from other groups obtained in young adults. However, our regression models did not indicate that HRV mediates the relationship between cognition and physical fitness in the elderly, suggesting that age-related degeneration of autonomic control can affect aspects of NVI in this population.

Keywords: cognition, executive functions, working memory, autonomic nervous system, heart rate variability, aging, physical fitness

OPEN ACCESS

Edited by:
Mario Bernardo-Filho,
Rio de Janeiro State University, Brazil

Reviewed by:
Douglas F. Watt,
Lesley University, United States
Eddy A. Van Der Zee,
University of Groningen, Netherlands

***Correspondence:**
Felipe de Oliveira Matos
fomatos@uem.br

Received: *15 August 2019*
Accepted: *17 February 2020*
Published: *06 March 2020*

Citation:
Matos FO, Vido A, Garcia WF, Lopes WA and Pereira A (2020) A Neurovisceral Integrative Study on Cognition, Heart Rate Variability, and Fitness in the Elderly. Front. Aging Neurosci. 12:51. doi: 10.3389/fnagi.2020.00051

INTRODUCTION

The neurovisceral integration (NVI) model (Thayer and Lane, 2000; Thayer et al., 2009) proposes that adaptive behavior depends on the integration of neural networks spanning both the central (CNS) and autonomic nervous systems (ANS) tasked with regulating cardiovascular function. Brain metabolism critically depends on the cardiovascular supply of cerebral blood flow (CBF) due to the limited availability of this organ's intracellular energy substrates. The crosstalk between CNS and ANS structures, necessary to provide the brain with adequate levels of oxygen and energy sources, is mediated by a network of brain areas known as the "central autonomic network" (CAN; Benarroch, 1993; Valenza et al., 2019), which includes the anterior cingulate cortex (ACC), insula, and the

ventromedial prefrontal cortex (vmPFC). Outputs from these regions eventually reach premotor neurons located in the lower brain stem and nucleus ambiguus in the medulla oblongata which contributes to the sympathetic and parasympathetic modulation of the heart (Thayer and Lane, 2000; Benarroch, 2012).

The heart rate variability (HRV) reflects the variation in the time interval between consecutive heartbeats (Saul, 1990; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996) and has been considered a surrogate parameter for the complex interaction between brain and cardiovascular system (Ernst, 2017). Both systems are simultaneously affected by the normal process of aging (Peters, 2006; Jandackova et al., 2016) and the result is a decline in the capacity of the CAN to adjust the CBF in response to environmental challenges while negatively influencing emotion control (Mather and Thayer, 2018) and cognitive performance (Thayer et al., 2009; Elias and Torres, 2017; Forte et al., 2019). Jandackova et al. (2016) showed a marked decline in HRV in middle-aged individuals which seems to be secondary to other pathological conditions. Cognitive dementia is the result of multiple age-related neuropathies (Power et al., 2018) associated with cardiovascular risk factors (Oishi et al., 2017), such as chronic brain hypoperfusion (Zheng et al., 2019), which particularly affects the frontal cortex (Sherwood et al., 2011).

Over the past decade, a large number of studies have investigated the role lifestyle activities play in decreasing the risk of cognitive decline in the elderly (for review, see Christie et al., 2017). Among these activities, physical exercise has gained prominence due to its neuroprotective effects against dementia and other neurodegenerative diseases (Phillips et al., 2014; Vecchio et al., 2018). For instance, research has shown that regular exercise promoted hippocampal neurogenesis (Erickson et al., 2009, 2011), local increases on the concentration of neurotrophins (such as BDNF; Leckie et al., 2014; Håkansson et al., 2017), neuroplasticity (Erickson and Kramer, 2009; Voss et al., 2013a,b; Erickson et al., 2014), angiogenesis (Bloor, 2005; Al-Jarrah et al., 2010), and adaptive changes in CBF (Dupuy et al., 2015; Jennings et al., 2015). Electrophysiological and neuroimaging studies have provided evidence that cardiorespiratory fitness is positively correlated with brain function, particularly in brain regions associated with the CAN, which has shown increased neuroplasticity after physical exercise interventions, improving both cardiovascular and cognitive control (Gomez-Pinilla and Hillman, 2013). However, relatively few studies have focused on the relationship between physical fitness and effects on NVI biomarkers such as HRV, particularly in the elderly (see Albinet et al., 2010, 2016; Dupuy et al., 2018). While Alderman and Olson (2014) demonstrated the role of physical fitness in improving autonomic and neurocognitive health in young adults, these authors failed to show HRV-mediated influences between cardiorespiratory fitness and cognitive performance, suggesting that other mediators may be more relevant in this population, which is at the apex of cognitive performance and with not much individual difference in cardiorespiratory fitness, compared to the elderly. In addition, other studies that investigated the relationship between cognition, HRV, and fitness from an NVI perspective have focused exclusively on aerobic capacity as an independent variable (Alderman and Olson, 2014; Dupuy et al., 2015).

Thus, the main purpose of the current study is to fill the gap in the literature regarding the association of physical fitness with cognitive performance and HRV in elderly subjects. Besides, we aim to verify how this relationship is associated with different physical abilities, such as strength, flexibility, dynamic balance, and aerobic capacity.

MATERIALS AND METHODS

Participants

Twenty-eight (28) subjects (24 women) aged 60 years and over (mean age 66.71 ± 7.64 years old) participated in the research. The volunteers were screened using the following inclusion criteria: being over 60 years old, be literate, possess normal or corrected visual acuity, possess familiarity with computers, and have medical clearance to perform physical activities.

The exclusion criteria were: smoking (<6 months), surgery (<6 months), official medical diagnosis of psychiatric, psychological or cardiac disease, use of medication that may impair cardiac autonomic control and/or cognitive functions, Mini-Mental State Examination (MMSE) score <24 (or <21 for subjects with lower schooling levels; Almeida, 1998), Geriatric Depression Scale-Short Form (GDS-SF) score>5.

All experimental procedures performed in this study were approved by the Research Ethics Committee of the State University of Maringá (1,161,402).

Outcome Measures

Cognitive Measurements

We used three tests to evaluate different dimensions of executive function, namely the N-back test (working memory), the Stroop task (selective attention and inhibitory control), and the Wisconsin Card Sorting Test (WCST; abstract reasoning and cognitive flexibility). All tests were performed in a computerized setup created with the software Presentation Version 20.2 (Neurobehavioral Systems, Inc., Berkeley, CA, USA).

The Wisconsin Card Sorting Test (WCST)

The WCST (Grant and Berg, 1948) is a problem-solving task for cognitive performance assessment. The test consists of matching test cards one by one with stimulus cards, following a rule that must be deduced by the subjects themselves. We used 64 test cards which had to be associated with four stimulus cards (1-one red triangle; 2- two green stars; 3- three yellow crosses; and 4- four blue circles). The match is made by pressing on the keyboard the number corresponding to the desired stimulus card. After each attempt, the participant received positive or negative feedback on their performance through the words "right" or "wrong" presented on the screen. Blocks of 10 or 11 stimuli were presented and at the end of each one, the rule changed. We used as a parameter to evaluate performance the total number of errors

and the number of perseverative errors. These scores were chosen because they are sensitive to the effects of aging (MacPherson et al., 2002; Guarino et al., 2019).

Stroop Color

We used the Victoria version of the Stroop test (Spreen and Strauss, 1998), which consists of 72 stimuli, distributed in three tasks with 24 items each. The task is divided into three blocks, starting after an instruction presented on the screen explains the task to be performed in each step. The stimuli are presented for 2 s and if no response is given within 5 s another stimulus is presented.

The first block was composed of colored rectangles in the colors green, pink, blue and brown; the second block consisted of neutral words (each, never, today, everything) written with the colors of the previous rectangles; the third block had stimuli with a word-color conflict. At each stimulus presented the subjects should press a key with a color corresponding to the stimulus presented on the screen.

We used response latency as a measure of performance.

N-back

We used a spatial n-back task adapted from Vermeij et al. (2014). The test has three levels, 0-back (control condition), 1-back (low working memory load) and 2-back (high working memory load). The test consisted of black square-shaped stimuli, which could appear at any one of 14 fixed locations on a monitor screen. Before each stimulus, a fixation point was presented at the center of the screen. Each trial lasted 2,500 ms and consisted of 2,000 ms of fixation and 500 ms of stimulus presentation. The task began after the subjects pressed a key indicating they had read the instructions on the screen. Each level consisted of 60 trials, with 17 target stimuli that should be identified by the subject. During instruction a screen containing the black squares in all possible positions appeared for 30 s, then a quick presentation of each stimulus was made for 500 ms in a sequence from left to right and bottom to top on the screen.

Test performance was evaluated by the percentages of 2-back hits, total hits, and misses.

Heart Rate Variability

Heart Rate was recorded with the subjects at rest in a comfortable and quiet room for 10 min. R-wave peaks from the ECG were recorded continuously using a Polar HRM V800 heart rate monitor (Polar OY, Finland) with a sampling rate of 1,000 Hz for subsequent analysis of HRV. R-R intervals (the interval between two successive R waves of the ECG's QRS signal) were visually inspected for ectopic beats and replaced by interpolated data from adjacent normal to normal (N-N) intervals. A period of 5 min with lowest variance was selected for analysis. We performed both time and frequency domain analysis of the ECG signal with the Kubios software (Kubios Oy). In the time domain, we calculated the mean HR, the standard deviation of the N-N intervals (SDNN) and the square root of the successive quadratic mean interval differences (RMSSD), which are the most common measures representing parasympathetic activity (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). In the frequency domain, we used Fourier transform to quantify spectral density power of low frequency (LF; 0.04–0.15 Hz) and high frequency (HF; 0.15–0.40 Hz) bands. Additional calculations included LF + HF, LF and HF expressed in normalized units and the LF/HF ratio. We used the Poincaré graph to quantify SD1 and SD2 (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996).

Physical Assessment

To assess physical abilities we used a functional fitness battery test for elderly people (Rikli and Jones, 1999a). Aerobic capacity was measured by the distance covered in meters during a 6-min walk test (6MWT). For measures of agility, speed, and dynamic balance, we used the Timed Up-and-Go test (TUG), in which the subjects should sit in a chair, stand up, walk to a cone 2.44 meters away, return, and sit again in the shortest time possible (time was measured in seconds). Flexibility was evaluated with the sit and reach test, measured in centimeters. Additionally, Handgrip strength was measured using a hand dynamometer with the dominant hand (North Coast Medical, Inc., Morgan Hill, CA, USA). Three trials from the dominant hand were calculated and the median was used for the analysis (Innes, 1999). Handgrip strength is expressed in kilograms (kg).

Data Collection Procedures

The evaluations were performed in three moments along 2 days. In moment 1, the participants signed the Informed Consent Form and answered the MMSE and GDS-SF questionnaires. Then, they went through a familiarization period with the computerized cognitive tests. Moments 2 and 3 occurred 1 week later to minimize learning effects on the performance of cognitive tests. In moment 2 we recorded the subjects' resting heart rate (HR) before they undertook tests of executive function. The moment 3 occurred soon after and consisted of physical fitness measures.

Statistical Analysis

We performed an exploratory analysis with the Shapiro–Wilks test. For variables with normal distribution, we used the Pearson correlation test and, for non-normal distributions, we used the Spearman correlation test. Finally, multiple linear regressions with the hierarchical method were used for the independent variables, in which the researchers chose the order of the variables inserted in the model. We used the Durbin–Watson test for the independance of residuals. We also tested for multicollinearity and no strong correlation coefficients were found between the independent variables. There were no outliers in the residual statistics (with standard deviations below −3 and +3). We used the SPSS 21.0 Software (IBM Corp.) for analysis.

RESULTS

Table 1 presents the results of the descriptive analysis of cognitive, HRV and physical variables. Correlations between cognitive measures and HRV parameters are shown in **Table 2**. We found significant correlations between the number of misses in the working memory task (N-back) and both mean HR (r = 0.406, p = 0.032) and mean RR intervals (r = − 0.383,

TABLE 1 | Descriptive values of cognitive performance, heart rate variability (HRV), and fitness.

	Mean (±SD)	Median	Minimum	Maximum
HRV				
Mean HR	78.18 (9.12)	77.50	59	98
Mean RR	777.21 (94.43)	772	613	1018
SDNN	17.94 (16.22)	12.55	7.8	81.5
RMSSD	21.16 (26.12)	13.55	4.6	116.8
LF (%)	49.62 (17.31)	53.15	10.10	77.45
LF (n.u.)	55.84 (20.13)	58.68	10.14	92.70
HF (%)	40.01 (20.02)	33.14	5.54	89.14
HF (n.u.)	43.82 (19.96)	39.86	7.26	89.49
Total Power	394.29 (906.52)	119.50	30	4781
LF/HF	2.06 (2.44)	1.48	0.113	12.77
SD1	14.99 (18.49)	9.55	3.3	82.7
SD2	19.76 (14.71)	15.700	8.0	80.4
SD1/SD2	1.77 (0.77)	1.70	0.68	3.98
Physical variables				
6MWT (m)	504.11 (78.7)	497.5	350	675
TUG (ms)	7,371.4 (2244.9)	6,595	4,970	15,600
Strength (Kg/f)	25.71 (8.7)	24	14	47
Flexibility (cm)	3.8 (9.7)	5.7	−22	18
Cognition				
N-back				
Hits 2-back	49.02 (21.9)	47.10	0.0	82.4
Hits N-back	67.75 (13.5)	70.60	44.1	91.2
Miss N-back	16.65 (8.4)	15.80	2.5	37.5
Stroop				
latency C_A (ms)	8,414.99 (22,329.81)	1,100.4	−8,838.3	84,365.6
latency C_B (ms)	1,538.90 (7414.47)	−69.0	−14,215.3	26,546.8
latency RT C_A (ms)	98.90 (223.83)	62.9	−248.9	910.8
latency RT C_B (ms)	101.86 (243.77)	47.6	−110.8	1,109.0
Cards of Wisconsin				
Miss	31.26 (7.52)	31.00	15	52
Perseverative Miss	9.59 (3.70)	10.00	4	16

p = 0.044). As for the WCST results, the number of misses correlated significantly with the following HRV indexes, RMSSD (r = 0.476, p = 0.012), LF (n.u.; r = −386, p = 0.046), HF (%; r = 0.426, p = 0.027), HF (n.u.; r = 0.389, p = 0.045), SD1 (r = 0.473, p = 0.013), and SD2/SD1 ratio (r = −0.465, p = 0.014). The performance on the Stroop test did not correlate with any of the HRV variables.

Working memory performance measured by the N-back correlated positively with aerobic performance (6MWT) and dynamic balance (TUG; **Figure 1**). Only the number of hits on the 2-back test did not show a significant correlation with the TUG (r = −332, p = 0.084). No significant correlation was found between working memory and either handgrip strength or flexibility. The other cognitive tests also showed no significant correlation ($p < 0.05$) with physical fitness variables.

Table 3 shows the correlations measures between HRV indexes and physical fitness parameters. No significant correlation ($p < 0.05$) was found between these variables in the present study.

Multiple Regression Model for Mean HR

We analyzed the impact of cognition variables (independent variables) N-back misses and C-A Latency on the HRV index and the Mean HR (dependent variables). Regarding the correlation coefficients between the independent variables, an acceptable

TABLE 2 | Correlations between HRV and cognitive parameters.

	Mean HR	Mean RR	SDNN	RMSSD	LF (%)	LF (n.u.)	HF (%)	HF (n.u.)	Total power	LF/HF	SD1	SD2	SD2
N-back													
Hit 2-back	−0.050	0.075	−0.023	0.082	−0.026	0.019	−0.022	−0.022	−0.070	−0.037	0.085	−0.087	−0.101
Hit N-back	−0.115	0.128	0.070	0.132	−0.090	−0.016	−0.009	0.016	0.004	−0.094	0.132	−0.019	−0.117
Miss N-back	0.406*	−0.383*	0.123	0.246	−0.204	−0.250	0.258	0.255	0.012	−0.256	0.248	0.010	−0.189
Stroop													
Latency C_A	0.153	−0.118	0.264	0.130	0.024	−0.027	0.054	0.031	0.182	−0.022	0.124	0.217	0.009
Latency C_B	−0.166	0.164	0.082	0.296	−0.052	−0.125	0.165	0.131	−0.018	−0.123	0.292	−0.050	−0.242
Latency RT C_A	−0.207	0.218	0.217	0.193	−0.008	−0.051	0.065	0.050	0.129	−0.045	0.188	0.142	−0.070
Latency RT C_B	−0.244	0.244	0.137	0.285	−0.037	−0.071	0.106	0.080	−0.069	−0.071	0.283	0.008	−0.226
Cards of Wisconsin													
Misses	0.109	−0.095	0.277	0.476*	−0.302	−0.386*	0.426*	0.389*	0.229	−0.379	0.473*	0.127	−0.465*
Perseverative Misses	0.106	−0.103	0.067	0.229	−0.095	−0.175	0.209	0.170	0.074	−0.192	0.228	0.011	−0.340

*$p < 0.05$.

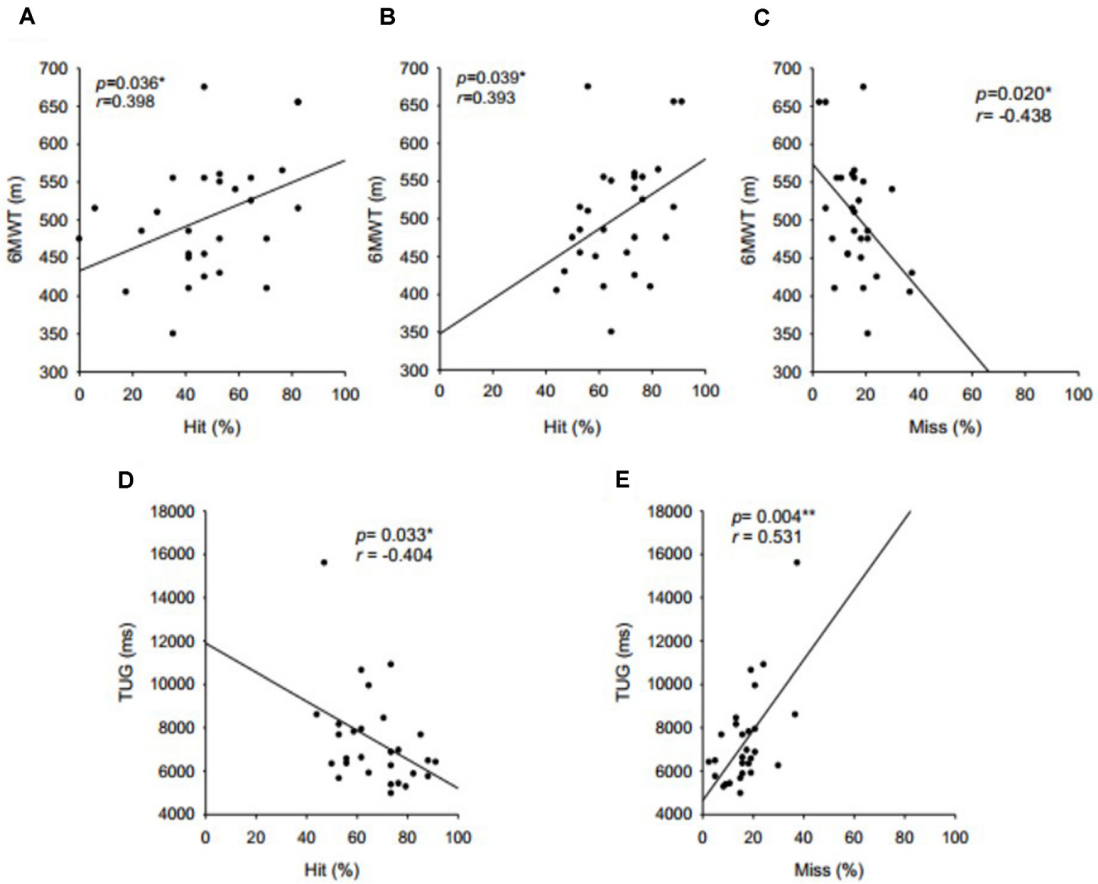


FIGURE 1 | Correlations between working memory, aerobic capacity, and dynamic balance. **(A)** Hits in 2-back level and walk, **(B)** total hits in N-back test and walk, **(C)** misses in N-back and walk, **(D)** total hits in N-back test and TUG, and **(E)** misses in N-back and walk.

TABLE 3 | Correlation between HRV and physical parameters.

	6MWT	TUG	Strength	Flexibility
Mean HR	−0.198	0.018	−0.173	0.071
Mean RR	0.215	−0.014	0.172	−0.099
SDNN	0.058	0.068	−0.301	0.058
RMSSD	0.148	0.130	−0.227	0.148
LF (%)	−0.027	−0.044	−0.134	0.151
LF (n.u.)	−0.057	−0.012	−0.121	0.044
HF (%)	0.090	−0.007	0.097	−0.018
HF (n.u.)	0.058	0.025	0.119	−0.043
Total power	0.025	−0.014	−0.153	0.018
LF/HF	−0.074	−0.015	−0.118	0.069
SD1	0.157	0.123	−0.218	0.138
SD2	0.031	−0.003	−0.231	−0.054
SD1/SD2	−0.164	0.054	0.063	−0.150

no correlation was statistically significant.

variation was verified regarding the absence of multicollinearity (correlation coefficient ranging from −0.327 to 0.406). The Durbin-Watson's diagnosis of independent residue verification was 2.458 and confirmed the quality of the model.

We observed that both models had moderate correlation coefficients ($0.406 < r < 0.530$), and the coefficients values increased by including more independent variables to the model (Model 2). Similarly, R^2 indicates that the percentage change in the dependent variable explained by the independent variables increased as they were input into the model ($0.165 < R^2 < 0.281$), suggesting that model 2, associated with N-back misses and AC latency in the Stroop test account for approximately 28% of the variation in mean HR. The change statistics revealed that the construction of the two models sequentially improved the model prediction, indicating statistical significance for the insertion of an independent variable in relation to a model without predictors ($p = 0.032$), improving the model by approximately 16% ($p = 0.016$; **Table 4**).

TABLE 4 | Impact analysis, change statistics and analysis of variance for models involving mean HR and cognition.

			Change statistics			ANOVA	
Models	***r***	***R*²**	***R*²**	***F***	***sig* F**	***F***	***p***
1	0.406	0.165	0.165	5.144	0.032*	5.144	0.032*
2	0.530	0.281	0.116	4.039	0.049*	4.892	0.016*

**Model 1: mean HR (dependent variable) × Miss Nback (independent variable). *Model 2: mean HR (dependent variable) × Miss Nback Latency C-A (independent variable). P < 0.05*.*

TABLE 5 | Regression coefficient for models involving mean HR variability and cognition variables.

				Collinearity	
Models	**Std.Coeff.**	***t***	***sig***	**Tolerance**	**VIF**
1					
Miss Nback	0.406	2.268	0.032*	1.000	1.000
2					
Miss Nback	0.496	2.838	0.009*	0.936	1.096
Latency C-A	0.352	2.010	0.049*	0.936	1.069

**Model 1: mean HR (dependent variable) × Miss Nback (independent variable). *Model 2: mean HR (dependent variable) × Miss Nback Latency C-A (independent variable). P < 0.05*.*

TABLE 6 | Impact analysis, change statistics and analysis of variance for the model involving miss N-back and mean RR.

			Change statistics			ANOVA	
Model	***r***	R^2	R^2	***F***	***sig* F**	***F***	***p***
1	0.383	0.147	0.147	4.472	0.044*	4.472	0.044*

**Model 1: mean_RR (dependent variable) × Miss Nback (independent variable). P < 0.05*.*

TABLE 7 | Regression coefficient for the model involving miss N-back and mean RR.

				Cofllinearity	
Model	**Std.Coeff.**	***t***	***sig***	**tolerance**	**VIF**
1					
Miss N-back	−0.383	−2.115	0.044*	1.000	1.000

**Model 1: mean HR (dependent variable) × Miss N-back (independent variable). P < 0.05*.*

Finally, ANOVA confirms that the fit of the model with predictors is better than the fit without predictors ($p < 0.032$).

Table 5 shows that the regression coefficients of both models have a significant impact on the understanding of the relationships between the independent and dependent variables. Model 2 shows that the significance values are all below 0.049, confirming the relevance of the variables N-back misses and C-A Stroop Latency for the model. It is noteworthy that the standardized coefficients reveal that the variable "N-back miss" has greater relevance to the model (coefficient = 0.496) when compared to "C-A Stroop latency" (coefficient = 0.0352).

Multiple Regression Model for Mean RR

We evaluated the impact of the cognitive variable "N-back misses" (independent variables) on the HRV variable "Mean RR" (dependent variable). Regarding the correlation coefficients between the independent variables, an acceptable variation was found regarding the absence of multicollinearity (correlation coefficient ranging from −0.383 to 0.045). The Durbin-Watson's diagnosis was 2.419, confirming the quality of the model.

Table 6 presents the analysis of the correlation coefficients and impact of N-back misses on mean RR, as well as the change coefficients and the ANOVA value for the model. The correlation coefficient of the model was moderate ($r = 0.383$) and R^2 (0.147) indicates that the percentage variation of mean RR explained by N-back was approximately 14%.

Table 7 shows that the regression coefficient has a significant impact on the understanding of the relationships between the independent variable and the dependent variable.

DISCUSSION

The purpose of the present study was to evaluate how physical fitness contributes to executive performance in the context of the NVI model in elderly subjects. Normal physiological aging is known to increase the probability of the occurrence of pathological changes in the brain (Fjell et al., 2014) which are associated with different trajectories of cognitive performance in the elderly, including dementia (Sorond et al., 2015). Even though recent studies have shown the benefits of physical exercise and the positive influence of increased aerobic capacity on mitigating the deleterious effects of aging on cognitive measures (Kramer et al., 2006; Miller et al., 2012), many questions remain regarding the relative impact of different exercise modalities and the indirect role played by cardiovascular improvements on those measures. While a recent study Alderman and Olson (2014) demonstrated the positive role of aerobic capacity in both autonomic and cognitive measures in young adults, the authors were not able to show a correlation between these outcomes with changes in HRV, suggesting that brain-cardiovascular interactions may not be the most important mediators of change in this population.

The physical fitness of our sample was within the expected values for their age, when compared to normative values available in the literature (for references see: Innes, 1999; Rikli and Jones, 1999b; Mazo et al., 2015). Our subjects also performed comparably to other reports in the literature that used the same working memory tasks (Vermeij et al., 2014). The only exception was the number of hits in the N-back test, which was considerably lower, probably due to cultural specificities and the low educational level of our sample when compared to other studies. Our results also corroborated previous findings on the relationship between cognitive performance and physical fitness (Hansen et al., 2004; Erickson and Kramer, 2009; Erickson et al., 2009; Albinet et al., 2010; Chang et al., 2012; Soares-Miranda et al., 2014; Håkansson et al., 2017; Northey et al., 2018). Specifically, we demonstrated that working memory is associated with the performance in the 6-min walk and TUG tests, which are reliable indicators of aerobic capacity and dynamic balance in the elderly (**Figure 1**). These results are in agreement with other studies that showed a direct correlation between aerobic capacity and performance on tasks relying on executive functions (Kramer and Erickson, 2007; Alderman and Olson, 2014; Dupuy et al., 2015, 2018). Hansen et al. (2004) found that after a period of 8 weeks of aerobic training individuals not only improved performance on cognitive tasks (working memory, sustained and selective attention), but that this result was accompanied by improvements in HRV. The authors then performed a detraining period and found that this manipulation caused a reduction in both cognitive performance and HRV, indicating that they were associated with physical fitness. However, another study that investigated the cognitive performance of athletes

throughout the sport season found that at times of large increased physical demand there was an associated increase in simple reaction time (slower cognitive processing) accompanied by reduced athletic performance, which indicated a dose-response relationship between exercise intensity and cognition (Matos et al., 2014). Surprisingly, these authors showed that changes in physical training did not induce significant changes in HRV, suggesting that other adjacent mechanisms may be involved in this interaction between cognition and physical capacity.

In our study, we also found a correlation between performance on cognitive tests and HRV (**Table 2**). For instance, there are positive correlations between N-back errors and mean HR and negative correlations between N-back errors and average RR intervals. This result indicates that higher parasympathetic activity is associated with better scores in working memory tasks. We also found a positive correlation between the number of errors in the WCST and heart indices associated with parasympathetic activity (RMSSD, percentage of HF, HF n.u and SD1) and a negative correlation between the number of errors with sympathetic activity (LF n.u. and SD2). This result leads us to consider that higher parasympathetic and lower sympathetic activity are associated with a decrease in behavioral flexibility. Other studies support the hypothesis that better performance in executive functions is associated with a higher parasympathetic activity as measured by HRV (Hansen et al., 2003; Duschek et al., 2009; Thayer et al., 2009). Apparently, this relationship between cognition and HRV is important for self-regulation under various behavioral conditions (Blasi et al., 2006; Thayer et al., 2009; Capuana et al., 2014; Mather and Thayer, 2018).

Regarding the hypothesis that HRV could act as a link between cognition and physical performance, none of our regression models showed a significant relationship between executive function and HRV-mediated physical fitness. These results reinforce the negative findings of a previous study that investigated whether the influence of cardiorespiratory fitness on cognition of young adults would be mediated by HRV (Alderman and Olson, 2014). The researchers attributed their findings to the low variability in the aerobic capacity of their young volunteers, indicating that studies in other age-groups could obtain different results. However, our results corroborated their findings, suggesting that other mechanisms not associated with the NVI model may be responsible for the relationship between cognition and physical fitness in the elderly. The list of possible mediators includes neurotrophin levels, such as BDNF, and changes in CBF due to regular physical exercise (Dinoff et al., 2016).

Cardiovascular adaptations that positively influence HRV are generally attributed in the literature to the practice of aerobic exercises (Carter et al., 2003). However, other training methods such as resistance training, are also shown to promote cognitive improvements in the elderly (Liu-Ambrose et al., 2010; Bherer et al., 2013; Kattenstroth et al., 2013; Gajewski and Falkenstein, 2016; Müller et al., 2017; Northey et al., 2018; Lin et al., 2019). Chang et al. (2012) reviewed the effect of resistance training on elderly cognition and proposed IGF-1-mediated increases in neurogenesis, vascular density and glucose utilization in the brain as possible mediators. In addition, BDNF and VEGF (vascular endothelial growth factor) are also suggested as possible targets for exercise-related brain adaptations, which would explain the absence of correlation between HRV and physical fitness shown in our study. Our results also showed a lack of relationship between strength capacity and cognitive performance, pointing to the need for further studies exploring this association.

Age-related impairments in cognition, especially executive functions, are also associated with reduced HRV, probably due to the detrimental effects of aging on vagal activity (Britton et al., 2008; Frewen et al., 2013; Forte et al., 2019). The vagal tank theory (VTT) proposed by Laborde et al. (2018), uses the metaphor of a "vagal tank," the level of which is determined by changes in cardiac vagal control activity and determines the efficiency of self-regulatory adaptations to cognitive, social, and emotional challenges. According to the VTT, risk of abnormal HRV, which is a measure of cardiac vagal control, is the impaired ability to cope with physical and emotional demands in daily life activities. One alternative to counteract these effects would be to engage in regular physical exercise, which improves both the basal "tank" volume and vagal reactivity (Hottenrott et al., 2019).

Even though it is widely known that physical activity improves cognitive and brain functions, there is still no consensus on the magnitude of its effects and which are the best strategies and adequate dosage (duration, intensity, and frequency; Kramer et al., 2005; Hillman et al., 2008; Erickson et al., 2019). Physical activity can promote either nonspecific or specific cognitive improvements, depending on the type of exercise, with executive functions being the most benefited (Gajewski and Falkenstein, 2016; Erickson et al., 2019). According to the Physical Activity, Cognition, and Brain Outcomes of the American College of Sports Medicine, training programs involving different components such as endurance, strength and coordination, from moderate to vigorous intensity over the long term, are the most adequate to provide cognitive benefits to older populations (>50 years; Erickson et al., 2019).

In summary, our results are consistent with the literature regarding the relationship between cognition and physical fitness. Our results also support the NVI model, now tested in elderly individuals, demonstrating the association between executive function performance and HR. However, the hypothesis of HRV acting as a link between cognition and physical fitness was not confirmed in our sample, which indicates that age-related degeneration of autonomic control can affect aspects of NVI in this population.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on reasonable request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Permanent Committee on Human

Research Ethics—COPEP, State University of Maringá. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FM and AP designed the study and wrote the manuscript. AV and FM collected the data. AV, FM, WG, WL, and AP analyzed the data. All authors read and commented on the manuscript.

ACKNOWLEDGMENTS

We thank CREAS (Reference Center Specializing in Social Assistance) from Lidianópolis (PR), in particular to Mrs. Lucia de Jesus Maia Buzato, Coordinator of Social Assistance.

REFERENCES

Albinet, C. T., Abou-Dest, A., André, N., and Audiffren, M. (2016). Executive functions improvement following a 5-month aquaerobics program in older adults: role of cardiac vagal control in inhibition performance. *Biol. Psychol.* 115, 69–77. doi: 10.1016/j.biopsycho.2016.01.010

Albinet, C. T., Boucard, G., Bouquet, C. A., and Audiffren, M. (2010). Increased heart rate variability and executive performance after aerobic training in the elderly. *Eur. J. Appl. Physiol.* 109, 617–624. doi: 10.1007/s00421-010-1393-y

Alderman, B. L., and Olson, R. L. (2014). The relation of aerobic fitness to cognitive control and heart rate variability: a neurovisceral integration study. *Biol. Psychol.* 99, 26–33. doi: 10.1016/j.biopsycho.2014.02.007

Al-Jarrah, M., Jamous, M., Al Zailaey, K., and Bweir, S. O. (2010). Endurance exercise training promotes angiogenesis in the brain of chronic/progressive mouse model of Parkinson's disease. *NeuroRehabilitation* 26, 369–373. doi: 10.3233/nre-2010-0574

Almeida, O. P. (1998). Mini exame dos estado mental e o diagnóstico de demência no brasil. *Arq. Neuropsiquiatr.* 56, 605–612. doi: 10.1590/s0004-282x1998000400014

Benarroch, E. E. (1993). The central autonomic network: functional organization, dysfunction, and perspective. *Mayo Clinic Proceedings* 68, 988–1001. doi: 10.1016/s0025-6196(12)62272-1

Benarroch, E. E. (2012). "Central autonomic control," in *Primer on the Autonomic Nervous System*, 3rd Edn. eds D. Robertson, I. Bioggioni, G. Burnstock, P. A. Low and J. F. R. Paton (Cambridge: Academic press), 9–12.

Bherer, L., Erickson, K. I., and Liu-Ambrose, T. (2013). A review of the effects of physical activity and exercise on cognitive and brain functions in older adults. *J. Aging Res.* 2013:657508. doi: 10.1155/2013/657508

Blasi, G., Goldberg, T. E., Weickert, T., Das, S., Kohn, P., Zoltick, B., et al. (2006). Brain regions underlying response inhibition and interference monitoring and suppression. *Eur. J. Neurosci.* 23, 1658–1664. doi: 10.1111/j.1460-9568.2006.04680.x

Bloor, C. M. (2005). Angiogenesis during exercise and training. *Angiogenesis* 8, 263–271. doi: 10.1007/s10456-005-9013-x

Britton, A., Singh-Manoux, A., Hnatkova, K., Malik, M., Marmot, M. G., and Shipley, M. (2008). The association between heart rate variability and cognitive impairment in middle-aged men and women. *Neuroepidemiology* 31, 115–121. doi: 10.1159/000148257

Capuana, L. J., Dywan, J., Tays, W. J., Elmers, J. L., Witherspoon, R., and Segalowitz, S. J. (2014). Factors influencing the role of cardiac autonomic regulation in the service of cognitive control. *Biol. Psychol.* 102, 88–97. doi: 10.1016/j.biopsycho.2014.07.015

Carter, J. B., Banister, E. W., and Blaber, A. P. (2003). Effect of endurance exercise on autonomic control of heart rate. *Sports Med.* 33, 33–46. doi: 10.2165/00007256-200333010-00003

Chang, Y.-K., Pan, C.-Y., Chen, F.-T., Tsai, C.-L., and Huang, C.-C. (2012). Effect of resistance-exercise training on cognitive function in healthy older adults: a review. *J. Aging Phys. Act.* 20, 497–517. doi: 10.1123/japa.20.4.497

Christie, G. J., Hamilton, T., Manor, B. D., Farb, N. A. S., Farzan, F., Sixsmith, A., et al. (2017). Do lifestyle activities protect against cognitive decline in aging? A review. *Front. Aging Neurosci.* 9:381. doi: 10.3389/fnagi.2017.00381

Dinoff, A., Herrmann, N., Swardfager, W., Liu, C. S., Sherman, C., Chan, S., et al. (2016). The effect of exercise training on resting concentrations of peripheral brain-derived neurotrophic factor (BDNF): a meta-analysis. *PLoS One* 11:e0163037. doi: 10.1371/journal.pone.0163037

Dupuy, O., Billaut, F., Raymond, F., Benraiss, A., Theurot, D., Bosquet, L., et al. (2018). Effect of acute intermittent exercise on cognitive flexibility: the role of exercise intensity. *J. Cogn. Enhanc.* 2, 146–156. doi: 10.1007/s41465-018-0078-z

Dupuy, O., Gauthier, C. J., Fraser, S. A., Desjardins-Crèpeau, L., Desjardins, M., Mekary, S., et al. (2015). Higher levels of cardiovascular fitness are associated with better executive function and prefrontal oxygenation in younger and older women. *Front. Hum. Neurosci.* 9:66. doi: 10.3389/fnhum.2015.00066

Duschek, S., Muckenthaler, M., Werner, N., and del Paso, G. A. R. (2009). Relationships between features of autonomic cardiovascular control and cognitive performance. *Biol. Psychol.* 81, 110–117. doi: 10.1016/j.biopsycho.2009.03.003

Erickson, K. I., and Kramer, A. F. (2009). Aerobic exercise effects on cognitive and neural plasticity in older adults. *Br. J. Sports Med.* 43, 22–24. doi: 10.1136/bjsm.2008.052498

Elias, M. F., and Torres, R. V. (2017). The renaissance of heart rate variability as a predictor of cognitive functioning. *Am. J. Hypertens.* 31, 21–23. doi: 10.1093/ajh/hpx150

Erickson, K. I., Hillman, C., Stillman, C. M., Ballard, R. M., Bloodgood, B., Conroy, D. E., et al. (2019). Physical activity, cognition, and brain outcomes: a review of the 2018 physical activity guidelines. *Med. Sci. Sports Exerc.* 51, 1242–1251. doi: 10.1249/mss.0000000000001936

Erickson, K. I., Leckie, R. L., and Weinstein, A. M. (2014). Physical activity, fitness, and gray matter volume. *Neurobiol. Aging* 35, S20–S28. doi: 10.1016/j.neurobiolaging.2014.03.034

Erickson, K. I., Prakash, R. S., Voss, M. W., Chaddock, L., Hu, L., Morris, K. S., et al. (2009). Aerobic fitness is associated with hippocampal volume in elderly humans. *Hippocampus* 19, 1030–1039. doi: 10.1002/hipo.20547

Erickson, K. I., Voss, M. W., Prakash, R. S., Basak, C., Szabo, A., Chaddock, L., et al. (2011). Exercise training increases size of hippocampus and improves memory. *Proc. Natl. Acad. Sci. U S A* 108, 3017–3022. doi: 10.1073/pnas.1015950108

Ernst, G. (2017). Heart-rate variability—more than heart beats? *Front. Public Health* 5:240. doi: 10.3389/fpubh.2017.00240

Fjell, A. M., McEvoy, L., Holland, D., Dale, A. M., and Walhovd, K. B. (2014). What is normal in normal aging? Effects of aging, amyloid and Alzheimer's disease on the cerebral cortex and the hippocampus. *Prog. Neurobiol.* 117, 20–40. doi: 10.1016/j.pneurobio.2014.02.004

Forte, G., Favieri, F., and Casagrande, M. (2019). Heart rate variability and cognitive function: a systematic review. *Front. Neurosci.* 13:710. doi: 10.3389/fnins.2019.00710

Frewen, J., Finucane, C., Savva, G. M., Boyle, G., Coen, R. F., and Kenny, R. A. (2013). Cognitive function is associated with impaired heart rate variability in ageing adults: the Irish longitudinal study on ageing wave one results. *Clin. Auton. Res.* 23, 313–323. doi: 10.1007/s10286-013-0214-x

Gajewski, P. D., and Falkenstein, M. (2016). Physical activity and neurocognitive functioning in aging—a condensed updated review. *Eur. Rev. Aging Phys. Act.* 13:1. doi: 10.1186/s11556-016-0161-3

Gomez-Pinilla, F., and Hillman, C. (2013). The influence of exercise on cognitive abilities. *Compr. Physiol.* 3, 403–428. doi: 10.1002/cphy.c110063

Grant, D. A., and Berg, E. (1948). A behavioral analysis of degree of reinforcement and ease of shifting to new responses in a weigl-type card-sorting problem. *J. Exp. Psychol.* 38, 404–411. doi: 10.1037/h0059831

Guarino, A., Favieri, F., Boncompagni, I., Agostini, F., Cantone, M., and Casagrande, M. (2019). Executive functions in Alzheimer disease: a systematic review. *Front. Aging Neurosci.* 10:437. doi: 10.3389/fnagi.2018.00437

Håkansson, K., Ledreux, A., Daffner, K., Terjestam, Y., Bergman, P., Carlsson, R., et al. (2017). BDNF responses in healthy older persons to 35 minutes of physical exercise, cognitive training, and mindfulness: associations with working memory function. *J. Alzheimers Dis.* 55, 645–657. doi: 10.3233/JAD-160593

Hansen, A. L., Johnsen, B. H., and Thayer, J. F. (2003). Vagal influence on working memory and attention. *Int. J. Psychophysiol.* 48, 263–274. doi: 10.1016/s0167-8760(03)00073-4

Hansen, A. L., Johnsen, B. H., Sollers, J. J. III., Stenvik, K., and Thayer, J. F. (2004). Heart rate variability and its relation to prefrontal cognitive function: the effects of training and detraining. *Eur. J. Appl. Physiol.* 93, 263–272. doi: 10.1007/s00421-004-1208-0

Hottenrott, L., Ketelhut, S., and Hottenrott, K. (2019). Commentary: Vagal Tank Theory: The Three Rs of Cardiac Vagal Control Functioning–Resting, Reactivity, and Recovery. *Front. Neurosci.* 13:1300. doi: 10.3389/fnins.2019.01300

Hillman, C. H., Erickson, K. I., and Kramer, A. F. (2008). Be smart, exercise your heart: exercise effects on brain and cognition. *Nat. Rev. Neurosci.* 9, 58–65. doi: 10.1038/nrn2298

Innes, E. (1999). Handgrip strength testing: a review of the literature. *Aust. Occup. Ther. J.* 46, 120–140. doi: 10.1046/j.1440-1630.1999.00182.x

Jandackova, V. K., Scholes, S., Britton, A., and Steptoe, A. (2016). Are changes in heart rate variability in middle-aged and older people normative or caused by pathological conditions? Findings from a large population-based longitudinal cohort study. *J. Am. Heart Assoc.* 5:e002365. doi: 10.1161/jaha.115.002365

Jennings, J. R., Allen, B., Gianaros, P. J., Thayer, J. F., and Manuck, S. B. (2015). Focusing neurovisceral integration: cognition, heart rate variability, and cerebral blood flow. *Psychophysiology* 52, 214–224. doi: 10.1111/psyp.12319

Kattenstroth, J.-C., Kalisch, T., Holt, S., Tegenthoff, M., and Dinse, H. R. (2013). Six months of dance intervention enhances postural, sensorimotor, and cognitive performance in elderly without affecting cardio-respiratory functions. *Front. Aging Neurosci.* 5:5. doi: 10.3389/fnagi.2013.00005

Kramer, A. F., Colcombe, S. J., McAuley, E., Scalf, P. E., and Erickson, K. I. (2005). Fitness, aging and neurocognitive function. *Neurobiol. Aging* 26, 124–127. doi: 10.1016/j.neurobiolaging.2005.09.009

Kramer, A. F., and Erickson, K. I. (2007). Effects of physical activity on cognition, well-being and brain: human interventions. *Alzheimers Dement.* 3, S45–S51. doi: 10.1016/j.jalz.2007.01.008

Kramer, A. F., Erickson, K. I., and Colcombe, S. J. (2006). Exercise, cognition, and the aging brain. *J. Appl. Physiol.* 101, 1237–1242. doi: 10.1152/japplphysiol.00500.2006

Laborde, S., Mosley, E., and Mertgen, A. (2018). Vagal tank theory: the three rs of cardiac vagal control functioning—resting, reactivity, and recovery. *Front. Neurosci.* 12:458. doi: 10.3389/fnins.2018.00458

Leckie, R. L., Oberlin, L. E., Voss, M. W., Prakash, R. S., Szabo-Reed, A., Chaddock-Heyman, L., et al. (2014). BDNF mediates improvements in executive function following a 1-year exercise intervention. *Front. Hum. Neurosci.* 8:985. doi: 10.3389/fnhum.2014.00985

Lin, S., Yang, Y., Qi, Q., Wei, L., Jing, N., Jie, Z., et al. (2019). The beneficial effect of physical exercise on cognitive function in a non-dementia aging chinese population. *Front. Aging Neurosci.* 11:238. doi: 10.3389/fnagi.2019.00238

Liu-Ambrose, T., Nagamatsu, L. S., Graf, P., Beattie, B. L., Ashe, M. C., and Handy, T. C. (2010). Resistance training and executive functions: a 12-month randomized controlled trial. *Arch. Intern. Med.* 170, 170–178. doi: 10.1001/archinternmed.2009.494

MacPherson, S. E., Phillips, L. H., and Della Sala, S. (2002). Age, executive function, and social decision making: a dorsolateral prefrontal theory of cognitive aging. *Psychol. Aging* 17, 598–609. doi: 10.1037/0882-7974.17.4.598

Mather, M., and Thayer, J. (2018). How heart rate variability affects emotion regulation brain networks. *Curr. Opin. Behav. Sci.* 19, 98–104. doi: 10.1016/j.cobeha.2017.12.017

Matos, F. O., Samulski, D. M., Lima, J. R. P., and Prado, L. S. (2014). Cargas elevadas de treinamento alteram funções cognitivas em jogadores de futebol. *Rev. Br. Med. Esporte* 20, 388–392. doi: 10.1590/1517-869220142005 01274

Mazo, G. Z., Petreça, D. R., Sandreschi, P. F., and Benedetti, T. R. B. (2015). Valores normativos da aptidão física para idosas brasileiras de 60 a 69 anos de idade. *Rev. Br. Med. Esporte* 21, 318–322. doi: 10.1590/1517-86922015210 4134470

Miller, D. I., Taler, V., Davidson, P. S. R., and Messier, C. (2012). Measuring the impact of exercise on cognitive aging: methodological issues. *Neurobiol. Aging* 33, 622.e29–622.e43. doi: 10.1016/j.neurobiolaging.2011.02.020

Müller, P., Rehfeld, K., Schmicker, M., Hökelmann, A., Dordevic, M., Lessmann, V., et al. (2017). Evolution of neuroplasticity in response to physical activity in old age: the case for dancing. *Front. Aging Neurosci.* 9:56. doi: 10.3389/fnagi.2017.00056

Northey, J. M., Cherbuin, N., Pumpa, K. L., Smee, D. J., and Rattray, B. (2018). Exercise interventions for cognitive function in adults older than 50: a systematic review with meta-analysis. *Br. J. Sports Med.* 52, 154–160. doi: 10.1136/bjsports-2016-096587

Oishi, E., Ohara, T., Sakata, S., Fukuhara, M., Hata, J., Yoshida, D., et al. (2017). Day-to-day blood pressure variability and risk of dementia in a general japanese elderly population: the hisayama study. *Circulation* 136, 516–525. doi: 10.1161/CIRCULATIONAHA.116.025667

Peters, R. (2006). Ageing and the brain. *Postgrad. Med. J.* 82, 84–88. doi: 10.1136/pgmj.2005.036665

Phillips, C., Baktir, M. A., Srivatsan, M., and Salehi, A. (2014). Neuroprotective effects of physical activity on the brain: a closer look at trophic factor signaling. *Front. Cell. Neurosci.* 8:170. doi: 10.3389/fncel.2014.00170

Power, M. C., Mormino, E., Soldan, A., James, B. D., Yu, L., Armstrong, N. M., et al. (2018). Combined neuropathological pathways account for age-related risk of dementia. *Ann. Neurol.* 84, 10–22. doi: 10.1002/ana.25246

Rikli, R. E., and Jones, C. J. (1999a). Development and validation of a functional fitness test for community-residing older adults. *J. Aging Phys. Act.* 7, 129–161. doi: 10.1123/japa.7.2.129

Rikli, R. E., and Jones, C. J. (1999b). Functional fitness normative scores for community-residing older adults, ages 60–94. *J. Aging Phys. Act.* 7, 162–181. doi: 10.1123/japa.7.2.162

Saul, J. (1990). Beat-To-beat variations of heart rate reflect modulation of cardiac autonomic outflow. *Physiology* 5, 32–37. doi: 10.1152/physiologyonline.1990.5.1.32

Sherwood, C. C., Gordon, A. D., Allen, J. S., Phillips, K. A., Erwin, J. M., Hof, P. R., et al. (2011). Aging of the cerebral cortex differs between humans and chimpanzees. *Proc. Natl. Acad. Sci. U S A* 108, 13029–13034. doi: 10.1073/pnas.1016709108

Soares-Miranda, L., Sattelmair, J., Chaves, P., Duncan, G., Siscovick, D. S., Stein, P. K., et al. (2014). Physical activity and heart rate variability in older adults: the cardiovascular health study. *Circulation* 129, 2100–2110. doi: 10.1161/CIRCULATIONAHA.113.005361

Sorond, F. A., Cruz-Almeida, Y., Clark, D. J., Viswanathan, A., Scherzer, C. R., De Jager, P., et al. (2015). Aging, the central nervous system and mobility in older adults: neural mechanisms of mobility impairment. *J. Gerontol. A Biol. Sci. Med. Sci.* 70, 1526–1532. doi: 10.1093/gerona/glv130

Spreen, O., and Strauss, E. (1998). *A Compendium of Neuropsychological Tests: Administration, Norms, and Commentary.* 2nd Edn. New York, NY: Oxford University Press.

Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. (1996). Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation* 93, 1043–1065. doi: 10.1161/01.cir.93.5.1043

Thayer, J. F., Hansen, A. L., Saus-Rose, E., and Johnsen, B. H. (2009). Heart rate variability, prefrontal neural function and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation and health. *Ann. Behav. Med.* 37, 141–153. doi: 10.1007/s12160-009-9101-z

Thayer, J. F., and Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disord.* 61, 201–216. doi: 10.1016/s0165-0327(00)00338-4

Valenza, G., Sclocco, R., Duggento, A., Passamonti, L., Napadow, V., Barbieri, R., et al. (2019). The central autonomic network at rest: uncovering functional MRI correlates of time-varying autonomic outflow. *NeuroImage* 197, 383–390. doi: 10.1016/j.neuroimage.2019.04.075

Vecchio, L. M., Meng, Y., Xhima, K., Lipsman, N., Hamani, C., and Aubert, I. (2018). The neuroprotective effects of exercise: maintaining a healthy brain throughout aging. *Brain Plast.* 4, 17–52. doi: 10.3233/bpl-180069

Vermeij, A., van Beek, A. H. E. A., Reijs, B. L. R., Claassen, J. A. H. R., and Kessels, R. P. C. (2014). An exploratory study of the effects of spatial working-memory load on prefrontal activation in low- and high-performing elderly. *Front. Aging Neurosci.* 6:303. doi: 10.3389/fnagi.2014.00303

Voss, M. W., Erickson, K. I., Prakash, R. S., Chaddock, L., Kim, J. S., Alves, H., et al. (2013a). Neurobiological markers of exercise-related brain plasticity in older adults. *Brain Behav. Immun.* 28, 90–99. doi: 10.1016/j.bbi.2012.10.021

Voss, M. W., Heo, S., Prakash, R. S., Erickson, K. I., Alves, H., Chaddock, L., et al. (2013b). The influence of aerobic fitness on cerebral white matter integrity and cognitive function in older adults: results of a one-year exercise intervention. *Hum. Brain Mapp.* 34, 2972–2985. doi: 10.1002/hbm.22119

Zheng, W., Cui, B., Han, Y., Song, H., Li, K., He, Y., et al. (2019). Disrupted regional cerebral blood flow, functional activity and connectivity in Alzheimer's disease: a combined asl perfusion and resting state fMRI study. *Front. Neurosci.* 13:738. doi: 10.3389/fnins.2019.00738

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Aging Neuroscience

BRIEF RESEARCH REPORT
published: 20 March 2020
doi: 10.3389/fnagi.2020.00058

Endogenous Apelin Is Protective Against Age-Associated Loss of Retinal Ganglion Cells in Mice

Yuki Ishimaru[1]*, *Akihide Sumino*[1,2], *Fumiya Shibagaki*[1], *Akiko Yamamuro*[1], *Yasuhiro Yoshioka*[1] and *Sadaaki Maeda*[1]*

[1]Laboratory of Pharmacotherapeutics, Faculty of Pharmaceutical Sciences, Setsunan University, Hirakata, Japan,
[2]Laboratory of Food Chemistry, Yokohama University of Pharmacy, Yokohama, Japan

Age-associated loss of retinal ganglion cells (RGCs) causes visual deficits, but there is not yet any therapeutic agent to prevent the loss of these cells. Herein, we report that apelin, an endogenous peptide ligand of APJ receptor, is protective against the age-related loss of RGCs in mice. The mRNA expression of apelin was reduced in the retina of old mice compared with that in young mice, whereas retinal APJ expression increased with age. Immunofluorescence staining showed that APJ was present in RGCs and their surrounding cells expressed apelin. In addition, both functional and histological analyses demonstrated that apelin deficiency accelerated the loss of RGCs associated with age in mice. These results suggest that endogenous apelin plays a protective role against the degeneration of RGCs and that the apelinergic axis may be a new target for preventing age-related visual impairment.

Keywords: retinal ganglion cell (RGC), apelin, aging, knock-out, cell loss, apelin receptor (APJ)

OPEN ACCESS

Edited by:
Mario Bernardo-Filho,
Rio de Janeiro State University, Brazil

Reviewed by:
Victoria Maneu,
University of Alicante, Spain
Bernard Masri,
Institut National de la Santé et de la Recherche Médicale (INSERM), France

***Correspondence:**
Yuki Ishimaru
ishimaru@pharm.setsunan.ac.jp
Sadaaki Maeda
smaeda@pharm.setsunan.ac.jp

Received: *19 July 2019*
Accepted: *19 February 2020*
Published: *20 March 2020*

Citation:
Ishimaru Y, Sumino A, Shibagaki F, Yamamuro A, Yoshioka Y and Maeda S (2020) Endogenous Apelin Is Protective Against Age-Associated Loss of Retinal Ganglion Cells in Mice. Front. Aging Neurosci. 12:58. doi: 10.3389/fnagi.2020.00058

INTRODUCTION

Age-related visual impairments can occur even in the absence of recognized eye diseases (Trick, 1987; Langrová et al., 2008). Retinal ganglion cells (RGCs) are neurons that transmit visual information from the retina to the brain and are more vulnerable to age-related loss than other retinal neurons (Cavallotti et al., 2001; Neufeld and Gachie, 2003). Therefore, the identification of protective factors against RGC loss with age should aid in the development of drugs that prevent visual deficits in the elderly.

Apelin is an endogenous peptide ligand of the G protein-coupled receptor APJ (Tatemoto et al., 1998). APJ stimulation by apelin in neurons results in activation of pro-survival signaling pathways that can afford neuroprotection (O'Donnell et al., 2007; Zeng et al., 2010; Cook et al., 2011). We previously reported that apelin deficiency in mice accelerated the loss of motor neurons in amyotrophic lateral sclerosis (Kasai et al., 2011), which is an age-related neurodegenerative disease. In addition, our recent study showed that APJ is expressed in RGCs of adult mice, and apelin deficiency increases the loss of RGCs induced by N-methyl-D-aspartate (Ishimaru et al., 2017). Recently, it was reported that the deletion of apelin or APJ in mice exhibits enhanced cardiovascular, renal, and reproductive aging (Rai et al., 2017), suggesting that the apelin-APJ system has a crucial role in cell and tissue homeostasis.

In the present study, we investigated whether endogenous apelin plays a protective role against the loss of RGC with age.

MATERIALS AND METHODS

Mice

All mice experiments were performed in accordance with protocols approved by the Committee for the Ethical Use of Experimental Animals and the Safety Committee for Recombinant DNA Experiments at Setsunan University. Two- and 12-months-old male wild-type (WT) and apelin-knockout (KO) mice on a C57BL/6N background were used. The generation of apelin-KO mice was described previously (Kidoya et al., 2008).

Genotyping

Five microliters of the blood sample was collected from the mouse tail vein and incubated in 50 μl of 50 mM sodium hydroxide solution for 30 min at room temperature, followed by neutralization with 5.5 μl of 1 M Tris-HCl (pH 8.0) and centrifugation to remove the insoluble fraction. Polymerase chain reaction (PCR) was performed with a Thermal Cycler T100 (BIO-RAD Laboratories, Hercules, CA, USA) and a KOD-FX DNA polymerase (TOYOBO, Osaka, Japan). Genotyping for apelin deficiency was performed by using the primers described in the previous study (Kasai et al., 2008). Reactions initially were denatured at 94°C for 2 min followed by 30 cycles at 98°C for 10 s, 70.3°C for 2 min and a final extension at 70.3°C for 2 min. Amplicons were separated using 1% agarose gel and visualized under UV light after staining with ethidium bromide. Genotyping for the rd8 mutation was performed by using the primers and methods described in the study of Mattapallil et al. (2012); however, PCR reactions were carried out for 35 cycles at 94°C for 30 s, 55.7°C for 30 s, and 72°C for 30 s, Amplicons were separated using 3% agarose gel.

Real-Time Reverse-Transcription Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated from the retinal tissues by using the SV Total RNA Isolation System (Promega, Madison, WI, USA). One microgram of total RNA was reverse transcribed with M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) and random primers (Invitrogen). One-twentieth of the total cDNA (50 ng of equivalent RNA) was used in each amplification reaction. Real-time RT-PCR was performed with a Thermal Cycler Dice Real-Time System (Takara, Ohtsu, Japan) and THUNDERBIRD Probe qPCR Mix (TOYOBO) or THUNDERBIRD SYBR qPCR Mix (TOYOBO). The following TaqMan probe was used for real-time RT-PCR assays: apln (apelin), Mm00443562_m1; and APLNR (APJ), Mm00442191_s1 (Applied Biosystems, Foster City, CA, USA). Reactions initially were denatured at 95°C for 1 min followed by 45 cycles at 95°C for 5 s, and 60°C for 30 s, The sequences of the gene-specific primers used are as follows: 36B4 (encodes acidic ribosomal phosphoprotein PO; forward, 5′-CACTGGTCTAGG ACCCGAGAAG-3′; reverse, 5′-GGTGCCTCTGGAGATTTT CG-3′) and glutamate aspartate transporter (GLAST; forward, 5′-GATCGGAAACATGAAGGAGC-3′; reverse, 5′-CAAGAAG AGGATGCCCAGAG-3′). Reactions initially were denatured at 95°C for 1 min followed by 40 cycles at 95°C for 10 s, 55°C for 20 s, and 72°C for 20 s, A melting curve analysis was carried out after amplification to verify the accuracy of the amplicon formation.

Immunofluorescence

Mice were anesthetized with an intraperitoneal injection of a mixture of medetomidine (0.3 mg/kg), butorphanol (5 mg/kg), and midazolam (4 mg/kg) and then perfused with saline and 4% paraformaldehyde in phosphate buffer. Enucleated eyes were sequentially fixed for 24 h in 4% paraformaldehyde solution, embedded in paraffin, and cut sagittally into sections of 3-μm thickness through the cornea and parallel to the optic nerve. The antigens in the retinal sections were retrieved by microwave heating in Tris-EDTA buffer (pH 8.0) containing 0.05% Tween-20. Then the sections were exposed to Tris-buffered saline (pH 7.4) including 0.5 or 5% skim milk, 40 μg/ml Fab fragment goat anti-mouse IgG (115-007-003, Jackson ImmunoResearch Laboratories Inc., West Grove, PA, USA), and 0.5% Triton-X 100 and then incubated with mouse anti-Brn-3a (sc-8429, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA, 1:50), rabbit anti-APJ (Kasai et al., 2010; 1:50), and mouse anti-apelin (013-25871, Wako Pure Chemical Ind., Limited, Osaka, Japan, 1:100) antibodies. The secondary antibody used was biotinylated goat anti-mouse IgG antibody (E0433, DAKO Corp., Carpinteria, CA, USA, 1:500). The biotinylated antibody was detected with streptavidin-conjugated with FITC (554060, BD Biosciences, San Diego, CA, USA, 1:500) or conjugated with Alexa Fluor 568 (S11226, Invitrogen, 1:500). Anti-APJ antibody was labeled with FITC by using a fluorescein labeling kit (DOJINDO Lab, Kumamoto, Japan). Nuclei were detected with Hoechst33342 (Sigma–Aldrich, St. Louis, MO, USA, 1:1,000). Photographs were taken with a fluorescence microscope (AZ-100M, Nikon, Tokyo, Japan). The number of RGCs for each eye was determined by counting the number of Brn-3a-positive cells per retinal section and averaging results from six sections per individual.

Electroretinography

Electroretinography was performed as previously described (Ishimaru et al., 2017). In brief, dark-adapted mice were anesthetized with a mixture of medetomidine (0.3 mg/kg), butorphanol (5 mg/kg), and midazolam (4 mg/kg), and then treated with a mydriatic agent (Mydrin P; Santen Pharmaceutical Company Limited, Osaka, Japan) and a corneal anesthetic drug (Benoxil 0.4% solution; Santen Pharmaceutical Company Limited, Osaka, Japan). A different electrode, an indifferent electrode, and a ground electrode were placed on the cornea, mouth, and tail of mice, respectively. Electroretinograms were evoked by a white light flash (3.0×10^{-5} cd·s/m^2) with an LS-100 (Mayo; Nagoya, Japan), a light-emitting device, and were recorded with a corneal electrode and Powerlab data acquisition system (AD Instruments, Mountain View, CA, USA). The amplitudes of the STR were quantified by measuring from the baseline to the maximum peak of the waveforms.

Statistics

Differences between groups were analyzed using unpaired student's *t*-test (**Figures 1A,B**) or two-way ANOVA with

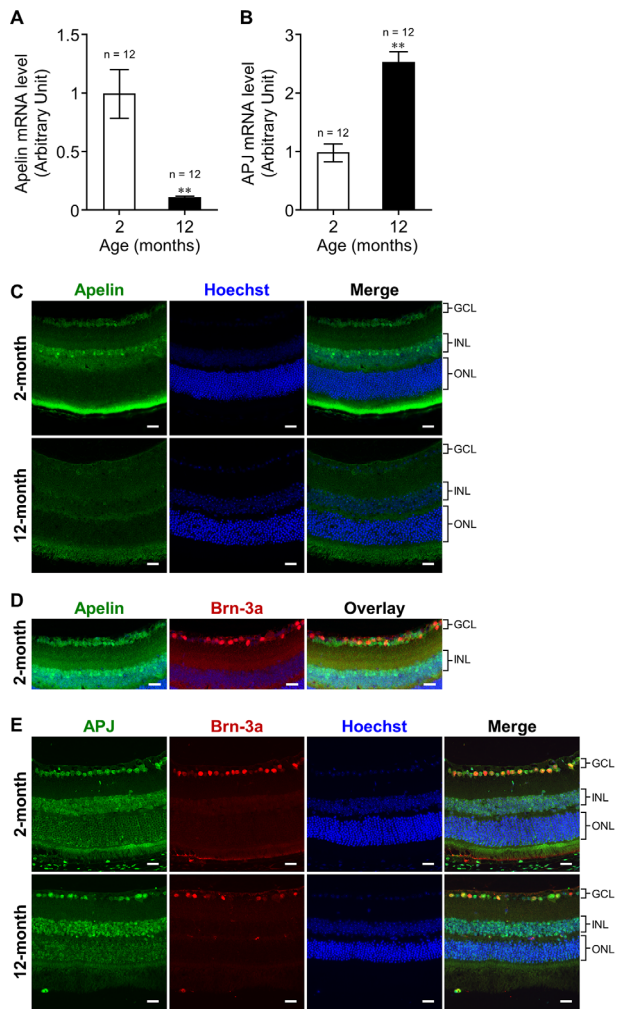


FIGURE 1 | Effect of aging on the expression of apelin and APJ in the retina of mice. **(A,B)** Apelin **(A)** and APJ **(B)** mRNA expression levels in the retinas of wild-type (WT) mice at 2 and 12 months of age were assessed by real-time polymerase chain reaction (RT-PCR; n = 12 mice per group). 36B4 (encodes acidic ribosomal phosphoprotein PO) was used as the internal standard to normalize mRNA levels. **(C,E)** Representative images show immunofluorescence of apelin **(C)** and double immunofluorescence of APJ and Brn-3a [an Retinal ganglion cell (RGC) marker; **E**] in retinal sections from WT mice at 2 and 12 months of age. **(D)** Immunostaining of apelin and Brn-3a was performed on adjacent sections from WT mice at 2 months of age. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer. Scale bars, 20 μm. Data were analyzed by unpaired student's t-test and represent the mean ± SEM. $^{**}p < 0.01$.

Bonferroni's *post hoc* test (**Figures 2B,D**). P-values < 0.05 were considered statistically significant. All results are expressed as the mean ± SEM.

RESULTS

Influence of Aging on Apelin and APJ Expressions in the Retina

To investigate the influence of aging on apelin and APJ expression in the retina, we measured their expression levels in the retinas of 2-months- and 12-months-old mice. Real-time RT-PCR demonstrated that the expression of retinal apelin mRNA was markedly lower in the old mice than that in the young ones (**Figure 1A**). In contrast, retinal APJ mRNA expression was significantly higher in the old mice than in the young ones (**Figure 1B**). To determine apelin and APJ expressing cells, we performed immunostaining for apelin and APJ in the retina of 2-months- and 12-months-old mice. Apelin immunoreactivity was observed in cells belonging to the ganglion cell layer (GCL) and cells located in the inner part of the inner nuclear layer (INL) in the retinas of 2-months- and 12-months-old mice (**Figure 1C**). The apelin immunoreactivities did not show overlap with Brn-3a- (an RGC marker) positive cells in adjacent sections (**Figure 1D**). APJ was detected in the Brn-3a-positive RGCs of the old mice, as well as in those of the young mice (**Figure 1E**).

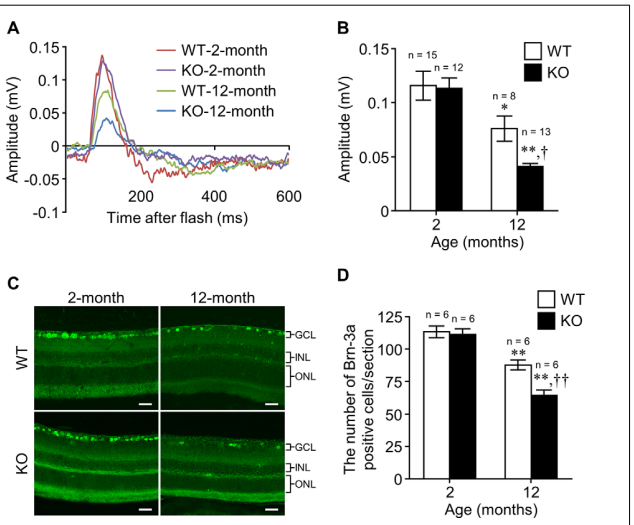


FIGURE 2 | Apelin deficiency accelerates the age-related loss of RGCs in mice. **(A)** Representative waveforms show the STR evoked by 3.0×10^{-5} cd·s/m^2 in the retinas of WT and apelin-knockout (KO) mice at 2 and 12 months of age. **(B)** The STR amplitudes were quantified by measuring from the baseline to the maximum peak in the waveforms (n = 15 mice per group for WT-2-months, n = 8 mice per group for WT-12-months, n = 12 mice per group for KO-2-months, n = 13 mice per group for KO-12-months). **(C)** Representative images show immunofluorescence of Brn-3a in retinal sections from WT and apelin-KO mice at 2 and 12 months of age. GCL, ganglion cell layer; INL, inner nuclear layer; ONL, outer nuclear layer. Scale bars, 20 μm. **(D)** The number of Brn-3a-positive cells per retinal section was counted (n = 6 mice per group). Data were analyzed by two-way ANOVA with Bonferroni's *post hoc* test and represent the mean ± SEM. $^{*}p < 0.05$ and $^{**}p < 0.01$ vs. 2-months, $^{\dagger}p < 0.05$ and $^{\dagger\dagger}p < 0.01$ vs. WT.

Apelin Deficiency Accelerates the Age-Related Loss of RGCs

To examine the effect of apelin deficiency on the decline of RGCs with age, we assessed the scotopic threshold response (STR), which mainly reflects the activity of RGCs determined by electroretinography, in WT and apelin-KO mice at 2 and 12 months of age. There was no significant difference in the STR between WT and apelin-KO mice at 2 months of age (**Figures 2A,B**). In contrast, the STR in the apelin-KO mice

at 12 months of age was markedly reduced compared with that in the age-matched WT mice (**Figures 2A,B**). Consistent with the results of the electroretinography, apelin deficiency enhanced the decrease in the number of Brn-3a-positive cells in the 12-months-old mice (**Figures 2C,D**). Eosin and Hematoxylin staining also showed that the number of the cells in the GCL was significantly decreased in aged apelin-KO mice (WT-2-months: 306.61 ± 1.93, KO-2-months: 293.11 ± 7.45, WT-12-months: 278.21 ± 1.01, KO-12-months: 246.14 ± 7.63).

DISCUSSION

Aging decreases the expression levels of apelin and APJ in multiple organs and tissues (Rai et al., 2017). In keeping with the report, apelin expression was markedly reduced in the retina of the old mice, indicating that senescence down-regulated the expression of apelin in the retina. Apelin immunoreactivity was detected in cells belonging to the GCL and cells located in the inner part of the INL, suggesting that the decrease of apelin expression is associated with the loss or dysfunction of cells present in the GCL and the loss of INL cells such as amacrine cells, which decrease with age-associated loss of RGCs (Samuel et al., 2011; Akopian et al., 2016). In contrast to apelin, the expression of APJ was up-regulated in the aging retina. Although we could not identify the retinal cells with increased expression of APJ with age, we showed that APJ was present in RGCs, whereas apelin was expressed in some cells surrounding RGCs in young mice. Moreover, we found that apelin deficiency in mice facilitated RGC degeneration with age in both histological and functional aspects. These results suggest that the up-regulation of APJ expression in the older mice might be a compensatory response to the decrease in apelin expression to promote RGC survival and that the apelin-APJ system contributes to RGC maintenance; however, further investigations are needed to elucidate the phenomenon.

Apelin deficiency facilitated the loss of RGCs in the old mice, but it did not affect that in the young ones, thus suggesting that chronic lack of apelin renders RGCs vulnerable to age-induced cell loss. Recent studies showed that long-term administration of apelin from periphery suppresses age-related cardiac hypertrophy and the degenerative loss of skeletal muscle (Rai et al., 2017; Vinel et al., 2018), indicating that apelin supplementation could rescue tissues and organs from senescence. In contrast to these tissues, the retina does not receive apelin from the bloodstream because of the blood-retinal barrier. Therefore, it is necessary to design APJ agonists that can cross the blood-retinal barrier and confirm their protective effect against the loss of RGCs with age in future work.

Although the data reported here provide that endogenous apelin plays a role in protecting RGCs against aging, the underlying mechanisms remain to be established. The loss of RGCs with age is considered to be induced by, at least in part, abnormal glutamate metabolism (Henneberry et al., 1989). Indeed, the expression of GLAST, which is a glutamate transporter expressed in Müller cells and regulates the synaptic activity in the inner retina, was markedly decreased in the aging retina (Young, 1.00 ± 0.08; Old, 0.14 ± 0.02). Our previous study showed that RGCs of apelin-KO mice are vulnerable to glutamate-induced excitotoxicity (Ishimaru et al., 2017). Therefore, endogenous apelin might protect against the age-related loss of RGCs by suppressing glutamate excitotoxicity induced by the decline of glutamate uptake into Müller cells. However, further investigations are required, given the role of apelin on oxidative stress, autophagy (Foroughi et al., 2019), and mitochondrial dysfunction (Zeng et al., 2010), all of which are associated with the degeneration of RGCs with age (Militante and Lombardini, 2004; Chrysostomou et al., 2010; Boya, 2017).

In the present study, we used apelin-KO mice and their WT controls mice maintained on a C57BL/6N background. C57BL/6N mice are a common strain used worldwide for the creation of single-gene KOs, but the mice carry an rd8 mutation and several ocular abnormalities (Mattapallil et al., 2012; Moore et al., 2018). We examined the rd8 mutation in our mice and observed the presence of the rd8 mutation in homozygous form in both WT mice and apelin-KO mice (**Supplementary Figure S1**). Although the aforementioned reports revealed that the rd8 mutation causes degeneration in the inner and outer nuclear layers, the outer plexiform layer, as well as the photoreceptor outer segments, there was no obvious change in the ganglion cell layer in C57BL/6N mice with or without the rd8 mutation. In addition, electroretinography in the rd8 mutation mice remains relatively stable for 1 year (Chang et al., 2002). Therefore, our data, taken together with others, imply that the accelerated degeneration of RGCs in apelin-KO mice at 12 months of age was due to apelin deficiency but not the rd8 mutation, it is necessary, however, to examine the effect of apelin deficiency on the aging retina under conditions of the rd8-free background.

In conclusion, to our knowledge, this is the first report showing that endogenous apelin plays a protective role in retinal tissue during aging and that the apelin-APJ system may be a new target for preventing age-related degeneration of RGCs; however, further studies, including the effect of APJ-KO and intravitreal injection of apelin on the aging retina, are necessary to determine the precise role of the apelin-APJ system on the loss of RGCs with age.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by the Committee for the Ethical Use of Experimental Animals and the Safety Committee for Recombinant DNA Experiments at Setsunan University. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

YI conceived the study and wrote the manuscript. YI, AS, and FS conducted the experiments and analyzed the data. YI, AS,

FS, AY, YY, and SM interpreted the results. SM assisted in the manuscript preparation.

FUNDING

This work was supported by Japan Society for the Promotion of Science (JSPS) KAKENHI Grant Numbers JP16K16612 (YI), JP19K18898 (YI), and JP19K07110 (SM). This work was also supported by the support project for Laboratory of Pharmacotherapeutics, Faculty of Pharmaceutical Sciences, Setsunan University.

ACKNOWLEDGMENTS

We thank Mr. Toru Kuriyama, Ms. Reina Miura, Ms. Hikari Chitose, and Mr. Akinori Tanimura at Setsunan University for their assistance in experiments.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2020.00058/full#supplementary-material.

REFERENCES

Akopian, A., Kumar, S., Ramakrishnan, H., Viswanathan, S., and Bloomfield, S. A. (2016). Amacrine cells coupled to ganglion cells *via* gap junctions are highly vulnerable in glaucomatous mouse retinas. *J. Comp. Neurol.* 527, 159–173. doi: 10.1002/cne.24074

Boya, P. (2017). Why autophagy is good for retinal ganglion cells? *Eye Lond.* 31, 185–190. doi: 10.1038/eye.2016.278

Cavallotti, C., Artico, M., Pescosolido, N., and Feher, J. (2001). Age-related changes in rat retina. *Jpn. J. Ophthalmol.* 45, 68–75. doi: 10.1016/s0021-5155(00)00294-x

Chang, B., Hawes, N. L., Hurd, R. E., Davisson, M. T., Nusinowitz, S., and Heckenlively, J. R. (2002). Retinal degeneration mutants in the mouse. *Vision Res.* 42, 517–525. doi: 10.1016/s0042-6989(01)00146-8

Chrysostomou, V., Trounce, I. A., and Crowston, J. G. (2010). Mechanisms of retinal ganglion cell injury in aging and glaucoma. *Ophthalmic Res.* 44, 173–178. doi: 10.1159/000316478

Cook, D. R., Gleichman, A. J., Cross, S. A., Doshi, S., Ho, W., Jordan-Sciutto, K. L., et al. (2011). NMDA receptor modulation by the neuropeptide apelin: implications for excitotoxic injury. *J. Neurochem.* 118, 1113–1123. doi: 10.1111/j.1471-4159.2011.07383.x

Foroughi, K., Khaksari, M., Rahmati, M., Bitaraf, F. S., and Shayannia, A. (2019). Apelin-13 protects PC12 cells against methamphetamine-induced oxidative stress, autophagy and apoptosis. *Neurochem. Res.* 44, 2103–2112. doi: 10.1007/s11064-019-02847-9

Henneberry, R. C., Novelli, A., Cox, J. A., and Lysko, P. G. (1989). Neurotoxicity at the N-methyl-D-aspartate receptor in energy-compromised neurons. An hypothesis for cell death in aging and disease. *Ann. N. Y. Acad. Sci.* 568, 225–233. doi: 10.1111/j.1749-6632.1989.tb12512.x

Ishimaru, Y., Sumino, A., Kajioka, D., Shibagaki, F., Yamamuro, A., Yoshioka, Y., et al. (2017). Apelin protects against NMDA-induced retinal neuronal death *via* an APJ receptor by activating Akt and ERK1/2 and suppressing TNF-alpha expression in mice. *J. Pharmacol. Sci.* 133, 34–41. doi: 10.1016/j.jphs.2016.12.002

Kasai, A., Ishimaru, Y., Kinjo, T., Satooka, T., Matsumoto, N., Yoshioka, Y., et al. (2010). Apelin is a crucial factor for hypoxia-induced retinal angiogenesis. *Arterioscler. Thromb. Vasc. Biol.* 30, 2182–2187. doi: 10.1161/atvbaha.110.209775

Kasai, A., Kinjo, T., Ishihara, R., Sakai, I., Ishimaru, Y., Yoshioka, Y., et al. (2011). Apelin deficiency accelerates the progression of amyotrophic lateral sclerosis. *PLoS One* 6:e23968. doi: 10.1371/journal.pone.0023968

Kasai, A., Shintani, N., Kato, H., Matsuda, S., Gomi, F., Haba, R., et al. (2008). Retardation of retinal vascular development in apelin-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* 28, 1717–1722. doi: 10.1161/atvbaha.108.163402

Kidoya, H., Ueno, M., Yamada, Y., Mochizuki, N., Nakata, M., Yano, T., et al. (2008). Spatial and temporal role of the apelin/APJ system in the caliber size regulation of blood vessels during angiogenesis. *EMBO J.* 27, 522–534. doi: 10.1038/sj.emboj.7601982

Langrová, H., Zrenner, E., Kurtenbach, A., and Seeliger, M. W. (2008). Age-related changes in retinal functional topography. *Invest. Ophthalmol. Vis. Sci.* 49, 5024–5032. doi: 10.1167/iovs.07-1309

Mattapallil, M. J., Wawrousek, E. F., Chan, C. C., Zhao, H., Roychoudhury, J., Ferguson, T. A., et al. (2012). The Rd8 mutation of the Crb1 gene is present in vendor lines of C57BL/6N mice and embryonic stem cells and confounds ocular induced mutant phenotypes. *Invest. Ophthalmol. Vis. Sci.* 53, 2921–2927. doi: 10.1167/iovs.12-9662

Militante, J., and Lombardini, J. B. (2004). Age-related retinal degeneration in animal models of aging: possible involvement of taurine deficiency and oxidative stress. *Neurochem. Res.* 29, 151–160. doi: 10.1023/b:nere.0000010444.97959.1b

Moore, B. A., Roux, M. J., Sebbag, L., Cooper, A., Edwards, S. G., Leonard, B. C., et al. (2018). A population study of common ocular abnormalities in C57BL/6N rd8 mice. *Invest. Ophthalmol. Vis. Sci.* 59, 2252–2261. doi: 10.1167/iovs.17-23513

Neufeld, A. H., and Gachie, E. N. (2003). The inherent, age-dependent loss of retinal ganglion cells is related to the lifespan of the species. *Neurobiol. Aging* 24, 167–172. doi: 10.1016/s0197-4580(02)00059-3

O'Donnell, L. A., Agrawal, A., Sabnekar, P., Dichter, M. A., Lynch, D. R., and Kolson, D. L. (2007). Apelin, an endogenous neuronal peptide, protects hippocampal neurons against excitotoxic injury. *J. Neurochem.* 102, 1905–1917. doi: 10.1111/j.1471-4159.2007.04645.x

Rai, R., Ghosh, A. K., Eren, M., Mackie, A. R., Levine, D. C., Kim, S. Y., et al. (2017). Downregulation of the apelinergic axis accelerates aging, whereas its systemic restoration improves the mammalian healthspan. *Cell Rep.* 21, 1471–1480. doi: 10.1016/j.celrep.2017.10.057

Samuel, M. A., Zhang, Y., Meister, M., and Sanes, J. R. (2011). Age-related alterations in neurons of the mouse retina. *J. Neurosci.* 31, 16033–16044. doi: 10.1523/jneurosci.3580-11.2011

Tatemoto, K., Hosoya, M., Habata, Y., Fujii, R., Kakegawa, T., Zou, M. X., et al. (1998). Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. *Biochem. Biophys. Res. Commun.* 251, 471–476. doi: 10.1006/bbrc.1998.9489

Trick, L. R. (1987). Age-related alterations in retinal function. *Doc. Ophthalmol.* 65, 35–43. doi: 10.1007/bf00162718

Vinel, C., Lukjanenko, L., Batut, A., Deleruyelle, S., Pradere, J. P., Le Gonidec, S., et al. (2018). The exerkine apelin reverses age-associated sarcopenia. *Nat. Med.* 24, 1360–1371. doi: 10.1038/s41591-018-0131-6

Zeng, X. J., Yu, S. P., Zhang, L., and Wei, L. (2010). Neuroprotective effect of the endogenous neural peptide apelin in cultured mouse cortical neurons. *Exp. Cell Res.* 316, 1773–1783. doi: 10.1016/j.yexcr.2010.02.005

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Medicine

ORIGINAL RESEARCH
published: 08 July 2020
doi: 10.3389/fmed.2020.00261

Rapid Geriatric Assessment Using Mobile App in Primary Care: Prevalence of Geriatric Syndromes and Review of Its Feasibility

Reshma Aziz Merchant[1,2], Richard Jor Yeong Hui[3], Sing Cheer Kwek[3], Meena Sundram[3], Arthur Tay[4], Jerome Jayasundram[1], Matthew Zhixuan Chen[1], Shu Ee Ng[1], Li Feng Tan[5] and John E. Morley[6]*

[1] Division of Geriatric Medicine, Department of Medicine, National University Hospital, National University Health System, Singapore, Singapore, [2] Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore, Singapore, [3] National University Polyclinics, National University Hospital System, Singapore, Singapore, [4] Department of Electrical and Computer Engineering, National University of Singapore, Singapore, Singapore, [5] Healthy Ageing Program, Alexandra Hospital, National University Health System, Singapore, Singapore, [6] Division of Geriatric Medicine, Saint Louis University School of Medicine, St. Louis, MO, United States

OPEN ACCESS

Edited by:
Trentham Furness, Australian Catholic University, Australia

Reviewed by:
Lindsay M. K. Wallace, Dalhousie University, Canada
Liang-Yu Chen, Taipei Veterans General Hospital, Taiwan

***Correspondence:**
Reshma Aziz Merchant
reshmaa@nuhs.edu.sg

Specialty section:
This article was submitted to Geriatric Medicine, a section of the journal Frontiers in Medicine

Received: *02 March 2020*
Accepted: *14 May 2020*
Published: *08 July 2020*

Citation:
Merchant RA, Hui RJY, Kwek SC, Sundram M, Tay A, Jayasundram J, Chen MZ, Ng SE, Tan LF and Morley JE (2020) Rapid Geriatric Assessment Using Mobile App in Primary Care: Prevalence of Geriatric Syndromes and Review of Its Feasibility. Front. Med. 7:261. doi: 10.3389/fmed.2020.00261

With the aging population and consequent increase in associated prevalence of frailty, dementia, and multimorbidity, primary care physicians will be overwhelmed with the complexity of the psychosocial and clinical presentation. Geriatric syndromes including frailty, sarcopenia, cognitive impairment, and anorexia of aging (AA) either in isolation or in combination are associated with an increased risk of adverse outcomes and if recognized early, and appropriately managed, will lead to decreased disability. Primary care practices are often located in residential settings and are in an ideal position to incorporate preventive screening and geriatric assessment with personalized management. However, primary care physicians lack the time, multidisciplinary resources, or skills to conduct geriatric assessment, and the limited number of geriatricians worldwide further complicates the matter. There is no one effective strategy to implement geriatric assessment in primary care which is rapid, cost-effective, and do not require geriatricians. Rapid Geriatric Assessment (RGA) takes <5 min to complete. It screens for frailty, sarcopenia, AA, and cognition with assisted management pathway without the need of a geriatrician. We developed RGA iPad application for screening with assisted management in two primary care practices and explored the feasibility and overall prevalence of frailty, sarcopenia, and AA. The assessment was conducted by trained nurses and coordinators. Among 2,589 older patients ≥65 years old, the prevalence of frailty was 5.9%, pre-frail 31.2%, and robust 62.9%. Fatigue was present in 17.8%, and among them, the prevalence of undiagnosed depression as assessed by the Patient Health Questionnaire (PHQ)-9 was 76.4% and 13.5% of total. The prevalence of sarcopenia was 15.4%, and 13.9% experienced at least one fall in the past year. AA was prevalent in 10.9%. The time taken to do the assessment with defined algorithm

was on average 5 min or less per patient, and 96% managed to complete the assessment prior to seeing their doctor in the same session. The RGA app is a rapid and feasible tool to be used by any healthcare professional in primary care for identification of geriatric syndrome with assisted management.

Keywords: geriatric syndrome, primary care, rapid geriatric assessment, iPad application, older adult, frailty, sarcopenia, anorexia of aging

INTRODUCTION

With the aging population and consequent increase in associated prevalence of frailty, dementia, and multimorbidity, primary care physicians will be overwhelmed with the complexity of the psychosocial and clinical presentation. There is evidence that geriatric syndromes, e.g., frailty, sarcopenia, falls, polypharmacy, anorexia/weight loss, cognitive impairment, and depression, if recognized early and appropriately managed will lead to decreased disability and mortality with better quality of life in older persons (1, 2). The World Health Organization Integrated Care for Older People guidelines recommends assessing older persons for declining physical and mental capacities with necessary interventions (3). Comprehensive geriatric assessment (CGA), initially introduced by Marjory Warren, extends beyond traditional medical history and incorporates an interdisciplinary diagnostic process to identify medical, functional, and psychosocial issues in order to develop a personalized care plan to maximize the well-being of the older adult (4, 5). The effectiveness of the CGA and evaluation was further supported by a meta-analysis which showed reduced hospitalization, death, and institutionalization (6). Geriatric assessment is often conducted by geriatricians or geriatric nurse practitioners and can take between 20 and 45 min (7). In the current acute-care centric setting and disease model of care, proactive screening for geriatric syndromes and case finding is done on *ad hoc* basis, and in many instances, it is not followed up with intervention (8, 9). While CGA is effective in hospitalized older adults presenting with falls, fracture, functional decline, and delirium and with shortage of geriatricians worldwide, preventive screening and geriatric assessment in primary care are the best possible solutions in the provision of upstream goal-directed person-centered care (7, 10, 11).

Primary care is the foundation of healthcare system in Singapore. More than 20 subsidized polyclinics and 1,700 general practice clinics are located island-wide in the residential setting and are in ideal position to do CGA with the necessary interventions (12). They are often the first line of contact and treat a whole range of conditions from upper respiratory tract infections to chronic diseases and keep the population healthy through preventive population health. The concept of teamlet was introduced in 2014 in the polyclinic where patient-centered care is provided for those with chronic diseases by a team of doctors, care coordinators, and supported by in-house allied healthcare staff (13). The teamlet focuses on patients' medical, functional, and psychological needs and provides holistic integrated care within the primary care setting. The focus has largely been on chronic diseases, and they work under constraints of limited time and limited multidisciplinary resources and may not have the necessary skills to conduct CGA. While not a problem locally, reimbursement issues in certain countries may be an obstacle for them to implement CGA as a routine practice. There is no one effective strategy to implement CGA in primary care which is rapid and cost-effective and does not require geriatricians or geriatric trained nurse clinicians to perform the assessments (10, 14). Many brief screening tools have been developed worldwide to be used in primary care including Gerontopôle Frailty Screening Tool (GFST) which includes a dedicated pathway for disability prevention, Kihon Checklist (KCL) which is a self-reported comprehensive health checklist, Vulnerable Elders Survey-13 (VES-13) which is a self-administered questionnaire with the aim of identifying those at risk of death or functional decline, Easycare Two-step Older persons Screening (Easycare-TOS) which is a brief standardized tool for assessing perceptions of older adults about their health and care needs, and the Electronic Frailty Index in the United Kingdom (11, 15, 16). Most assessment tools are predominantly screening or case finding tools with limited or no interventions recommended.

Rapid Geriatric Assessment (RGA) is one of the most practical tools developed at St. Louis University and takes <5 min to screen for frailty, sarcopenia, anorexia of aging (AA), and cognition and does not require a geriatrician to administer the assessment (1, 16). RGA comprises of four screening tools including the FRAIL scale for frailty, the SARC-F scale for sarcopenia, the Simplified Nutritional Appetite Questionnaire (SNAQ) for AA, and the Rapid Cognitive Screen (RCS) for cognition (17) (**Supplementary 1**).

The aim of this study was to explore the feasibility and implementation of RGA iPad application in two busy primary care practices and to determine overall prevalence of frailty, sarcopenia, and AA.

MATERIALS AND METHODS

An iPad mobile application for RGA was developed in English and Chinese, and screening was carried out in two primary care practice teamlets in the Western region of Singapore from April 2019 to December 2019 (**Figure 1**). Screening was done by trained care coordinators and/or nurse for 2,710 older patients ≥65 years old, and 2,589 had complete data collected. The assessment was carried out for those who had appointments to see their doctor on the same day. There are no copyright issues with RGA as the questionnaires belong to John E. Morley.

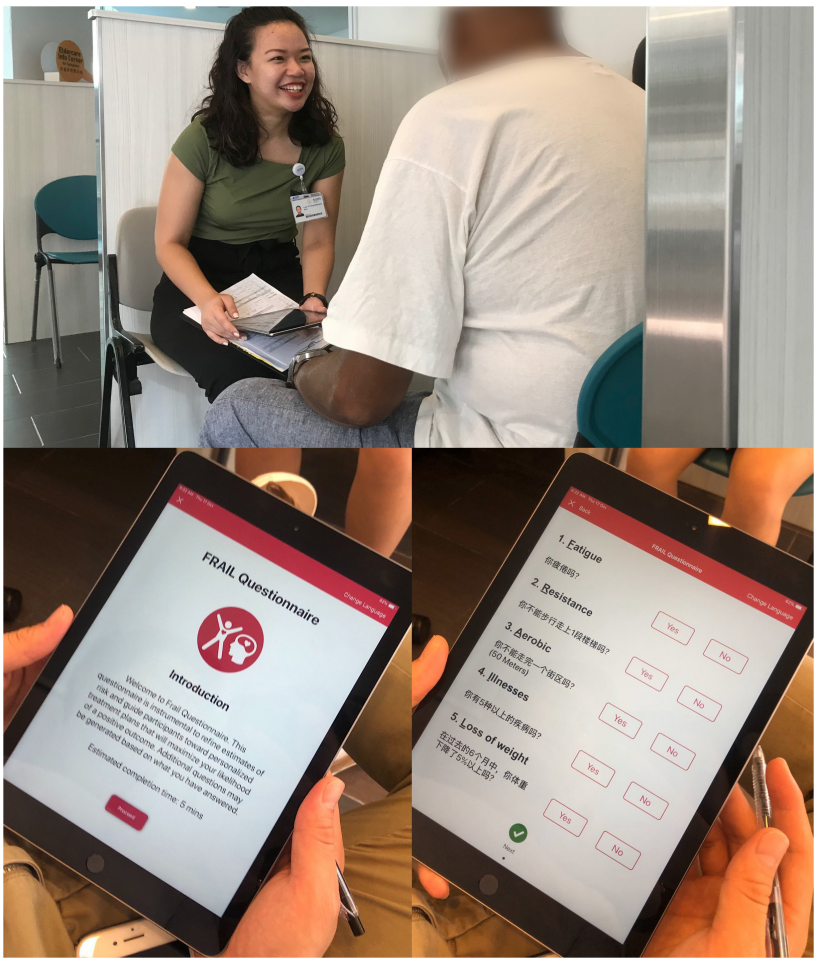


FIGURE 1 | RGA mobile iPad app and screening.

As part of the RGA, frailty was screened using the FRAIL scale with dedicated care pathway, sarcopenia using SARC-F, nutrition status using the SNAQ, and cognition using the RCS (**Figure 2**) (18). The assessment tool in RGA has been validated in many different countries and clinical conditions and has been shown to predict adverse outcomes such as mortality, functional decline, falls, and hospitalization (19). The five-item FRAIL scale (Fatigue, Resistance, Aerobic, Illness, and Loss of Weight) screens for frailty. The scores range from 0 to 5, where scores of 1–2 are considered pre-frail and 3–5 represent frail. The SARC-F screens for sarcopenia and comprises of five questions including strength, rise from a chair, assistance with walking, climbing stairs, and falls in the past year. A total score of ≥4 indicates sarcopenia. SARC-F has sensitivity ranging from 25 to 50% and specificity ranging from 90 to 98% (20, 21). The SNAQ includes four questions on appetite, taste of food, portion consumed, early satiety, and number of meals consumed daily. The total SNAQ score ranged from 4 to 20, and score of ≤14 predicts at least 5% of weight loss within 6 months with a sensitivity of 81.5% and a specificity of 76.4% (22). RCS includes four questions to screen for mild cognitive impairment or dementia. It includes five-item recall, clock drawing, and story recall. A total score of ≤5 indicates dementia, 6–7 mild cognitive impairment, and 8–10 indicates normal cognition (23).

The assisted management pathway algorithm is shown in **Figure 2**. For those who screened positive for fatigue, additional questions included screening for sleep apnea and depression. Depression was assessed using the Patient Health Questionnaire (PHQ-9) which is a self-administered version of the PRIME-MD diagnostic instrument for common mental disorders. It consists of nine items, and each item is assessed on a four-point scale (0 = not at all, 1 = several days, 2 = more than half the days, 3 = nearly every day) with scores ranging from 0 to 27 (24). PHQ-9 has been locally validated in Asian primary care setting (25).

While most information on the iPad app was self-explanatory, the coordinators and nurses were trained in administering the RGA. There was no prior CGA carried out in the primary care setting. Feasibility and practicality were measured using the average time taken to complete the assessment including the algorithm and the completeness of the assessment prior

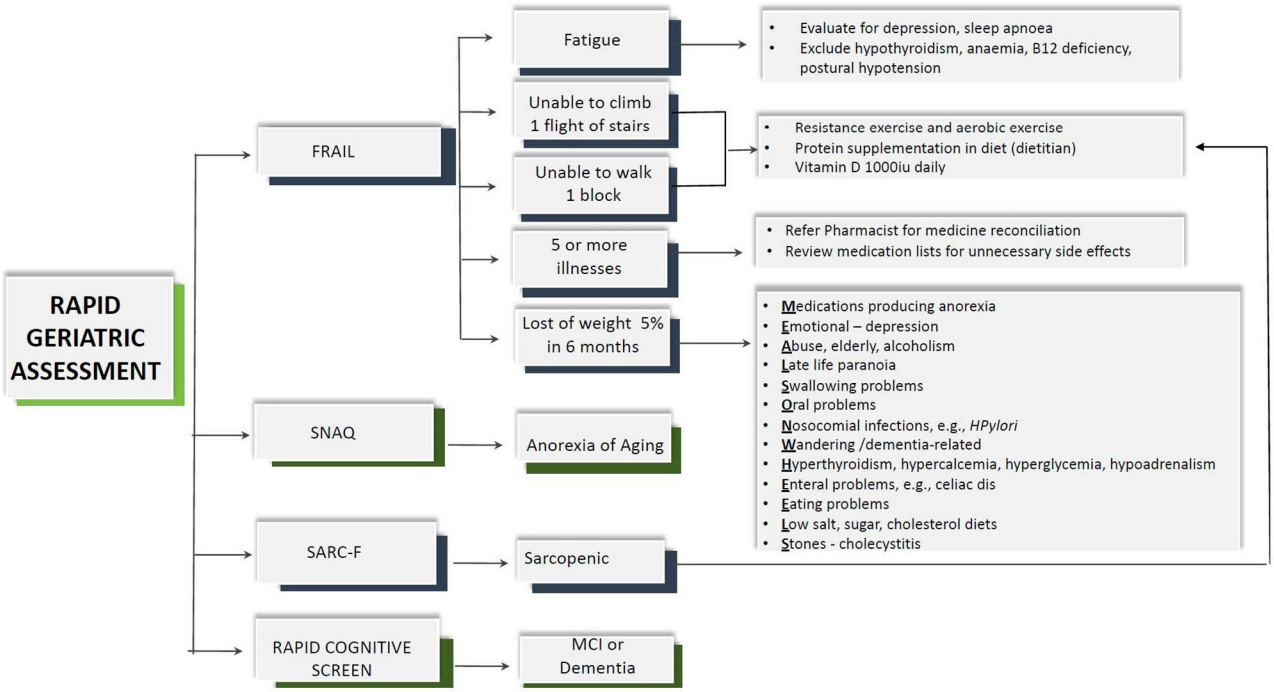


FIGURE 2 | Rapid geriatric assessment and personalized management.

to seeing their doctor on the same day. There was no additional time allocated nor were the patients advised to come early for the assessment. The patients were given a piece of paper incorporating the assessment findings and scores to be handed over to their doctor on the same day. Outcome and recommended interventions of RGA were not evaluated.

Data were analyzed using SPSS Version 25.0 (IBM Corp., 2017). Characteristics of participants were presented as mean, standard deviation for continuous variables, while categorical variables were presented as frequencies (percentages). Differences between genders were assessed using two-sample *t*-test (when normality and homogeneity assumptions were satisfied); otherwise, Mann–Whitney *U*-test was used for continuous variables, while chi-square test was used for categorical variables. Statistical significance was set at *p*-value of <0.05.

As this was performed as part of the routine clinical care in the teamlets within primary care, no ethics approval was necessary. The ethics approval was obtained, and consent was required if the older patients who were identified as pre-frail or frail were interested to participate in a subsequent intervention study.

RESULTS

Among the 2,589 older patients, 1,358 (52.5%) were women, and overall mean age was 73.1 years (**Table 1**). For living arrangements, almost two thirds were still living with family, with only 116 (8.5%) women and 77 (6.3%) men living alone. Among them, 579 (22.4%) were still working with only 13.2% in full-time employment. The overall prevalence of frailty was 5.9%, pre-frail 31.2%, and robust 62.9%. There was a significant difference between men and women where 6% of women compared with 4.2% of men were frail. The prevalence of sarcopenia was 15.4, and 20.6% of women compared with 9.6% of men were assessed to be sarcopenic. AA was prevalent in 283 (10.9%) older patients with prevalence in women being almost double that of men. RCS has not been validated locally, and hence the data for cognition is only available for 190 older patients. One third of the older patients screened had evidence of underlying cognitive impairment.

Figure 3 is the breakdown of geriatric syndromes among the old (65–79 years old) and old-old ($\geq$80 years old). Prevalence of frailty among the old-old was 15.4%, which is almost five times more compared with 3.8% of the young old. More than one in three of the old-old were sarcopenic compared with one in 10 of the old group. Prevalence of dementia as assessed using the RCS was 35.9% among the old-old.

FRAIL has five items (Fatigue, Resistance, Aerobic, Illness, and Loss of Weight) and as shown in **Figure 2**, each item has personalized care management pathway. Fatigue was present in 17.8%:15.4% of men and 20% of women (**Table 2A**). Among those who were fatigued, the prevalence of sleep apnea was 4.8%. Depression as assessed by the PHQ-9 was prevalent in 352 (76.4%) older patients who were feeling fatigued and 13.6% of total. Almost half had minimal depression, and one in 10 had moderate to severe depression which had not been diagnosed prior to the implementation of RGA. One in seven older patients was unable to climb one flight of stairs, and similar numbers

TABLE 1 | Demographics of participants.

	Overall N = 2,589	Men 1,226 (47.4)	Women 1,358 (52.5)	*P*-value
Age (mean, SD)	73.1, 6.5	72.8, 6.2	73.3, 6.7	**0.030**
Living arrangement				**<0.001**
Alone	193 (7.5)	77 (6.3)	116 (8.5)	
Spouse	699 (27.0)	423 (34.6)	275 (20.3)	
Family (Spouse/Children/ Others/Friend)	1,651 (63.7)	716 (58.4)	930 (68.5)	
Domestic Helper	30 (1.2)	4 (0.3)	26 (1.9)	
Landlord/Tenant	17 (0.7)	6 (0.5)	11 (0.8)	
Employment				**<0.001**
Retired	1,042 (40.2)	789 (64.4)	558 (41.1)	
Full time	341 (13.2)	250 (20.4)	186 (13.7)	
Part time	238 (9.2)	124 (10.1)	115 (8.5)	
Home maker/housewife	821 (31.7)	24 (2.0)	418 (30.8)	
Unemployed	148 (5.7)	39 (3.2)	81 (6.0)	
Smoking				**<0.001**
Never smoked	2,090 (80.7)	770 (62.8)	1,315 (96.8)	
Current smoker	164 (6.3)	144 (11.7)	19 (1.4)	
Past smoker	336 (13.0)	312 (25.4)	24 (1.8)	
Frailty Status				**<0.001**
Robust	1,628 (62.9)	821 (67.0)	818 (60.2)	
Pre-frail	809 (31.2)	349 (28.5)	459 (33.8)	
Frail	151 (5.9)	56 (4.1)	81 (6.0)	
Sarcopenia	399 (15.4)	118 (9.6)	280 (20.6)	**<0.001**
Anorexia of Aging	283 (10.9)	96 (7.8)	185 (13.6)	**<0.001**
Cognitive Impairment[1]	190	97 (51.1)	92 (48.9)	0.060
Normal	125 (65.8)	70 (72.2)	55 (59.8)	
MCI	26 (13.7)	14 (14.4)	12 (13.0)	
Dementia	39 (20.5)	13 (13.4)	26 (27.2)	

Values are n (%) unless otherwise noted.

[1] Rapid Cognitive Screen was conducted in 190 participants; percentages are of remaining men and women participants. Bold implies significance.

were also unable to walk one bus-stop distance which is about 50 m. Overall, 252 (9.8%) older patients had five or more chronic illnesses, which they were treated for, with only 7.9% of women compared with 11.8% of men. Loss of 5% or more weight in the last 6 months was reported by 3.6% of men and 4.6% of women. Among those with weight loss, additional questions on eating problems found that 8.3% reported issues with choking or coughing on eating, and 6.5% have issues with chewing or lack of teeth (**Supplementary 2**).

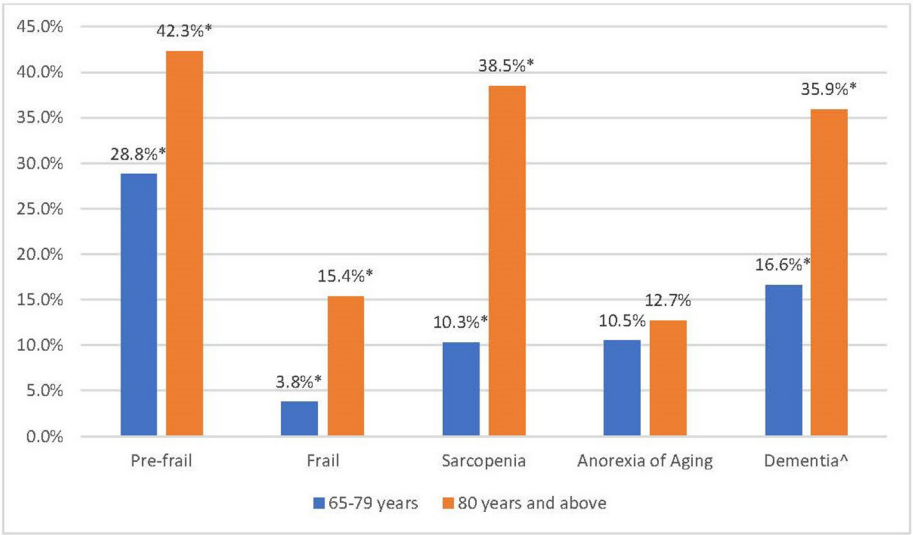
Sarcopenia was assessed using SARC-F comprising of five questions including strength, rise from a chair, assistance with walking, climbing stairs, and falls in the past year. Overall, one in four older patients had difficulties carrying 4.5 kg of weight, with one in three women and one in six men reporting difficulties (**Table 2B**). More women (17.3%) compared with men (9.9%) reported difficulties walking across the room. More than one in four had difficulties transferring from chair to bed, and similar numbers also had difficulties climbing one flight of stairs, with prevalence being much higher in women; 33.2% had difficulty transferring from a chair or bed, and 31.9% had difficulty climbing a flight of 10 stairs. The overall prevalence of one or more falls was 13.9% affecting 142 (11.6%) of men and 218 (16.1%) women.

The time taken to do the assessment and generate the above assessment was on average 5 min or less per patient as time was shown on the iPad screen. Time taken to complete was evaluated both by the authors for their own clinic patients before rolling out officially, and feedback was obtained from care coordinators and nurses. Not all patients were required to complete the full algorithm. As there was no additional time allocated, and patients usually came on time for their appointment, 2,710 patients were assessed, and full assessment was available for 2,589 patients. The rest could not complete assessments as they were called in by their doctors. Overall, 96% managed to complete the assessment prior to seeing their doctor.

DISCUSSION

Geriatric syndromes including frailty, sarcopenia, cognitive impairment, and AA either in isolation or combination are associated with increased risk of adverse outcomes including hospitalization, falls, functional decline, institutionalization, and mortality (26–29). Current healthcare is based on traditional disease-based model which is no longer sustainable in countries with a fast aging population where rise in multimorbidity and associated consequences will require proactive, upstream, person-centered care, multidisciplinary primary care approach to early identification of geriatric syndrome, assessment, and management (30).

Frailty is characterized by diminished strength and endurance due to accumulation of multiple deficits and known to be due to loss of harmonic interactions between different dimensions including psychological, socioeconomic, genetic, biological, and functional domains contributing to higher adverse outcomes (31). It is a state of gradual functional decline over 5–10 years eventually leading to disability during which there are many opportunities for early case finding and intervention before the onset of disability. The prevalence of frailty increases with age, and half of frail older adults are still independent (32, 33). Sarcopenia is an age-related loss of strength, skeletal muscle mass, and function. SARC-F is the recommended screening tool for sarcopenia in many international guidelines and has been validated in many countries worldwide and has a good predictive value for future dependency (34–36). Nutrition, frailty, and sarcopenia are closely related, and a potentially modifiable factor for prevention or delaying onset of frailty and sarcopenia. AA is a precursor for geriatric syndromes and malnutrition. The SNAQ has been validated worldwide in different diseases and settings to identify those at risk of malnutrition. The concurrent assessment for frailty, sarcopenia, AA, and cognition is important for upstream interventions even before the onset of geriatric syndromes. The above conditions share similar risk



*indicates significant difference between the two age groups

65-79 years old 2117 (81.8%), ≥80 years 472 (18.2%)

^ Rapid Cognitive Screen conducted in 190 patients, 65-79 years old 151 (79.5%), ≥80 years 38 (20.5%)

FIGURE 3 | Prevalence of geriatric syndromes in the old and old–old in primary care. *indicated significant between the two age groups. 65–79 years old 2,117 (81.8%), ≥80 years 472 (18.2%). ^Rapid cognitive screen conducted in 190 patients, 65–79 years old 151 (79.5%), ≥80 years 38 (20.5%).

factors which are reversible with early intervention including polypharmacy, low physical activity, nutrition, chronic diseases including diabetes, depressive symptoms, poor social network, and undiagnosed cognitive impairment (37–40).

While it may be preferred for screening tools in primary care to have high sensitivity for the diagnosis of frailty and other geriatric syndromes, screening tools in RGA incorporating assisted management pathway provide not just diagnosis but intervention even before the onset of geriatric syndromes which makes it unique. A person with falls identified with SARC-F and at risk of AA may not screen positive for sarcopenia or frailty, but early intervention and assessment may delay the onset of sarcopenia and frailty. Sarcopenia can lead to physical frailty, causing poor balance, low muscle strength, falls, and fracture.

Primary care is the core component of healthcare where preventive healthcare is most feasible due to the accessibility and location within the community setting. In addition to assessing for frailty, the British Geriatric Society (BGS) recommends referral to the geriatric team for CGA (41). Due to limited numbers of geriatricians and the fact that frailty, sarcopenia, AA, and cognitive impairment are often difficult to diagnose in earlier stages, a fast and practical tool is needed to facilitate case finding in primary care (42). Primary care practices worldwide are often overwhelmed with multitudes of clinical issues ranging from neonates to frail older adults, and CGA is often not on their cards as it is time-consuming. Despite advances worldwide in developing practical tools in primary care, the overall take-up has been low due to multiple reasons including lack of skills, time, and reimbursement (9, 16). In addition, in countries like Singapore where we have different ethnic groups, there's often a language barrier in conducting CGA. To address these issues, RGA app was developed in English and Chinese. In addition, primary care physicians were not required to do CGA or RGA; they were just told to manage relevant positive findings.

TABLE 2A | Frailty and subcomponents.

Frailty	Overall 2,589	Men 1,226 (47.4)	Women 1,358 (52.5)	*P*-value
Fatigue	461 (17.8)	189 (15.4)	272 (20.0)	**0.003**
Sleep apnea*	21 (4.8)	11 (5.8)	10 (3.6)	**0.007**
Depression*	352 (76.4)	126 (66.7)	226 (83.1)	0.199
Minimal depression	192 (48.0)	69 (45.7)	123 (49.4)	
Mild depression	119 (29.8)	45 (29.8)	74 (29.7)	
Moderate to severe depression	41 (10.3)	12 (7.9)	29 (11.6)	
Unable to climb 1 flight of stairs	351 (13.6)	103 (8.4)	248 (18.2)	**<0.001**
Unable to walk 1 Bus Stop	358 (13.8)	126 (11.8)	232 (17.1)	**<0.001**
Five or more chronic illnesses	252 (9.8)	145 (11.8)	107 (7.9)	**0.001**
Loss of weight	107 (4.1)	44 (3.6)	63 (4.6)	0.181

Values are n (%) unless otherwise noted.
**Percentages are of remaining men and women participants who complained of fatigue.*
Bold implies significance.

Fatigue in the FRAIL scale is a well-known presenting complaint for those who are depressed, suffering from sleep apnea, or have underlying medical conditions such as anemia, hypothyroidism, hypotension, and B12 deficiency. Fatigue, depression, and B12 deficiency also accelerates cognitive decline.

TABLE 2B | Sarcopenia and subcomponents.

SARC-F	Overall 2,589	Men 1,226 (47.4)	Women 1,358 (52.5)	*P*-value
Sarcopenia	399 (15.4)	118 (9.6)	280 (20.6)	<0.001
Difficulty lifting and carrying 4.5 kg	658 (25.4)	199 (16.2)	458 (33.7)	<0.001
Difficulty walking across a room	357 (13.8)	121 (9.9)	236 (17.3)	<0.001
Difficulty transferring from a chair or bed	705 (27.2)	252 (20.6)	452 (33.2)	<0.001
Difficulty climbing a flight of ten stairs	663 (25.6)	229 (18.7)	433 (31.9)	<0.001
Falls in the last year	361 (13.9)	142 (11.6)	218 (16.1)	0.005

Values are n (%) unless otherwise noted.

The "R" and "A" component of FRAIL and sarcopenia can be managed with resistance exercise and a high-protein diet. The "I" refers to more than five illnesses which can be managed by reviewing medications and reducing inappropriate prescribing. Loss of weight and/or AA has many treatable factors (**Figure 2**). In addition, SARC-F also has a question on falls, and if screened positive can be referred for additional evaluation which includes vision.

The prevalence of frailty in primary care was 5.1% which is very similar to community prevalence (32). In addition to prevalence of frailty, among those who were fatigued, the prevalence of undiagnosed depression was 76.4%, which makes it 13.6% overall. While the number is significant, the prevalence is much lower than that in other studies where it ranged from 29.9 to 37.2% (43, 44). However, the prevalence in our study is similar to other studies done locally reporting the prevalence between 3.7 and 13.3% (25, 45, 46). Depression in older adults in primary care often goes unrecognized and carries a poor prognosis, and RGA made it possible to screen for depression using a simple question of fatigue where almost three quarters were screened to have depression (47). Sleep apnea has been associated with fatigue, cognitive impairment, falls, and functional impairment (48). The overall self-reported prevalence in our patients was 4.8% which is much lower than the prevalence reported using polygraphy in the population-based cohort study not restricted to older adults (49, 50). The prevalence of sleep apnea increases with age and often under-recognized by patients and caregivers (51).

The prevalence of sarcopenia in our older patients in primary care was 15.4%, significantly higher in women. More importantly, RGA was able to identify those with difficulties transferring and climbing one flight of stairs and those who suffered a fall. As there is no gold standard for diagnosis of sarcopenia, the prevalence within community varies from 4 to 23% (52).

Malnutrition and AA are often used interchangeably, when in fact one is a precursor of the other and early interventions may prevent malnutrition, weight loss, and cachexia (53–55). Slightly more than half of older adults at risk of malnutrition are known to be either frail or pre-frail (56). The prevalence of AA ranges between 10.7 and 25% in community-dwelling older adults (57, 58). Despite its high prevalence and significance, it is often overlooked by healthcare professionals and attributed to normal aging. The prevalence of AA in our older patients in primary care was 10.9%, being significantly higher in women, which is very similar to the Japanese (58). Only 4.1% of the older patients reported significant weight loss.

The RGA app was not intended to replace assessment by primary care clinicians but to enhance and enable case finding among those at risk. There were initial concerns among primary care doctors on consultation time if additional issues needed addressing. While the additional issues were flagged up to managing primary care physician, we do not have data on interventions offered and outcome including the development of geriatric syndrome and/or healthcare utilization. In addition, we do not have data on behavior change or knowledge on geriatric syndromes among primary care physicians. Longitudinal follow-up of these patients will be required. However, the process was seamless, and it was eventually accepted as usual practice. The RGA app is unique compared to other geriatric assessment tools developed for primary care in that it includes personalized management assistance pathway and can be administered by any health care professional and/or coordinator. The time taken was evaluated initially before and just after rolling out, and feedback was obtained from the team subsequently. It was both feasible, requiring on average 5 min or less per patient, and practical where it did not require additional resources, and complete data were obtained for 96% of the patients assessed. RGA with management assistance iPad application is rapid and practical and can be used in any primary care practice for identification and management of geriatric syndromes without the need of additional time or resources.

CONCLUSION

The RGA app is a rapid and feasible tool to be used in primary care for identification of geriatric syndrome and assisted management. It can be used by any healthcare professional to identify the at-risk population. Psychosocial aspects especially loneliness can be incorporated in the future including the use of artificial intelligence to further fine-tune the identification of at-risk seniors (59).

DATA AVAILABILITY STATEMENT

The datasets for this article are not publicly available as this is more of a feasibility study using an app to enroll participants in intervention study. Requests to access the datasets should be directed to Reshma Aziz Merchant, reshmaa@nuhs.edu.sg.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by NHG DSRB (National Healthcare Group Domain

Specific Review Board). The written informed consent wasn't obtained for this study as Rapid Geriatric Assessment was done as a routine care protocol in primary care, and willingness to answer the questions was deemed as an informed consent. Written informed consent was obtained from the participants of the study (as required by the ethics committee) once they were enrolled in any intervention after screening, and written informed consent was obtained from the individuals for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

RM, RH, SK, MS, AT, JJ, MC, SN, LT, and JM contributed to conceptualization. RM, RH, and SK contributed to the formal analysis. RM, MC, and LT contributed to funding acquisition. RM, AT, JJ, and JM contributed to app design and creation. RM, RH, SK, MS, and JM contributed to methodology and project administration. RM contributed to writing the original draft. RH, SK, MS, AT, JJ, MC, SN, LT, and JM contributed to review and editing. All authors contributed to the article and approved the submitted version.

FUNDING

This study is part of a larger project that has been funded by the Ministry of Health of Singapore: Healthy Aging Innovation Grant under the National Innovation Challenge on Active and Confident Aging (Award No: MOH/NIC/HAIG02/2017).

ACKNOWLEDGMENTS

The authors wish to thank Lim Jia Yi, Nethania Low, Jinming Zhang, Qiu Ming Tian, and Fatimah Endut for administering assessment in Primary Care and Research Logistics.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2020.00261/full#supplementary-material

REFERENCES

1. Morley JE. Rapid geriatric assessment: secondary prevention to stop age-associated disability. *Clin Geriatr Med.* (2017) 33:431–40. doi: 10.1016/j.cger.2017.03.006
2. Rubenstein LZ, Josephson KR, Wieland GD, English PA, Sayre JA, Kane RL. Effectiveness of a geriatric evaluation unit. A randomized clinical trial. *N Engl J Med.* (1984) 311:1664–70. doi: 10.1056/NEJM198412273112604
3. Thiyagarajan JA, Araujo de Carvalho I, Pena-Rosas JP, Chadha S, Mariotti SP, Dua T, et al. Redesigning care for older people to preserve physical and mental capacity: WHO guidelines on community-level interventions in integrated care. *PLoS Med.* (2019) 16:e1002948. doi: 10.1371/journal.pmed.1002948
4. Ellis G, Whitehead MA, Robinson D, O'Neill D, Langhorne P. Comprehensive geriatric assessment for older adults admitted to hospital: meta-analysis of randomised controlled trials. *BMJ.* (2011) 343:d6553. doi: 10.1136/bmj.d6553
5. St John PD, Hogan DB. The relevance of Marjory Warren's writings today. *Gerontologist.* (2014) 54:21–9. doi: 10.1093/geront/gnt053
6. Stuck AE, Siu AL, Wieland GD, Adams J, Rubenstein LZ. Comprehensive geriatric assessment: a meta-analysis of controlled trials. *Lancet.* (1993) 342:1032–6. doi: 10.1016/0140-6736(93)92884-V
7. Gudmundsson A, Carnes M. Geriatric assessment: making it work in primary care practice. *Geriatrics.* (1996) 51:55–65.
8. Theile G, Winter A, Hummers-Pradier E, Junius-Walker U. [Use and acceptance of a basic geriatric assessment in primary care setting]. *Z Gerontol Geriatr.* (2012) 45:323–30. doi: 10.1007/s00391-011-0265-3
9. Shreve ST, Kumanyika S. Geriatric assessment: can primary care fill the void? *J Am Geriatr Soc.* (1997) 45:1407–8. doi: 10.1111/j.1532-5415.1997.tb02946.x
10. Garrard JW, Cox NJ, Dodds RM, Roberts HC, Sayer AA. Comprehensive geriatric assessment in primary care: a systematic review. *Aging Clin Exp Res.* (2020) 32:197–205. doi: 10.1007/s40520-019-01183-w
11. Morley JE, Arai H, Cao L, Dong B, Merchant RA, Vellas B, et al. Integrated care: enhancing the role of the primary health care professional in preventing functional decline: a systematic review. *J Am Med Dir Assoc.* (2017) 18:489–94. doi: 10.1016/j.jamda.2017.03.015
12. Primary Healthcare Services. Available online at: https://www.moh.gov.sg/home/our-healthcare-system/healthcare-services-and-facilities/primary-healthcare-services.
13. Cheong TY. Transforming Care. *SMA News.* (2014) 2. Available online at: https://www.sma.org.sg/UploadedImg/files/Publications%20-%20SMA%20News/4611/Insight%202.pdf
14. Counsell SR, Callahan CM, Buttar AB, Clark DO, Frank KI. Geriatric resources for assessment and care of elders (GRACE): a new model of primary care for low-income seniors. *J Am Geriatr Soc.* (2006) 54:1136–41. doi: 10.1111/j.1532-5415.2006.00791.x
15. Clegg A, Bates C, Young J, Ryan R, Nichols L, Ann Teale E, et al. Development and validation of an electronic frailty index using routine primary care electronic health record data. *Age Ageing.* (2016) 45:353–60. doi: 10.1093/ageing/afw039
16. Fougere B, Cesari M, Arai H, Woo J, Merchant RA, Flicker L, et al. Editorial: involving primary care health professionals in geriatric assessment. *J Nutr Health Aging.* (2018) 22:566–8. doi: 10.1007/s12603-018-1001-0
17. Morley JE, Adams EV. Rapid geriatric assessment. *J Am Med Dir Assoc.* (2015) 16:808–12. doi: 10.1016/j.jamda.2015.08.004
18. Morley JE, Little MO, Berg-Weger M. Rapid geriatric assessment: a tool for primary care physicians. *J Am Med Dir Assoc.* (2017) 18:195–9. doi: 10.1016/j.jamda.2016.11.017
19. Diaz de Leon Gonzalez E, Gutierrez Hermosillo H, Martinez Beltran JA, Chavez JH, Palacios Corona R, Salinas Garza DP, et al. Validation of the FRAIL scale in Mexican elderly: results from the Mexican Health and Aging Study. *Aging Clin Exp Res.* (2016) 28:901–8. doi: 10.1007/s40520-015-0497-y
20. Yang M, Hu X, Xie L, Zhang L, Zhou J, Lin J, et al. Screening sarcopenia in community-dwelling older adults: SARC-F vs SARC-F combined with calf circumference (SARC-CalF). *J Am Med Dir Assoc.* (2018) 19:277 e1–e8. doi: 10.1016/j.jamda.2017.12.016
21. Bahat G, Oren MM, Yilmaz O, Kilic C, Aydin K, Karan MA. Comparing SARC-F with SARC-CalF to screen sarcopenia in community living older adults. *J Nutr Health Aging.* (2018) 22:1034–8. doi: 10.1007/s12603-018-1072-y
22. Wilson MM, Thomas DR, Rubenstein LZ, Chibnall JT, Anderson S, Baxi A, et al. Appetite assessment: simple appetite questionnaire predicts weight loss in community-dwelling adults and nursing home residents. *Am J Clin Nutr.* (2005) 82:1074–81. doi: 10.1093/ajcn/82.5.1074
23. Malmstrom TK, Voss VB, Cruz-Oliver DM, Cummings-Vaughn LA, Tumosa N, Grossberg GT, et al. The Rapid Cognitive Screen (RCS): a point-of-care screening for dementia and mild cognitive impairment. *J Nutr Health Aging.* (2015) 19:741–4. doi: 10.1007/s12603-015-0564-2
24. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med.* (2001) 16:606–13. doi: 10.1046/j.1525-1497.2001.016009606.x

25. Sung SC, Low CC, Fung DS, Chan YH. Screening for major and minor depression in a multiethnic sample of Asian primary care patients: a comparison of the nine-item Patient Health questionnaire (PHQ-9) and the 16-item quick inventory of depressive symptomatology - self-report (QIDS-SR16). *Asia Pac Psychiatry.* (2013) 5:249–58. doi: 10.1111/appy.12101
26. Cesari M, Prince M, Thiyagarajan JA, De Carvalho IA, Bernabei R, Chan P, et al. Frailty: an emerging public health priority. *J Am Med Dir Assoc.* (2016) 17:188–92. doi: 10.1016/j.jamda.2015.12.016
27. Fried LP, Tangen CM, Walston J, Newman AB, Hirsch C, Gottdiener J, et al. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci.* (2001) 56:M146–56. doi: 10.1093/gerona/56.3.M146
28. Bottle A, Kim D, Hayhoe B, Majeed A, Aylin P, Clegg A, et al. Frailty and co-morbidity predict first hospitalisation after heart failure diagnosis in primary care: population-based observational study in England. *Age Ageing.* (2019) 48:347–54. doi: 10.1093/ageing/afy194
29. Cox NJ, Ibrahim K, Sayer AA, Robinson SM, Roberts HC. Assessment and treatment of the anorexia of aging: a systematic review. *Nutrients.* (2019) 11:144. doi: 10.3390/nu11010144
30. Whitty CJM, MacEwen C, Goddard A, Alderson D, Marshall M, Calderwood C, et al. Rising to the challenge of multimorbidity. *BMJ.* (2020) 368:l6964. doi: 10.1136/bmj.l6964
31. Pilotto A, Custodero C, Maggi S, Polidori MC, Veronese N, Ferrucci L. A multidimensional approach to frailty in older people. *Ageing Res Rev.* (2020) 60:101047. doi: 10.1016/j.arr.2020.101047
32. Merchant RA, Chen MZ, Tan LWL, Lim MY, Ho HK, van Dam RM. Singapore healthy older people everyday (HOPE) study: prevalence of frailty and associated factors in older adults. *J Am Med Dir Assoc.* (2017) 18:734.e9–e14. doi: 10.1016/j.jamda.2017.04.020
33. Shamliyan T, Talley KM, Ramakrishnan R, Kane RL. Association of frailty with survival: a systematic literature review. *Ageing Res Rev.* (2013) 12:719–36. doi: 10.1016/j.arr.2012.03.001
34. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyere O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing.* (2019) 48:16–31. doi: 10.1093/ageing/afy169
35. Chen LK, Woo J, Assantachai P, Auyeung TW, Chou MY, Iijima K, et al. Asian working group for sarcopenia: 2019 consensus update on sarcopenia diagnosis and treatment. *J Am Med Dir Assoc.* (2020) 21:300–7.e2. doi: 10.1016/j.jamda.2019.12.012
36. Dent E, Morley JE, Cruz-Jentoft AJ, Arai H, Kritchevsky SB, Guralnik J, et al. International clinical practice guidelines for sarcopenia (ICFSR): screening, diagnosis and management. *J Nutr Health Aging.* (2018) 22:1148–61. doi: 10.1007/s12603-018-1139-9
37. Aznar-Tortonda V, Palazón-Bru A, la Rosa DMF, Espínola-Morel V, Pérez-Pérez BF, León-Ruiz AB, et al. Detection of frailty in older patients using a mobile app: cross-sectional observational study in primary care. *Br J Gen Pract.* (2019) 70:e29–e35. doi: 10.3399/bjgp19X706577
38. Ding YY, Kuha J, Murphy M. Multidimensional predictors of physical frailty in older people: identifying how and for whom they exert their effects. *Biogerontology.* (2017) 18:237–52. doi: 10.1007/s10522-017-9677-9
39. Hajek A, Brettschneider C, Posselt T, Lange C, Mamone S, Wiese B, et al. Predictors of frailty in old age - results of a longitudinal study. *J Nutr Health Aging.* (2016) 20:952–7. doi: 10.1007/s12603-015-0634-5
40. Bauer J, Morley JE, Schols A, Ferrucci L, Cruz-Jentoft AJ, Dent E, et al. Sarcopenia: a time for action. An SCWD position paper. *J Cachexia Sarcopenia Muscle.* (2019) 10:956–61. doi: 10.1002/jcsm.12483
41. Turner G, Clegg A, British Geriatrics S, Age UK, Royal College of General Practioners. Best practice guidelines for the management of frailty: a British Geriatrics Society, Age UK and Royal College of General Practitioners report. *Age Ageing.* (2014) 43:744–7. doi: 10.1093/ageing/afu138
42. Fougere B, Sirois MJ, Carmichael PH, Batomen-Kuimi BL, Chicoulaa B, Escourrou E, et al. General practitioners' clinical impression in the screening for frailty: data from the FAP study pilot. *J Am Med Dir Assoc.* (2017) 18:193.e1–e5. doi: 10.1016/j.jamda.2016.11.008
43. Kronfly Rubiano E, Rivilla Frias D, Ortega Abarca I, Villanueva Villanueva M, Beltran Martinez E, Comellas Villalba M, et al. [Risk of depression in 75 years or older persons, comprehensive geriatric assessment and associated factors in primary care: cross sectional study]. *Aten Primaria.* (2015) 47:616–25. doi: 10.1016/j.aprim.2014.09.012
44. Boongird C, Thamakaison S, Krairit O. Impact of a geriatric assessment clinic on organizational interventions in primary health-care facilities at a university hospital. *Geriatr Gerontol Int.* (2011) 11:204–10. doi: 10.1111/j.1447-0594.2010.00671.x
45. Niti M, Ng TP, Kua EH, Ho RC, Tan CH. Depression and chronic medical illnesses in Asian older adults: the role of subjective health and functional status. *Int J Geriatr Psychiatry.* (2007) 22:1087–94. doi: 10.1002/gps.1789
46. Subramaniam M, Abdin E, Sambasivam R, Vaingankar JA, Picco L, Pang S, et al. Prevalence of depression among older adults-results from the well-being of the Singapore elderly study. *Ann Acad Med Singapore.* (2016) 45:123–33.
47. Cole MG, Bellavance F, Mansour A. Prognosis of depression in elderly community and primary care populations: a systematic review and meta-analysis. *Am J Psychiatry.* (1999) 156:1182–9.
48. Tsai MS, Li HY, Huang CG, Wang RYL, Chuang LP, Chen NH, et al. Risk of Alzheimer's disease in obstructive sleep apnea patients with or without treatment: real-world evidence. *Laryngoscope.* (2020). doi: 10.1002/lary.28558. [Epub ahead of print].
49. Bauters FA, Hertegonne KB, De Buyzere ML, Joos GF, Chirinos JA, Rietzschel ER. Phenotype and risk burden of sleep apnea: a population-based cohort study. *Hypertension.* (2019) 74:1052–62. doi: 10.1161/HYPERTENSIONAHA.119.13452
50. Tan A, Hong Y, Tan LWL, van Dam RM, Cheung YY, Lee CH. Validation of NoSAS score for screening of sleep-disordered breathing in a multiethnic Asian population. *Sleep Breath.* (2017) 21:1033–8. doi: 10.1007/s11325-016-1455-4
51. Ernst G, Mariani J, Blanco M, Finn B, Salvado A, Borsini E. Increase in the frequency of obstructive sleep apnea in elderly people. *Sleep Sci.* (2019) 12:222–6. doi: 10.5935/1984-0063.20190081
52. Masanes F, Rojano ILX, Salva A, Serra-Rexach JA, Artaza I, Formiga F, et al. Cut-off points for muscle mass - not grip strength or gait speed - determine variations in sarcopenia prevalence. *J Nutr Health Aging.* (2017) 21:825–9. doi: 10.1007/s12603-016-0844-5
53. Payette H, Boutier V, Coulombe C, Gray-Donald K. Benefits of nutritional supplementation in free-living, frail, undernourished elderly people: a prospective randomized community trial. *J Am Diet Assoc.* (2002) 102:1088–95. doi: 10.1016/S0002-8223(02)90245-2
54. Malafarina V, Uriz-Otano F, Iniesta R, Gil-Guerrero L. Effectiveness of nutritional supplementation on muscle mass in treatment of sarcopenia in old age: a systematic review. *J Am Med Dir Assoc.* (2013) 14:10–7. doi: 10.1016/j.jamda.2012.08.001
55. Cox NJ, Morrison L, Ibrahim K, Robinson SM, Sayer AA, Roberts HC. New horizons in appetite and the anorexia of ageing. *Age Ageing.* (2020) afaa014. doi: 10.1093/ageing/afaa014
56. Bollwein J, Volkert D, Diekmann R, Kaiser MJ, Uter W, Vidal K, et al. Nutritional status according to the mini nutritional assessment (MNA(R)) and frailty in community dwelling older persons: a close relationship. *J Nutr Health Aging.* (2013) 17:351–6. doi: 10.1007/s12603-013-0034-7
57. Donini LM, Dominguez LJ, Barbagallo M, Savina C, Castellaneta E, Cucinotta D, et al. Senile anorexia in different geriatric settings in Italy. *J Nutr Health Aging.* (2011) 15:775–81. doi: 10.1007/s12603-011-0048-y
58. Tsutsumimoto K, Doi T, Makizako H, Hotta R, Nakakubo S, Makino K, et al. Aging-related anorexia and its association with disability and frailty. *J Cachexia Sarcopenia Muscle.* (2018) 9:834–43. doi: 10.1002/jcsm.12330
59. Ambagtsheer RC, Shafiabady N, Dent E, Seiboth C, Beilby J. The application of artificial intelligence (AI) techniques to identify frailty within a residential aged care administrative data set. *Int J Med Inform.* (2020) 136:104094. doi: 10.1016/j.ijmedinf.2020.104094

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership