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Non-pharmacological interventions in healthy and pathological aging: Facts and perspectives

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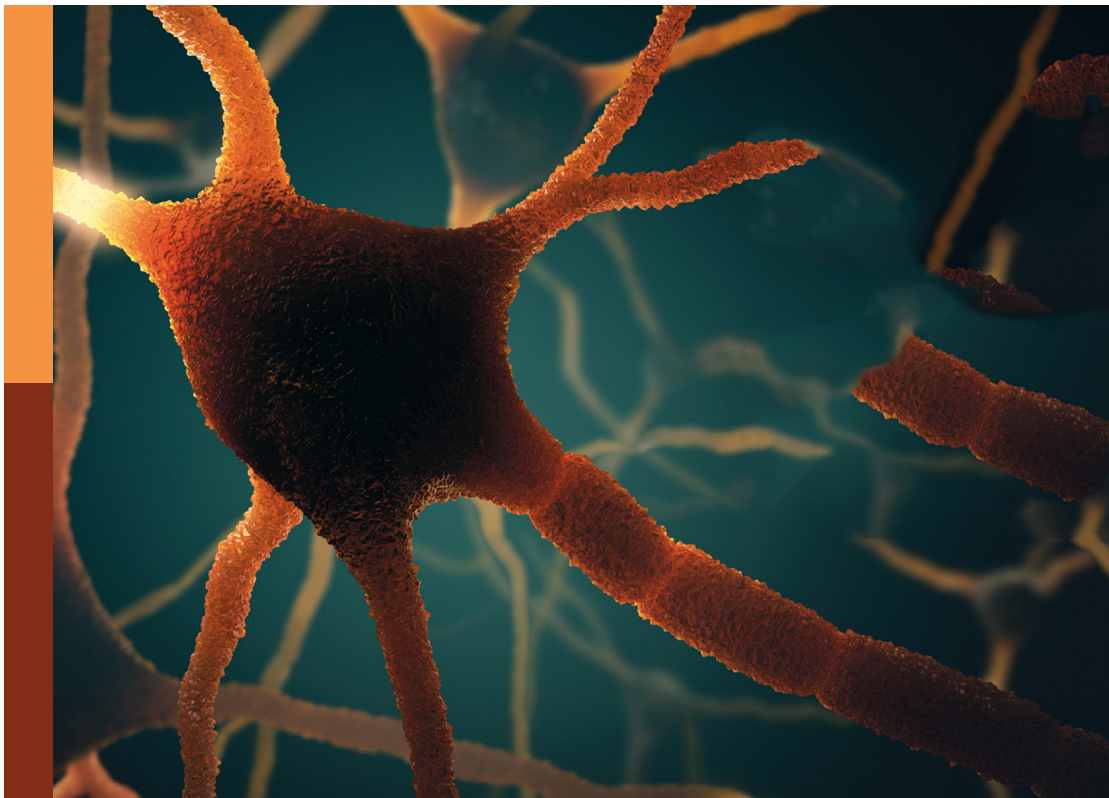

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Non-pharmacological interventions in healthy and pathological aging: Facts and perspectives

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Editorial: Non-pharmacological interventions in healthy and pathological aging: Facts and perspectives

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Editorial on the Research Topic
Non-pharmacological interventions in healthy and pathological aging: Facts and perspectives

Introduction

The elderly population is defined as people aged 60 years and older according to the World Health Organization. The concept "aging population" is, from a historical point of view, a contemporary issue. The number of older people has been increasing, mainly in developing countries. There is data reporting that in 2019 this number was 1 billion and probably will increase to 1.4 billion in 2030 and even 2.1 billion in 2050, considering the world population. In fact, in 2019 only 9% of the world population was aged ≥65 years but the figures are expected to increase to 16% by 2050 (United Nations, 2019; Rudnicka et al., 2020; OECD, 2023).

Aging is a physiological phenomenon which can be related to healthy or pathological processes. The decline of physical and mental conditions, related to locomotor, cognition, and bodily functions, is associated with frailty syndrome and, consequently, with mortality. To the development of health aging, i.e., developing and maintaining the functional ability that enables wellbeing in older age, it is necessary to establish healthy habits, considering physical exercise, diet, mental health, quality of sleep, and other approaches throughout the life cycle. The environment in which people live can influence health and, consequently, the aging process. Cognitive functions and mood can be negatively affected during aging, increasing the risk of development of depression, dementia, and the deterioration of brain functions due to neurodegenerative diseases. Other health conditions are more prevalent in older people, such as obesity, benign prostatic hyperplasia, cardiovascular disease, stroke, and urinary incontinence (Ayensa and Calderon, 2011; Araujo et al., 2014; Salthouse, 2019; Badal et al., 2020; Bae, 2021; Arnoldy et al., 2023; Kim et al., 2023; Liu et al., 2023).

Therefore, strategies that promote healthy aging and thereby preventing impairments in quality of life, are important. The use of non-pharmacological interventions in the management of physical and/or mental impairments is desirable as they can be considered minimally invasive, effective and, generally, have are low-cost. Consequently, a better understanding of the mechanisms involved in these approaches is crucial (Sá-Caputo et al., 2014; Chen et al., 2015; Biagi et al., 2016; Conelea et al., 2017; Bennett et al., 2019; Boehme et al., 2021; Arauz et al., 2022; Cardoso et al., 2022; Tseng et al., 2023).

In this context, research involving health strategies such as dietary interventions, physical exercise, and cognitive exercise is needed as these interventions are expected to improve muscle strength, functionality, quality of life, quality of sleep, cognitive function and to help manage phenomena as depression, cardiovascular-, and urinary-related conditions, among other common health issues in older people. New approaches as whole-body vibration, fecal microbiota transplantation and transcranial magnetic stimulation have been reported as important strategies to prevent and manage health conditions in older people. Good adherence, easy application, reduced adverse effects and costs are reported advantages of these non-pharmacological interventions (Basso et al., 2019; Gaitán et al., 2020; Dal Farra et al., 2021; Liu et al., 2022; Nawrat-Szołtysik et al., 2022). However, non-pharmacological interventions serving health strategies are still understudied.

The aim of this Research Topic was to publish original papers and reviews describing the mechanisms related to the use of non-pharmacological interventions to prevent and to manage health conditions through the life cycle. Moreover, we focused on the presentation of data that can develop a better understanding regarding neuroscience aspects related to it, promoting evidence-based clinical practice.

In this special issue, ten articles addressing those questions are included. We summarize their major contributions according to the subject categories. One Brief Research, seven Original Research Papers, one Study Protocol and one Systematic Review. The *Brief Research* reported the similarities and differences regarding the antidepressant effect of repetitive transcranial magnetic stimulation in younger and older adults (Cotovio et al.). Seven *Original Research Papers* reported the effects of sulforaphane intake on processing speed and negative moods in healthy older adults (Nouchi et al.), the association between social engagement and depressive symptoms in middle-aged and elderly people (Yang et al.), the association of sleep quality with lower urinary tract symptoms/benign prostatic hyperplasia (Li et al.), the effect of regular fecal microbiota transplantation and the effect of whole-body vibration as a passive alternative to exercise after myocardial damage in middle-aged in mice (Zhang et al.), the effects of a specific Tai Chi concept on trunk postural control after stroke (Cui et al.), and the effects of a multidisciplinary body weight reduction program on physical and mental health and fatiguability of older people with obesity (Usubini et al.). The *Study Protocol* of a trial aimed to assess the effects of a 6-month multi-domain exercise program combining multiple exercise modalities, meditation, and social interaction on memory and brain function, in cognitively healthy late middle-aged and older adults (Chang et al.). The *Systematic Review* compared the efficacy and acceptability of treatments for depressive symptoms in people with cognitive impairment (Jin et al.). Taken these studies together it is made clear that progress is being made and new avenues lie ahead of us in the use of non-pharmacological interventions.

Conclusion

As the number of people with old age has increased worldwide, the promotion of knowledge and strategies to achieve healthy aging is desirable and necessary. Considering the impact of health conditions during the aging process, approaches with a minimum of side effects, with good adherence, and low cost are needed to manage the negative impact on health and to promote healthy aging. Thus, research on the effectiveness of non-pharmacological interventions is relevant and increasingly important, given the urgency of increasing numbers of older people worldwide. Non-pharmacological approaches reported in this Research Topic, such as "repetitive transcranial magnetic stimulation", "sulforaphane intake", "physical exercise", "meditation", "social interaction", "fecal microbiota transplantation", "whole-body vibration", "physiological treatments", "music therapy", "Tai Chi", "body weight reduction programs", "rehabilitation programs", and "dietary interventions" seem to support improvements in cognitive, mental and physical function and, therefore, promote healthy aging and manage the consequences related to pathological aging.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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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.

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BRIEF RESEARCH REPORT
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In Older Adults the Antidepressant Effect of Repetitive Transcranial Magnetic Stimulation Is Similar but Occurs Later Than in Younger Adults

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Background: Treatment resistant depression is common in older adults and treatment is often complicated by medical comorbidities and polypharmacy. Repetitive transcranial magnetic stimulation (rTMS) is a treatment option for this group due to its favorable profile. However, early influential studies suggested that rTMS is less effective in older adults. This evidence remains controversial.

Methods: Here, we evaluated the rTMS treatment outcomes in a large international multicenter naturalistic cohort of >500 patients comparing older vs. younger adults.

Results: We show that older adults, while having similar antidepressant response to younger adults, respond more slowly, which may help to explain differences from earlier studies when the duration of a treatment course was shorter.

Conclusions: Such evidence helps to resolve a long-standing controversy in treating older depressed patients with rTMS. Moreover, these findings provide an important data point in the call to revise policy decisions from major insurance providers that have unfairly excluded older adults.

Keywords: transcranial magnetic stimulation, depression, older adults, naturalistic study, efficacy

INTRODUCTION

Major Depressive Disorder (MDD) is a leading cause of disability worldwide, and commonly affects older adults (Beekman et al., 1995). Older adult patients are more likely to have treatment resistant depression (Little et al., 1998) and treatment is particularly challenging due to comorbidities and polypharmacy (Tedeschini et al., 2011; Kok and Reynolds, 2017). Brain stimulation strategies, namely repetitive transcranial magnetic stimulation (rTMS), have been considered an effective antidepressant treatment for those who do not respond or tolerate other treatment strategies (Mutz et al., 2019). This approach has also been supported in preclinical studies, with evidence

of antidepressant-like effects in animal models (De Risio et al., 2020). Due to its favorable side-effects profile (Machii et al., 2006), lower likelihood of drug–drug interaction (George, 2010), and potential cognitive enhancing effects (Martin et al., 2017; Chou et al., 2019), rTMS is a particularly interesting antidepressant treatment modality for older patients with depression. However, clinical efficacy of rTMS in older patients has been controversial. In fact, while older age has been considered a predictor of poorer rTMS antidepressant efficacy (Fregni et al., 2006), a recent metanalysis of randomized controlled trials supported that rTMS is a clinically effective antidepressant treatment in older patients (Valiengo et al., 2022), and there are suggestions that rTMS has similar antidepressant efficacy in the older and younger patients (Conelea et al., 2017; Sackeim et al., 2020). However, evidence to support either of these views is still lacking. This still has important clinical implications since some major health insurance providers, mainly in USA, have policies that limit coverage of rTMS for older adults. Here, we aim to assess if rTMS antidepressant response differs in overall efficacy between the older and younger patients, and if the trajectory of response differs.

METHODS

We conducted an international multicenter naturalistic retrospective study, with data from adult patients treated with rTMS for a major depressive episode in the context of MDD or bipolar disorder type II, irrespective of medication status, at three different rTMS clinical centers: Berenson-Allen Center for Noninvasive Brain Stimulation (Boston, USA), University of Iowa Center for Noninvasive Brain Stimulation (Iowa City, USA), and Champalimaud Foundation Neuropsychiatry Unit (Lisbon, Portugal), from 2000 to 2021, in compliance with each site's local Internal Review Board (IRB) policies for analysis and publication of clinical data. All adult patients treated with DLPFC rTMS for depression at Boston and Iowa centers were considered for inclusion since informed consent for retrospective clinical data inclusion in the current analysis was exempted by local IRBs. For Lisbon center, only patients who signed informed consent were considered. Depression was considered as any major depressive episode in the context of either MDD or bipolar disorder type II, according to the Diagnostic and Statistical Manual of Mental Disorders (American Psychiatric Association, 1994, 2000, 2013). Patients were eligible if treated with Magstim, Neuronetics, or Magventure devices. Patients were excluded if age or motor threshold at first session could not be retrieved, if treatment device changed during treatment, if less than 10 sessions were conducted, or if average interval between consecutive sessions was ≥2.5 days. The following data were extracted from electronic clinical databases: age, sex, baseline (week 1), and weekly self-report depression severity scores (Beck Depression Inventory, BDI: Boston and Lisbon cohorts; Patient Health Questionnaire, PHQ-9: Iowa City cohort), stimulation parameters such as treatment device, stimulation side, and stimulation protocol (1, 10, 18, or 20 Hz rTMS; intermittent theta burst stimulation, iTBS), including the total number of pulses per treatment, and weekly resting motor threshold. Patients were considered younger when less than 65 years-old, and older when 65 years-old or older. Clinical response to rTMS was calculated as the percent reduction of self-reported depression severity scores at each measurement relative to baseline (week 1). Data for continuous measurements are presented as mean ± standard error of the mean (SEM). Patients were considered responders when a reduction of severity of at least 50% relative to baseline (week 1) was observed, with data presented as percent of patients (%).

For statistical analyses, continuous variables were analyzed using longitudinal mixed effects regression analyses, where the dependent variables were the percent reduction of self-reported depression severity scores, or the absolute difference from baseline in BDI or PHQ-9 scores. The independent variables in the longitudinal mixed effects regression models included week of treatment, age group and their interaction term. *Post-hoc* exploratory unpaired two sample *t*-tests at each time point were performed and corrected for multiple comparisons using False Discovery Rate (FDR) of 0.05, according to Benjamini and Hochberg (1995). Additional longitudinal mixed effects regression analyses were performed, similar to those mentioned above, controlling for potential confounding effects of sex, TMS device, stimulation side, stimulation protocol (1, 10, 18, or 20 Hz rTMS or iTBS), treatment site, year of treatment, and total number of pulses per treatment, as well as baseline self-report depression severity when appropriate. Differences in response status between age groups were analyzed using chi-squared tests and corrected for multiple comparisons as above. All data were analyzed using StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC.

RESULTS

Data were collected and analyzed from a total of 546 patients, 58.6% of whom were women, including 442 <65 years-old and 104 ≥65 years-old. Mean (±SEM) baseline BDI and PHQ9 were 29.3 (±0.6) and 17.8 (±0.4), respectively (see **Table 1** for further details). At baseline (week 1), when comparing <65 and ≥65 years-old age groups, depression severity in younger adults was higher, when assessed with BDI, and similar, when assessed with PHQ-9, when compared to older adults (baseline BDI: 29.9 ± 0.6 vs. 26.9 ± 1.3, $p = 0.03$; baseline PHQ-9: 18.0 ± 0.4 vs. 17.0 ± 1.0, $p = 0.3$). We did not find a difference between the two age groups regarding the percentage of responders (**Figure 1A**) when assessed at the most common TMS clinical trial endpoints, i.e., weeks 4 ($N = 372$ vs. 91), 5 ($N = 318$ vs. 84), and 6 (N = 303 vs. 80). Regarding trajectory of response to treatment, measured according to % reduction of depression severity in self-report scales, we found that severity decreased across time (ß = −7.8 ± 0.8, $p < 0.0001$; **Figure 1B**; **Table 2**) during the rTMS treatment. While no differences were observed between the two age groups, there was a significant interaction between time and age group (ß = 5.5 ± 1.9, $p = 0.003$), with depression severity reducing more rapidly among younger than in older adults. Similar results were obtained when controlling for potential confounding effects of

TABLE 1 | Demographic and clinical characteristics of study sample.

Characteristic	Total sample (*N* = 546)	Study centers			Age group	
	Mean ± SEM or %	Boston (*N* = 346)	Iowa City (*N* = 165)	Lisbon (*N* = 35)	<65 (*N* = 442)	≥65 (*N* = 104)
		Mean ± SEM or %	Mean ± SEM or %	Mean ± SEM or %	Mean ± SEM or %	Mean ± SEM or %
Age group					N.A.	N.A.
<65 years-old	80.9	79.8	82.4	85.7		
≥65 years-old	19.1	20.2	17.6	14.3		
Age	49.2 ± 0.7	50.1 ± 0.8	47.6 ± 1.3	48.6 ± 2.8	44.3 ± 0.6	70.4 ± 0.4
Sex (% female)	58.6	59.4	58.5	51.4	59.8	53.9
Depression						
Unipolar	92.9	88.8	100.0	80.0	92.8	93.2
Bipolar	7.1	11.2	0.0	20.0	7.2	6.8
Stimulation side (% left)	91.3	89.1	96.4	88.6	91.6	90.2
Study protocol						
1 Hz rTMS	8.4	10.8	3.6	11.4	8.3	9.0
10 Hz rTMS	41.2	44.8	43.6	0.0	38.9	51.7
18 Hz rTMS	0.6	1.0	0.0	0.0	0.3	2.3
20 Hz rTMS	25.6	43.4	0.0	0.0	27.6	16.9
iTBS	4.2	0.0	52.7	88.6	25.1	20.2
Stimulation device						
Magstim	46.7	73.7	0.0	0.0	46.6	47.1
Neuronetics	16.7	26.3	0.0	0.0	15.8	20.2
Magventure	36.6	0.0	100.0	100.0	37.6	32.7
Pulses per treatment	1,929.0 ± 44.1	2,231.0 ± 40.9	1,669.1 ± 91.9	668.6 ± 32.7	1,885.2 ± 48.5	2,125.4 ± 103.6
Baseline HAMD[a]	21.0 ± 0.4	21.0 ± 0.4	N.A.	N.A.	22.1 ± 0.5	21.4 ± 0.7
Baseline MADRS[a]	28.7 ± 0.5	N.A.	29.4 ± 0.5	25.4 ± 1.5	28.6 ± 0.6	29.1 ± 1.4
Baseline BDI[b]	29.3 ± 0.6	29.5 ± 0.6	N.A.	28.1 ± 1.9	29.9 ± 0.6	26.9 ± 1.3
Baseline PHQ9[b]	17.8 ± 0.4	N.A.	17.8 ± 0.4	N.A.	18.0 ± 0.4	17.0 ± 1.0

BDI, Beck Depression Inventory; HAMD, Hamilton Depression Rating Scale; Hz, Hertz; iTBS, Intermittent Theta Burst Stimulation; MADRS, Montgomery–Åsberg depression rating scale; N, number of subjects; N.A., non-applicable; PHQ9, Patient Health Questionnaire; rTMS, repetitive transcranial magnetic stimulation; SEM, standard error of the mean.

[a] *HAMD was used to assess clinitian-rated depression severity in Boston while MADRS was used in Iowa city and Lisbon.*

[b] *BDI was used to assess self-reported depression severity in Boston and Lisbon while PHQ9 was used in Iowa city.*

sex, TMS device, stimulation side, stimulation protocol (1, 10, 18, or 20 Hz rTMS or iTBS), treatment site, year of treatment, and total number of pulses per treatment (**Table 2**). The *post-hoc* comparisons between the two age groups revealed differences in every week of assessment, favoring larger decrease of depression severity in younger when compared to older patients, until week 5, but no differences observed in week 6. A somewhat similar pattern was also found when considering absolute difference in BDI scores from baseline (**Figure 1C**; **Table 2**), with a significant reduction of depression severity across rTMS treatment (ß = -2.8 ± 0.2, $p < 0.0001$), and significant effects of age group (ß = -1.3 ± 0.6, $p = 0.04$) and the interaction between time and age (ß = 1.4 ± 0.4, $p < 0.0001$). When controlling for the potential confounders mentioned above, as well as baseline BDI, similar effects were obtained for time and interaction, but without a significant effect for age group (**Table 2**). The *post-hoc* comparisons between the two age groups revealed differences only in weeks 3 and 5 of assessment, with larger decreases of depression severity in younger patients. In analyses of absolute difference from baseline with PHQ-9 (**Figure 1D**; **Table 2**), used alternatively to BDI in one of the centers, there was a significant effect of time (ß = -1.1 ± 0.1, $p < 0.0001$), but no effect of age group or interaction between both time and age group. Similar effects were observed when controlling for confounders, including baseline PHQ-9 (**Table 2**), with no differences found between the two age groups in *post-hoc* comparisons.

DISCUSSION

These results provide evidence that rTMS has similar efficacy for treating depression in older and younger adults, as studied in a large naturalistic sample. However, the data suggest that the antidepressant response trajectory differs between these two groups, favoring a slower antidepressant response in older patients. Hence, our results suggest a discrepancy in TMS efficacy between age groups observed in the first weeks of treatment,

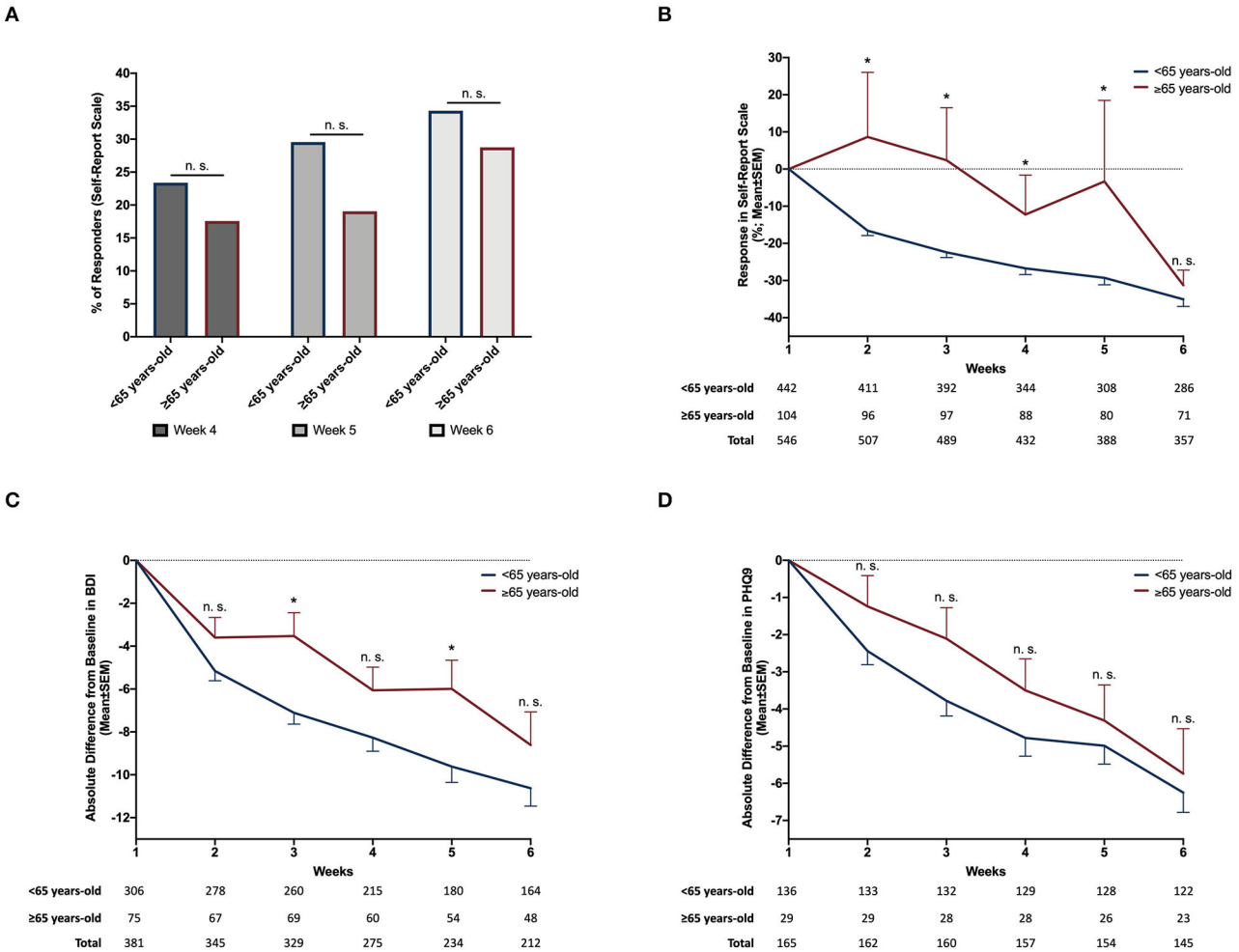

FIGURE 1 | Differences in antidepressant response across rTMS treatment between <65 and ≥65 years-old patients. When comparing the percentage of responders between <65 years-old and ≥65 years-old group using a chi-squared test, no differences were observed in any of the tested time points: 4th week−23.4 vs. 17.6%, $p = 0.2$|5th week−29.6 vs. 19.1%, $p = 0.06$|6th week−34.3% vs. 28.8%, $p = 0.3$ **(A)**. When analyzed using longitudinal mixed effects regression model, % change of depression severity in self-report scale was found to decrease across time ($ß = -7.8 \pm 0.8$, $p < 0.0001$). No differences were observed between the two age groups ($ß = 2.8 \pm 3.6$, $p = 0.4$) but we found a significant interaction between time and age group ($ß = 5.5 \pm 1.9$, $p = 0.003$). *Post-hoc t*-tests revealed larger decreases of depression severity in <65-years-old when compared to ≥65 years-old until week 5 **(B)**. When considering absolute difference of BDI scores relative to baseline (week 1), we also observed that depression severity decreased across time ($ß = -2.8 \pm 0.2$, $p < 0.0001$) as well as a significant effect of age group ($ß = -1.3 \pm 0.6$, $p < 0.04$) and interaction ($ß = 1.4 \pm 0.4$, $p < 0.0001$). *Post-hoc t*-tests revealed larger decrease of depression severity in <65-years-old only in week 3 and 5 **(C)**. When considering absolute difference of PHQ9 scores from baseline (week 1), there was a significant effect of time ($ß = -1.1 \pm 0.1$, $p < 0.0001$), but no effect of age group ($ß = 0.8 \pm 0.5$, $p < 0.1$) or interaction ($ß = 0.002 \pm 0.2$, $p < 1.0$). No differences were found in *post-hoc* comparisons between the two age groups **(D)**. For plots **(B–D)**, while total sample mostly drops across time due to attrition, there are increases in some instances due to reductions in missing values. *$p < 0.05$, *post-hoc* exploratory *t*-tests corrected for multiple comparison using False Discovery Rate (FDR) of 0.05, according to Benjamini and Hochberg (1995); BDI, Beck Depression Inventory; n.s., non-significant; PHQ9, Patient Health Questionnaire; SEM, Standard Error of the Mean.

but that is no longer significantly present when treatment is completed. In fact, this finding could justify why age was previously considered a poor predictor, since only 2 weeks of treatment were considered in that predictive analysis (Fregni et al., 2006). In fact, if we had only considered this shorter treatment period, treatment responses would be significantly different between older and younger patients, with amelioration of mean depression severity only in the younger group. However, when at least a full 6-week rTMS treatment was offered, no differences in treatment response were observed between the two age groups, suggesting that more sessions are needed in older adult patients to improve depression severity. These findings are consistent with our previously published work (Valiengo et al., 2022), where a meta-regression also suggested that more rTMS sessions were associated with enhanced antidepressant response in older adults. Other authors have previously suggested that improved efficacy of rTMS in older adults could be achieved with higher stimulation intensities, to overcome a greater distance

TABLE 2 | Statistical models of antidepressant response across transcranial magnetic stimulation treatment.

Independent variable	Percentage reduction of self-report depression severity				Absolute difference of BDI scores from baseline				Absolute difference of PHQ9 scores from baseline			
	Non-adjusted		Adjusted[a]		Non-adjusted		Adjusted[a]		Non-adjusted		Adjusted[a]	
	ß ± SEM	*p*-value	ß ± SEM	*p*-value	ß ± SEM	*p*-value	ß ± SEM	*p*-value	ß ± SEM	*p*-value	ß ± SEM	*p*-value
Age group	2.8 ± 3.6	0.4	3.6 ± 4.1	0.4	−1.3 ± 0.6	0.04	−1.4 ± 0.7	0.06	0.8 ± 0.5	0.1	0.7 ± 0.5	0.2
Week	−7.8 ± 0.8	<0.0001	−7.8 ± 0.9	<0.0001	−2.8 ± 0.2	<0.0001	−2.9 ± 0.2	<0.0001	−1.1 ± 0.1	<0.0001	−1.1 ± 0.1	<0.0001
Age group x Week	5.5 ± 1.9	0.003	6.0 ± 2.1	0.005	1.4 ± 0.4	<0.0001	1.4 ± 0.4	0.001	0.002 ± 0.2	1.0	−0.0007 ± 0.2	1.0
Sex (Ref.: Female)	N.A.	N.A.	−1.4 ± 3.2	0.7	N.A.	N.A.	0.3 ± 0.4	0.4	N.A.	N.A.	−0.1 ± 0.4	0.8
TMS Device (Ref.: Magstim)	N.A.	N.A.			N.A.	N.A.	–		N.A.	N.A.	N.A.	N.A.
Neuronetics			−2.4 ± 4.6	0.6			0.06 ± 0.5	0.9				
Magventure			−5.5 ± 8.6	0.5			1.2 ± 2.0	0.6				
Stimulation side (Ref.: Right)	N.A.	N.A.	6.3 ± 23.7	0.8	N.A.	N.A.	1.9 ± 2.6	0.5	N.A.	N.A.	0.5 ± 1.0	0.6
Stim. protocol (Ref.: 1Hz rTMS)	N.A.	N.A.			N.A.	N.A.			N.A.	N.A.		
10 Hz rTMS			−2.3 ± 56.4	0.9			−1.8 ± 2.6	0.5			−0.9 ± 0.5	0.07
18 Hz rTMS			−6.6 ± 34.2	0.8			−1.8 ± 3.4	0.6			N.A.	N.A.
20 Hz rTMS			−8.2 ± 24.2	0.7			−2.1 ± 2.7	0.4			N.A.	N.A.
iTBS			−5.4 ± 39.3	0.9			−3.6 ± 3.2	0.3			Omitted[d]	Omitteddd[d]
Site (Ref.: Boston)	N.A.	N.A.			N.A.	N.A.			N.A.	N.A.	N.A.	N.A.
Iowa City			2.0 ± 6.5	0.8			N.A.	N.A.				
Lisbon			Omitted[b]	Omitted[b]			Omitted[b]	Omitted[b]				
Year of treatment	N.A.	N.A.	−0.2 ± 0.8	0.8	N.A.	N.A.	−0.004 ± 0.09	1.0	N.A.	N.A.	−0.2 ± 0.2	0.3
Pulses per treatment	N.A.	N.A.	−0.002 ± 0.04	1.0	N.A.	N.A.	Omitted[c]	Omitted[c]	N.A.	N.A.	Omitted[e]	Omitted[e]
Baseline BDI	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	−0.03 ± 0.02	0.09	N.A.	N.A.	N.A.	N.A.
Baseline PHQ9	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	−0.07 ± 0.03	0.06

BDI, Beck Depression Inventory; Hz, Hertz; iTBS, Intermittent Theta Burst Stimulation; N.A., not applicable; PHQ9, Patient Health Questionnaire; Ref., Reference; rTMS, repetitive transcranial magnetic stimulation; SEM, standard error of the mean; Stim., stimulation; TMS, transcranial magnetic stimulation.

[a]Models are adjusted for potential confounding effects of sex, TMS device, stimulation side, stimulation protocol (1Hz, 10Hz, 18Hz, or 20Hz rTMS or iTBS), treatment site, year of treatment, total number of pulses per treatment.

[b]Omitted because of collinearity with TMS device.

[c]Omitted because of collinearity with stimulation protocol and study center.

[d]Omitted because of collinearity with stimulation side.

[e]Omitted because of collinearity with stimulation protocol.

from coil to brain, secondary to prefrontal atrophy in this population (Nahas et al., 2004). However, increasing stimulation intensity in the presence of atrophy can also lead to greater shunting of the induced current through the cerebrospinal fluid, and thus lower focality of the stimulation (Wagner et al., 2007). Thus, we argue for the potentially safer and more effective approach of allowing for a greater number of sessions in older adults, and hypothesize that in this population, longer rTMS courses, up to 8- or 10-weeks long, may be even more effective. This hypothesis, while supported by the greater antidepressant response at 6 relative to 4-weeks of rTMS treatment (O'Reardon et al., 2007), requires further empirical support.

Our study is not without limitations that should be considered. First, this study has a retrospective, naturalistic, and multicenter design. Accordingly, different socio-demographic or clinical variables, such as education, medication, and illness duration, were not available to collect or analyze, which may potentially limit the interpretation of the findings. While this study design can be regarded as a limitation, it also has some strengths. In fact, clinical research environments that enroll highly selected patients may lack external validity (Fagiolini et al., 2017). This is usually not the case with naturalistic studies, which may more closely reflect the general population of patients, and thus be more generalizable. Additionally, we have analyzed data from a wide range of time, from three different centers. This approach typically further improves the generalizability of results and, if bias occurs, it is most likely non-differential information/misclassification (Ahrens and Pigeot, 2014). Such type of bias favors the null hypothesis across analysis (Ahrens and Pigeot, 2014), which was not the case in the present study, where we found significant differences between age groups, namely in the first stages of treatment, even when adjusting the analysis for potential socio-demographic and clinical confounders available for analysis, and/or correcting for multiple comparisons. Finally, we have also repeated all statistical models, while adjusting for potential confounders, extracted

or all available socio-demographic and clinical variables, and have confirmed results of the original models. Second, since we found close to significant differences in treatment response between age groups at week 5 (**Figure 1A**), it is possible that we may have lacked statistical power to detect significant differences in response rates between groups. Nevertheless, while the difference between % responders in younger and older adults was apparently of larger magnitude at 5 weeks, it still did not reach significance, albeit a reasonably large sample size. Furthermore, this possible difference was markedly diminished at 6 weeks. These findings support that clinical meaningful efficacy is equivalent at the end of treatment. We believe that our results provide important evidence for efficacy of TMS in older adults even in light of these potential limitations.

In conclusion, our results demonstrate that rTMS is an effective treatment for depression in older patients but that longer rTMS therapeutic courses of at least 6-weeks should be provided. This conclusion is of particular importance when considering that some major insurance providers, namely in the USA, have policies that preclude insurance coverage of rTMS for older adults or that make extension of the treatment course beyond 4 weeks dependent on the efficacy achieved up to that point. We expect that the evidence presented here will lead to a revision of such policies, to ensure maximal benefit for older adults who are in particular need of effective treatment option when afflicted by a depressive episode.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because it was generated from different participating centers. Requests to access the datasets should be directed to AJO-M (albino.maia@neuro.fchampalimaud.org) and/or AP-L (apleone@hsl.harvard.edu).

ETHICS STATEMENT

These studies were reviewed and approved by the Champalimaud Foundation Ethics Committee and the Beth Israel Deaconess Medical Center and Iowa University local Institutional Review Boards (IRBs). Champalimaud Foundation patients/participants provided their written informed consent to participate in this study. Beth Israel Deaconess Medical Center and Iowa University IRBs exempted informed consent for retrospective clinical data.

AUTHOR CONTRIBUTIONS

GC, AB, DP, AJO-M, and AP-L conceived and designed the work, acquired the data, and analyzed and interpreted data. GC, AJO-M, and AP-L drafted the manuscript that was critically revised by the remaining authors for important intellectual content. AJO-M and AP-L had full access to all the data in the study and taken the responsibility for the integrity of the data and the accuracy of the data analysis. All authors approved the final version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Conflict of Interest: AJO-M was the national coordinator for Portugal of a non-interventional study (EDMS-ERI-143085581, 4.0) to characterize a Treatment-Resistant Depression Cohort in Europe, sponsored by Janssen-Cilag, Ltd (2019–2020), is the recipient of a grant from Schuhfried GmBH for norming and validation of cognitive tests, and is the national coordinator for Portugal of trials of psilocybin therapy for treatment-resistant depression, sponsored by Compass Pathways, Ltd (EudraCT number 2017-003288-36), and of esketamine for treatment-resistant depression, sponsored by the Janssen-Cilag, Ltd (EudraCT NUMBER: 2019-002992-33). AP-L is a co-founder of Linus Health and TI Solutions AG; serves on the scientific advisory boards for Starlab Neuroscience, Magstim Inc., Radiant Hearts, and MedRhythms; and is listed as an inventor on several issued and pending patents on the real-time integration of non-invasive brain stimulation with electroencephalography and magnetic resonance imaging. None of the aforementioned agencies or companies had a role in the design and conduct of the study, in the collection, management, analysis, and interpretation of the data, in the preparation, review, or approval of the manuscript, nor in the decision to submit the manuscript for publication.

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.

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Effects of sulforaphane intake on processing speed and negative moods in healthy older adults: Evidence from a randomized controlled trial

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Background: Recent studies have reported that sulforaphane (SFN) intake with cognitive training had positive effects on cognitive functions. However, it is still unknown whether SFN intake alone has beneficial effects on cognition as well as mood. We investigated whether a SFN intake intervention improved cognitive performance and mood states in healthy older adults.

Methods: In a 12-week, double-blinded, randomized controlled trial (RCT), we randomly assigned 144 older adults to a SFN group or a placebo group. We asked the participants to take a supplement (SFN or placebo) for 12 weeks. We measured several cognitive functions, mood states, and biomarkers before and after the intervention period.

Results: The SFN group showed improvement in processing speed and a decrease in negative mood compared to the placebo group. In addition, the SFN group exhibited a higher SFN-*N*-acetyl-L-cysteine (NAC) level compared to the placebo group. However, there were no significant results in other biomarkers of oxidant stress, inflammation, or neural plasticity.

Discussion: These results indicate that nutrition interventions using SFN can have positive effects on cognitive functioning and mood in healthy older adults.

KEYWORDS

sulforaphane, nutrition, processing speed, mood state, biomarker

Introduction

Aging is a global phenomenon and negatively affects cognitive functioning (Salthouse, 2019) and mood state (Karel, 1997). Cognitive decline and negative mood are associated with challenges in everyday behavior and social communication (de Paula et al., 2015). For example, older adults with lower cognitive functioning and depressive

symptoms engaged in fewer activities of daily living (ADLs) (de Paula et al., 2015). Thus, it is vital to enhance cognitive functioning and mood state in this population.

Nutrients are critical for better cognitive and mental health in older adults. Phytochemicals contain color, aroma, and flavor and are found in fruits, vegetables, grains, beans, and other plants; examples include carotenoids, flavonoids, and isothiocyanates. Their consumption generally provides beneficial health effects (Tan and Norhaizan, 2019; Rapposelli et al., 2022). Several randomized controlled trials (RCTs) found that carotenoids and flavonoids had benefits on cognitive functions and mood states (Nouchi et al., 2020b; Tanprasertsuk et al., 2021; Yagi et al., 2021; Park et al., 2022). However, few studies have investigated the beneficial outcomes of isothiocyanate on cognitive functioning and mood state in healthy older adults (Nouchi et al., 2021a). Hence, we aimed to examine the influence of isothiocyanate on cognitions and mood state in healthy older adults.

Sulforaphane (SFN) is an isothiocyanate found in cruciferous vegetables such as cauliflower and broccoli. Only two RCTs with human participants have explored the effects of SFN intake on cognitive functioning and mood (Shiina et al., 2015; Nouchi et al., 2021a). SFN intake has shown beneficial effects in cognitive performance and reduced symptoms in schizophrenia patients (Shiina et al., 2015). Further, another study involving healthy older adults revealed that cognitive training combined with SFN enhanced several cognitive functions (Nouchi et al., 2021a). In addition, previous animal studies have found that SFN reduced depressive behaviors in mice (Wu et al., 2016; Zhang et al., 2017; Ferreira-Chamorro et al., 2018). Hence, SFN intake could have beneficial effects on cognitive functioning and mood state. However, it is still unknown whether SFN alone could improve cognitive functioning and mood state in healthy older adults. As such, we conducted an RCT to examine the benefits of SFN intake on cognitive functioning and mood state in healthy older adults.

For several reasons, we hypothesized that SFN intake would improve processing speed, working memory, and negative mood state. First, previous studies using older adults have demonstrated that SFN intake, combined with cognitive training, boosted processing speed performance, and working memory capacity (Nouchi et al., 2021a). Second, the intake of fruits and vegetables has been correlated with lower negative mood in older adults (Ford et al., 2013). Taken together, we expected that SFN intake would heighten the performance of processing speed and the capacity of working memory and reduce negative mood.

To consider the biological mechanism of the effects of SFN, we measured blood biomarkers [oxidative stress: heme oxygenase-1 (HO-1) and glutathione S-transferase (GST)], inflammation [tumor necrosis factor-α (TNF-α)], neural plasticity [brain derived neurotrophic factor (BDNF)], and a urine biomarker [SFN absorption: sulforaphane *N*-acetyl-L-cysteine (SFN-NAC)]. Previous animal studies have reported the effects of SFN on the biomarkers of oxidative stress, inflammation, and neural plasticity (Dwivedi et al., 2016; Wang et al., 2016; Jhang et al., 2018). In addition, prior studies with human participants found that SFN intake led to changes in the SFN-NAC level (Nouchi et al., 2021a). Thus, we expected that SFN intake would alter the levels of HO-1, GST, TNF-α, and BDNF. Moreover, we expected the SFN intake group to exhibit a greater SFN absorption level compared to the placebo group, whose members took placebo supplements without SFN (please see "Sulforaphane and placebo supplements" section).

Materials and methods

Design and setting of the randomized controlled trial

We carried out an RCT from March 2021 to June 2021 in Tokyo, Japan. The protocol was approved by the Ethics Committee of KAGOME CO., LTD. and the Ethics Committee of Nihonbashi Cardiology Clinic. Further, we registered the RCT with the University Hospital Medical Information Network Clinical Trial Registry (UMIN000042666).

This RCT was a double-blinded RCT using an SFN group and a placebo group. We blinded participants and testers to the specific research hypotheses related to cognitive functions and mood and their group membership. We used several cognitive, emotional, and biological measures. The primary outcome measures were the performance of a processing speed test and working memory scores. Further, we employed the Consolidated Standards of Reporting Trials statement (see **Supplementary Table 1**)[1] to report the study structure. The RCT design is shown in **Figure 1**.

Participants

We recruited participants *via* e-mail from a pool of individuals listed by L. Smile Co., Ltd. Subsequently, 321 individuals were invited to participate in a screening. After receiving written informed consent from each participant, the testers checked whether they were eligible for the study. All participants underwent the screening, which included a blood test, the Japanese version Mini-Mental State Examination (MMSE-J) (Folstein et al., 1975), the Frontal Assessment Battery at bedside (FAB) (Dubois et al., 2000), the Japanese Reading Ability Test (JART) (Matsuoka et al., 2006), and the Geriatric Depression Scale-15 (GDS) (Sugishita et al., 2017). We excluded

1 http://www.consort-statement.org/home/

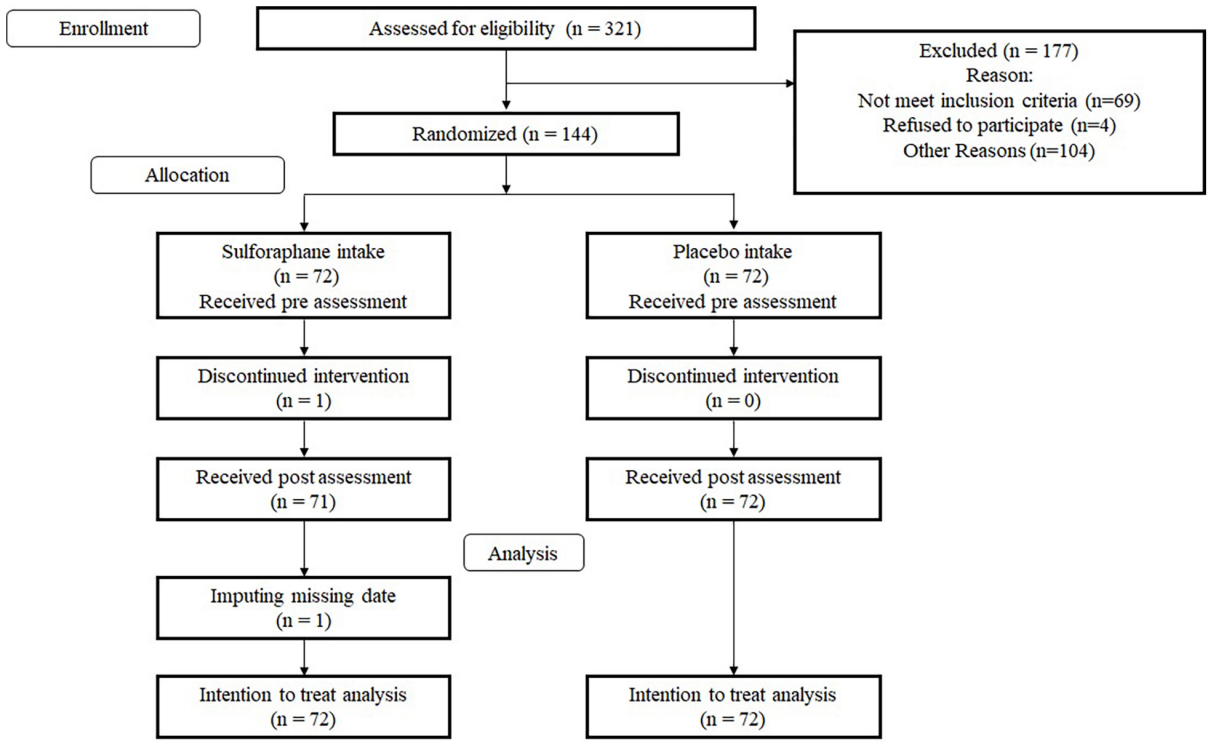

FIGURE 1
CONSORT diagram.

69 participants based on the results of the screening. Four individuals refused to participate, and 104 did not take part due to other reasons. Finally, 144 individuals (73 men, 71 women) participated. Average age of all participants was 66.82 years (standard deviation, *SD* = 4.29). One person in the SFN group did not complete the intervention due to an inability to adhere to the study's schedule. **Table 1** shows the baseline characteristics in each group.

TABLE 1 The baseline characteristics in each group.

	SFN	PL
Age	66.89	66.75
	(4.44)	(4.17)
MMSE-J	28.67	28.71
	(1.20)	(1.13)
FAB	15.10	15.13
	(1.94)	(1.88)
JART	109.78	109.58
	(9.06)	(8.04)
GDS	1.42	1.43
	(1.30)	(1.34)

SFN, sulforaphane; PL, placebo; MMSE, Japanese version's Mini-Mental State Examination; FAB, Frontal Assessment Battery at bedside; JART, Japanese Reading Test; GDS, Geriatric Depression Scale.

Inclusion and exclusion criteria

Based on our earlier studies (Nouchi et al., 2012, 2016a,b; Nozawa et al., 2015; Kawata et al., 2022), we set the following inclusion criteria: participants should be healthy native Japanese (60–80 years of age). They agreed to receive medical and physical checkups and to have samples of their blood taken. The exclusion criteria were as follows: Participants (1) were suffering from mental illness, diabetes, neurological disease, heart (cardiac) disease, or another serious illness; (2) using medications that interfere with cognitive functioning (including benzodiazepines, antidepressants, or other central nervous system agents); (3) had a history of serious illness and problems that precluded them from participation; (4) had an MMSE-J score of less than 26; (5) had a GDS score higher than 5; (6) had an FAB score of less than 12; (7) had an IQ score lower than 85; (8) had food allergy concerns (especially allergies to broccoli, milk, eggs, wheat, buckwheat, peanuts, shrimp, and crab); (9) drank more than 60 g of alcohol per day on average for a week; (10) had taken part in other intervention-based studies within the past 2 months; (11) were unable to refrain from using medicines and health foods (including supplements, foods for specified health use, foods with health claims, aojiru, energy drinks) during the intervention period; or (12) were unable to ingest the SFN or placebo supplements as

instructed during the intervention period and to fill out the life diary. It noted that aojiru is a powdered health food in Japan, which made mainly from kale, a cruciferous vegetable contained glucoraphanin. Finally, we excluded participants whom the principal investigator judged to be inappropriate for the study.

Sample size calculation

Using symbol search (SS), a prior study using older adults found that 12 weeks of SFN intake improved processing speed performance (Nouchi et al., 2021a). In the previous study, the effect size was a medium (Cohen' d = 0.48, the SFN group vs. the control group). Therefore, we assumed that the SFN group would have a greater effect in SS compared to the placebo group in this study. The sample size was calculated using G*power which was a tool to estimate a sample size (Faul et al., 2009). We used the general liner model [processing speed score at the baseline, MMSE-J, gender, and age as the covariates, a one-tailed test, $\alpha = 0.025$, power = 0.80, effect size (f^2) = 0.05]. We computed the f^2 based on the abovementioned study (Nouchi et al., 2021a). Finally, we set 144 as the sample size.

Randomization

After recruitment, we randomly assigned the 144 eligible participants into the two groups using JMP software, Version 5.01 (SAS Institute, Cary, NC). The cognitive functions were the primary outcome measures, with sex affecting several aspects of cognitive performance (McCarrey et al., 2016). Thus, we stratified the participants based on sex; in addition, we adjusted other attributes (age, pre-MMSE-J) as moderating factors, with an allocation ratio of 1:1.

Sulforaphane and placebo supplements

The participants took three capsules of the SFN or placebo supplements per day for 12 weeks. Participants took the three capsules with water at once. The SFN supplements contained 30 mg of glucoraphanin, which is converted to SFN *via* gut microbial thioglucosidase (Fahey et al., 2012). The placebo supplements contained 0 mg of glucoraphanin (Fahey et al., 2012). KAGOME CO., LTD. provided both the supplements (Nagoya, Japan). Researchers, participants, and testers were blinded to the supplements until the study was completed.

Cognitive functioning

Firstly, we assessed the participants' cognitive status using the MMSE-J, FAB, and JART during the screening. To obtain a comprehensive picture of cognitive status, we used the MMSE-J which gauges individuals' memory, attention, language, and visuospatial abilities (Folstein et al., 1975). Moreover, to ascertain participants' reading ability and IQ, we used JART (Matsuoka et al., 2006), the Japanese version of the National Adult Reading Test. JART depicts 25 kanji (Chinese characters) compound words, and the participants were asked to write down the pronunciation of each kanji compound word. The FAB is a simple screening test that measures frontal lobe functioning (Dubois et al., 2000).

We examined executive functioning, processing speed, visual, and verbal episodic memory performance, verbal short-term memory performance, and a capacity of working memory using the standardized cognitive tests. In this study, processing speed was measured by SS and digit symbol coding (Cd) from the Wechsler Adult Intelligence Scale, 3rd edition (WAIS-III) (Wechsler, 1997). In the SS, participants were asked to check whether the target symbols existed in the other symbol pool. In the Cd, participants were asked to draw the symbol corresponding numbers. For executive functioning, we used Trail making test (TMT) (Japan Society for Higher Brain Dysfunction, Brain function test committee, 2019) and two types of Stroop tests such as Stroop task (ST) and reverse Stroop task (rST) (Hakoda and Watanabe, 2004). In the TMT, participants were asked to link numbers or letters. Short-term memory was measured by digit span (DS) (Wechsler, 1997). The digit span requires participants to memorize the series of numbers in the same order or in the reverse order. Working memory was measured by letter number sequence (LNS) (Wechsler, 1997). In LNS, participants listened to the combination of numbers and letters. Then, they were asked to recall numbers and letters in the ascending order. For episodic memory, we used logical memory (LM), design memory (DM), visual paired associates (VPA), and visual reproduction (VR) from the Wechsler Memory Scale-Revised (WMS-R) (Wechsler, 1987). The LM was the verbal episodic memory task. In the LM, participants were asked to memorize the short story and recall the story immediately and after about 30 min delay. The DM, the VPA, and the VR were visual episodic memory task. Participants were required to memorize the abstract figures in the DM, the pairs of figures and colors in the VPA, and geometric shapes in the VR. The details of each cognitive measure were shown in **Supplementary File 1**.

Emotional state

For the participant's emotional state, we used a short version of the Profile of Mood State Second Edition (POMS2) (Heuchert and McNair, 2012; Yokoyama and Watanabe, 2015). In the POMS2, participants were asked to rate the six emotional sates [depression–dejection (D), confusion–bewilderment (C–B), friendliness (F), vigor–activity (V), fatigue–inertia (F–I), anger–hostility (A–H), tension–anxiety (T–A)] in the prior

week. The total mood disturbance (TMD) scores was estimated using the six emotional states (Nouchi et al., 2021a).

We measured quality of life using the World Health Organization-Five WellBeing Index (WHO-5). In WHO-5, participants were asked to rate their mental wellbeing within 2 weeks. A higher score in WHO-5 means good mental wellbeing (Nouchi et al., 2021a).

Blood biomarkers

We measured antioxidant response (GST and HO-1), neuroplasticity (BDNF), and the neuroinflammation (TNF-α) blood parameter. Experienced nurses collected blood through venipuncture in 5 mL separator tubes for serum and 5 mL EDTA tubes for plasma (Nipro Corporation, Osaka, Japan). The obtained serum and plasma samples were aliquoted and stored at –80°C.

BDNF has three different forms such as prodomain BDNF, mature BDNF, and precursor BDNF (Hempstead, 2015). The mature BDNF has neurotrophic and neuroprotective functions. In this study, we measured the serum levels of mature BDNF using a mature BDNF rapid ELISA kit (Biosensis, Thebarton, Australia). The serum levels of TNF-α were measured with and a Quantikine HS ELISA kit (R&D systems, Minneapolis, MN, United States), respectively, following the manufacturer's instructions. The enzyme activity of GST was determined with 1-chloro-2,4-dinitrobenzene as the substrate, as described previously (Kikuchi et al., 2015). The plasma level of HO-1 was determined using a StressXpress HO-1 ELISA kit (StressMarq Biosciences, Victoria, BC, Canada) following the manufacturer's instructions.

Urine biomarker

We used the excreted level of SFN-NAC from urine samples. The SFN-NAC is a main metabolite of SFN. The urine sample was collected each participant 10–12 h after the intake of the SFN or placebo at the last day. The time was when urinary excretion of isothiocyanate metabolites reached a maximum level (Ushida et al., 2015). The urinary levels of SFN-NAC were determined using a LC-MS/MS system. In this study, the urinary levels of SFN-NAC were standardized to creatinine levels (Nouchi et al., 2021a). The details of the urine analysis were described in **Supplementary File 2**.

Analyses

We performed all analyses using the software R (Version 4.12). One person in the SFN group did not take the tests after the intervention period. Therefore, all cognitive, emotional, and biomarker measures of the one participant were missing data. First, we imputed missing data using the multiple imputation method (predictive mean matching, $m = 20$) *via* the "mice" function in the mice package (van Buuren and Groothuis-Oudshoorn, 2011). Next, we calculated the changes in scores for cognitive functioning, emotional states, and biomarkers (the post-intervention score minus the pre-intervention score). Second, we used a permutation general linear model (GLM) for all changes in scores in cognition, emotional states, and biomarkers. In the model, the change score in cognition, emotion or biomarkers was the dependent variable, and the supplement factor (SFN or placebo) was the independent variable. In the GLM for measuring cognitive functioning, we used the pre-scores for the dependent variable, MMSE score, age, and sex as covariates. In the GLM for measuring emotional states, we employed the pre-scores for the dependent variable, age, and sex as covariates. In the GLM for measuring biomarkers using blood samples, we used the pre-scores for the dependent variable (age and sex) as covariates. In addition, to check an absorbed level of SFN, we conducted permutation GLM (SFN vs. placebo) for SFN-NAC urinary levels. We performed all GLMs with permutation tests using the "lmp" function in the lmPerm package.[2] The permutation GLM was the same as in previous studies (Nouchi et al., 2020a, 2021a,b; Kawata et al., 2021). Finally, based on our hypotheses, we harnessed a gatekeeping procedure based on Holm methods to adjust all of the pooled p-values. We carried out the gatekeeping procedure using the "pargateadjp" in multxpert package. We deemed the adjusted value $p < 0.05$ to be significant for multiple comparison methods.

Results

We checked participants' adherence to intake of supplements using a permutation test. The analysis did not reveal any significant differences between groups ($p > 0.05$) for the number of supplement-intake days (maximum = 84 days: SFN (*mean* = 83.9, *SD* = 0.4) or the placebo (*mean* = 83.9, *SD* = 0.2). In addition, we ensured there was no significant difference of the baseline measures between the groups (**Table 1**).

We analyzed the data based on the ITT (intention to treat) rule. For cognitive functioning, we found significant group differences in SS ($t = 2.14$, *uncorrected* $p = 0.01$, *adjusted* $p = 0.03$; **Table 2**). The processing speed performance of the SFN group improved compared to the placebo group (**Table 2**). For mood state, we found significant group differences in TMD ($t = 2.15$, *uncorrected* $p = 0.01$, *adjusted* $p = 0.03$; **Table 2**). Moreover, supplementary analyses for the subscale of POMS2 indicated

2 http://cran.r-project.org/web/packages/lmPerm/index.html

that SFN reduced A-H (t = 1.53, *uncorrected* p = 0.02) and C-B (t = 1.51, *uncorrected* p = 0.02) compared to the placebo (see **Supplementary Table 2**).

For blood biomarkers, we did not find any significant group differences in oxidative stress (HO-1 and GST), inflammation (TNF-α), or neural plasticity (BDNF) (**Table 3**). However, we confirmed that the SFN group showed a greater SFN-NAC level compared to the placebo group (t = 8.68 *uncorrected* $p < 0.01$, *adjusted* $p < 0.01$, **Figure 2**).

Discussion

We aimed to reveal the beneficial effects of SFN intake on cognitive functioning and mood state in healthy older adults. We formulated three hypotheses related to cognitive functioning, mood state, and biomarkers. We expected that SFN intake would improve processing speed, decrease negative mood, and alter biomarkers. We derived three key findings corresponding to these hypotheses. For cognitive functioning, the SFN group exhibited a significant improvement in processing speed performance (measured by SS) compared to the placebo group. For emotional states, the SFN group significantly decreased negative mood (measured by TMD) compared to the placebo group. For the biomarkers, we found significant group differences in urinary SFN-NAC levels. In contrast, we did not observe any significant changes in the other biomarkers. We separately discuss each outcome below.

First, we noted a beneficial impact of SFN on processing speed performance. This result partially supports our hypothesis; it is consistent with previous cohort studies and RCTs. For example, one cohort study reported that people who consumed a large amount of cruciferous vegetables had better processing speed performance (Nurk et al., 2010). In addition, this result was consistent with a RCT using SFN intake and video game training. The previous study used 4 groups with 2 (nutrition factor; SFN intake or placebo intake) by 2 (video game factor: brain training game or puzzle game) factorial design. The previous study revealed both brain training and puzzle games groups with SFN intake led to a significant improvement of processing speed performance in healthy older adults (Nouchi et al., 2021a). In the previous study, it did not exclude an effect of playing video gaming. However, the current study is the first to demonstrate direct evidence that SFN intake alone enhances processing speed in healthy older adults. Taken together, these findings indicate that SFN intake can produce beneficial effects for processing speed performance.

Second, SFN intake decreased negative mood (measured by TMD). In addition, supplementary analyses for the subscale of POMS revealed that SFN intake selectively reduced the A-H and C-B scores. These results also support our hypothesis. The current finding is in line with previous evidence from animal and patient studies. Prior animal studies found that SFN treatment suppressed depression-like behaviors in mice (Wu et al., 2016; Zhang et al., 2017; Ferreira-Chamorro et al., 2018). Moreover, past studies on autism spectrum disorder (ASD) demonstrated that SFN reduced aberrant behaviors related to negative mood (Singh et al., 2014; Bent et al., 2018). The present study can expand existing findings to demonstrate that SFN intake can reduce the TMD score in healthy older adults.

For the biomarkers, we noted significant group differences in urinary SFN-NAC levels, but not in other biomarkers. This result partially supports our hypothesis related to SFN-NAC. The urinary SFN-NAC level is an indicator of SFN absorption. The increased level of SFN-NAC in the SFN group confirmed the compliance with taking the SFN or placebo supplements. In addition, we replicated the previous work, which showed increasements in SFN-NAC levels after SFN intake combined with cognitive training (Nouchi et al., 2021a). This study used the same method (participants, SFN supplements, intervention period) as the previous study. Taken together, this indicates that 12 weeks of intervention using SFN supplements containing 30 mg of glucoraphanin is enough to increase SFN absorption in healthy older adults.

We did not observe any significant effects of SFN on the blood biomarkers of antioxidant response, neural plasticity, or the neuroinflammation blood parameter. These results do not support our hypothesis. However, past human studies discovered that SFN intake did not change the antioxidant response level of HO-1 (Wise et al., 2016), the neuroinflammatory parameters of C-reactive protein (e.g., interleukin-6, interleukin-8) (Wise et al., 2016), or neural plasticity parameters (e.g., BDNF) (Shiina et al., 2015). In contrast, a prior study revealed a positive impact of SFN on antioxidant response, measured using GST (Egner et al., 2014). It should note that there are several methodological differences (biomarkers, intervention period, and participants) between the current study and the aforementioned ones.

It is important to consider the biological mechanism of SFN's beneficial effects on cognitive functioning and mood state. SNF activates the Nrf2 antioxidant response, but may also have Nrf2-independent actions. The anti-oxidative and anti-inflammatory properties of SFN are key functions in explaining improvements in cognitive functioning and mood state. SFN has anti-oxidative and anti-inflammatory functions (Juge et al., 2007). Previous studies showed that the inflammation and oxidative stress levels were associated with cognitive functioning (Sartori et al., 2012; Hajjar et al., 2018) and negative mood state (Salim, 2014; Jones et al., 2020). It has been hypothesized that SFN intake will reduce oxidative and inflammatory levels, thereby enhancing cognitive functioning and mood state. However, our results do not directly support this hypothesis since we found no significant changes in the biomarkers of the antioxidant and anti-inflammatory levels following SFN intake. As mentioned before, there are some methodological limitations. It is difficult to conclude

TABLE 2 Baseline and change scores in cognitive functions and mood states in each group.

	Baseline score			Change score			
	PL	SFN	*p*-value	PL	SFN	*p*-value	Adjusted *p*-value
Cd	74.88	73.54	0.24	2.54	3.28	0.33	0.83
(Processing speed)	(14.26)	(14.09)		(6.64)	(7.37)		
SS	37.46	37.17	0.62	1.29	1.99	0.01	0.03
(Processing speed)	(7.34)	(5.79)		(4.76)	(3.83)		
ST	31.85	31.33	1.00	3.08	2.92	0.45	1
(Executive function)	(9.81)	(9.44)		(5.90)	(8.15)		
rST	44.79	45.68	0.37	2.50	2.90	0.26	1
(Executive function)	(9.13)	(7.75)		(4.65)	(5.63)		
TMT	46.08	43.32	0.40	−1.67	−0.93	0.36	1
(executive function)	(29.53)	(18.99)		(32.98)	(25.46)		
DS	17.21	17.04	0.52	0.56	0.07	0.08	0.2
(Verbal short-term memory)	(3.21)	(3.65)		(2.97)	(1.80)		
LNS	11.39	11.61	0.60	−0.08	−0.30	0.13	1
(Working memory capacity)	(2.23)	(2.70)		(2.32)	(2.25)		
LM immediately	22.99	24.31	0.27	2.51	2.41	0.50	1
(verbal episodic memory)	(6.74)	(7.06)		(4.34)	(4.73)		
LM delay	19.01	20.00	0.36	2.83	3.72	0.13	1
(Verbal episodic memory)	(7.36)	(7.60)		(4.71)	(5.69)		
DM	7.06	6.99	0.41	0.21	−0.03	0.12	1
(visual episodic memory)	(1.54)	(1.41)		(1.54)	(1.87)		
VPA	13.85	13.40	0.86	0.61	1.23	0.02	1
(Visual episodic memory)	(3.50)	(3.76)		(2.88)	(3.13)		
VR	33.67	34.08	0.94	0.83	0.32	0.08	1
(Visual episodic memory)	(4.58)	(3.30)		(3.72)	(3.17)		
TMD	−0.54	−1.44	0.76	1.60	−0.11	0.01	0.03
(Mood state)	(9.11)	(8.78)		(9.40)	(6.95)		
WHO-5	17.60	16.79	0.37	0.36	0.82	0.64	1
(Quality of life)	(3.85)	(3.14)		(3.33)	(3.04)		

Standard deviation (SD) in parentheses. SFN, sulforaphane; PL, placebo; Cd, digit symbol coding; SS, symbol search; ST, Stroop task; rST, reverse Stroop task; TMT, trail making test; DS, digit span; LNS, letter number sequence; LM, logical memory; DM, design memory; VPA, visual paired associates; VR, visual reproduction; TMD, total mood disturbance; WHO-5, World Health Organization-five wellbeing index.

whether SFN's anti-oxidative and anti-inflammatory properties can definitively explain improvements in cognitive functioning and mood. Hence, to confirm this hypothesis, future research should investigate the effects of SFN's antioxidant and anti-inflammatory properties on cognitive functioning and mood using other populations and biomarkers.

Other proposed biological mechanisms include changes in neurotransmitter release and mitochondrial quality control (Huang et al., 2019). It is well known that neurotransmitters are associated with cognitive functioning and negative mood (Jenkins et al., 2016). In addition, the dysfunction of mitochondrial quality control is an essential hallmark of age-related cognitive decline (Apaijai et al., 2020) and negative mood, such as depressive symptoms (Allen et al., 2018). Previous studies, using 12-week periods of SFN intake for children with ASD, reported a significant correlation between improved clinical symptoms and a change in several urinary neurotransmitter-related metabolites, such as serotonin and homovanillate (HMV), which represent the normal end product of dopamine degradation (Bent et al., 2018). In addition, several previous animal studies found effects of SFN on brain mitochondria (Jardim et al., 2020). Thus, it is possible for changes in neurotransmitter release and modulations in mitochondrial quality control *via* SFN intake to enhance cognitive functioning and alleviate negative mood. However, we did not measure the functions of neurotransmitters or mitochondria. To prove these hypotheses, future research should investigate the role of neurotransmitters and mitochondria in boosting cognitive functioning and mood.

This study has some limitations. First, the intervention period: We set the length of the intervention period (12 weeks) based on a prior study (Nouchi et al., 2021a). However,

TABLE 3 Baseline and change scores in biomarkers in each group.

	Baseline score			Change score			
	PL	SFN	*p*-value	PL	SFN	*p*-value	Adjusted *p*-value
GST	9.09	8.90	0.99	4.63	3.35	0.18	1
	(6.75)	(6.40)		(15.54)	(14.25)		
HO-1	0.64	0.45	0.35	−0.13	−0.04	0.09	1
	(1.53)	(0.32)		(0.82)	(0.24)		
TNF-α	0.44	0.41	0.42	−0.05	0.03	0.20	1
	(0.31)	(0.29)		(0.29)	(0.33)		
BDNF	26.55	26.28	0.78	−2.25	−2.80	0.99	1
	(6.77)	(7.28)		(5.80)	(7.95)		
SFN-NAC	–	–	–	0.18	1.94	<0.01	<0.01
	–	–	–	(0.46)	(1.67)		

Standard deviation (SD) in parentheses. SFN, sulforaphane; PL, placebo; GST, glutathione S-transferase; HO-1, heme oxygenase-1; TNF-α, tumor necrosis factor-α; BDNF, brain derived neurotrophic factor; SFN-NAC, sulforaphane N-acetyl-L-cysteine. SFN-NAC was only measured after the intervention period.

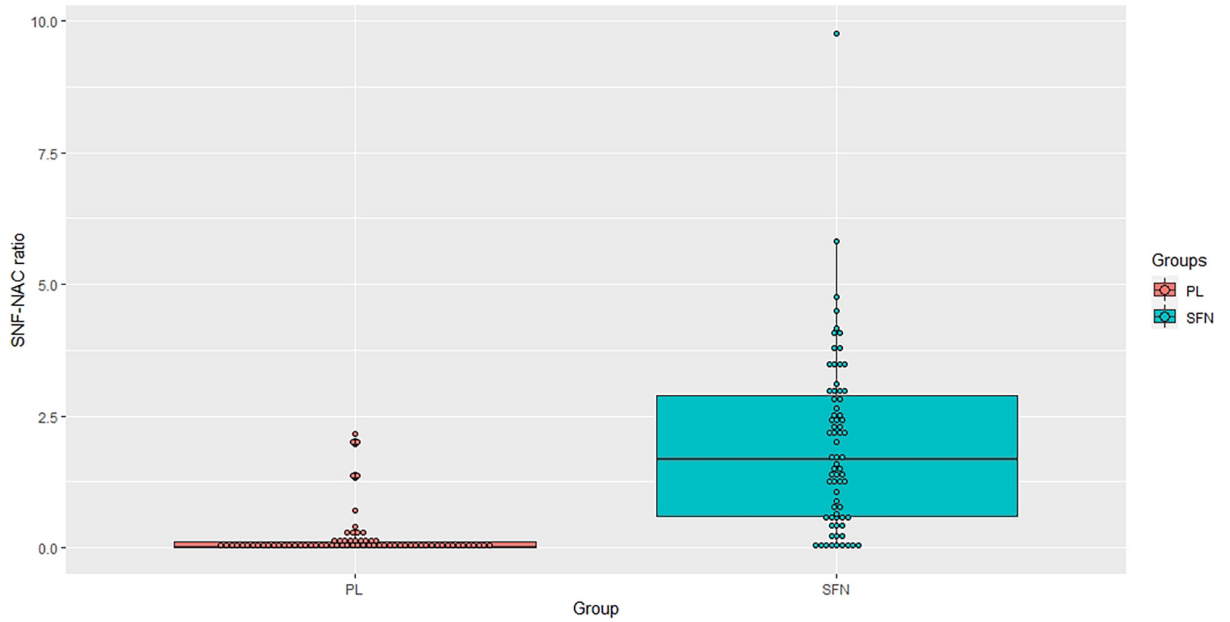

FIGURE 2
SNF-NAC ratio in each group.

it is important to examine whether shorter or longer SFN intervention periods would have positive outcomes for cognitive functioning and emotional states. In addition, a peak level of some biomarkers would occur earlier in the intervention period. The level of the biomarkers would be reduced over the intervention period. Therefore, it would be also important to check the biomarker multiple times. Second, the participants were healthy older adults. To generalize the findings, it is necessary to test whether SFN intake would have beneficial effects for young and middle-aged adults as well. Third, for cognitive functions, we found the significant improvements in VPA in the SFN group compared to the placebo group without adjustment of the *p*-value. However, the result did not survive after Bonferroni correction. It indicates that SFN would have a possibility to improve the visual episodic memory performance in older adults. Further study should be needed to evaluate the beneficial effect of SFN on the visual episodic memory. Finally, we did not control the amount of SFN intake from vegetables consumed daily before the intervention. The baseline difference in SFN intake can affect improvements in cognitive functioning and mood state. In future research, the amount of SFN intake should be controlled.

Conclusion

We explored the beneficial effects of SFN intake on cognitive functioning and mood in healthy older adults. We found that 12 weeks of SFN intake boosted processing speed, reduced overall negative mood, and increased the SFN-NAC level compared to the placebo group, whose members took supplements without SFN. Although we did not find any significant changes in antioxidant response, neural plasticity, or the neuroinflammation blood parameter, these results indicate that nutrition interventions using SFN can have positive effects on cognitive functioning and mood in healthy older adults.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of KAGOME CO., LTD. (Ref. 2020-R11) and the Ethics Committee of Nihonbashi Cardiology Clinic (Ref. NJI-020-10-01). The patients/participants provided their written informed consent to participate in this study.

Author contributions

RN and QH: conceptualization, methodology, investigation, data curation, writing—original draft preparation, visualization, and project administration. RN: formal analysis and funding acquisition. RN, QH, YU, HS, and RK: writing—review and editing. RK: supervision. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

QH, YU, and HS were full-time employees of KAGOME CO., LTD.

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.

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Supplementary material

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The multi-domain exercise intervention for memory and brain function in late middle-aged and older adults at risk for Alzheimer's disease: A protocol for Western–Eastern Brain Fitness Integration Training trial

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Background: Aging is associated with cognitive decline, increased risk for dementia, and deterioration of brain function. Modifiable lifestyle factors (e.g., exercise, meditation, and social interaction) have been proposed to benefit memory and brain function. However, previous studies have focused on a single exercise modality or a single lifestyle factor. Consequently, the effect of a more comprehensive exercise program that combines multiple exercise modalities and lifestyle factors, as well as examines potential mediators and moderators, on cognitive function and brain health in late middle-aged and older adults remains understudied. This study's primary aim is to examine the effect of a multi-domain exercise intervention on memory and brain function in cognitively healthy late middle-aged and older adults. In addition, we will examine whether apolipoprotein E (*ApoE*) genotypes, physical fitness (i.e., cardiovascular fitness, body composition, muscular fitness, flexibility, balance, and power), and brain-derived neurotrophic factor (BDNF) moderate and mediate the exercise intervention effects on memory and brain function.

Methods: The Western-Eastern Brain Fitness Integration Training (WE-BFit) is a single-blinded, double-arm, 6-month randomized controlled trial. One hundred cognitively healthy adults, aged 45–70 years, with different risks for Alzheimer's disease (i.e., *ApoE* genotype) will be recruited and randomized into either a multi-domain exercise group or an online educational course control

group. The exercise intervention consists of one 90-min on-site and several online sessions up to 60 min per week for 6 months. Working memory, episodic memory, physical fitness, and BDNF will be assessed before and after the 6-month intervention. The effects of the WE-BFit on memory and brain function will be described and analyzed. We will further examine how *ApoE* genotype and changes in physical fitness and BDNF affect the effects of the intervention.

Discussion: WE-BFit is designed to improve memory and brain function using a multi-domain exercise intervention. The results will provide insight into the implementation of an exercise intervention with multiple domains to preserve memory and brain function in adults with genetic risk levels for Alzheimer's disease.

Clinical trial registration: ClinicalTrials.gov, identifier: NCT05068271.

KEYWORDS

***ApoE* gene, BDNF, fitness, meditation, memory, social interaction, brain function**

Background

The rapid growth of the aged population has dramatically shifted the global demographic structure (United Nations., 2019). Advancing age has been associated with cognitive decline (Salthouse, 2019), progressive brain deterioration (Li et al., 2020), and increased incidence of various types of dementia, particularly Alzheimer's disease (AD) (Livingston et al., 2020). Considering the significant impact of age-related cognitive decline and AD on individual and societal levels (Roberts et al., 2015; Alzheimer's Association., 2021), as well as the limited curative effects of approved drugs on the progression of AD (Mehta et al., 2017), identifying potentially modifiable lifestyle factors for cost-effective, non-pharmaceutical preventative strategies has been a priority to lessen the adverse impact of aging on cognitive function and brain function.

Exercise, memory, and brain function

Exercise, a planned, structured, and repetitive form of physical activity that aims to improve or maintain one or more components of physical fitness (e.g., cardiovascular and muscular fitness, balance, coordination, and power) (Caspersen et al., 1985), might be a promising non-pharmaceutical preventative approach for decelerating the trajectory of age-related cognitive decline (Chang et al., 2017b; Northey et al., 2018; Chen et al., 2020a) and the deterioration of brain function (Chen et al., 2020b), as well as for preventing or delaying the onset of AD (Valenzuela et al., 2020). Empirical research has revealed that exercise could beneficially alter the metabolomic profiles related to memory (Gaitán et al., 2021), and greater physical fitness levels associated with exercise have been linked to superior cognitive function (Netz, 2019) and to activation of the prefrontal cortex (Chen et al., 2019). Notably, epidemiological research has indicated that around 2% of dementia cases might be prevented by modifying the lifestyle of physical inactivity (Livingston et al., 2020), reflecting the beneficial role of non-pharmaceutical effects of exercise or higher physical fitness levels.

Exercise has been linked to improvements in working memory (Rathore and Lom, 2017; Chen et al., 2020a), which has been classically characterized as the capacity to dynamically store, update, and manipulate incoming information over short periods of time (Baddeley, 2012), and involves activation of cortical networks including the inferior frontal gyrus, anterior cingulate gyrus, hippocampus, and thalamus (Yin et al., 2013; Gutierrez-Garralda et al., 2014; Toepper et al., 2014). Additionally, deficits in working memory in particular accompany aging and AD (Kirova et al., 2015; Salthouse, 2019), and impaired working memory has been associated with increased psychological disorders (Chai et al., 2018). Fortunately, systematic reviews and meta-analyses have revealed that exercise training, such as aerobic exercise and resistance training, significantly improves working memory performance in cognitively healthy older adults and in older adults with mild cognitive impairment (MCI) (Northey et al., 2018; Chen et al., 2020a). Neuroimaging evidence has further described the beneficial effects of chronic exercise on brain function related to working memory in late middle-aged and older adults, reflected by greater activation in the prefrontal lobe, anterior circulate, and hippocampus (Chen et al., 2019), as well as attenuated aging effects on hippocampal volume (Erickson et al., 2011).

There are fewer studies that have examined the effects of exercise on episodic memory. Episodic memory is long-term, retrospective memory bound to temporal and spatial contexts (Tulving, 2002; Yonelinas et al., 2019) and has been closely linked to the hippocampus and surrounding medial temporal lobe structures (Yonelinas et al., 2019). Using direct (Hayes et al., 2016) or estimated (Boots et al., 2015; Freudenberger et al., 2016) measures of cardiovascular fitness, higher cardiovascular fitness levels have been linked to superior episodic memory in cognitively healthy older adults (Hayes et al., 2016) and late middle-aged adults at risk for AD (Boots et al., 2015). Similar findings have been reported in neuroimaging research. For instance, using the episodic associative learning task, a task that assesses hippocampal-dependent relational binding, Cole et al. (2020) indicated that healthy older adults with higher levels of cardiovascular fitness had larger hippocampal volumes and an enhanced rate of relational memory acquisition. Of note, the protective effects of cardiovascular fitness on episodic memory and brain health might be moderated by sex for individuals at risk for AD, such that positive correlations between cardiovascular fitness and hippocampus volume were observed in older adult women but not men, whereas positive correlations were observed between cardiovascular fitness and episodic memory in older adult men but not women (Dougherty et al., 2017).

Exercise intervention with multiple exercise modalities, memory, and brain function

Current evidence has highlighted that exercise training or increased physical fitness might function as a non-pharmaceutical strategy against the deleterious effects of aging and AD on memory capacity and brain function; however, it should be noted that the majority of studies have focused on a single exercise modality (e.g., aerobic or resistance training) (Chen et al., 2020a,b). Systematic and meta-analytic reviews have revealed that exercise programs combining multiple exercise modalities (e.g., resistance training combined with aerobic exercise) might potentially evoke even greater benefits on cognitive functions (Kramer and Colcombe, 2018; Tomporowski and Pesce, 2019; Chen et al., 2020a). Notably, health-related physical fitness (e.g., cardiovascular fitness and muscular fitness) and skill-related physical fitness (e.g., balance, coordination, and power) (American College of Sports Medicine., 2018) might impact cognitive functions (Netz, 2019) and activation patterns of the frontal gyrus and premotor cortex regions (Voelcker-Rehage et al., 2010) differentially, reflecting a potential mediating role of type of fitness on cognitive function and brain regions. These results also suggest that the effects of the exercise interventions might vary according to the exercise modalities included in the program. For instance, although an exercise intervention combining aerobic and resistance exercise showed no significant effects on working memory in healthy older adults (Linde and Alfermann, 2014; Gajewski and Falkenstein, 2018), a 6-month exercise program combining multiple fitness modalities including aerobic, strength, flexibility, balance, and coordination maintained working memory performance in older women (Klusmann et al., 2010). Meanwhile, a multi-domain exercise intervention, combining aerobic and resistance exercises, balance, and flexibility training (two 1-h on-site sessions and three 20-min home-based sessions per week), showed no significant differences in episodic memory performance in a sample of older adults who suffered from subject memory complaints or objective/clinical apparent memory impairment (Fissler et al., 2017). However, a similar 6-month multi-domain exercise intervention, which consisted of aerobic, resistance, flexibility, balance, and coordination training, enhanced episodic memory in cognitively healthy older women aged 70–93 years (i.e., Mini-Mental State Examination scores $\geq$26) (Klusmann et al., 2010), suggesting that variations in participant characteristics might have impacted the outcomes.

In addition to the Western-style exercise, evidence has suggested that Eastern mind-body exercise (e.g., Tai Chi Chuan/Tai Ji Quan/Taiji) has cognitive benefits in older adults (Chang et al., 2010). Most Eastern mind-body exercise includes complex coordinated sequential movements and multiple forms of exercise (e.g., aerobic fitness, muscular fitness, balance, flexibility, and coordination), as well as emotional and psychosocial components (e.g., mental concentration, breathing control, and meditation) (Chang et al., 2014). Eastern mind-body exercises have been linked to stimulating multiple aspects of cognitive functioning and have been recommended for preventing age-related cognitive decline in older adults (Chang et al., 2010). For instance, improved delayed recall on an episodic memory task has been reported in older adults with MCI after a 6-month Tai Chi intervention (Sungkarat et al., 2018). Evidence from systematic and meta-analytic research has also revealed improved performance in episodic memory in older adults with MCI (Zou et al., 2019), as well as working memory and episodic memory in older adults (Ye et al., 2021), suggesting that there are benefits of mind-body exercise on various memory domains among older adults, even among those with cognitive impairment. Neuroimaging research using resting-state functional magnetic resonance imaging (rs-fMRI) has provided further evidence showing the benefits of mind-body exercise on memory (Tao et al., 2016). For instance, intervention research using high-resolution fMRI showed that the Tai Chi group had better memory performance which was correlated with increased resting-state functional connectivity (rs-FC) between the hippocampus and medial prefrontal cortex in cognitively healthy older adults (Tao et al., 2016). While evidence has suggested benefits of exercise on memory, prior

interventions have not combined both Eastern and Western-style exercise using multiple modalities. At the same time, the effects of interventions on cognitive function and brain function might vary according to the exercise modalities and the characteristics of the participants examined. Accordingly, whether an integrated intervention encompassing both Western style of physical fitness training and Eastern mind-body exercise affects memory and brain function in adults at risk for dementia remains less examined and requires further investigation.

Other modifiable lifestyle factors, memory, and brain function

Other non-pharmaceutical interventions or modifiable lifestyle factors (e.g., meditation and social interaction) have been proposed to mitigate age-related cognitive decline (Livingston et al., 2020). Meditation (e.g., mindfulness, transcendental meditation, and Vihangam yoga), a self-regulatory technique for maintaining attention and concentration on a single aspect of sensation and focusing on the present moment (Fox et al., 2014), has been considered a cognitively stimulating activity (Gallant, 2016). Research focusing on the effects of meditation-based interventions on memory has revealed promising results (Levi and Rosenstreich, 2019). Interventional-based research comparing the effects of meditation practice on operation span test performance found improved working memory in young adults (Mrazek et al., 2013). The benefits of meditation on working memory have been reported in one recent meta-analytic study of cognitively healthy and impaired older adults aged 60 years or above (Chan et al., 2019), suggesting the potential benefits of mindfulness training for improving working memory capacity in older adults. Additionally, the beneficial effects of meditation on episodic memory have been observed (Van Vugt et al., 2012; Basso et al., 2019; Nyhus et al., 2019), suggesting the potential benefits of meditation for improving episodic memory capacity.

The long-term practice of meditation has been related to changes in memory-related brain function. Increased cortical activity in the Default Mode Network (DMN) (Froeliger et al., 2012) in experienced meditators relative to non-meditators has been reported. Findings from interventional research in older adults also suggested altered connectivity within the DMN, and between the DMN and other nodes (Cotier et al., 2017). Considering the DMN has been linked to working memory (Piccoli et al., 2015) and episodic memory (Huo et al., 2018), these findings might imply superior memory in older adults who meditate. Indeed, reviews and meta-analyses indicated that extensive meditation practice was associated with changes in the frontoparietal network and higher brain efficiency during tasks involving memory (Levi and Rosenstreich, 2019).

Social interaction might also be involved in mitigating age-related cognitive decline (Evans et al., 2019; Livingston et al., 2020). Kelly et al. (2017) conducted a meta-analysis on the associations between social relationships and cognitive functions of healthy older adults by examining the effects of different social relationships (i.e., social activities, social networks, and social support) on working memory and episodic memory, showing that greater social activity and social support were associated with superior working memory and episodic memory, respectively. Evidence for the importance of social relationships has also been supported by research examining the effects of social isolation on cognitive functioning (Evans et al., 2019), and social isolation indexed by the social network index and social activity has been correlated with poor late-life memory. Finally, findings from cohort and longitudinal research (Berkman et al., 2000; Sommerlad et al., 2019), as well as systematic reviews and meta-analyses, have suggested the detrimental effects of less social engagement on risk for dementia (Penninkilampi et al., 2018; Saito et al., 2018; Sundström et al., 2020).

Collectively, while meditation and social interaction benefit memory and enhance brain function, whether meditation and social interactions in combination with multi-domain exercise positively affect neurocognition remains unknown and requires further investigation.

The moderators and mediators of exercise, cognitive function, and brain function

Age-related cognitive decline and late-onset AD might be influenced by several risk factors, such as the apolipoprotein E (*APOE*) gene (Martins et al., 2005). *ApoE* has three common allelic isoforms (i.e., epsilon (ε)2, ε3, and ε4). Among them, the *ApoE* ε4 allele(s) has been closely associated with cognitive impairment (Martins et al., 2005; Wang et al., 2011). The *ApoE* ε4 allele(s) is overrepresented in late-onset AD, and it has been reported that more than 48% of the AD population carries one or more *ApoE* ε4 alleles (Ward et al., 2012). *ApoE* ε4 allele(s) status has also been associated with disease progression from MCI to AD, such that the onset of AD could be reported 8–16 years earlier among individuals with the *ApoE* ε4 allele(s), compared to non-*ApoE* ε4 allele carriers (Liu et al., 2013), indicating as well even faster rates of cognitive decline (Martins et al., 2005).

The *ApoE* ε4 allele(s) might also influence the effects of exercise on cognitive functioning and the risk for AD (Hamer and Chida, 2009; Wang et al., 2011). Higher physical activity levels are more strongly associated with superior memory among older adults with one or two *ApoE* ε4 allele(s) (Smith et al., 2011). Alternatively, research has suggested a positive correlation between cardiovascular fitness levels and verbal learning memory in older adults, irrespective of the status of *ApoE* ε4 allele(s) (Boots et al., 2015). Interestingly, a positive correlation between cardiovascular fitness and performance on

the Rey Auditory Verbal Learning Test was only observed in older adult men at risk for AD, indicating that there might be a gender-specific effect of cardiovascular fitness on episodic memory in older adults at risk for AD (Dougherty et al., 2017). Finally, compared to sedentary older adults with the *ApoE* ε4 allele(s), older *ApoE* ε4 carriers with higher exercise levels had better performance on the Sternberg task (Deeny et al., 2008), suggesting the benefits of exercise on working memory among those with greater genetic risk for AD. Notably, while evidence suggests that the *ApoE* genotype moderates the influence of exercise and physical fitness levels on memory, no investigation thus far has examined the role of the *ApoE* genotype in an integrated intervention composed of Western-style physical fitness training and Eastern mind-body exercise.

Exercise-evoked brain-derived neurotrophic factor (BDNF) might be a candidate biological mechanism for improving cognitive function (Miranda et al., 2019; Heinze et al., 2020). BDNF, a member of the neurotrophic family with the highest levels found in hippocampal neurons (Murer et al., 2001), has been considered a putative mediating factor with multiple aspects influencing neuronal survival and maintenance (Benarroch, 2015), as well as hippocampal-related memory formation and maintenance (Bramham and Messaoudi, 2005; Miranda et al., 2019). While BDNF is the most pervasive neurotrophin in the developed adult brain (Song et al., 2015), correlations between decreased plasma BDNF concentration, increasing age (Lommatzsch et al., 2005), and poor memory performance in healthy older adults (Erickson et al., 2010) and older adults with MCI and AD dementia (Borba et al., 2016) have been observed. Research that has explored exercise training and BDNF levels in relation to the memory has revealed improved spatial memory and increased hippocampal volume following an aerobic exercise intervention, and these exercise-induced changes in hippocampal volume were correlated with elevated serum BDNF levels (Erickson et al., 2011). Meta-analytic research has further indicated that regular exercise might not only elevate resting peripheral BDNF concentrations (Szuhany et al., 2015; Dinoff et al., 2016), but also intensify BDNF responsivity immediately after both acute and regular exercise (Szuhany et al., 2015). Notably, prior studies mainly applied a single type of exercise, leaving the effects of multi-domain exercise on BDNF and memory in older adults unknown.

Aims of the study

Several studies have examined the independent effects of chronic exercise, meditation, or social interaction on memory and brain health; however, research has yet to examine the influence of multi-domain exercise with the consideration of moderators and mediators on memory in adults at risk for AD. The current study entitled Western-Eastern Brain Fitness Integration Training (WE-BFit) Trial aims to utilize a randomized controlled design to examine the effects of a 6-month multi-domain exercise program combining multiple exercise modalities (i.e., aerobic exercise, resistance exercise, coordinative, and flexibility exercise), meditation, and social interaction on working memory and episodic memory, as well as brain function, in cognitively healthy late middle-aged and older adults. Additionally, whether the effects of this intervention will be affected by *ApoE* genotype, the status of physical fitness, or neurotrophic changes will be further examined (Figure 1).

Methods

Study design and schedule

The multi-domain interventional trial of WE-BFit is designed as a double-arm, randomized controlled trial (RCT) targeting potential factors for promoting working memory, episodic memory, and brain function in cognitively healthy late middle-aged and older adults who are and who are not at genetic risk for AD. The trial is planned to start in April 2022 and aims to recruit 100 eligible participants who will be randomly assigned into two parallel groups (i.e., the multi-domain exercise group, $n = 50$; the control group, $n = 50$). *ApoE* genotypes of eligible participants will be examined at the baseline assessment. Additionally, other primary outcomes (i.e., working memory, episodic memory, and brain function) and secondary outcomes (i.e., multiple components of physical fitness and BDNF) will be assessed before and after the 6-month intervention. This trial will be led primarily by the Faculty of Physical Education and Sport Sciences of the National Taiwan Normal University. A flow chart of the current trial is presented in Figure 2.

Participants

To ensure a sufficient sample size, the sample size calculation for the current study was conducted using G*Power version 3.1.9.4 (ANOVA; repeated measures, within-between interaction). Multiple recruitment strategies will be utilized, including online social media advertising and word-of-mouth referrals, as well as posters and flyers placed or distributed in local community centers and organizations. Finally, individuals from previous studies will also be screened for recruiting potential participants. Before participating in the current study, potential participants will be screened by phone, followed by an on-site interview to ensure they meet the inclusion criteria.

Eligible participants (half of them are *ApoE* ε3/ε4 or *ApoE* ε4/ε4 carriers) will be provided sufficient information, and informed consent relating to the study will be provided prior to the initiation of the study. Recruitment, enrollment, and

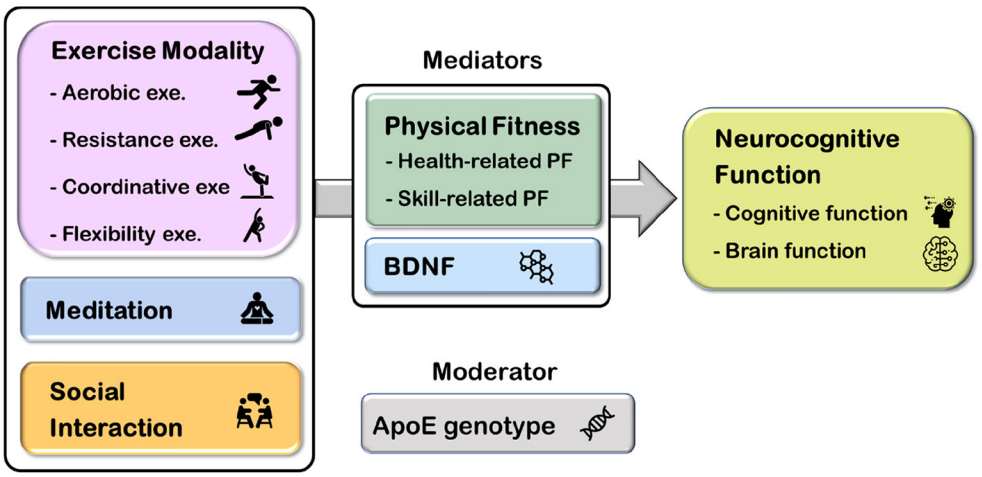

FIGURE 1
The overall picture of the Western-Eastern Brain Fitness Integration Training (WE-BFit) Trial. *ApoE*, apolipoprotein E; BDNF, brain-derived neurotrophic factor; PF, physical fitness; exe., exercise.

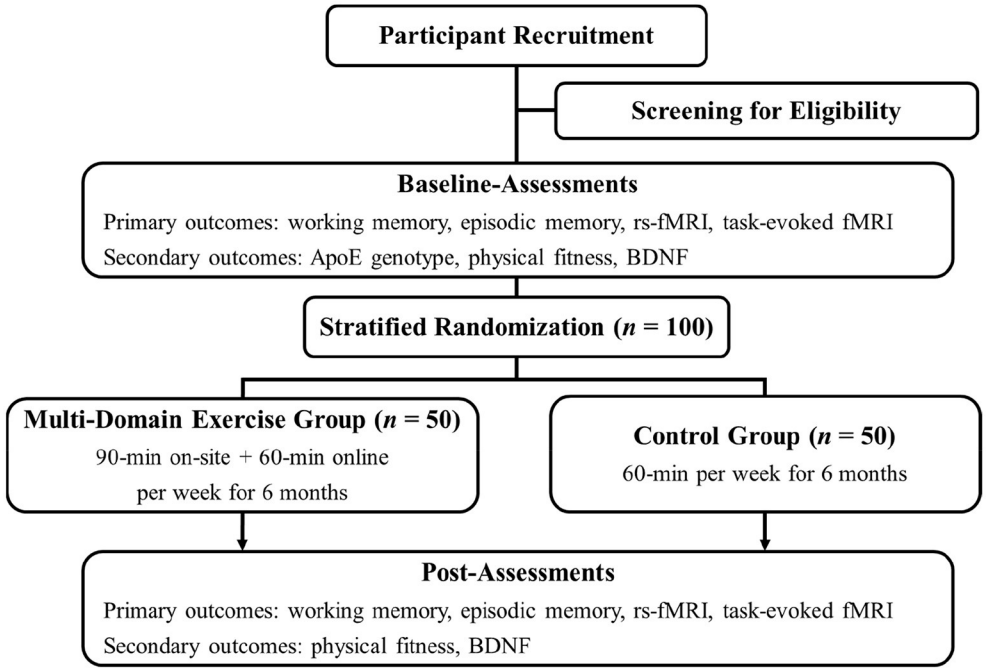

FIGURE 2
Study flowchart. *ApoE*, apolipoprotein E; BDNF, brain-derived neurotrophic factor; fMRI, functional magnetic resonance imaging; rs-fMRI, resting-state functional magnetic resonance imaging.

randomization will occur on a rolling basis. Finally, a total of 100 community-dwelling, cognitively healthy adults aged 45–70 years who are able to participate in moderate-intensity exercise will be recruited in a 1-year time period in Taipei, Taiwan. The inclusion and exclusion criteria are detailed in Table 1.

Randomization and blinding

To minimize the potential bias of individual differences or covariates, eligible participants will be recruited and randomly assigned to either a multi-domain exercise group or an online educational courses control group with a 1:1 allocation

TABLE 1 Inclusion and exclusion criteria for participating in the study.

Inclusion criteria	Exclusion criteria
- Adults aged from 45 to 70 years	- Diagnosed or self-reported cognitive problems (e.g., mild cognitive impairment or dementia)
- Normal or corrected-to-normal vision	- The diagnosed or self-reported physical disease (e.g., untreated hypertension and chronic heart disease, stroke, brain tumor, musculoskeletal disorders, other exercise contradictions)
- Able to speak and read Chinese	- Diagnosed or self-reported major psychiatric illness (e.g., major depression, schizophrenia)
- Scores of MMSE $\geq$ 25	- Diagnosed or self-reported neurodegenerative disease (e.g., AD and other dementias, PD and PD-related disorders, Huntington's disease)
- PAR-Q score = 0	- History of severe alcohol or drug abuse
- Able to conduct the exercise with moderate intensity	- History of chemotherapy
- Meet the criteria to undergo MRI	- Unable to complete the MRI scan
	- Traveling consecutively for 3 weeks or more during the study
	- Unwillingness to be randomized to one of the two groups
	- Currently participating in another study trial

AD, Alzheimer's disease; MMSE, Mini-Mental Status Examination; MRI, magnetic resonance imaging; PAR-Q, Physical Activity Readiness Questionnaire; PD, Parkinson's disease.

ratio using a computerized permuted block randomization algorithm with stratification (Lim and In, 2019). Additionally, the randomization will be stratified by *ApoE* genotype (High-risk: *ApoE* ε3/ε4 and *ApoE* ε4/ε4 vs. Low-risk: *ApoE* ε2/ε2, *ApoE* ε2/ε3, *ApoE* ε2/ε4, and *ApoE* ε3/ε3) to confirm equal allocation to both groups based on these criteria.

The randomization list for the assignment sequence will be created using the IBM SPSS Statistics for Windows (SPSS Inc., Chicago, IL, USA). Except for staff involved in the multi-domain exercise program and online educational program, assessors and data analyzers will be kept blind to participants' group assignments.

Intervention protocols

Eligible individuals will participate all together in either the multi-domain exercise group or the control group for 6 months.

Multi-domain exercise group

The exercise program for the multi-domain exercise group is designed by senior academic psychologists with input from a team of professional exercise experts experienced in working with late middle-aged and older adults. The multi-domain exercise group consists of a 6-month intervention, including one 90-min on-site and several online sessions, for up to 60 min per week. The program primarily focuses on multiple exercise modalities (i.e., aerobic exercise, resistance exercise, and coordinative exercise), with two additional lifestyle domains (i.e., meditation and social interaction). Specifically, each exercise course includes five stages:

1. Stage 1: 15 min of warm-up and aerobic exercise.
2. Stage 2: 25 min of resistance and flexibility exercise from the "Western exercise" perspective.
3. Stage 3: 30 min of the main exercise, named as Bagua Daoyin, consisting of resistance exercise, coordination, and flexibility exercise from the "Eastern exercise" perspective. This exercise, including eight forms of sequential movements, emphasizes muscular strength and endurance of the upper and lower trunk, trunk rotation, weight shifting, and coordination of visual and musculoskeletal systems. Additionally, awareness of the proprioception with mental focus from different parts of the body will be heightened.
4. Stage 4: 10 min of social interaction exercise. The exercise program will take place in a group format, and practice in pairs or small groups will be frequently encouraged, providing sufficient social interactions among participants.
5. Stage 5: 10 min of cool down and meditation. After the cessation of the main exercise session, a cool down and mindfulness-based meditation will be conducted. The mindfulness-based meditation is based on a mindfulness program utilized previously, focusing on the components of mindful breathing, awareness, nonjudgement, and acceptance (Nien et al., 2020).

Each on-site exercise course will be conducted at one recreational center in Taipei, Taiwan, supervised by experienced exercise instructors, who will instruct the movements designed for the multi-domain exercise program.

Control group

The control group will not receive any exercise program and will be informed to maintain their lifestyles. In addition, participants will be invited to attend one 60-min online group educational course regarding the effects of exercise on cognitive function and general instructions for cognitive promotions per week to eliminate the experimental expectation effect. The participants will be required to provide their physical activity behavior once per month for 6 months (see Figure 2). After data collection at the Post-Assessments, the participants can voluntarily join the multiple-domain exercise program for the next 3 months as compensation for their participation.

TABLE 2 Assessments.

	Baseline-assessments Recruitment (< 3 weeks before the intervention)			6-month intervention			Post-assessments (< 3 weeks after the intervention)	
	Screening	$ADay_1$	$ADay_2$	Initial intervention	Middle intervention	Final intervention	$ADay_3$	$ADay_4$
Information of study	X							
Review of criteria	X							
Medical history, General health status, MMSE, PAR-Q	X							
Informed consent form	X							
Battery of psychosocial measures	X						X	
Blood extraction		X					X	
Battery of physical fitness tests		X					X	
Magnetic resonance imaging			X					X
rs-fMRI								
fMRI: n-back task, RISE task								
Attendance				X	X	X		

ADay, Assessment days; MMSE, Mini-Mental Status Examination; PAR-Q, Physical Activity Readiness Questionnaire; rs-fMRI, resting-state functional magnetic resonance imaging; RISE, relational and item-specific encoding.

Assessments and outcome measures timeline

All participants will undergo multiple assessments before (Baseline-Assessments) and after (Post-Assessments) the intervention. The Baseline-Assessments will be organized into two assessment days (ADay) in the following order: (1) $ADay_1$: psychosocial measures, blood extraction, and physical fitness assessment, and (2) $ADay_2$: fMRI scans. The same assessment order ($ADay_3$ and $ADay_4$) will be carried out in the Post-Assessments. Assessments will be carried out at National Taiwan Normal University, and fMRI scans will be performed in the Imaging Center for Integrated Body, Mind, and Culture Research in National Chengchi University, Taipei, Taiwan, within 3 weeks of completion of the initial screening and the cessation of the 6-month intervention (Table 2). The timeline for the trial is visualized in Figure 3.

Primary outcome assessments

N-back working memory task

A modified n-back working memory task (Li et al., 2014) will be programmed using E-Prime to examine participants' working memory. The n-back task contains a sequence of single-digit numbers (i.e., 1–9) presented with a duration of 500 ms and a fixed inter-stimulus interval of 2,000 ms. All participants will be instructed to respond if the current stimulus matches the one from *n* steps earlier in the sequence. In addition, all participants will be required to respond by pressing a button on a standard keyboard when the stimulus matches and pressing another button when the stimulus does not match. Both 1-back and 2-back conditions will be included in the task. A total of four blocks with 16 trials will be conducted, in which the stimuli will appear in random order. The total task time is about 7 min, and reaction time and accuracy will be recorded as indices of behavioral performance.

Relational and item-specific encoding task

The computerized Relational and Item-Specific Encoding (RISE) task is modified from the original RISE task (Ragland et al., 2012, 2015; Erickson et al., 2019) and will be utilized to assess episodic memory. Briefly, the task consists of two phases. During the first phase (i.e., the encoding phase), pairs of item-specific objects or relational objects will be alternatively presented. Participants will be instructed to identify whether the object is living or nonliving (item-specific encoding response trials) or whether one item could fit inside the other item in real life (relational encoding response trials). During the second phase (i.e., the recognition phase), participants will determine whether the item has been presented previously or

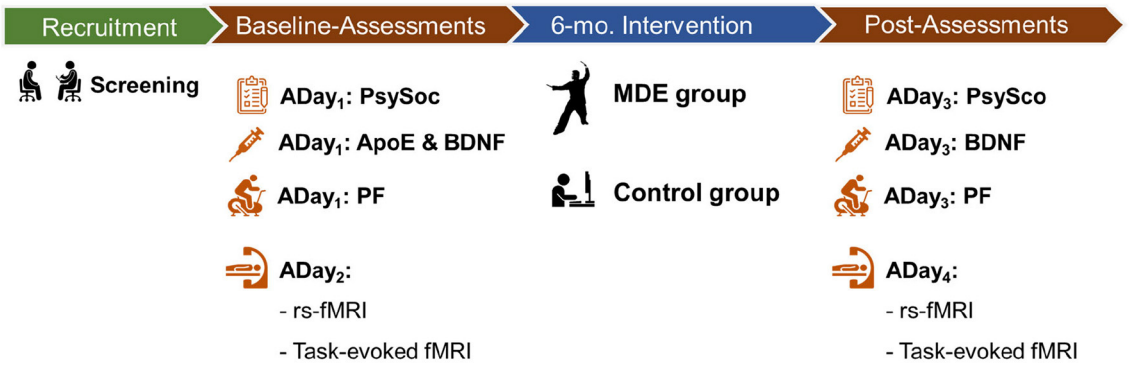

FIGURE 3
Experimental timeline. $ADay_1$, $ADay_2$, $ADay_3$, and $ADay_4$, assessment day 1 to assessment day 4, respectively; BDNF, brain-derived neurotrophic factor; fMRI, functional magnetic resonance imaging; MDE, multi-domain exercise; PF, physical fitness assessments; PsySoc, psychosocial measures; rs-fMRI, resting-state functional magnetic resonance imaging.

never presented (item recognition). Stimuli will be presented for 3 and 2 s for the encoding phase and item recognition phase of the task, respectively, and participants are encouraged to respond as quickly and accurately as possible. The total task duration will be ∼30 min. Reaction time and accuracy during both encoding and recognition phases will be recorded as the indices of behavioral performance.

Functional magnetic resonance imaging acquisition and analysis

All participants will undergo a series of whole-brain functional magnetic resonance imaging (fMRI) before and after the intervention. The data acquired will include resting-state fMRI (rs-fMRI) and task-evoked fMRI using a Siemens 3.0 T MRI scanner (Magnetron Prisma, Siemens, Germany) with a 32-channel head coil at the Imaging Center for Integrated Body, Mind, and Culture Research in National Chengchi University, Taipei, Taiwan. The parameters for MRI screening will be adapted from previous studies (Castells-Sánchez et al., 2019; Erickson et al., 2019).

The resting-state fMRI scan session (rs-fMRI)

A single-shot T2*-weighted gradient echo-planar image (EPI) sequence will be applied (TE/TR/flip angle = 30 ms/2,000 ms/90°, 64 contiguous axial slices with a slice thickness of 3.0 mm).

Task-evoked fMRI scan sessions (task-evoked fMRI)

Participants will be instructed to perform two fMRI tasks during the task-evoked fMRI scans. The two fMRI tasks will include n-back working memory task and the RISE task as described above. A single shot T2*-weighted gradient EPI sequence will be applied to the two fMRI tasks with the following parameters: TE/TR/flip angle = 30 ms/2,000 ms/90°, and 64 contiguous axial slices will be acquired with a slice thickness of 3.0 mm.

Secondary outcome assessments

Cardiovascular fitness

All participants will be informed to refrain from high-intensity exercise for 8 h and from eating or drinking any beverage for 2 h before the cardiovascular fitness test. Participants will be asked to wear the heart rate (HR) monitor (Polar HR monitor, Mode V800, Finland) to monitor their HR during the entire testing protocol. Cardiovascular fitness will be assessed by the YMCA cycling ergometer test (Golding et al., 1989), which is a widely recommended way to estimate maximal oxygen uptake (i.e., VO_{2max}) for adults with Class A risk stratifications (Fletcher et al., 2001). The YMCA cycling ergometer test comprises two to four 3-min consecutive stages on an electronically braked cycle ergometer (Corival IV CPET, Lode, Netherlands) and is targeted to reach the participants' HR according to their 85% age-predicted maximal HRs [$HR_{max} = 206 - (0.67 \times age_{years})$ (Gellish et al., 2007)]. Specifically, participants are instructed to pedal at a speed of 50 rpm throughout the test. In the initial stage, participants are asked to cycle at a workload of 150 kgm/min (25 W) for 3 min. The workloads of the following two stages will depend on each participant's HR recorded during the second and third minute of the initial stage (e.g., HR < 80 bpm, the workloads for the second and third stages will be 600 kgm and 750 kgm, respectively; HR >100 bpm, the workloads for second and third stages will be 300 kgm and 450 kgm, respectively). The process will be terminated if the participant's HR reaches their 85% age-predicted maximum HR. Finally, VO_{2max} of each participant

will be estimated based on the slope of HRs, the workload, and their body weight.

Other fitness indices

In addition to cardiovascular fitness, other indices associated with health-related physical fitness (i.e., body composition, muscular fitness, and flexibility) and skill-related physical fitness (i.e., balance and power) will be assessed.

Regarding health-related physical fitness, body composition [e.g., body water, body fat, skeletal muscle mass, body mass index (BMI), and percentage of body fat] will be assessed using a multifrequency, whole body, and segmental body composition analyzer (ACCUNIQ BC380 Body Composition Analysis, SELVAS Healthcare Inc., Daejeon, Korea) with bioelectrical impedance analysis (BIA) technology. Compared to the reference values of dual-energy x-ray absorptiometry, the measuring device demonstrates high correlation coefficients for lean body mass (kg) in Asian men and women ($r = 0.983$ and $r = 0.957$, respectively), as well as high correlation coefficients for percent of body fat in Asian men and women ($r = 0.881$ and 0.893, respectively) (Yang et al., 2018). Each measurement will take around 2 min. Muscular fitness will be assessed using push-ups, and the scores will be recorded as the number of push-ups completed in 30 s. Finally, flexibility will be assessed using the sit-and-reach test, in which participants will be instructed to reach as far as possible with their palms facing down while sitting down on the floor with legs stretched straight out. Further distance indicates better lower back and hip joint flexibility.

As for skill-related physical fitness, participants' balance will be assessed using the Single-Leg Stand (30 s) assessment. Participants will be instructed to perform the test three times with and without their eyes closed. The length of time the participant can maintain their balance will be recorded as the index of balance performance. Finally, the participant's leg power will be assessed using the distance of the Standing Long Jump. The length between the takeoff line and the nearest point of contact on the landing will be recorded as the power performance index.

Genotype and blood assays

ApoE genotype

Blood samples from the antecubital veins will be collected by licensed medical technicians/nurses during participants' first visit to the laboratory before the random assignment. The genotypes will be determined using a polymerase chain reaction method with modification. Two (rs429358: *ApoE* C112R; rs7412: *ApoE* R158C) genes will determine identification of the *ApoE* allele(s) (*ApoE* ε2, *ApoE* ε3, and *ApoE* ε4). Based on the genotypes, participants will be categorized as high risk (*ApoE* ε3/ε4 and *ApoE* ε4/ε4) or low risk (*ApoE* ε2/ε2, *ApoE* ε2/ε3, *ApoE* ε2/ε4, and *ApoE* ε3/ε3) for AD occurrence.

Neurotrophic measure BDNF

All participants will be asked to avoid exercising 8 h before the blood test. Following an overnight fast, participants' blood antecubital veins will be drawn by licensed medical technicians/nurses during the Baseline- and Post-Assessments. Approximately, 6 ml of venous blood will be collected by vacutainers (CAT, BD Vacutainer). The blood will then be separated using a centrifuge at 3,000 rpm for 15 min, and the supernatant fluid will be stored at −80°C for serum marker assays. The peripheral serum BDNF levels will be assayed using a ChemiKineTM BDNF Sandwich ELISA Kit (Millipore, Billerica, MA, USA). The procedure has been employed in our previous studies (Chang et al., 2017a).

Psychosocial measures

Mindfulness

Mindfulness will be assessed by the Chinese version of the Mindful Attention Awareness Scale (CMAAS) (Nien et al., 2020). The CMAAS (Chang et al., 2011) was based on the Mindful Attention Awareness Scale (MAAS) (Brown and Ryan, 2003). The MAAS assesses levels of dispositional mindfulness. The CMAAS is a 15-item questionnaire. Participants respond on a 6-point Likert scale from 1 (almost always) to 6 (almost never). Higher scores are associated with higher dispositional mindfulness. The CMAAS has been shown to have high internal consistency, with Cronbach's alphas at 0.88.

Other psychosocial measures

Potential confounding factors associated with psychosocial factors will be assessed using the following questionnaires: sleep *via* a Chinese version of the Pittsburgh Sleep Quality Index (Tsai et al., 2005) and the Chinese version of short forms of the Geriatric Depression Scale (GDS-15) (Sheikh and Yesavage, 1986; Liu et al., 1998), and health-related quality of life *via* the WHOQOL-OLD-Taiwan (Yao et al., 2017).

Retention and adherence

To maximize participants' adherence throughout the entire study period, participants will be frequently contacted by the investigators or research staff *via* telephone and e-mail. Participants will also be encouraged to contact the research staff or leave messages if they have any inquiries or concerns about the study. Participants will receive financial reimbursement for each of the assessments (∼20 US dollars per hour).

Power analysis

The study will recruit a total sample size of 100 participants for final statistical analyses. The sample size is estimated from

an *a priori* power analysis using G*Power 3.1.9.4 (power = 0.80, alpha = 0.05) and the effect size (Hedges' $g = 0.30$) based upon our previous meta-analysis that examines the effects of exercise interventions on executive function, including working memory in adults of age 55–65 years (Chen et al., 2020a).

Statistical analysis

To address whether the 6-month multi-domain exercise program affects working memory or episodic memory performance and brain functioning, we will employ a two-way repeated-measure analysis of variances (ANOVA) of mixed design with group status (i.e., multi-domain exercise group vs. control group) as the between-subject variable and time points (i.e., Baseline-Assessment vs. Post-Assessment) as the within-subject variable, with the Greenhouse–Geisser correction, where deemed appropriate. The two-way ANOVA will be employed individually for each primary outcome. Multiple *t*-test comparisons will be conducted as follow-up by setting the familywise alpha levels at 0.05, prior to a Bonferroni correction.

To address the moderating role of *ApoE* genotype on the effect of the multi-domain exercise program on working memory and episodic memory performance, as well as brain function assessed from neuroimaging metrics, we will employ the three-way repeated-measure ANOVA of mixed design with group status as the between-subject variable, and *ApoE* genotype [i.e., high-risk candidates (*ApoE* ε3/ε4 and *ApoE* ε4/ε4) vs. the low-risk candidates (*ApoE* ε2/ε4, *ApoE* ε2/ε3, *ApoE* ε2/ε2, and *ApoE* ε3/ε3)] and time points as the within-subject variable, with a Greenhouse–Geisser correction where deemed appropriate. In addition, multiple *t*-test comparisons will be conducted as follow-ups by setting the familywise alpha levels at 0.05 prior to a Bonferroni correction.

To address whether the effects of the multi-domain exercise program on working memory, episodic memory, and brain function are mediated by physical fitness and BDNF, we will employ Pearson product-moment correlations to examine relationships between change in physical fitness, BDNF, and the primary and secondary outcomes. Separate mediation analyses will be conducted using PROCESS software for SPSS. Statistical significance of mediators will be considered if the 95% bias-corrected bootstrap confidence interval (5,000 bootstrap samples) does not include zero. For all analyses, age, sex, BMI, and educational levels will be controlled for, and the alpha value will be set at 0.05, prior to statistical adjustment for all analyses.

Ethics statement

The protocol has been proven by the Institutional Review Board of the National Taiwan Normal University, Taiwan (REC number: 20212HM023) and has been registered on ClinicalTrials.gov (NCT05068271). All participants will be given informed consent according to the Declaration of Helsinki; the purpose, methodological approaches, and potential risks of the current study will be fully explained prior to participating in the study.

Discussion

Aging has been associated with cognitive decline and an increased risk for AD, as well as deterioration of brain function (Salthouse, 2019; Li et al., 2020). Since the curative effects of pharmaceutical interventions are limited (Mehta et al., 2017), identifying cost-effective non-pharmaceutical intervention strategies for preserving memory and brain function has been prioritized in the field. Notably, while several factors (e.g., exercise, physical fitness, meditation, and social interaction) have been described as beneficial for cognitive and brain function, no multi-domain intervention trials combining the Western style of physical fitness training and the Eastern mind-body exercise components of meditation and social interaction have been conducted for the prevention of age-related decline in memory and brain function in late middle-aged and older adults with and without a genetic risk for AD.

Accordingly, this WE-BFit trial will be the first RCT to evaluate the effectiveness of this type of intervention for promoting memory and brain function in this population while considering the moderators and mediators (e.g., *ApoE* genotype, BDNF, and physical fitness). The results of WE-BFit could provide valuable insight regarding the effectiveness of different components of an intervention program on cognitively healthy late middle-aged and older adults, such as the number of sessions per week, the length of each session, and the aspects of the exercise program (e.g., exercise modalities, meditation, and social interaction).

Several challenges of this study are worth mentioning. For instance, the current trial will recruit 50 cognitively healthy participants with a genetic risk for AD. Nevertheless, the recruitment of sufficient participants is challenging, given that the prevalence of *ApoE* ε4 allele(s) in Taiwan is only around 20% (Hong et al., 1996; Wang et al., 2011). Unfortunately, this challenge will be further escalated by the occurrence of the COVID-19 pandemic. Although this challenge might be partially alleviated through recruiting *ApoE* ε4 allele(s) carriers from our previous research and the progressive ease of the COVID-19 pandemic in Taiwan, we still expect the inclusion of a sufficient number of participants, especially the *ApoE* ε4 allele(s) carriers, will be difficult. Drop-out and adherence will be other challenges in the current study. Two strategies will be applied to curtail these challenges. First, frequent contacts and regular

newsletters will be delivered to all participants from the research group. Furthermore, the multi-domain exercise group can attend two out of 10 optional exercise sessions each week instead of adhering to a fixed exercise schedule. These approaches enhance the personal connection to the study and provide user-friendly access to the exercise program, and we expect this to enhance participants' adherence and minimize the risk of them withdrawing from the intervention (Robiner, 2005). Finally, the current trial collects a considerable amount of outcome measures, and many of them have to be collected in a time-sensitive manner. For instance, to accurately reflect the relationships between physiological conditions and brain health, physical fitness, BDNF levels, cognitive tasks, and fMRI assessments will be completed within 3 weeks before and after the 6-month exercise program, respectively. To overcome this potential challenge, a limited number of participants (e.g., five people) will be grouped as one intervention unit, and the unit will coordinate the most suitable time for initiating the assessments and exercise program.

In summary, the WE-BFit program will offer significant insight into the health and societal impacts of a multi-domain exercise program (e.g., Western style of physical fitness training and Eastern mind-body exercise, meditation, and social interaction) on memory and brain function in late middle-aged and older adults. This study will provide valuable results informing healthcare professionals, gerontology investigators, and healthcare policymakers about the effectiveness of a non-pharmaceutical intervention on cognitive and brain function.

Ethics statement

The studies involving human participants were reviewed and approved by Institutional Review Boards from the National Taiwan Normal University. The patients/participants will provide their written informed consent to participate in this study.

Author contributions

Conceptualization: Y-KC, KE, S-HC, C-MH, and C-HC. Methodology: KE, F-TC, R-HL, J-RS, and C-HC. Writing: Y-KC, SA, F-TC, S-HC, C-MH, and C-HC. Visualization: R-HL, J-RS, and S-HC. Supervision: Y-KC, KE, and C-MH. Reviewing and editing: Y-KC, KE, SA, F-TC, R-HL, J-RS, S-HC, C-MH, and C-HC. All authors contributed to the article and approved the submitted version.

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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.

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The association between social engagement and depressive symptoms in middle-aged and elderly Chinese: A longitudinal subgroup identification analysis under causal inference frame

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Background: Studies have suggested that there is a significant association between social engagement and depression symptoms. However, this association may differ in people with different features such as different sociodemographic characteristics and health conditions.

Methods: Research data were obtained from the CHARLS database. The causal inference was performed with the propensity score. We used the linear mixed-effects model tree algorithm under the causal inference frame for subgroup identification analysis.

Results: We included 13,521 participants, and the median follow-up time is 4 years. Under the casual inference frame, the association between social engagement and depression symptoms is confirmed for all included individuals (OR = 0.957, $P = 0.016$; 95%CI: 0.923–0.992). Using the linear mixed-effects model tree, we found two subgroups, including middle-aged and elderly residents who live in rural areas with <6 h of sleep and those living in urban areas, could benefit more from social engagement. After using the propensity score method, all the two subgroups selected are statistically significant ($P = 0.007$; $P = 0.013$) and have a larger effect size (OR = 0.897, 95%CI: 0.830–0.971; OR = 0.916, 95%CI: 0.854–0.981) than the whole participants. As for sex difference, this associations are statistically significant in male (OR: 0.935, $P = 0.011$, 95%CI: 0.888–0.985) but not in female (OR: 0.979, $P = 0.399$, 95%CI: 0.931–1.029).

Conclusions: Our findings indicate that social engagement may reduce the risks of depressive symptoms among all individuals. The identified subgroups of middle-aged and elderly residents who live in rural areas with <6 h of sleep and those who live in urban areas may benefit more from the social engagement than the whole participants.

KEYWORDS

social engagement, depressive symptoms, middle-aged and elderly Chinese, subgroup identification, causal inference

Introduction

The latest statistics of the World Health Organization (WHO) showed that about 322 million people suffered from depression worldwide, and the peak incidence appeared among the elderly (World Health Organization., 2017). Nearly half of the people with depressive symptoms lived in Southeast Asia and the Western Pacific regions (World Health Organization., 2017). China is the most populous country in the Asia Pacific region; with the rapid aggravation of China's aging society, the number of senior citizens with depressive symptoms (DSs) may further increase in the future (Ren et al., 2020). DS not only contributes to a bad mood, negative attitudes, poor sleep, and quality of life (Cui, 2015) but also increases suicide rates (Dong et al., 2018). It has been confirmed that DS can cause lower working efficiency and higher medical costs, resulting in huge socioeconomic losses (Murray and Lopez, 1996). Therefore, it is urgent to find effective methods to prevent and treat DS.

Current treatments for patients with confirmed DS mainly include antidepressants and psychotherapy (Schuch and Stubbs, 2019). Although antidepressants are effective for about half of the patients (Pigott, 2015), all available antidepressants have side effects of various degrees, such as weight gain, increased diabetes risk, and sexual dysfunction (Schuch and Stubbs, 2019). Meanwhile, psychological therapies, such as cognitive behavioral therapy, have limited effects on the treatment of DS (Cuijpers et al., 2014). Therefore, the way to treat depressed patients after the fact is far less helpful than preventing their disease from the beginning.

Many studies have indicated that social engagement (SE) was associated with a low risk of DS (Glass et al., 2006; Isaac et al., 2009; Lou et al., 2013). As early as 2006, Glass et al. used longitudinal data from a cohort study of the New Haven elderly population to investigate whether SE could prevent the elderly from developing depressive symptoms (Glass et al., 2006). Their results indicated that SE was negatively associated with DS (Glass et al., 2006). In long-term residential care settings, Lou et al. analyzed six waves of data collected in the Hong Kong Longitudinal Study and found that a higher level of SE was associated with fewer DS (Lou et al., 2013).

However, the associations between SE and DS mentioned above were based on the estimation of average effects at a general population-wide level. However, in fact, the association between SE and DS tends to differ across subgroups with different characteristics (different sociodemographic characteristics, health conditions, etc.). Furthermore, several longitudinal studies have already suggested that the association between SE and DS was limited to specific populations (Takagi et al., 2013; Hajek et al., 2017). For example, evidence from a multicenter prospective cohort study in Germany showed that SE was associated with decreased DS only in women, but not men (Hajek et al., 2017).

Since SE is associated with a low risk of DS, promotion of SE can provide a protective effect of preventing and mitigating the initiation and progression of DS at the lowest cost (Solomonov et al., 2019; Bae, 2021). However, as the studies mentioned above, the effect of SE is not homogenous for all subgroups of people. Therefore, for the conduction of effective and precise prevention of the development and progression of DS, it is necessary to detect those subgroups of people with a stronger association between SE and low risk of DS. However, few studies paid much attention to this issue. In this case, the methodology of subgroup identification might be a useful approach to achieve this purpose since it can identify those specific subgroups with a stronger association between SE and lower DS risk.

In addition, for observational studies, the control of confounders is very important, and the results obtained without control of confounders are likely to be misleading (Streeter et al., 2017). The presence of confounders would influence the estimated association between SE and DS and lead to misleading results. Therefore, in order to properly estimate the association between SE and DS in different subgroups, the control of confounders must be taken into account, and one of the most effective ways to control confounders at present is the frame of causal inference.

Therefore, in this study, we aim to (1) investigate whether SE is associated with DS in the middle-aged and elderly Chinese population and (2) identify whether there are subgroups of middle-aged and elderly residents in China who show stronger or weaker patterns of association between SE and DS, while possible confounding variables are controlled by using a causal inference framework.

To take the time-constant heterogeneity into account, we use longitudinal data from the China Health and Retirement Longitudinal Survey (CHARLS) database (Zhao et al., 2014) instead of cross-sectional data to obtain more reliable results (Hajek et al., 2017). Since the data are longitudinal, we have chosen the generalized linear mixed-effects model tree (GLMM tree) algorithm to perform subgroup identification. In this study, SE and DS were defined as continuous variables, and all analysis was conducted under the framework of causal inference to ensure the correctness of the conclusion.

Methods

Data and sample

The data were obtained from the China Health and Retirement Longitudinal Survey (CHARLS) database (https://charls.charlsdata.com/pages/data/111/zh-cn.html). The CHARLS study was approved by the Biomedical Ethics Review Committee of Peking University in June 2008. All participants signed an informed consent form when participating. Zhao et al. (2014) have provided more detailed information about the

design and implementation of the CHARLS study (Zhao et al., 2014).

Our study used baseline (2011) (Zhao et al., 2013) and three-year follow-up data (2013, 2015, and 2018) (Zhao et al., 2014). More specifically, in order to take the "longitudinal effect" of SE on DS into account, the DS outcome from the waves of 2013, 2015, and 2018 was used. And that the SE from waves baseline, 2013 and 2015 were used.

Missing data are inevitable for cohort studies, and it is not recommended to be imputed when it appears in the primary outcome and key research factors (Engels and Diehr, 2003). Therefore, we selected cases that had complete data in SE and DS and imputed the missing data in other variables.

Thus, based on the requirement of the model and our research targets, we included participants according to the following criteria: (1) providing complete information about SE and DS during follow-ups; (2) aged over 45. For more detailed information, see Figure 1.

Measurement

Measurement of depressive symptoms

The Center for Epidemiological Studies Depression Scale-10 (CES-D-10), effective measurement of depressive symptoms in Chinese people (Cheng et al., 2016), was used in the CHARLS study. The scale consisted of 10 items and measured the feelings and behaviors of participants. Respondents were required to answer the frequency of symptoms described in each item in the past week. Each item has four options: (1) rarely or none of the time (<1 day); (2) some or a little of the time (1-2 days); (3) occasionally or a moderate amount of the time (3–4 days); (4) most or all of the time (5–7 days), corresponding to 0–3 points, among which "I felt hopeful about the future" and "I was happy" are reverse scoring problems. The total score range of the scale is 0–30. In our study, we regarded DS as a continuous variable. The *Cronbach's α* coefficient for the CES-D-10 is 0.761 in 2013, 0.798 in 2015, and 0.804 in 2018.

Measurement of social engagement

Questions including "Have you done any of these activities in the last month" and "Frequency of activity in the last month" were used to measure the SE of participants. The former question includes 12 options: (1) Interacted with friends; (2) Played Ma-jong, chess, or cards, or went to a community club; (3) Provided help to family, friends, or neighbors who did not live with you and who did not pay you for the help; (4) Went to a sport, social, or other kinds of club; (5) Took part in a community-related organization; (6) Did voluntary or charity work; (7) Cared for a sick or disabled adult who does not live with you and who did not pay you for the help; (8) Attended an educational or training course; (9) Invested in stocks; (10) Used the Internet; (11) other; (12) None of these. Participants who chose options 1-11 of the former question were qualified to answer the latter question which includes three options: (1) Almost daily; (2) Almost every week; (3) Not regularly.

In our study, we combined the information mentioned above to form a continuous variable. Participants who chose options "(1) Almost daily, (2) Almost every week, (3) Not regularly" of the latter question were recorded as 3, 2, and 1 point, respectively. And those choosing option 12 were recorded as 0 points. The *Cronbach's* α coefficient of SE is 0.591 in 2011, 0.601 in 2013, and 0.609 in 2015. We also performed exploratory factor analysis, and detailed results of it were provided in Supplementary material 1. Based on the results of *Cronbach's α* coefficient and exploratory factor analysis, we may conclude that the measurements of SE used in this study were reliable.

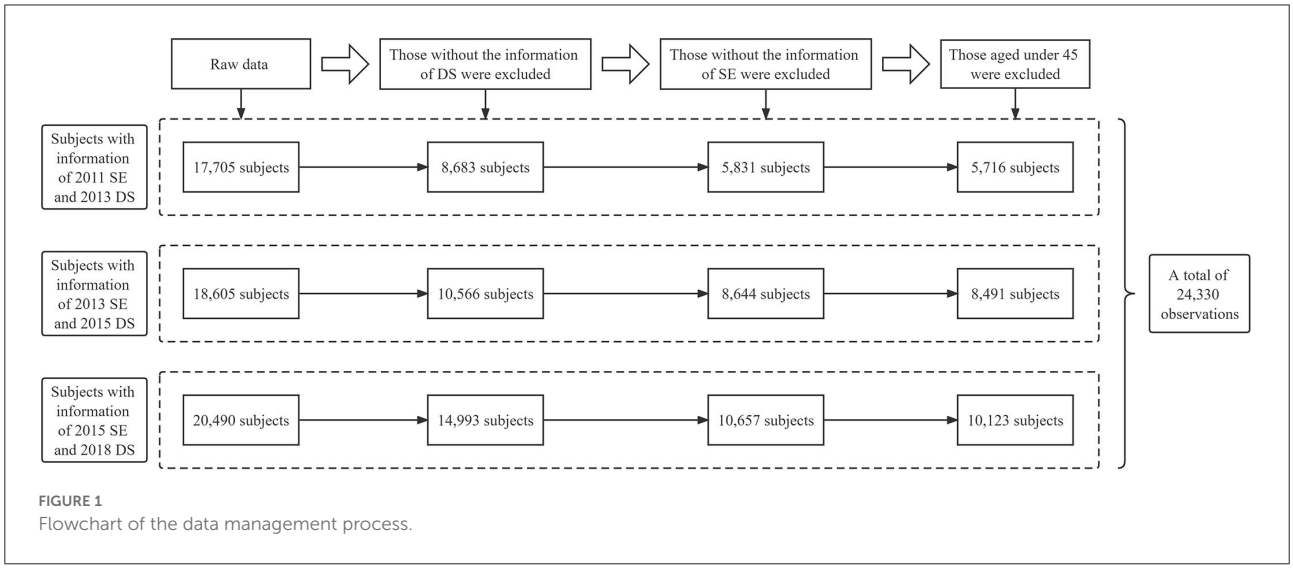

FIGURE 1
Flowchart of the data management process.

Identification of covariate

Following previously published studies (Lei et al., 2014), we considered potentially covariates associated with DS disparity. Possible covariates include gender; residential region; education level; marital status; wearing dentures; chronic disease; satisfaction; insurance; sleeping time; nap time; eyesight; drinking; hearing; smoking. Details about the covariates assignment table are in Supplemental material 2.

Chronic diseases included hypertension, dyslipidemia, diabetes or high blood sugar, cancer or malignant tumor, chronic lung disease, liver disease, heart problems, stroke, kidney disease, stomach or other digestive diseases, emotional or nervous or psychiatric problems, memory-related disease, arthritis or rheumatism, and asthma. Chronic disease, eyesight, and hearing information were obtained by self-reporting.

Self-reported life satisfaction was also taken into consideration. The CHARLS used the problem "Please think about your life-as-a-whole. How satisfied are you with it" to measure satisfaction. In our study, this item was replaced by the variable "satisfaction" in short.

Although covariates including "income," "the type of jobs," "retirement," "pain," "physical activity," and "cognition" were available in the database, however, they were dropped out because the missing rates are too high. Missing rates of these covariates are 63.97, 89.38, 20.69, 71.51, 65.91, and 37.36% in order.

Statistical analysis

Missing data imputation

According to previous literature (Li et al., 2021b), more than 20% of the missing follow-up data, even if filled, the bias is relatively large. So, covariates with more than 20% missing values, including "income," "work," "retirement," "pain," "physical activity," and "cognition," were dropped out, and covariates with <20% missing values were imputed.

Since the K nearest-neighbors imputation (KNN) method is almost unaffected by the distribution of data and is suitable for categorical variables (Beretta and Santaniello, 2016), in this study it was used to impute missing data. The algorithm was implemented with R package "DMwR2" version 0.0.2 (Torgo, 2016). Based on previous research, when using KNN to fill in longitudinal data, imputation parameter k should be >10, so the parameter of KNN imputation was set as "$k = 11$," which means the nearest 11 records were used (Beretta and Santaniello, 2016).

Description and comparison of basic characteristics

Count (proportion) was used for the presentation of categorical variables and mean (SD) for continuous variables. For comparison of characteristics across different years, analysis of variance was used for continuous variables, chi-square tests were applied for categorical variables, and nonparametric tests were used for ordinal variables.

Causal inference

Under the frame of causal inference, we can control confounding variables well and then more accurately estimate the association between SE and DS. In our analysis, confounders were identified by DAG (directed acyclic graphs). DAG is a tool for causal studies. And causal relationships are represented by arrows between the variables, pointing from cause to effect (Williams et al., 2018).

The specific process for determining the confounders is as follows: (1) using the linear mixed-effects regression (LMER) model to determine the associations among variables; (2) using DAG to visualize the relationships of variables; (3) using the Backdoor Criterion to identify confounders: (1) no vertex in confounders is a descendent of SE, and (2) confounders d-separates every path between SE and DS that has an incoming arrow into SE (backdoor path). Once confounding factors have been identified, the propensity score (PS) can be estimated.

In this study, the causal inference analysis (the control of confounders) was conducted through the propensity score (PS) method. PS method, which can be expressed as a function of multiple covariates as in equation 1, was first proposed by Rosenbaum and Rubin (Rosenbaum and Rubin, 1983). PS is the conditional probability of the i-th individual entering the observation group calculated based on the value of the known confounders (Rosenbaum and Rubin, 1983). In equation 2, the function $P(X)$ is called the propensity score, that is, the propensity toward exposure to treatment 1 given the observed covariates x.

$$PS = P(X) = P(T = 1|X) \quad (1)$$

Under the frame of linear mixed-effects regression (LMER), we included the estimated PS as a covariate to perform causal inference (to control the confounders) (Vansteelandt and Daniel, 2014) and then estimated the association between DS and ES among all participants.

Subgroup identification analysis

The longitudinal data obtained from the CHARLS database includes a baseline (2011) and three follow-up waves (2013, 2015, and 2018) of the survey, thus subgroup identification must consider the longitudinal structure of the data. To solve this issue, we used the generalized linear mixed-effects model trees (GLMM trees) algorithm for subgroup identification analysis which allows for taking the longitudinal structure into account.

The GLMM tree algorithm builds on model-based recursive partitioning (MOB) which uses a parameter instability test

for choosing partitioning variables (Fokkema et al., 2018). Because traditional MOB could not deal with longitudinal data, the GLMM tree was developed to take random effects into consideration (Fokkema et al., 2018).

The Generalized linear model (GLM) tree is a node-specific model. To estimate a GLMM tree, an iterative approach is taken (Fokkema et al., 2018). The interactive approaches alternate between (1) assuming random effects known, allowing for an estimate of fixed-effects by the GLM tree, and (2) assuming the GLM tree known, allowing for estimation of the random-effects parameter by GLMM (Fokkema et al., 2018).

The model can be expressed as in equation (2). In which, β_j is fixed effect whose value depends on terminal node j, g () is the link function, in this study the link function is defined as identity. The subscript i denotes individual observation, and x_i represents the column vector of fixed-effects predictor variables of observation i (variable details were presented in Supplemental material 1), and the random effects b are estimated globally.

$$g\left(\mu_{ij}\right) = x_i^T \beta_j + z_i^T b \tag{2}$$

The standardized average probability difference (*Cohen's d*) of DS in the terminal node (j) is estimated by the method of the GLMM tree. The practical meaning of *Cohen's d* is that, for people belonging to a specific subgroup in the terminal node (j), there is a difference (which can be measured by *Cohen's d*) in the DS scores between those who have different scores of SE.

We incorporated the potential partition variables into the GLMM tree model and then get the *Cohen's d* in all subgroups explored by the GLMM tree. Since the GLMM tree can only explore subgroups with stronger associations between SE and DS while it cannot control confounders effectively, it is necessary to estimate the association between SE and DS under the frame of causal inference.

So, once the subgroups were identified by the GLMM tree, we would stratify the whole sample into different subgroups and test the association between DS and ES among subgroups identified by the GLMM tree. Finally, compare the differences in results between subgroups and the whole population.

Because women are generally at greater risk of DS (Gater et al., 1998), but are more likely to be engaged in social activities than men (Bernard, 1982), we clarified the gender differences in the associations between SE and DS.

In this study, the function "lmertree()" nested R package "glmertree" version 0.2-0 (Fokkema et al., 2018) was used to estimate the GLMM tree model and conduct the subgroup identification analysis. The waves of subjects were specified using the cluster argument of the "lmertree()" function. In our analysis, the level of significance was set as 0.05.

Statistical software

All statistical analysis was performed using the R programming language (R Core Team, 2021) and RStudio (Version 1.1.463, RStudio Inc., 250 Northern Ave, Boston, MA 02210) software.

Results

Basic characteristics

In total, 24,330 responders (13,521 senior adults were non-duplicate individuals) were included in our analysis and the median follow-up time is 4 years. Table 1 presents the basic characteristics of the responders in different years (survey waves). The results show that the majority of the participants lived in rural areas and were married. The mean SE scores of observations were 1.44 (*SD* 1.910) in 2011, 1.87 (*SD* 2.306) in 2013, and 1.92 (*SD* 2.505) in 2015, respectively, (Table 1). The mean DS scores of observations were 7.98 (*SD* 5.794) in 2013, 8.16 (*SD* 6.431) in 2015, and 8.88 (*SD* 2.505) in 2018. Detailed information are presented in Table 1.

Identification of confounders

DAG was used to represent and better understand the associations between factors and outcomes (Figure 2). According to the DAG and the backdoor criterion, variables including residential region, hearing, and sleeping time were identified as confounders.

Overall analysis under causal inference frame

The propensity score was then estimated with the identified confounders. With the GLMM analysis, we found the association between DS and SE was statistically significant ($P = 0.016$) among all individuals under the causal inference frame and the odds ratio (OR) was 0.957 (95%*CI*: 0.923–0.992). Detailed results are presented in Table 2.

Subgroup identification

The subgroup identification analysis was conducted through the GLMM tree, and the survey wave was defined as the random effect. We incorporated all possible partition variables, including "Hearing," "Residential region," "Sleeping time," "Gender," "Insurance," "Naptime," "Drinking," "Wearing dentures," "Education level," "Marital status," "Eyesight," "Smoking," "Chronic disease," and "Age," into the tree building

TABLE 1 The basic characteristic of the study population.

Characteristics		Year			*P-value*
		2011 *n* (%)	2013 *n* (%)	2015 *n* (%)	
NO.		5,716 (100)	8,491 (100)	10,123 (100)	
Residential region[a] (N, %)	Rural	4,704 (82.3)	6,742 (79.4)	8,396 (82.9)	<0.001
	Urban	1,012 (17.7)	1,749 (20.6)	1,727 (17.1)	
Gender[a] (N, %)	Male	2,716 (47.5)	4,068 (47.9)	4,875 (48.2)	0.442
	Female	3,000 (52.5)	4,423 (52.1)	5,248 (51.8)	
Education level[c] (N, %)	Primary school graduate or below	4,063 (71.1)	5,853 (68.9)	6,893 (68.1)	<0.001
	Middle school, high school, or technical secondary school	1,580 (27.6)	2,473 (29.1)	3,045 (30.1)	
	Undergraduate or above	73 (1.3)	165 (1.9)	185 (1.8)	
Marital status[b] (N, %)	Married	4,983 (87.2)	7,392 (87.1)	8,843 (87.4)	0.060
	Separated	34 (0.6)	30 (0.4)	29 (0.3)	
	Divorced	699 (12.2)	1,069 (12.6)	1,251 (12.4)	
Wearing denture[a] (N, %)	Yes	509 (8.9)	435 (5.1)	431 (4.3)	<0.001
	No	5,207 (91.1)	8,056 (94.9)	9,692 (95.7)	
Chronic disease[c] (N, %)	No	3,928 (68.7)	5,603 (66.0)	7,154 (70.7)	<0.001
	One	1,022 (17.9)	1,574 (18.5)	1,628 (16.1)	
	Two or more	766 (13.4)	1,314 (15.5)	1,341 (13.2)	
Insurance[a] (N, %)	Yes	5,327 (93.2)	8,179 (96.3)	9,833 (97.1)	<0.001
	No	389 (6.8)	312 (3.7)	290 (2.9)	
Sleeping time[a] (N, %)	<6 h	1,672 (29.3)	2,884 (34.0)	3,122 (30.8)	<0.001
	6 h or more	4,044 (70.7)	5,607 (66.0)	7,001 (69.2)	
Naptime[a] (N, %)	<30 min	3,348 (58.6)	4,435 (52.2)	5,065 (50.0)	<0.001
	30 min or more	2,368 (41.4)	4,056 (47.8)	5,058 (50.0)	
Eyesight[c] (N, %)	Good	716 (12.5)	1,431 (16.9)	1,637 (16.2)	<0.001
	Fair	1,365 (23.9)	1,640 (19.3)	1,517 (15.0)	
	Poor	3,635 (63.6)	5,420 (63.8)	6,969 (68.8)	
Drinking[a] (N, %)	Yes	1,896 (33.2)	2,905 (34.2)	3,579 (35.4)	0.018
	No	3,820 (66.8)	5,586 (65.8)	6,544 (64.6)	
Hearing[c] (N, %)	Good	829 (14.5)	1,543 (18.2)	1,870 (18.5)	<0.001
	Fair	1,672 (29.3)	1,926 (22.7)	1,846 (18.3)	
	Poor	3,215 (56.2)	5,022 (59.1)	6,405 (63.3)	
Smoking[b] (N, %)	Never smoke	3,450 (60.4)	6,460 (76.1)	5,979 (59.1)	<0.001
	Still smoke	1,775 (31.1)	1,457 (17.2)	2,858 (28.2)	
	Totally quit	491 (8.6)	574 (6.8)	1,286 (12.7)	
Age[c] (N, %)	45–59	3,127 (54.7)	2,786 (32.8)	5,088 (50.3)	<0.001
	60–79	2,426 (42.4)	4,879 (57.5)	4,693 (46.4)	
	Over 80	163 (2.9)	826 (9.7)	342 (3.4)	
SE[d](Mean ± SD)		1.44 ±1.910	1.87 ± 2.306	1.92 ± 2.505	<0.001
		Year			
DS[d](Mean ± SD)		2013	2015	2018	<0.001
		7.98 ± 5.794	8.16 ± 6.431	8.88 ± 6.928	

[a]Chi-square test using a 3 × 2 table. [b]Chi-square test using a 3× 3 table. [c]Nonparametric tests. [d]Analysis of variance. SE, social engagement; DS, depressive symptoms.

TABLE 2 Subgroups analysis and estimation of the OR between SE and DS with LMER under causal inference frame.

Population	*Cohen's d* [a]	OR (SE)	95%CI (SE)	*P*-value (SE)
Overall analysis with whole samples	/	0.957	(0.923, 0.992)	0.016
Subgroup a	−0.1288313	0.897	(0.830, 0.971)	0.007
Subgroup b	0.0008699542	1.008	(0.960, 1.059)	0.742
Subgroup c	−0.09828412	0.916	(0.854, 0.981)	0.013
Male	/	0.935	(0.888, 0.985)	0.011
Female	/	0.979	(0.931, 1.029)	0.399

OR, odds ratio; CI, confidence interval; SE, social engagement. The symbol alphabet [a] means the standardized average probability difference of DS in the terminal nodes.

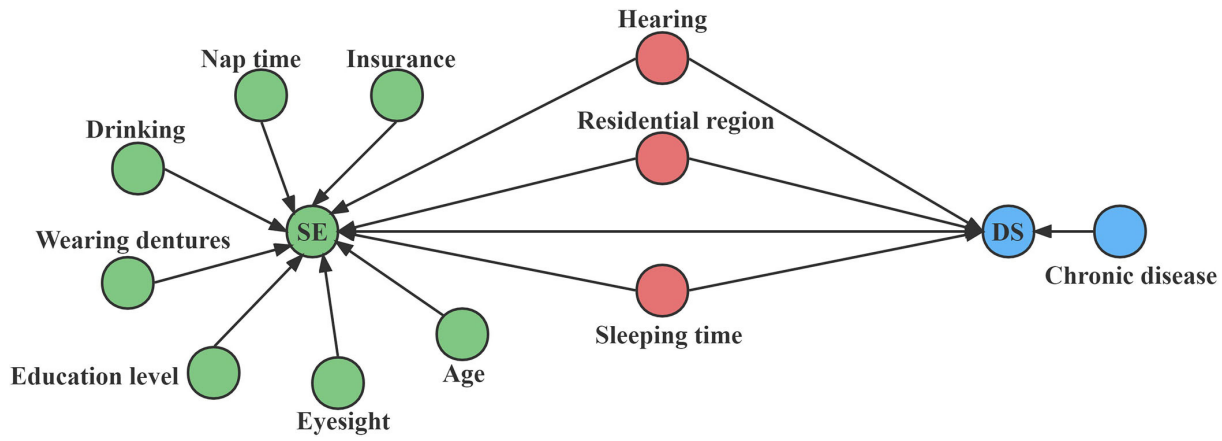

FIGURE 2
DAG analysis for selecting confounding variables. Causal relationships are represented by arrows between the variables, pointing from cause to effect. The green circle represents SE and its associated covariates, the blue circle represents DS and its related covariates, and the red circle represents the confounding variables.

process. The GLMM tree (Figure 3) selected "Residential region" as the first partitioning variable and "Sleeping time" as the second partitioning variable.

The terminal nodes presented in Figure 3 show a double factor–subgroup interaction: for participants who live in rural areas and sleep <6 h per day (Subgroup A), improving SE scores can reduce the DS scores (*Cohen's d* = −0.1288313). For people who live in rural areas and sleep more than 6 h per day (Subgroup B), SE can provide more or less the same change in DS scores (*Cohen's d* = 0.0008699542). For individuals who live in urban areas (Subgroup C), high SE scores can lead to low DS scores (*Cohen's d* = −0.09828412).

Subgroup analysis under causal inference frame

In subgroups A and C, the associations between DS and SE are also statistically significant under the causal inference frame. ORs of these two subgroups are 0.897 (95%*CI*: 0.830–0.971) and 0.916 (95%*CI*: 0.854–0.981), respectively, as shown in Table 2. The associations between SE and DS in these subgroups are more significant than in the whole samples; similarly, their ORs are smaller.

In subgroup B, the association between DS and SE is not statistically significant under the causal inference frame ($P = 0.742$), which is different from the whole people (Table 2).

The associations between DS and SE are statistically significant in males (OR: 0.935, $P = 0.011$, 95%CI: 0.888–0.985) but not statistically significant in females (OR: 0.979, $P = 0.399$, 95%CI: 0.931–1.029).

Discussion

Preventing DS is an important prerequisite for improving the quality of life, especially for middle-aged and senior citizens. Published studies indicated that SE was closely related to DS and could act as a key factor in the prevention of DS (Solomonov et al., 2019; Bae, 2021). Results of previously published studies indicated that there were differences in

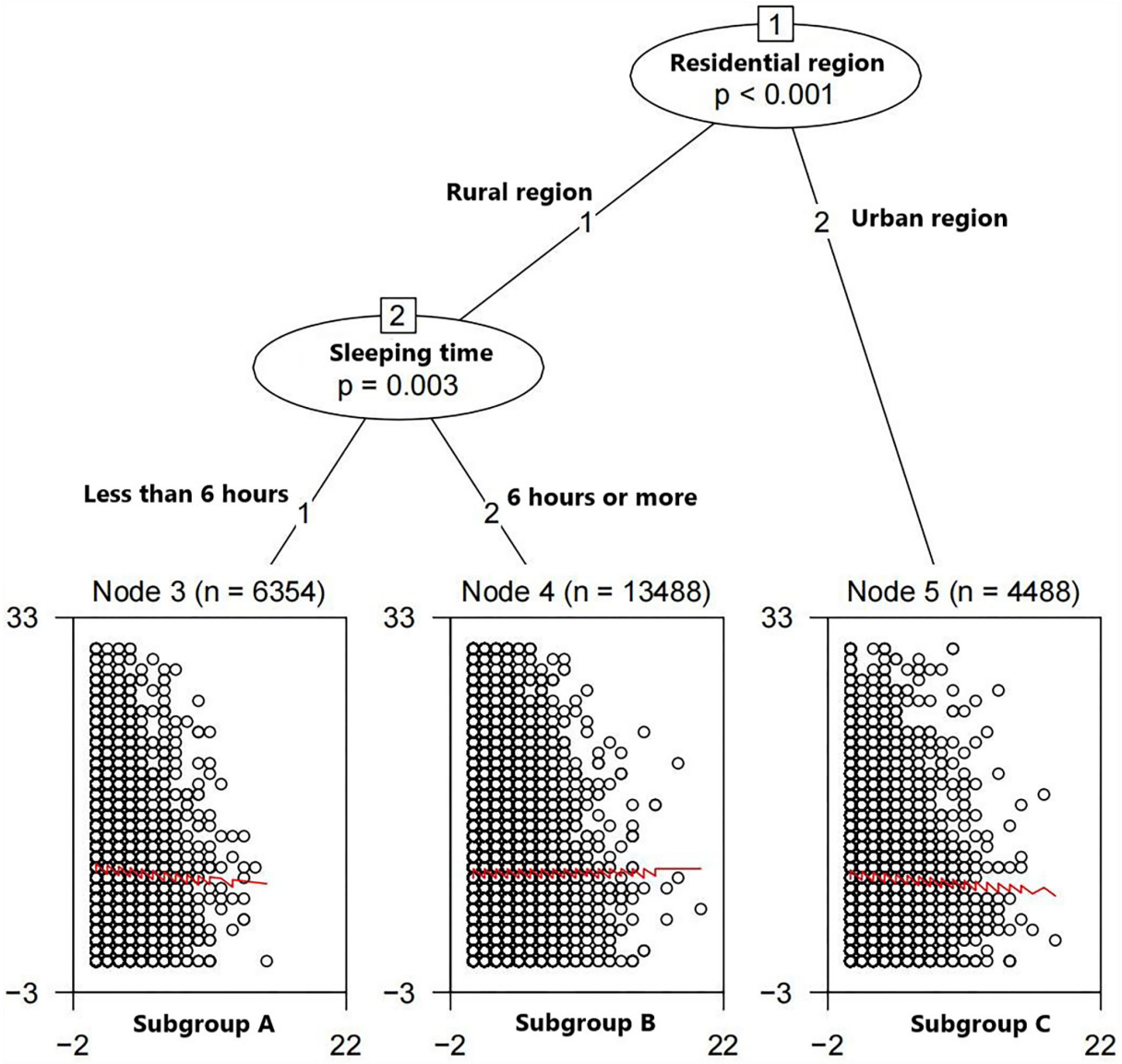

FIGURE 3
GLMM tree fitted. The selected partitioning variables are residential region and level of sleeping time. Terminal nodes: y axes represent CES.D-10 scores, and x axes represent SE scores.

the preventive effects of SE on depression in people with different characteristics (Min et al., 2016). Therefore, in order to deeper understand the heterogeneity of effects of the SE in subgroups of people with different characteristics, we launched this study. In general, we found that SE was associated with decreased risk of DS. Furthermore, in subgroup analysis, we found that SE may be more effective in preventing DS for people who live in the rural area with <6 h of sleep per day, and who live in the urban area. However, for people who live in rural areas and sleep more than 6 h per day, we did not find a significant association between SE and DS.

Sleep disturbances increase the risk of depression. The published study suggested that individuals with severe insomnia are more likely to develop depression (Schramm et al., 1995). In China, Jiang et al. (2020) used data from Henan Rural Cohort and found that short night sleep duration (<6 h) was the risk factor for DS in rural regions (Jiang et al., 2020). At the same time, sleep disorders are a typical symptom of most people with depression (Fang et al., 2019). In other words, depression can

lead to sleep disturbances. In addition, manipulation of sleep–wake cycles, such as sleep deprivation or early sleep periods, can alleviate depressive symptoms. This evidence suggests a strong bidirectional relationship between sleep, sleep changes, and depression (Riemann et al., 2001).

In addition, sleep is correlated with both the quantity and quality of social relationships (Gordon et al., 2021), and lacking sleep leads to an increase in loneliness and social isolation (ben Simon and Walker, 2018). Ben Simon and Walker conducted a longitudinal study and demonstrated that insufficient sleep leads to a neural and behavioral phenotype of social withdrawal and loneliness (ben Simon and Walker, 2018). Moreover, daytime sleepiness caused by poor sleep quality at night also increases social withdrawal (Holding et al., 2020). Therefore, a vicious cycle of sleep deprivation, daytime sleepiness, and social withdrawal may be key causes of depression. Increasing social interaction or improving sleep quality may be a key measure to break the vicious cycle and, by extension, prevent DS. However, rural residents with sufficient sleep time (>6 h) were not in this vicious circle, and our study did not confirm the association between SE and DS in this subgroup. Therefore, further research may be needed to further explore the association between SE and DS in this particular subgroup.

Retirement problems may cause the results that SE is more effective among the urban elderly in preventing DS. Unlike the rural residents engaged in agricultural work which was characterized by a "small-scale peasant economy" (based on the family as a unit and individual ownership of the means of production, which relies entirely or mainly on its own labor to meet its own consumption), the aged people in urban are more likely to face retirement problems. Sudden changes in social networks (caused by retiring) may make them out of their comfort zone and increase the risk of DS. The result of a systematic review and meta-analysis containing a total of 25 longitudinal studies between 1980 and 2020 showed that retirement is associated with more DS (Li et al., 2021a). Essentially, a possible cause for this is the change in the social relationship after retiring. More specifically, retirement is a process of losing or weakening a working (or social) role (Riley et al., 1994), and people are forced to change their life roles, which can cause psychological distress (Rohwedder and Willis, 2010). Therefore, increased SE can improve their mental health, and previous studies have shown that volunteering or working after retirement can be beneficial to mental health (Chen, 2012).

As for middle-aged adults in China, a previous study has found that SE can reduce the incidence of DS (Bhattacharya et al., 2016). Middle age is a special period in life when they shift their focus from exploring new social relationships to cultivating existing ones (Kiesow et al., 2021), such as interacting with close family members or friends (Bhattacharya et al., 2016). Therefore, maybe a good social connection with families or friends can help middle-aged people to enhance good mental health and prevent DS.

Previous studies have shown that DS is more common in women than men (Altemus et al., 2014), and our study confirms this conclusion. Besides, we also found that SE reduced the risk of depression in men, but not in women. While women are more likely to participate in social activities than men, a study that separates social networks' stress and support components found that the former is more closely related to women's mental health than the latter (Bernard, 1982). This suggests that attending social events makes it easier for women to get stress rather than stress relief. Therefore, men should be more involved in social activities than women to reduce the incidence of DS. In addition, it is recommended that families provide positive feedback in daily life to reduce the pressure that social activity places on women.

In our study, we found that from 2013 to 2018, the scores of DS showed an upward trend. With the rapid development of China's society and economy and the increasing degree of aging, the incidence of depression among the middle-aged and elderly Chinese is inevitable and may continue to rise in the future (United Nations, 2019). It is urgent to solve the problem of depression in the middle-aged and elderly Chinese. However, China has a large aging population, so the implementation of full coverage of the prevention is bound to bring a huge public health burden. Therefore, cost-effective interventions are important for the prevention of DS.

Our findings suggest that enhancing SE may be useful for reducing the risks of DS for the middle-aged and elderly in China, and generally speaking, SE is a part of everyday life and a modifiable factor in daily life. The cost of enhancing the SE of people through family and community efforts is relatively low. Thus, we could conclude that enhancing SE may be potentially a cost-effective way in preventing the initiation or the progression of DS.

Besides, in this study, we also found that two subgroups of people, including those rural people who sleep <6 h per day and those who live in urban areas, may benefit more in preventing DS or relieving the severity of it through improving SE. From the perspective of precision prevention, if sufficient attention can be given to these two subgroups, better DS prevention effects may be obtained and resource savings can be achieved. Therefore, in order to accurately prevent DS, communities and families are supposed to maintain a good community environment to ensure that there is a good environment suitable for the middle-aged and elderly adults to sleep at night. Besides, families are suggested to provide maximum social support to the middle-aged and elderly members, and the middle-aged and elderly people should also actively take measures to deal with the problem of retirement, develop good living styles, and avoid sleeping loss.

At present, the definition of SE varies, and a review article defines SE as "the action of being involved in community life,

socially or politically and structured by the environment, which places can be shared, and which are significant" by summarizing the relevant literature from 2009 to 2020 (Levasseur et al., 2022). The inclusion of social activities in our study directly follows the relevant questions defined in the CHARLS questionnaire. It divided SE into four categories, including activities for the purpose of leisure, activities that aim to provide pro bono service, activities through the Internet, and other activities. The inclusion of social engagement in this study is consistent with the definition mentioned above.

This study has several strengths. First, the effects of SE and DS are not short term, and the longitudinal data structure could ensure the evaluation of the longtime effects. Therefore, the results of longitudinal studies are more reliable than cross-sectional studies. Then, this study used baseline and 3 years of follow-up data, including the follow-up data of 2018 newly published in 2020. With a large sample size, nationwide sampling, and a seven-year time span, the research objects of this study are more representative. Besides, in subgroup analysis, a large sample size prevented the problem of excessively sparse subgroups and ensure the robustness of the results. Furthermore, compared with linear models, tree-based methods that generate hypotheses are more suitable for exploratory research on subgroup identification, and the GLMM tree shows higher accuracy and statistical power in subgroup identification (Beretta and Santaniello, 2016). Finally, causal inference analysis has rapidly evolved for generating scientific evidence (Hernán and Robins, 2020) since confounders were almost unavoidable in observational studies. In this study, we applied the PS adjustment to control possible confounding factors, and the conclusions drawn under the framework of causal inference are reliable.

There are some limitations to this study. This study was conducted based on the observational survey data obtained from the CHARLS study, and a prospective interventional study is still needed to verify the association between SE and DS. Moreover, DS is not based on clinical diagnosis but on scale measurement. Although the CES-D-10 scale has been validated for its effectiveness in Chinese populations, the judgment of DS may be biased to some extent. Measurements of SE consist of a series of questions. Based on the results of *Cronbach's α* coefficient and exploratory factor analysis, we may conclude that the measurements of SE used in this study were reliable. In addition, variables "the type of jobs" and "physical activity" are related to the occurrence of DS, but the missing rates of them are 89.38% and 65.91%. Variables missing more than 20% are not recommended to be imputed (Li et al., 2021b); thus, these two covariates were excluded. Finally, the effects of different kinds of SE were not included in the study. In the future, it should be determined what kind of social activities can minimize DS and obtain more quantitative results on this issue.

Conclusions

In general, we explored the association between SE and DS based on the longitudinal follow-up data obtained from the CHARLS study, and the results indicate that they are longitudinal and negatively associated. Our findings suggest that the enhanced SE may be useful for reducing the risks of DS for the middle-aged and elderly in China, especially for those middle-aged and elderly residents living in rural areas with less than six hours of sleep and those living in urban areas.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://charls.charlsdata.com/pages/data/111/zh-cn.html.

Author contributions

FC: the conception and design of this study, critical revision of the manuscript, and supervision of the study. YY and YL: data acquisition and management, statistical analysis, and interpretation of the results. YY: drafted the manuscript and assisted in the revision of the manuscript. PZ and JW: helped with data analysis. BM, LP, and YZ: assisted with the interpretation of the results and helped with the revision of the manuscript. All authors have reviewed and approved the manuscript before submission.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.934801/full#supplementary-material

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Regular fecal microbiota transplantation to Senescence Accelerated Mouse-Prone 8 (SAMP8) mice delayed the aging of locomotor and exploration ability by rejuvenating the gut microbiota

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Recent evidence points out the role of the gut microbiota in the aging process. However, the specific changes and relevant interventions remain unclear. In this study, Senescence Accelerated Mouse-Prone 8 (SAMP8) mice were divided into four groups; young-FMT-group transplanted fecal microbiota from young donors (2–3°months old) and old-FMT-group transplanted from old donors (10–11°months old); additionally, other two groups either adult mice injected with saline solution or untreated mice served as the saline and blank control groups, respectively. All mice were intervened from their 7-months-old until 13-months-old. The open field test at 9 and 11°months of age showed that the mice transplanted with gut microbiota from young donors had significantly better locomotor and exploration ability than those of transplanted with old-donors gut microbiota and those of saline control while was comparable with the blank control. 16S rRNA gene sequencing showed that the gut microbiome of recipient mice of young donors was altered at 11°months of age, whereas the alternation of the gut microbiome of old-donor recipient mice was at 9°months. For comparison, the recipient mice in the blank and saline control groups exhibited changes in the gut microbiome at 10°months of age. The hallmark of aging-related gut microbiome change was an increase in the relative abundance of *Akkermansia*, which was significantly higher in the recipients transplanted with feces from older donors than younger donors at 9°months of age. This study shows that

fecal microbiota transplantation from younger donors can delay aging-related declines in locomotor and exploration ability in mice by changing the gut microbiome.

KEYWORDS

aging, fecal microbiota transplantation, gut microbiota, *Akkermansia*, SAMP8

Introduction

Aging is inherently accompanied by the decline of physical and mental abilities, including locomotor, cognition, and bodily functions, to subsequently cause frailty syndrome, neurodegenerative diseases, and other age-related diseases, which reduce the quality of life of the aging population (Hou et al., 2019). Aging mechanisms and anti-aging interventions have long been a major focus of biomedical research, which is particularly relevant given the rapidly aging society.

The gut is a major organ for nutrients absorption, metabolism, and immunity, and contains hundreds of millions of microorganisms and their metabolites, which comprise the gut microbiota (Heintz and Mair, 2014) that interacts with host cells and tissues (Huang et al., 2021). Our previous study reported continuous changes in the gut microbiome of centenarians during their transition from a healthy status to death. The most significant changes of gut microbial communities in the period were found to occur at 7°months prior to death, suggesting that this may be a turning point of significant changes in the gut microbiome of centenarians (Luan et al., 2020). Recent studies have revealed an important relationship between the gut microbiome and aging-related diseases such as Alzheimer disease (Ticinesi et al., 2018; Haran and McCormick, 2021), suggesting that the gut microbiome plays an essential role in the aging process. Several cross-sectional comparative studies of different age groups of humans and animals have found that the β-diversity and microbiota composition of the gut microbiome vary according to aging status (Langille et al., 2014; Biagi et al., 2016; Kong et al., 2016; Odamaki et al., 2016; Piazzon et al., 2019; Reveles et al., 2019; Adriansjach et al., 2020). However, research on persistent longitudinal microbiome changes in the same organism in an aging condition is lacking.

Given the accumulating evidence of the role of the gut microbiota on overall health, life quality and/or expectancy could potentially be improved by remodeling the gut microbiome through interventions for probiotic/prebiotic regulation or fecal microbiota transplantation (FMT) (Smith et al., 2017; Vaiserman et al., 2017; Barcena et al., 2019; Stebegg et al., 2019). Ni et al. (2019) supplemented aged C57 mice with *Lactobacillus casei LC122* or *Bifidobacterium longum BL986* for 12°weeks and observed improvements in muscle strength, metabolism, and peripheral inflammation. Barcena et al. (2019) found that age-matched Hutchinson-Gilford progeria syndrome (HGPS) model mice (LmnaG609G/G609G) had gut microbiota dysbiosis, such as increased abundances of Proteobacteria and Cyanobacteria and decreased abundance of Verrucomicrobia compared with those of adult (4°months old) wild-type mice. Remodeling the gut microbiota of HGPS mice with that of wild-type mice improved the metabolic state and prolonged the life expectancy of HGPS mice (Barcena et al., 2019). Some animal studies report remodeling the middle-aged and elder gut microbiome through transplantation of younger gut microbiota can also improve health and prolong life expectancy. Smith et al. (2017) transplanted the gut microbiome of 3-week-old African turquoise killifish into 9.5-week-old African turquoise killifish, which resulted in prolonged life expectancy. Stebegg et al. (2019) transplanted the gut microbiota of 3-month-old C57BL/6 or BALB/C mice into isogenic 21-month-old mice, which caused the proliferation of intestinal Peyer's patches in the corresponding aged mice. Other animal studies have found that remodeling the young gut microbiome through transplantation of the aging gut microbiome can also have benefits. Kundu et al. (2019) found that transplantation of the gut microbiome of donor mice aged 24°months into germ-free recipient mice aged 5–6°weeks resulted in an increase in the number of hippocampal neurons and increased the gut length, whereas transplantation of the gut microbiome from young donor mice to age-matched recipients did not produce this effect. Although these studies show that regulation or remodeling of the gut microbiome has beneficial effects on the body, they have primarily been based on an experimental setting with antibiotic treatment and germ-free animals, which is not conducive to clinical translation to humans.

Senescence Accelerated Mouse-Prone 8 (SAMP8) is an animal model of aging with the characteristics of naturally occurring accelerating aging. In contrast to D-galactose-induced or gene knockout-related animal models of aging, SAMP8 mice eliminate the potential interfering effects of D-galactose and gene knockout on the gut microbiome (Lamas et al., 2016; Han et al., 2021). SAMP8 mice have neurodegenerative characteristics similar to those of Alzheimer disease that develops during aging in humans, thereby offering a valuable model for studying aging-related neurodegenerative diseases (Fernandez et al., 2021). SAMP8 mice enter a stage of

rapid aging at 7°months of age and have an average life expectancy of 10–17°months compared with 24°months of wild-type laboratory mice (Butterfield and Poon, 2005; Dutta and Sengupta, 2016). The gut microbiome of SAMP8 mice is different from that of their isogenic senescence-accelerated mouse resistant 1 (SAMR1) mice with a normal life expectancy. The α-diversity was lower in SAMP8 mice, and β-diversity was significantly different than the SAMR1 mice (Zhan et al., 2018). Detailed analysis of the microbiome structure of SAMP8 mice showed lower relative abundance of 27 species of gut bacteria, including Deferribacteres (Zhan et al., 2018). In addition, the health of SAMP8 mice could be improved by regulating the gut microbiome. For example, Yang et al. (2020) supplemented 9-month-old SAMP8 mice with a mixture of four probiotics for 12 consecutive weeks and found improvements in memory and inflammation.

However, these previous studies in SAMP8 mice have focused mainly on neurodegeneration-related mechanisms and probiotic interventions of the gut microbiota, with a lack of studies on the association of natural aging-related characteristics with such interventions like fecal microbiota transplantation. Therefore, we used the SAMP8 model to investigate the evolution and characteristics of the gut microbiome throughout the naturally aging process. Fecal microbiota transplants obtained from fecal samples of young (2–3°months) and old (10–11°months) SAMP8 mouse donors were transplanted into 7-month-old recipient SAMP8 mice, and their effects on the life expectancy and locomotor and exploration abilities of the recipient mice were studied in this research, as well as the potential bacterial marker of aging.

Materials and methods

Study design

Seven-month-old SAMP8 mice (male, n = 120) purchased from Laboratory Animal Science of Peking University Health Science Center (Beijing, China) were randomly divided into four groups, including the blank control group, saline group, young FMT group, and old FMT group, with 30 mice per group (**Figure 1**). The blank control group was not given any intervention, while the normal saline group, young FMT group, and old FMT group were administered normal saline, the fecal microbiota transplants of young (2–3°months old) donor mice, and the fecal microbiota transplants of old (10–11°months old) donor mice, respectively. One mouse in the old FMT group died the second day after randomization and was excluded. The study continued for 6°months, and SAMP8 mice were from 7 to 13°months old. To avoid coprophagy and microbe transfer *via* the skin, all mice were raised individually in ventilated cages at Chinese PLA General Hospital, maintained at room temperature (25℃) and 66–70% humidity. This study was approved by the Ethical Committee on Animal Experimentation of the Chinese PLA General Hospital.

Sample collection and fecal microbiota transplantation

Twenty-one 2-month-old donor SAMP8 mice were raised and their fecal pellets were collected at 2–3°months old and 10–11°months old. Each mouse was placed in an individual cylinder cup sterilized with 75% alcohol, and pellets were collected after the alcohol evaporated completely. The pellets of each mouse were weighed, individually placed in cryotubes. The feces of donor mice would be put at room temperature for some time from the collection to the freeze. As referred to in the previous study, the time was controlled within 2 h, which was majorly decided by the duration of mice defecation (Guo et al., 2016). A total of three mice were selected as the donor mice depending their total pellets weight was above 24°g at their 2–3°months old and 10–11°months old respectively. The feces of multiple mice would provide sufficient and uniform materials to conduct the long-lasting FMT in this study. The multi-donor could be used in FMT, which is in reference to the study of Paramsothy et al. (2017).

Fecal pellets of the three donor mice were mixed with saline at a ratio of 0.1°g to 1°ml, and the mixture was shaken until the pellets completely dissolved in the saline. The suspension was filtered and the liquid was collected for FMT. The gavage volume was based on the weight of the mouse according to a ratio of 0.1°ml liquid (microbiota transplants or saline) for 10°g of body weight. The liquid of FMT or saline was injected to the corresponding recipient mice once a week, at the same time.

The fecal pellets of the recipient mice and the mice in control group were collected at the day before the gavage, collection and preservation were same to the donor.

Open field tests

The open field tests were performed in reference to the previous study (Larke et al., 2017; Shieh and Yang, 2019, 2020) on 7, 9, and 11°months old of SAMP8 mice. The apparatus was consisted of a 45 × 45 × 45°cm chamber with the bottom painted black and the surroundings left transparent and one circular object was put in the center of the chamber. The SAMP8 mice were placed in the bottom left of the apparatus, recorded their movements by a digital camera, and analyzed by the Supermaze system purchased from Shanghai XinRuan Information Technology Co., Ltd., (Shanghai, China) for 5 min. The average velocity (total distances/5 min) and still time of the mice were used to evaluate the locomotor activity of the mice as described previously (Kraeuter et al., 2019; Miller et al., 2021). The Latency to the central zone and the explorative numbers of

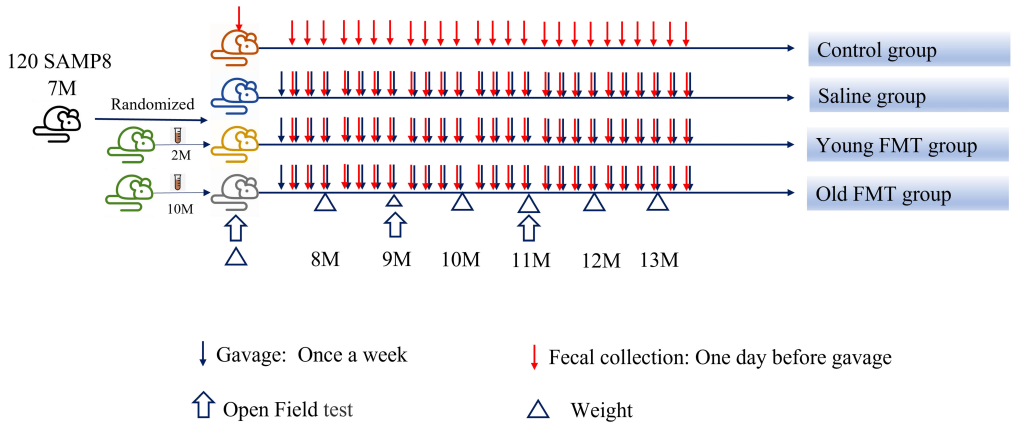

FIGURE 1
Scheme of the study procedure. FMT, fecal microbiota transplantation.

the object were used to determine the explorative behavior of the mice (Boehme et al., 2021). Sniffing, licking, or touching was all considered as exploring the objects.

16S rRNA gene sequencing and analysis

This research aimed to clarify the long-term aging trajectory of the gut microbiota, mice that did not survived at 13°months old were excluded. There were 10, 6, 8, and 8 mice in the Control group, Saline group, Young FMT group, and Old FMT group at 13°months old, respectively. We selected mice based on the group with the minimum number of mice at 13°months old, which was six in the saline group. However, the stool of mice decreased because of aging, we did not collect enough feces from one of these six mice at its 13°months old to conduct the 16s rRNA gene sequencing. After, five mice were randomly selected in each group, and the fecal pellets for those mice at the corresponding seven time points (7–13°months old, 7°months and 140 fecal samples in total) were conducted for 16S V3+V4 rRNA gene sequencing. Sequencing libraries were prepared by the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) and sequenced on the Illumina NovaSeq platform (Illumina, USA), and 250-bp paired-end reads were generated. The software package QIIME 2 (Quantitative Insights Into Microbial Ecology)[1] and the R package (version 4.1.0) were used for diversity analysis and taxonomic analysis for paired-end reads. Errors of amplified sequences were first corrected using DADA2 (*via* q2-dada2) with default parameters to generate a feature table and a feature sequences information file. Then, features of less than 200 (frequency less than 0.001) and samples with fewer than 10,000 reads were discarded to avoid low-abundance features and low biomass of the sample resulting in a low DNA extraction yield. The phylogenetic tree was generated *via* q2-phylogeny and alpha diversity was calculated using Faith's Phylogenetic Diversity metric with the q2-diversity package. PCoA was used to analyze the beta diversity according to the unweighted UniFrac distance, also with the q2-diversity package.

The taxonomic composition of the samples was classified (q2-feature-classifier) using pre-trained Silva classifiers (reference sequences clustered at 99% sequence similarity) (Bokulich et al., 2018; Robeson et al., 2021). The amplicon sequence variants were aggregated at the genus level.

Statistical analysis

GraphPad PRISM (version 8; GraphPad Inc., San Diego, CA, USA) was used for statistical analysis of original data and data visualization. One-way ANOVA and one-way repeated-measures ANOVA were used for analysis when the data were normally distributed and showed homogeneity of variance; otherwise, the non-parametric Kruskal-Wallis *H* test was used. Multiple comparisons were corrected by the Turkey *post-hoc* test. Kaplan-Meier and Log-rank test were used for survival analysis. Faith phylogenetic diversity was compared by Kruskal-Wallis *H* test, multiple comparisons were adjusted by the false discovery rate. Permutational multivariate analysis of variance (PERMANOVA, Adonis) based on the unweighted UniFrac distance was used to evaluate differences in beta diversity between groups with the "adonis" function. In addition, the "adonis. pair" function from the "EcolUtils" package was used for pairwise comparisons. LEfSe was used to identify the differentially abundant taxa across sample groups with default

1 https://qiime2.org

settings at the galaxy module. The top 10 most abundant genera within bacterial communities were obtained from all samples by comparing the relative abundance, and the stacked histogram showed these ten genera within each group.

Results

Effects of fecal microbiota transplantation on locomotor and exploration ability of aging mice

Senescence Accelerated Mouse-Prone 8 (SAMP8) mice were separately divided into four groups, recipients of FMT of young donor (n = 30), recipients of FMT of old donor (n = 29), control with saline treatment (n = 30), and untreated group (n = 30). The feces for young and old FMT were collected from three SAMP8 mice donors separately at their 2–3°months of age and 10–11°months of age. The intervention of FMT and saline treatments were started at 7°months of age, once every week, to the death of mice (Figure 1). During the study, the number of mice was lost because of aging. Therefore, the number of mice included in the subsequent statistical analysis was less than the initial number.

At 7, 9, and 11°months old, the four groups of SAMP8 mice were subject to an open field test to evaluate their locomotor and exploration ability. Firstly, we conducted the longitudinal comparison within each group by the self-pairwise comparison, and then multiple comparisons using the Turkey test. The results were summarized in Table 1.

In the blank control group, the locomotor ability (measured by average velocity and still time) of SAMP8 mice declined with aging, and the significant difference emerged in the 11-months test (7-months test vs. 11-months test, P = 0.0039; 9-months test vs. 11-months test, P = 0.0008; 7-months test vs. 9-months test, P = 0.82); while the exploration ability (measured by explorative numbers of objects) deceased but the difference between 7-month and 11-month was not significant. It should be noted that in the saline group, the declining trend of locomotor and exploration ability was more rapid than the control group (significantly different in 9°months and 11°months, respectively), suggesting the gavage had an effect on the locomotor and exploration ability with aging.

We found that the young FMT group had a slowly decreasing trend of performance with aging in the open field test, which was comparable with the control group but greatly exceeded the saline group and old FMT group (Table 1). The average movement speed of SAMP8 mice in saline group (P = 0.002) and old FMT group (P = 0.021) showed significant decrease as early as 9-month-old when compared with their 7-month-old, yet the average movement speed increased in young FMT group at 9-month-old despite not significantly (P = 0.42). For the aspect of still time, the mice in saline and old FMT group

TABLE 1 The locomotor activities and exploration abilities declined during aging.

Group	Average velocity (mm/s)			Still time (s)			Explorative numbers			Latency to central zone (s)		
	7M	9M	11M	7M	9M	11M	7M	9M	11M	7M	9M	11M
Control group (n = 20)	69.22 ± 15.41	71.07 ± 16.73	49.76 ± 30.97**	98.96 ± 17.40	106.5 ± 20.35	142.0 ± 48.79***	6.9 ± 3.8	7.1 ± 2.9	5.7 ± 4.8	272.6 ± 63.85	234.4 ± 100.7	277.4 ± 58.89
Saline group (n = 14)	74.11 ± 16.34	54.69 ± 9.28**	45.1 ± 13.21***	87.90 ± 20.13	113.3 ± 16.13**	136.5 ± 31.80***	9.0 ± 3.6	6.3 ± 4.2	4.0 ± 3.5**	256.6 ± 72.40	300.0 ± 0.04	282.8 ± 49.50
Young FMT Group (n = 16)	66.87 ± 21.14	73.03 ± 16.28	50.25 ± 22.01**	107.8 ± 27.27	95.09 ± 21.70	134.90 ± 52.70	6.0 ± 3.2	9.8 ± 7.9	7.7 ± 4.3	258.4 ± 71.72	230.1 ± 90.79	237.7 ± 91.64
Old FMT Group (n = 12)	71.13 ± 10.88	59.14 ± 17.3*	48.19 ± 19.99**	94.65 ± 17.56	116.4 ± 25.25**	139.3 ± 42.66**	6.5 ± 3.2	4.8 ± 2.5	3.4 ± 2.8*	291.7 ± 28.61	296.0 ± 13.83	292.3 ± 26.58

Data are Means ± SD, One-way Repeated Measures ANOVA, *P < 0.05, **P < 0.01, ***P < 0.001 (Compared with 7°months old).

at 9-month-old, compared to 7-month-old, had a significant increase (averagely > 22.9%, $P < 0.005$), and slightly increase in control group (averagely 7.62%, $P = 0.055$). Comparably, the young FMT group exhibited an exceptionally decrease of 11.8% in still time at 9-month-old, although not significant ($P = 0.09$). We observed similar results of exploration ability changing with the aging in the four groups. Compared with their 7-month-old, the explorative numbers of objects at 11-month-old decreased significantly in the saline group ($P = 0.001$) and old FMT group ($P = 0.031$) and insignificantly in the control group ($P = 0.186$); however, young FMT group increased albeit insignificantly ($P = 0.499$). Longitudinal comparison of the latency to central zone within group at 7-, 9- and 11-month-old showed no significant difference.

Besides the longitudinal comparison within each group, we also conducted the inter-group comparisons at the same months of age. The results also showed that the locomotor and exploration ability of mice in the young FMT group exceeded the other three groups at 9-month-old, 2°months after the FMT began. As shown in **Figure 2A**, the average movement speed of the 9-month-old mice in the young FMT group was significantly higher than that in the saline group ($P = 0.049$) and the old FMT group ($P = 0.020$) and was comparable with that in the control group ($P = 0.97$). For the still time (**Figure 2B**), significant difference was found between the young and old FMT groups ($P = 0.049$), while the young FMT mice had the lower but not significantly average still time than the control and the saline groups ($P > 0.19$). For the exploration ability, the young FMT group also showed the highest explorative numbers on average among the four group at 9-month-old (**Figure 2C**). The average latency to the central zone was comparable between the young FMT and control group, and both exceeded the saline and old FMT group (**Figure 2D**).

At the 11-month age, the excess of the young FMT group was not as obvious as in the 9-month-old. We observed that the young FMT mice still had significantly more numbers of object explorations than the old FMT mice at 11-month age ($P = 0.049$). For the other metrics, the differences were not significant (**Supplementary Figure 1**).

Moreover, considering the still time may influence the average movement speed (5 min included the mobile time and still time), we also calculated the average velocity excluding the still time. As shown in **Supplementary Table 1**, the results were consistent with the average movement speed that was calculated by total distances/5 min.

Effects of fecal microbiota transplantation on the life expectancy and weight of mice

The Kaplan-Meier survival curves up to 13°months of age of the young FMT, old FMT, saline and blank control groups were obtained for a life expectancy comparison. The survival difference among the four groups was not significant (**Figure 3A**). Nonetheless, the average survival period of the young FMT mice (333.3 days, SD ± 73.2) was longer than those in the saline group (325.4 days, SD ± 68.8) and old FMT group (325.1, SD ± 75.0). The average survival period was the longest in the control group (346.4 days, SD ± 76.7), implying the influence of gavage on survival.

A comparison of the body weight of mice among four groups at the same ages showed insignificant difference (**Supplementary Figure 2**). We selected the mice survived at 13-month-old in each group, and longitudinal compared their body weights. As shown in **Figure 3B**, the average weights of blank control group ranged from 28.6 to 29.6 g in the period of 7-month to 12-month-old, and decreased significantly to 26.3°g at 13-months-old (compared with 7-month-old, $P = 0.0028$). Similarly, the significant decrease of mean body weights in the young and old FMT mice was also observed at their 13-months age ($P <= 0.029$, **Figures 3C,D**). We did not observe decrease of mean body weight in the saline group, possibly due to the insufficient number of mice in this group survived at 13-months (**Figure 3E**, $P = 0.198$).

Effects of fecal microbiota transplantation on gut microbiome diversity during aging

16S rRNA gene sequencing analysis was performed on the gut microbiome of the four groups of SAMP8 mice. In each group, five mice that survived to 13°months were selected for gut microbiome analysis to monthly fecal samples. As shown in the **Supplementary Table 2**, the behavior of these selected mice was not aberrant from those unselected, namely those that did not survive the end of the study. Fecal samples were collected once in a month at the same time point for the groups, and before the FMT or saline treatment.

We obtained the α-diversity of the microbiome, measured by the faith phylogenetic diversity and the observed species, at each month for the four groups (**Figures 4A–D**, **Supplementary Figure 3**). Within each group, pair-wise comparison between months showed no significant differences (FDR adjusted, $P > 0.05$). However, comparison among the 7°months showed overall significantly different α-diversities in control groups (Kruskal-Wallis H test, the faith phylogenetic diversity: $P = 0.039$; the observed species: $P = 0.0197$), indicating the control mice had a remarkable fluctuation in α-diversity especially at 11- to 13-month ages.

Then, we analyzed the β-diversity (measured by unweighted unifrac distance) of samples to investigate the microbiome changes with age. As shown in **Supplementary Figure 4** and **Supplementary Table 3**, we found the phenomenon that

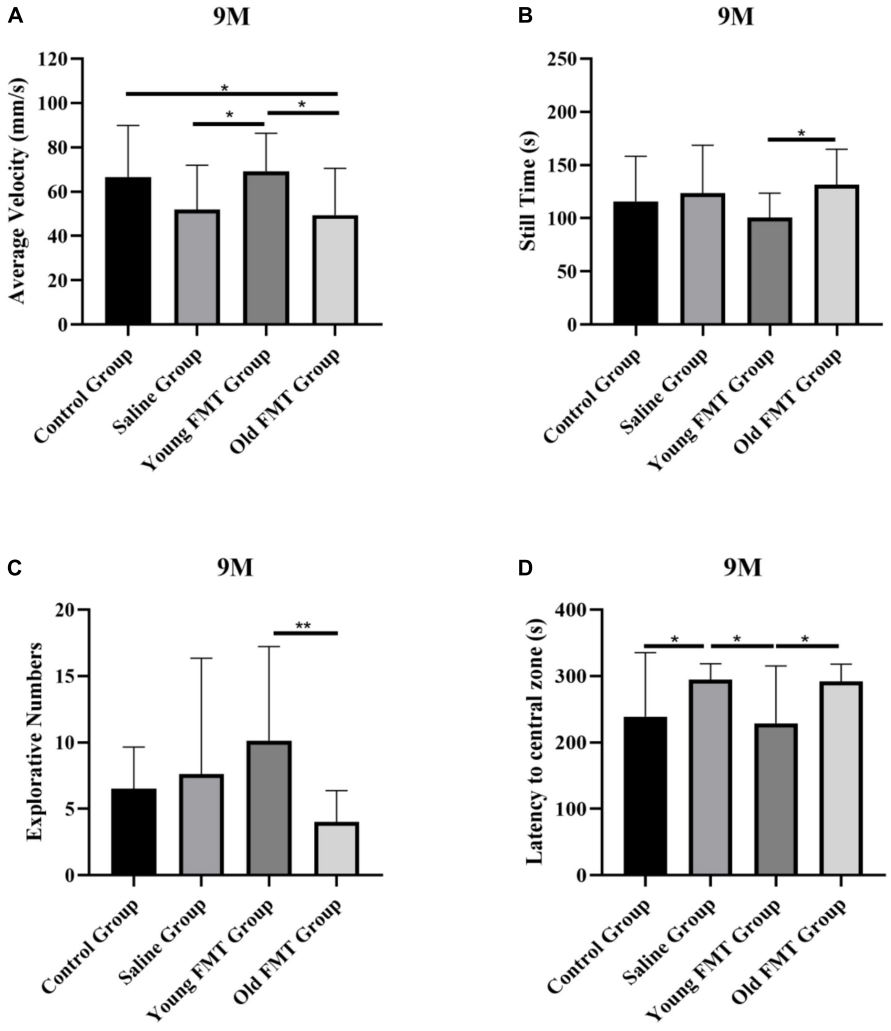

FIGURE 2
Effects of fecal microbiota transplantation on locomotor and exploration ability. **(A)** The average movement speed of Senescence Accelerated Mouse-Prone 8 (SAMP8) mice at 9-month-old in four groups. **(B)** The still time of SAMP8 mice at 9-month-old in four groups. **(C)** The exploring times of SAMP8 mice at 9-month-old in four groups. **(D)** The latency to central zone of SAMP8 mice at 9-month-old in four groups. Control group (n = 24), Saline group (n = 20), Young FMT group (n = 21), Old FMT group (n = 19). One-way ANOVA. All Data were shown by means ± SD, *$P < 0.05$, **$P < 0.01$.

7, 8, and 9°months were all significantly different from 12 to 13°months without considering the group factor, which suggested the existence of β-diversity shift with aging. Then considering the coexistence of factors containing the group and age, we conducted the PCoA and showed different groups on different plots (Figures 4E–H). The control mice had a remarkable and significant shift of community composition at the 10-month-old (Adonis, P = 0.001, Figure 4E and Supplementary Table 4). Interestingly, we found that the young FMT mice also had such shift (P = 0.001, Figure 4G), were at their 11-month-old (Supplementary Table 3). In contrast, for the saline and old FMT group, the shifts were not obvious (Supplementary Table 4). However, based on the relative distance of β-diversity, the shift appeared to begin at the 10-month-old for the saline group (Figure 4F), and 9-month for the old FMT group (Figure 4H).

We also compared the β-diversity among four groups at each month and no significant difference was found at the start of the study (Supplementary Figure 5, 7-month-old, unweighted Unifrac distances, Adonis, P = 0.207), while the 9-month-old (P = 0.003) and 10-month-old (P = 0.039) showed significant differences. However, after multiple comparisons and FDR adjustments, the differences between each two groups were insignificant (Supplementary Table 5). And when disregarding the blank controls, the significant differences existed in 9-month (P = 0.011), 10-month (P = 0.048), and 11-month (P = 0.045). The differences between each two groups still showed no

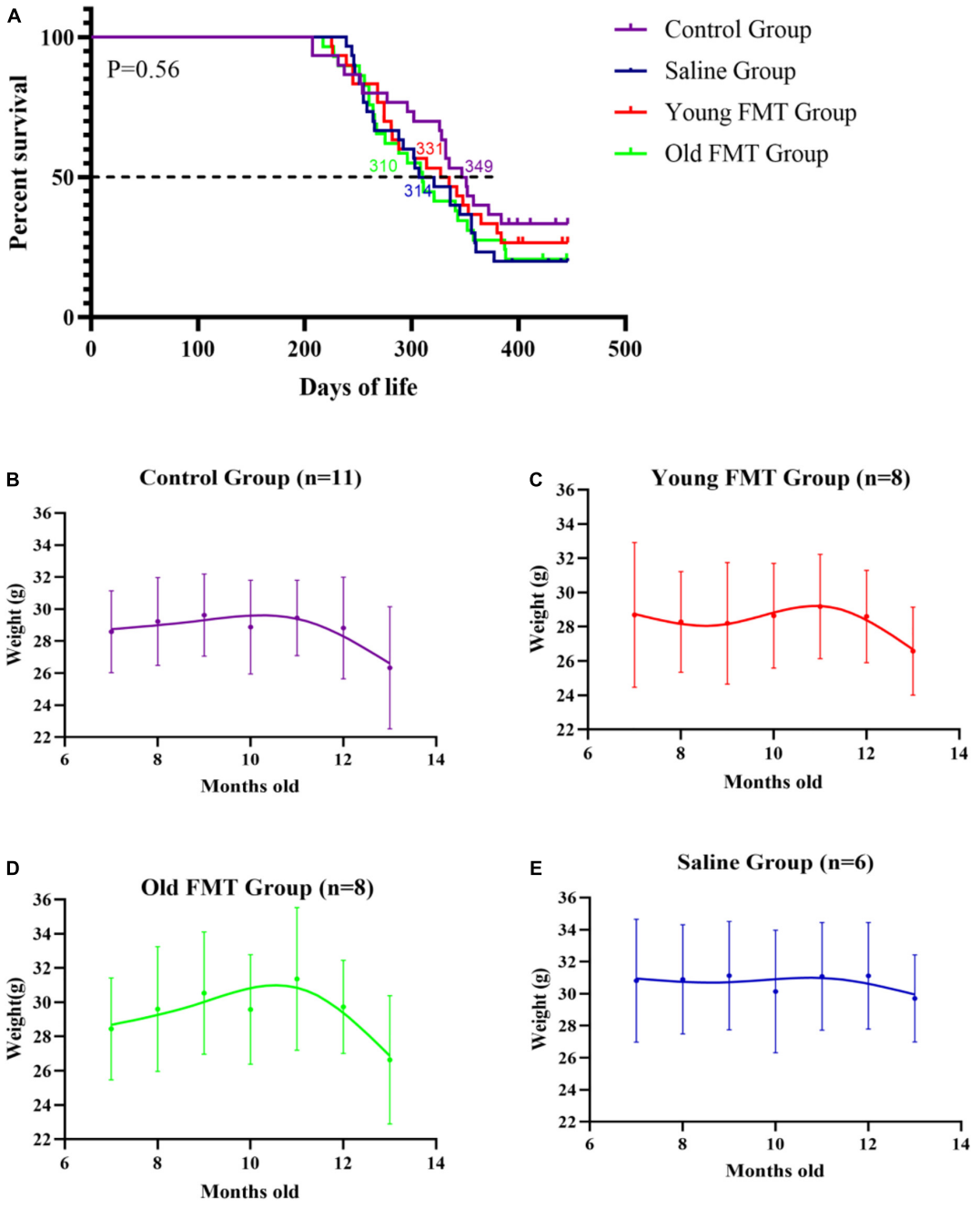

FIGURE 3
Effects of fecal microbiota transplantation on lifespan and weight change with aging. **(A)** Percentage survival of Senescence Accelerated Mouse-Prone 8 (SAMP8) mice in four groups. Differences were analyzed with the log-rank Mantel–Cox test. Median survival is indicated in the Kaplan–Meier plot. With the end of the study (13°months-old), the death events in Control group, Saline group, Young FMT group and Old FMT group were 20, 24, 22, and 23, respectively. **(B)** The longitudinal comparison of weight from 7°months old to 13°months old within control group (n = 11). **(C)** The longitudinal comparison of weight from 7°months old to 13°months old within young FMT group (n = 8). **(D)** The longitudinal comparison of weight from 7°months old to 13°months old within old FMT group (n = 8). **(E)** The longitudinal comparison of weight from 7°months old to 13°months old within saline group (n = 6). One-way repeated measures ANOVA.

significant after multiple comparisons and FDR adjustments (Supplementary Table 6). Interestingly, these time points among groups were coincident with these when microbial community shift appeared.

As for the diversity of donor-recipient gut microbiota, we showed in Supplementary Figures 6A–F. And the microbial community composition and structure of recipients were shifted toward a donor-like status. We also compared the β-diversity

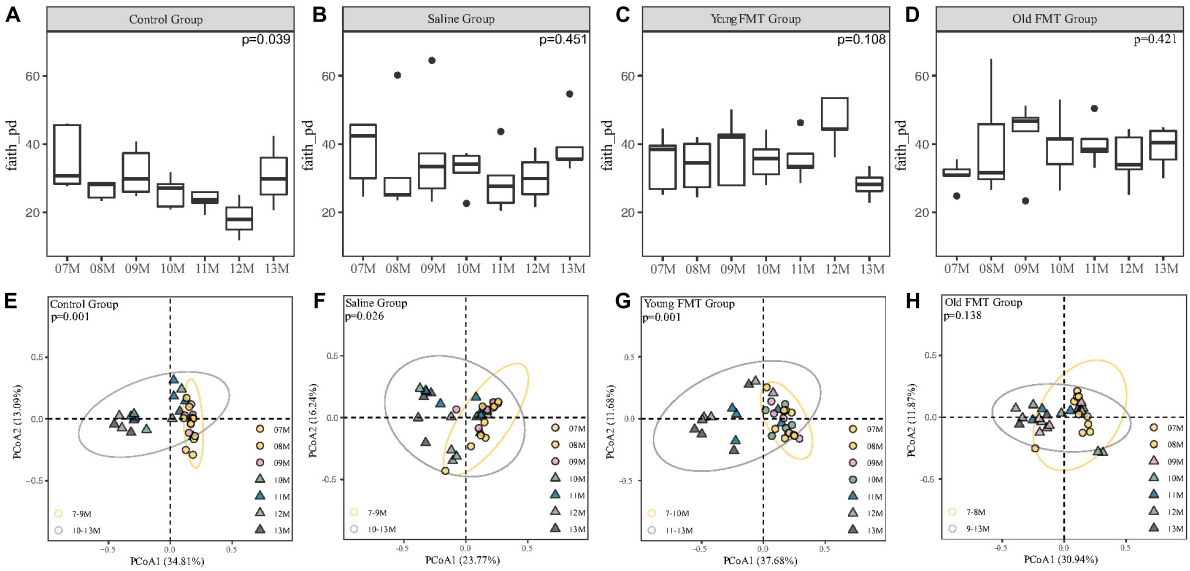

FIGURE 4
Effects of fecal microbiota transplantation (FMT) on Alpha and Beta diversity during aging. **(A–D)** The vertical comparison of Faith Phylogenetic Diversity index from 7 to 13°months old within group (Kruskal-Wallis *H* test). **(E–H)** Principal component analysis of Unweighted unifrac distance from 7 to 13°months old within group (PERMANOVA, Yellow Circle: the months of age before the turning point; Gray Circle: the months of age after the turning point). *P*-value was shown on the top left-hand corner. 7–11°months old, *n* = 5 per group; 12°months old, *n* = 3 (Control group), 4 (Saline group), 5 (Young FMT group), 5 (Old FMT group); 13°months old, *n* = 4 (Control group), 5 (Saline group), 5 (Young FMT group), 4 (Old FMT group).

between donor and recipients regardless of the age factor, and showed insignificant difference (**Supplementary Figures 6G,H**, Adonis, young donor vs. young FMT recipient, $P = 0.176$; old donor vs. old FMT recipient, $P = 0.254$).

Akkermansia abundance change is a marker of aging in Senescence Accelerated Mouse-Prone 8 mice

Next, we investigated the specific bacterial taxa that may be related to the shift in microbial community composition. The microbial communities at the genus level are illustrated in **Figure 5A**, and the control group slightly differed from the other three groups, again suggesting the effect of gavage. Intriguingly, the time points at which the relative abundance of *Akkermansia* increased in each group, were consistent with the shift point of microbial composition revealed by the β-diversity (**Figure 5B** and **Supplementary Table 6**). The relative abundance of *Akkermansia* was rare at 7-month-old and became dominating with aging in all four groups, despite still lower than 5% in some aging samples. Moreover, the shift of the abundance of *Akkermansia* occurred at ∼10-month-old for the control and saline group, 11-month-old for the young FMT mice, and ∼8- or 9-month for the old FMT mice. We further analyzed *Akkermansia* at 9-month-old using the linear discriminant analysis of effect size (LEfSe) and one-way analysis of variance (ANOVA). Both methods showed that the old FMT group had the significantly higher relative abundance of *Akkermanisa* as early as 9-month-old than that in the other three groups (**Figures 5C,D**).

Moreover, we also conducted the Spearman correlation analysis between the *Akkermanisa* and the behavior performance. As shown in **Figure 6**, We found the relative abundance of *Akkermanisa* was in significantly negative correlation with the average velocity ($r = -0.395$, $P = 0.0018$; excluding still time: $r = -0.380$, $P = 0.0028$) and explorative numbers ($r = -0.289$, $P = 0.0252$) and in significantly positive correlation with the still time ($r = 0.352$, $P = 0.0058$). However, the latency to the central zone showed an insignificantly positive correlation with the relative abundance of *Akkermanisa* ($r = 0.115$, $P = 0.382$).

Discussion

The gut microbiota plays an essential role in the aging process. Previous studies have compared gut microbiome characteristics in people of different ages, demonstrating differences in the composition in the aging body from that of younger controls. However, these results are dependent on race, diet, living conditions, and other individual differences, and there is a lack of consistency among studies (Biagi et al., 2016;

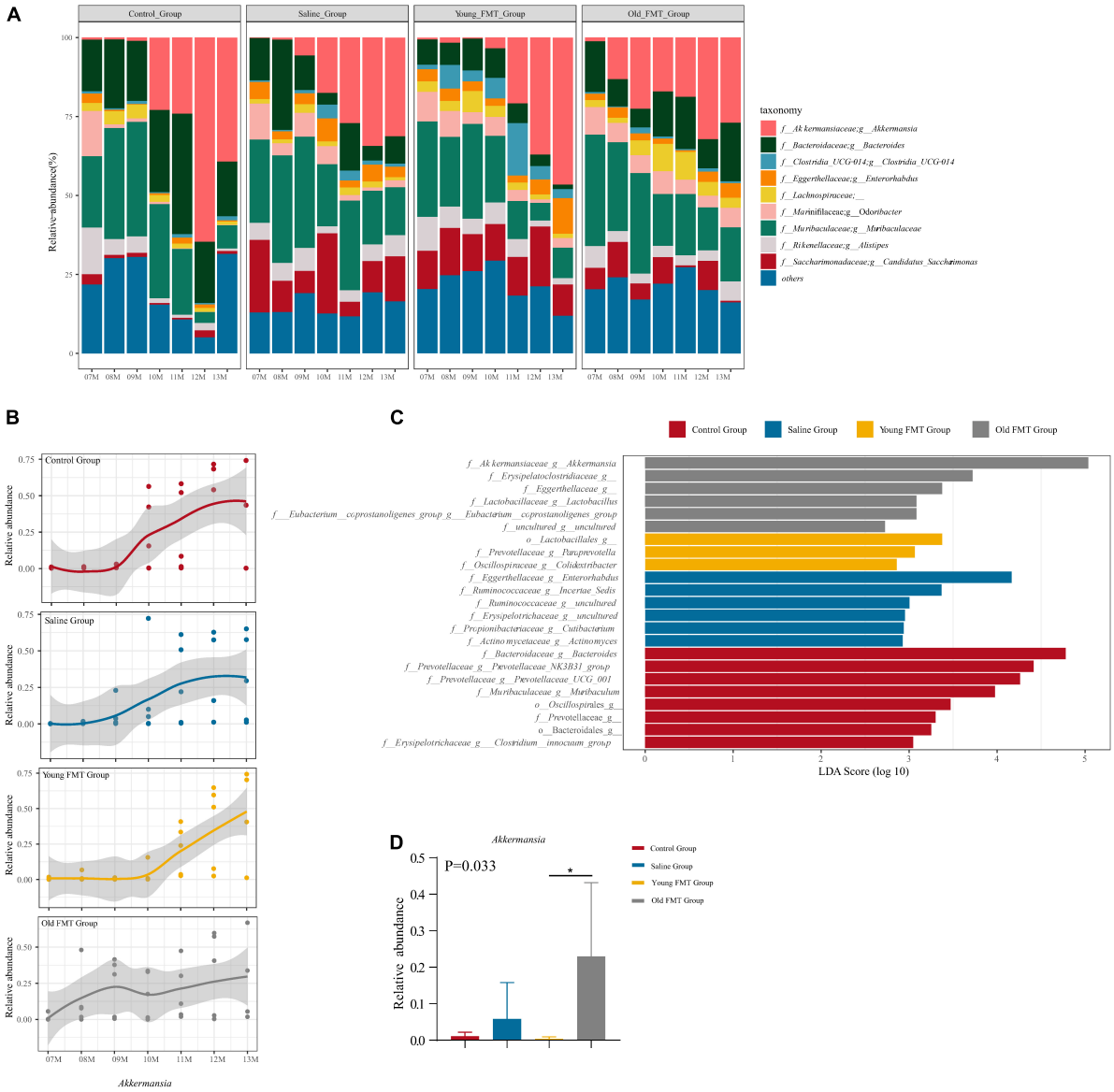

FIGURE 5
Akkermansia is the potential biomarker of aging. **(A)** The stacked histogram of the top 10 most abundant microbiota at genus level in relative abundance from 7 to 13°months old sequencing mice sample within the group. **(B)** The Nonlinear regression (95% confidence intervals) of the relative abundance of *Akkermansia* from 7 to 13°months old within group. The scatters represented the relative abundance of *Akkermansia* in samples. **(C)** The LDA score of different taxa obtained by the LEfSe analysis within 4 groups at 9°months old (Kruskal-Wallis *H* test, $P < 0.05$, LDA ≥ 3.0). **(D)** The relative abundance of *Akkermansia* within four groups at 9°months old (Kruskal-Wallis *H* test, $P < 0.05$*). Red: Control group; Blue: Saline group; Yellow: Young FMT group; Gray: Old FMT group; 7–11°months old: $n = 5$ per group; 12°months old: $n = 3$ (Control group), 4 (Saline group), 5 (Young FMT group), 5 (Old FMT group). 13°months old: $n = 4$ (Control group), 5 (Saline group), 5 (Young FMT group), 4 (Old FMT group).

Kong et al., 2016; Odamaki et al., 2016) as well as a lack of data on the longitudinal dynamic changes in the gut microbiome of the same organism throughout aging. In this study, the SAMP8 mouse model was used to systematically investigate the longitudinal trajectory of the gut microbiome during aging. Moreover, we explored the impact of FMT from old and young donors on the locomotor and exploration ability and lifespan of SAMP8 mice during aging, as gut microbiota interventions have been shown to improve health and prolong life expectancy. Boehme et al. (2021) transplanted the gut microbiota of C57BL/6 mice aged 3–4°months to isogenic mice aged 19–20°months, which resulted in improvements in neuroinflammation and cognitive behavior of the recipients. However, previous interventional studies were based on antibiotic treatment or germ-free mice, which is not conducive to human clinical translation. In the present study, the fecal

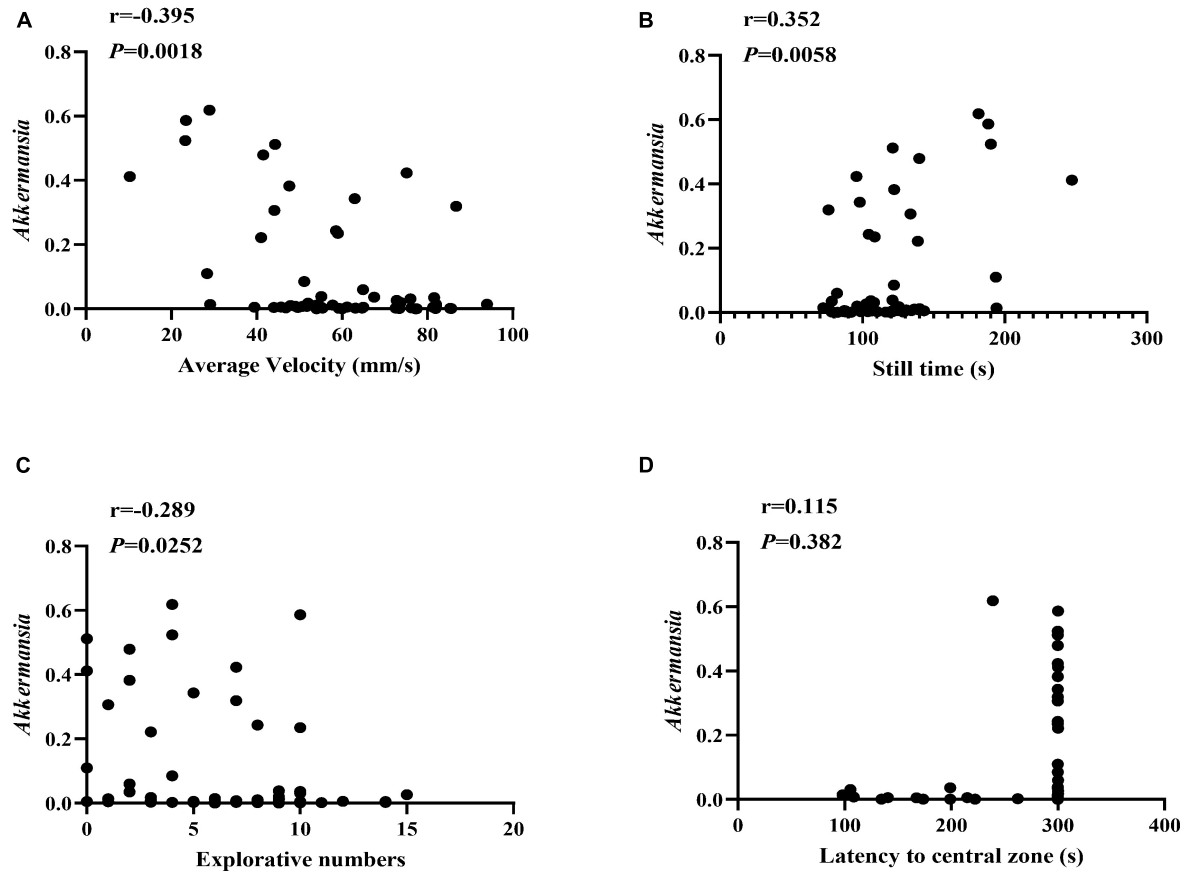

FIGURE 6
The correlation between *Akkermansia* and the behavior performance. **(A–D)** The Spearman correlation analysis between the relative abundance of *Akkermansia* and the average velocity, still time, explorative numbers, and latency to the central zone, respectively. The correlation coefficients (r) and the *P* value were shown in the top left.

microbiota transplants of SAMP8 mice aged 2–3°months and aged 10–11°months were used as interventions in 7-month-old SAMP8 mice to determine their effects on aging organisms that have not been treated with antibiotics.

In this study, we considered the entire aging period of SAMP8 mice between 7 and 13°months old, demonstrating that the locomotor and exploration ability gradually decreased during this time, partly revealing the decrease in skeletal muscle function and curiosity. Moreover, significant weight loss was found in SAMP8 mice at 13°months of age, implying that weight loss may mark significant deterioration in health; indeed, weight loss is associated with an increased incidence of various chronic diseases (Calderón-Larrañaga et al., 2021). This study found the young FMT intervention had played a role in slowing the decreasing trend of locomotor and exploration ability with aging and better performance than saline and old FMT intervention. Constructing a younger gut microbiota of aged SAMP8 mice by the gut microbiota transfer may be feasible for delaying the decline of physiological function with aging. And several animal studies have also demonstrated the beneficial effects of young FMT from the aspects such as improving cognition, improving inflammation, and prolonging life expectancy (Smith et al., 2017; Barcena et al., 2019; Ma et al., 2020). Unlike Smith et al., who found that young FMT significantly prolonged the lifespan of middle-aged African turquoise killifish, the present study did not find the significant extension of SAMP8 mice lifespan by young FMT. However, the median survival days were longer in SAMP8 mice that received the young FMT than saline or old FMT. Gut microbiota transplantation did not significantly affect the change in body weight during aging in SAMP8 mice. Considering that significant changes in body weight may be a major sign of the end of life (Calderón-Larrañaga et al., 2021), which occurs later than changes in behavior performance, this change may be more difficult to delay or reverse with gut microbiome transplantation.

We found no significant changes in the α-diversity of the gut microbiome with SAMP8 mouse aging, which was consistent among four groups, despite a possibly remarkable fluctuation in α-diversity of mice in the control group especially at 11- to 13-month ages. However, the β-diversity shifted

significantly at specific months old (10-month-old) of SAMP8 mice. Consistently, our previous study showed that the α-diversity of gut microbiome during the aging of centenarians had no significance, and the β-diversity changed significantly 7°months prior to death (Luan et al., 2020). The significant shift of microbial communities may cause by decreased food intake and physical activity.

This study found the relative abundance of *Akkermansia* increased at 10-month-old during the SAMP8 mice aging process. The increase of *Akkermansia* may be related to the decrease of some core taxa of the elder. Xu et al. (2019) have reported some beneficial genera lost with advanced ages. Badal et al. (2020) and Zhang et al. (2021) both find that *Akkermansia* is positively related to age. Palmas et al. (2022) also report that one of the main biomarkers of centenarians is *Akkermansia*. Moreover, as a metabolizing beneficial bacterium (Vallianou et al., 2020; Yoon et al., 2021), the increase of *Akkermansia* may also increase the resilience to metabolic aging of the body. Some validation work about *Akkermansia muciniphila*, which is a member of *Akkermansia*, also finds that supplementing the *Akkermansia muciniphila* to the mice could alleviate intestinal inflammation, improve the metabolism, and regulate the immune system of the aging (Greer et al., 2016; van der Lugt et al., 2019; Grajeda-Iglesias et al., 2021; Cerro et al., 2022). However, the increase of *Akkermansia* is also related to neurodegenerative diseases like Alzheimer's and Parkinson's diseases (Fang et al., 2020). Amorim Neto et al. (2022) also find that *Akkermansia muciniphila* could induce mitochondrial calcium overload and α-synuclein aggregation of the enteroendocrine cell, which may be an important mechanism of Parkinson's diseases. We hypothesized that the increase of *Akkermansia* may play a beneficial role in aging, yet it also increases the risk of neurodegenerative disorders. This study found the change in the relative abundance of the *Akkermansia* was significantly correlated with the locomotor and exploration ability with aging, which may also provide evidence for the hypothesis. To validate this hypothesis, we also will isolate strains of *Akkermansia* from feces samples of SAMP8 mice and cultivate muscle cell, brain cell, intestinal epithelium cell, and enteroendocrine cell in the strains-conditioned medium in subsequent studies as referred to the study of Amorim Neto et al. (2022).

The remarkable shift of the gut microbial communities and the corresponding increase in the relative abundance of *Akkermansia* were relatively delayed in the young FMT group to 11°months of age and appeared earlier in the old FMT group, at 9°months of age. Moreover, the earlier shift of β-diversity and increase of *Akkermansia* abundance showed the premature aging of gut microbiota in SAMP8 mice received the FMT from older donors. Shin et al. (2021) also overviews the studies about promoting healthy aging by regulating the aged microbial communities and reports it is feasible to conduct the FMT from young donors or supplement *Akkermansia*. This study found young FMT played a role in slowing the decreasing trend of locomotor and exploration ability with aging and delaying the increase of *Akkermansia*. However, the relationships between FMT and *Akkermansia*, and subsequent *Akkermansia* and aging, seem complex and need to be furtherly studied.

Regular FMT by gavage for a long time was found in this study as a potentially confounding factor affecting the SAMP8 mice during aging. The average survival days were longest in the untreated control group, and the microbial communities at the genus level also showed differences from other groups that received the gavage. However, few studies have reported the gavage effects on the gut microbiota. Further studies need to take the effects of gavage into consideration, such as the stress caused by the gavage and impact of gavage on food consumption.

In summary, the present study demonstrated key changes in the gut microbiome during aging and suggested *Akkermansia* as a key microbial marker of the individual aging process. However, this study was only conducted in the SAMP8 mouse model, and long-term gavage was also an important stressor to aged mice. Therefore, further studies are needed to explore whether other animal models or humans exhibit similar changes in their microbiomes and a better way to remodel the aged gut microbiota than gavage. In addition, the number of samples used for gut microbiome gene sequencing was small. Thus, subsequent studies are needed in larger samples with a variety of animal models and humans for validation and to search for relevant mechanisms. Further research in these directions is expected to help realize the transformation of healthy aging from the laboratory to the clinic by rejuvenating gut microbiota.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA806381/.

Ethics statement

The animal study was reviewed and approved by the Ethical Committee on Animal Experimentation of the Chinese PLA General Hospital.

Author contributions

YY and MN were responsible for the idea, outlined, and prepared the initial and final drafts of the manuscript. YY and MN funded this study. NZ, RM, HZ, XL, and LW performed the experiment. NZ, YZ, and MN analyzed the data. NZ and YZ drafted the manuscript. ZW, FP, RR, ZPL, ZWL, LP, GS, MN,

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.991157/full#supplementary-material

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Association of sleep quality with lower urinary tract symptoms/benign prostatic hyperplasia among men in China: A cross-sectional study

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Objective: As the population aged, voiding dysfunction has been steadily rising among males during the past decade. Increasing evidence showed that sleep disorders are associated with an increasing risk of various diseases, but the association between sleep disorders and lower urinary tract symptoms (LUTS)/benign prostatic hyperplasia (BPH) among Chinese males have not been well characterized.

Materials and methods: We conducted a cross-sectional analysis of data from West China Natural Population Cohort Study (WCNPCS) 2019–2021. Sleep quality was assessed by Pittsburgh sleep quality index (PSQI) in Chinese version. LUTS/BPH as a dependent variable of a binary variable, assessed by a self-reported questionnaire. Multivariate logistic regression analysis were performed to evaluate the correlation between sleep disorders and the risk of LUTS/BPH after adjusting for confounding factors.

Results: 11,824 eligible Chinese men participated in this cross-sectional survey. In multivariate logistic regression analysis, after adjusting for confounding variables, global PSQI score (OR: 1.257, 1.119–1.411, $p < 0.001$) and its six compounds (Subjective sleep quality: OR: 1.376, 1.004–1.886, $p = 0.048$; Sleep latency: OR: 0.656, 0.557–0.773, $p < 0.001$; Sleep duration: OR: 1.441, 1.189–1.745, $p < 0.001$; Habitual sleep efficiency: OR: 1.369, 1.193–1.570, $p < 0.001$; Daytime dysfunction: OR: 1.702, 1.278–2.267, $p < 0.001$) except the use of sleep drug subgroup were significantly positively correlated with LUTS/BPH prevalence. Significant interaction effects were observed in age subgroups (age-young group: age < 51; age-middle group: $51 \leq$ age ≤ 61;

age-older group: age > 61) ($P < 0.05$). Among older participants, sleep disorders were more significantly associated with the risk of LUTS/BPH.

Conclusion: There was a significant association between poor sleep quality and increased prevalence of LUTS/BPH, especially among the elderly male population, suggesting an important role of healthy sleep in reducing prostate disease burden.

KEYWORDS

sleep disorder, WCNPCS, LUTS/BPH, risk factor, Pittsburgh sleep quality index (PSQI)

Introduction

Benign prostatic hyperplasia (BPH) is the most common benign disease among the causes of micturition disorders in elderly men. It usually occurs after the age of 40. The prevalence rate of men over the age of 50 is 50%, and it is as high as 83% at the age of 80 (Berry et al., 1984; Gu et al., 1994). Wang et al. (2015) showed that the prevalence of BPH in Chinese population gradually increased from 2.9 to 69.2% among 40–80 years old. It is obvious that the prevalence of BPH has increased sharply with the age of Chinese men. The high prevalence of BPH greatly increases the health and economic burden of our society and the pain of these patients (Speakman et al., 2015).

Benign prostatic hyperplasia is due to the proliferation of stromal cells and epithelial cells stimulated by hormones and their active metabolites, causing the formation of anatomical hypertrophy prostate, which is manifested as urodynamic bladder outlet obstruction (BOO), resulting in lower urinary tract symptoms (LUTS), such as urinary retention, weak urine flow, etc. (Chughtai et al., 2016; Pejčić et al., 2017). In this study, LUTS/BPH refers to LUTS mainly manifested as obstruction caused by BPH.

Some studies have confirmed that sleep ensures the completion of important physiological functions by promoting the development of the central nervous system and the recovery of physical functions, which is a key factor in maintaining health (Tufik et al., 2009; Li et al., 2021). Researches involving both animals and humans have shown that sleep restriction can cause cognitive, immune, metabolic and hormonal disorders (Spiegel et al., 1999; Everson and Crowley, 2004; Hipólide et al., 2006). Previous cohort studies conducted by Koskderelioglu et al. (2017) and Branche et al. (2018) showed that poor sleep quality was associated with prostate disease.

However, the correlation between sleep quality and the risk of LUTS/BPH in Chinese men remains uncertain. Therefore, the purpose of this study is to analyze the potential association between sleep quality (measured by PSQI score) and LUTS/BPH in Chinese men. Importantly, the results may support the discovery of new strategies to prevent LUTS/BPH and improve the quality of life of aging population.

Materials and methods

Study population

The current research is a cross-sectional analysis based on the data of the West China Natural Population Cohort Study (WCNPCS) collected from May 2019 to June 2021. The sampling method of this study is cluster sequential sampling and the data was collected from three regions of Sichuan Province, the most populous province in Western China, including Mianzhu, Longquan, and Pidu. This study aimed to establish a large-scale prospective follow-up natural population cohort and collect various information of community participants in order to evaluate the health status of the general population in Western China.

A total of 36,075 participants aged 18–65 were included in this study. Participants were further excluded for lack of sleep quality and LUTS/BPH disease information (n = 1,102) and sleep quality score data (n = 23,149). Our final analysis sample was 11,824 participants (**Figure 1**). Specific general information (e.g., demographics, social-economic, education level and physical activities) was obtained through face-to-face interviews. Participants were further recruited for physical examination to collect biological samples, which were conducted by trained medical personnel in specially equipped mobile examination centers (MECs). Participants were recruited on a voluntary basis, and each participant signed and obtained informed consent before the survey. All research protocols were in accordance with the 1975 Helsinki Declaration and the applicable amendments at the time of the survey. The study protocol was approved by the ethics committee of West

Abbreviations: BPH, benign prostatic hyperplasia; BOO, bladder outlet obstruction; BMI, body mass index; Cr, creatinine; DM, diabetes mellitus; GAD-7, generalized anxiety disorder-7; LUTS, lower urinary tract symptoms; MECs, mobile examination centers; PSQI, Pittsburgh Sleep Quality Index; PHQ-9, patient health questionnaire-9; WCNPCS, West China Natural Population Cohort Study; WHR, waist-to-hip ratio.

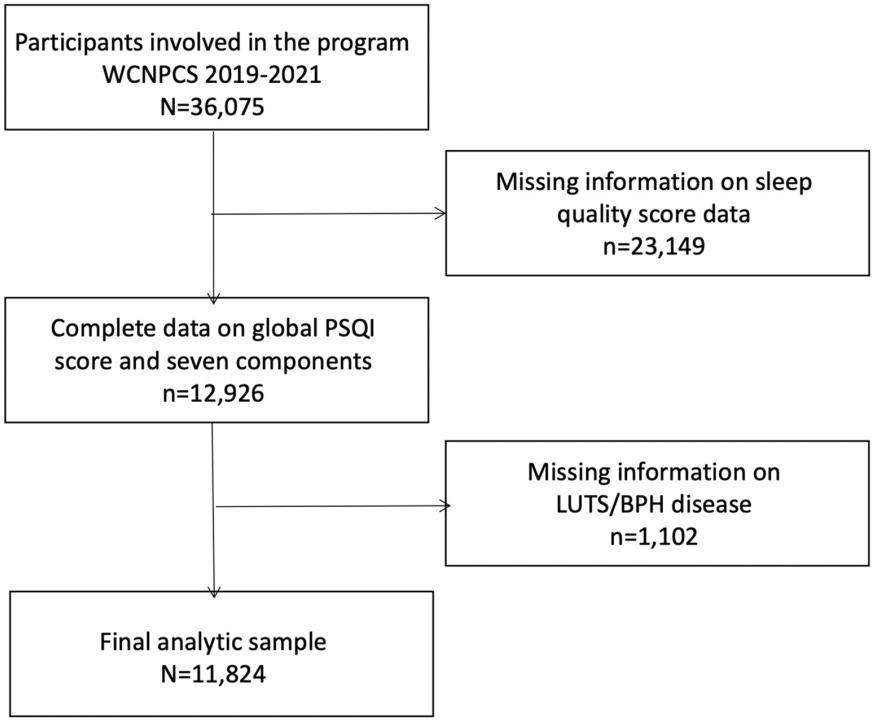

FIGURE 1
Flowchart of participant inclusion and exclusion for analysis.

China Hospital of Sichuan University. The study was registered in China Clinical Trial Registration Center (Registration No. ChiCTR1900024623, 2019/07/19).

Measurement of sleep quality

A PSQI questionnaire translated into Chinese was used to evaluate sleep quality. It is a standard self-report, including a 19-item questionnaire designed to collect a person's subjective feelings about sleep habits for more than 1 month (Buysse et al., 1989). Each item is divided into four levels, with scores ranging from 0 to 3. PSQI has been used to diagnose sleep disorders in many clinical applications and has been proved to have good reliability, validity, and sensibility (Tsai et al., 2005; Mollayeva et al., 2016). It estimates several different aspects of sleep, which affect seven aspects of sleep problems, including subjective sleep quality, sleep latency, sleep duration, habitual sleep frequency, sleep disorders, use of sleep drugs, and daytime dysfunction (Buysse et al., 1989). The sum constitutes the global sleep quality score (ranging from 0 to 21), and the higher score mean the worse sleep quality. The global PSQI score is divided by 7 points, which can distinguish poor or good sleep. It has high diagnostic sensitivity and specificity in Chinese population (98.3 and 90.2% respectively) (Li et al., 2021).

Measurement of lower urinary tract symptoms/benign prostatic hyperplasia

In addition, males were asked, "Have you ever been diagnosed with prostate hyperplasia?" Related symptoms of prostatic hyperplasia, including difficulty urinating, increased nocturia, urinary incontinence, were explained to all the participants. In WCNPCS study, symptoms were mainly assessed based on participant self-report, which was also commonly used in previous studies (Verhamme et al., 2002; Xiong et al., 2020; Zhang et al., 2022). The current study showed that men with sleep disorders are more likely to report daytime LUTS (Fantus et al., 2018). In order to make the results more reliable and exclude the direct impact of nocturia on sleep, We introduced daytime LUTS, which means symptoms of LUTS/BPH except nocturia.

Covariates

Information about sociodemographic characteristics and lifestyle factors was collected through questionnaire survey. For continuous covariates including age (year), body mass index (BMI, kg/m2), waist-to-hip ratio (WHR), the patient

TABLE 1 Baseline characteristics of participants.

Variable	Total	LUTS/BPH		*P*-value
	(*N* = 11,824)	Yes (*n* = 2,901)	No (*n* = 8,923)	
Age (year)	57.81 ± 12.40	60.51 ± 11.81	56.93 ± 12.46	<0.001
BMI (kg/m^2)	24.79 ± 3.21	24.76 ± 3.17	24.80 ± 3.22	0.676
WHR	0.89 ± 0.06	0.89 ± 0.06	0.88 ± 0.06	<0.001
PHQ-9	0.814 ± 2.00	0.94 ± 2.19	0.78 ± 1.93	<0.001
GAD-7	0.76 ± 2.09	0.85 ± 2.27	0.73 ± 2.03	<0.001
Cr (μmoI/L)	72.62 ± 24.19	71.91 ± 15.80	72.85 ± 26.35	0.002
Educational level				<0.001
Primary school	3,371 (34.16%)	985 (41.65%)	2,386 (31.80%)	
Junior school	3,785 (38.36%)	912 (38.56%)	2,873 (38.29%)	
High school	1,543 (15.64%)	307 (12.98%)	1,236 (16.47%)	
College	1,144 (11.59%)	157 (6.64%)	987 (13.16%)	
Graduate	25 (0.25%)	4 (0.17%)	21 (0.28%)	
Marital status				<0.001
Married	11,006 (93.83%)	2,680 (93.32%)	8,326 (93.99%)	
Unmarried	190 (1.62%)	29 (1.01%)	161 (1.82%)	
Divorced	209 (1.78%)	54 (1.88%)	155 (1.75%)	
Widowed	136 (1.16%)	47 (1.64%)	89 (1.01%)	
Smoking status				<0.001
Current	5,053 (42.79%)	1,267 (43.68%)	3,786 (42.51%)	
Occasionally	352 (2.98%)	69 (2.38%)	283 (3.18%)	
Ever	1,541 (13.05%)	450 (15.51%)	1,091 (12.25%)	
Never	4,862 (41.18%)	1,115 (38.44%)	3,747 (42.07%)	
Drinking status				0.044
Yes	6,072 (51.43%)	1,445 (49.83%)	4,627 (51.95%)	
Ever	868 (7.35%)	238 (8.21%)	630 (7.07%)	
No	4,866 (41.22)	1,217 (41.97%)	3,649 (40.97%)	
Coffee drinking				<0.001
1–2 times per week	752 (6.37%)	141 (4.86%)	611 (6.86%)	
3–5 times per week	75 (0.64%)	10 (0.35%)	65 (0.73%)	
>5 times per week	74 (0.63%)	21 (0.72%)	53 (0.60%)	
No	10,901 (92.37%)	2,727 (94.07%)	8,174 (91.81%)	
Tea drinking				<0.001
1–2 times per week	1,699 (14.40%)	350 (12.07%)	1,349 (15.15%)	
3–5 times per week	925 (7.84%)	186 (6.42%)	739 (8.30%)	
>5 times per week	5,147 (43.61%)	1,320 (45.53%)	3,827 (42.99%)	
No	4,031 (34.16%)	1,043 (35.98%)	2,988 (33.56%)	
Comorbidity index				<0.001
0	5,737 (48.53%)	1,256 (43.30%)	4,481 (50.24%)	
1	5,646 (47.76%)	1,500 (51.71%)	4,146 (46.48%)	
2	422 (3.57%)	139 (4.79%)	283 (3.17%)	
3	16 (0.14%)	6 (0.21%)	10 (0.11%)	
DM				0.005
Yes	893 (7.58%)	259 (8.97%)	634 (7.13%)	
Prediabetes	147 (1.25%)	36 (1.25%)	111 (1.25%)	
No	10,739 (91.17%)	2,594 (89.79%)	8,145 (91.62%)	
Physical activity				<0.001
Sufficient	5.877 (49.77%)	1,470 (50.67%)	4,407 (49.48%)	
Not sufficient	1,550 (13.13%)	303 (10.45%)	1,247 (14.00%)	
Inactive	4,381 (37.10%)	1,128 (38.88%)	3,253 (36.52%)	

BMI, body mass index; WHR, waist-to-hip ratio; PHQ-9, the patient health questionnaire-9; GAD-7, generalized anxiety disorder-7; Cr, creatinine; DM, diabetes mellitus.

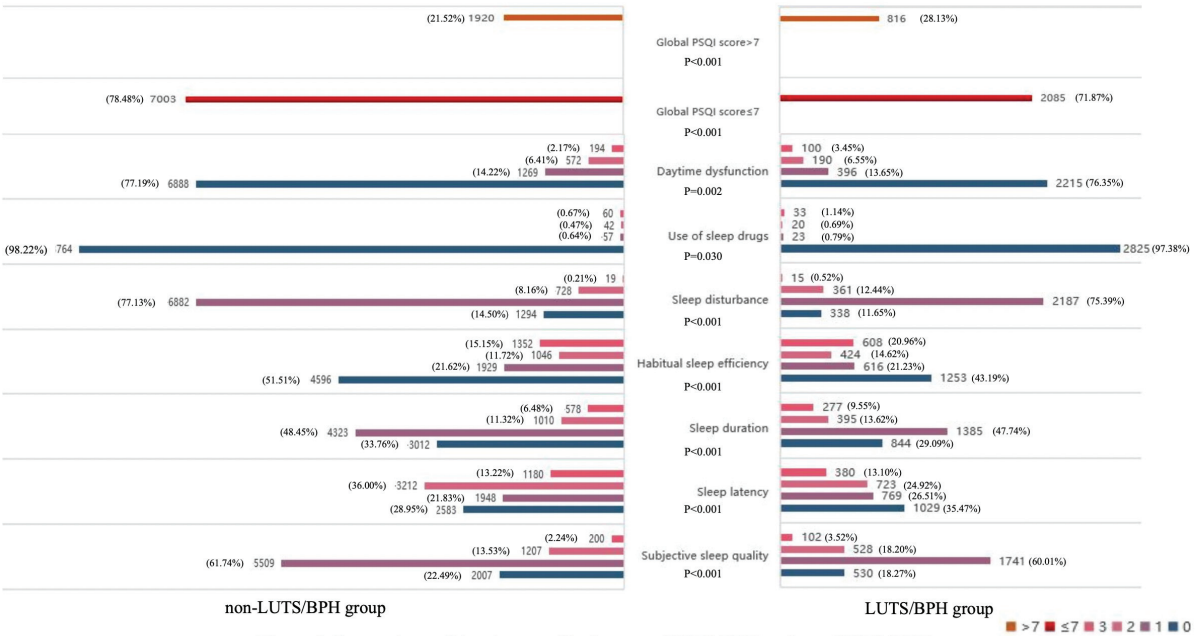

Figure 2 Comparison of the sleep quality between LUTS/BPH and non-LUTS/BPH groups

FIGURE 2
Comparison of the sleep quality between LUTS/BPH and non-LUTS/BPH groups.

TABLE 2 Associations between sleep disorder and LUTS/BPH and daytime LUTS/BPH.

	Non-adjusted OR (95%CI)	*P*-value	Adjusted I OR (95%CI)	*P*-value	Adjusted II OR (95%CI)	*P*-value
N	**11,824**		**11,623**		**9,266**	
LUTS/BPH						
Sleep disorder						
No	1		1		1	
Yes	1.427 (1.298, 1.570)	<0.001	1.372 (1.245, 1.512)	<0.001	1.257 (1.119, 1.411)	<0.001
Global PSQI score	1.043 (1.029, 1.057)	<0.001	1.037 (1.023, 1.051)	<0.001	1.021 (1.004, 1.038)	0.013
Daytime LUTS/BPH						
Sleep disorder						
No	1		1		1	
Yes	1.605 (1.458, 1.767)	<0.001	1.534 (1.390, 1.691)	<0.001	1.343 (1.193, 1.512)	<0.001
Global PSQI score	1.072 (1.058, 1.086)	<0.001	1.064 (1.050, 1.078)	<0.001	1.044 (1.026, 1.061)	<0.001

Non-adjusted: no covariates were adjusted.
Adjusted I: adjusted for age.
Adjusted II: adjusted for age, BMI, educational level, marital status, smoking status, drinking status, coffee intake, tea intake, WHR, PHQ-9, GAD-7, comorbidity index, DM, physical activity, Cr.

health questionnaire-9 (PHQ-9), generalized anxiety disorder-7 (GAD-7) and creatinine (Cr μmol/L). For categorical covariates including education level (primary school, junior school, high school college, or graduate), marital status (married, unmarried, divorced, or widowed), smoking status (current, occasionally, ever or never), drinking status (yes, ever or no), coffee and tea intake (1–2 times/week, 3–5 times/week or >5 times/week), comorbidity index, diabetes mellitus (DM) (yes, prediabetes or no) and physical activity (sufficient, not sufficient or inactive). Diabetes mellitus, congestive heart failure,

coronary artery disease, chronic obstructive pulmonary disease (chronic bronchitis and/or emphysema) and hypertension, cancer consisted of comorbid conditions. The number of subjects reported conditions were then combined to generate an ordinal comorbidity index. Exclusive diabetes was excluded and the total number of reported diseases was merged to create a sequential comorbidity index (Fantus et al., 2018). Individuals with a PHQ-9 score $\geq$ 10 are considered to have depressive symptoms (Kroenke et al., 2001). Calculate the average intake of coffee or tea in the dietary interview

TABLE 3 Regression analysis between PSQI components and LUTS/BPH regression analysis.

	Non-adjusted OR (95%CI)	*P*-value	Adjusted I OR (95%CI)	*P*-value	Adjusted II OR (95%CI)	*P*-value	*P* for trend
PSQI domain scores							
Subjective sleep quality							<0.001
0	1		1		1		
1	1.197 (1.072, 1.336)	0.001	1.163 (1.040, 1.300)	0.008	1.130 (0.995, 1.284)	0.060	
2	1.657 (1.440, 1.906)	<0.001	1.635 (1.418, 1.886)	<0.001	1.535 (1.299, 1.814)	<0.001	
3	1.931 (1.494, 2.497)	<0.001	1.800 (1.384, 2.342)	<0.001	1.376 (1.004, 1.886)	0.048	
Sleep latency							<0.001
0	1		1		1		
1	0.991 (0.887, 1.107)	0.872	1.000 (0.894, 1.118)	0.994	0.893 (0.783, 1.018)	0.089	
2	0.565 (0.507, 0.630)	<0.001	0.603 (0.540, 0.673)	<0.001	0.500 (0.439, 0.569)	<0.001	
3	0.808 (0.705, 0.926)	0.002	0.833 (0.726, 0.957)	0.010	0.656 (0.557, 0.773)	<0.001	
Sleep duration							<0.001
0	1		1		1		
1	1.143 (1.037, 1.260)	0.007	1.137 (1.030, 1.255)	0.011	1.120 (0.999, 1.255)	0.052	
2	1.396 (1.214, 1.604)	<0.001	1.276 (1.107, 1.470)	0.001	1.218 (1.035, 1.432)	0.017	
3	1.710 (1.454, 2.012)	<0.001	1.537 (1.303, 1.814)	<0.001	1.441 (1.189, 1.745)	<0.001	
Habitual sleep efficiency							<0.001
0	1		1		1		
1	1.171 (1.049, 1.308)	0.005	1.099 (0.982, 1.230)	0.099	1.135 (0.999, 1.289)	0.051	
2	1.487 (1.307, 1.692)	<0.001	1.362 (1.194, 1.554)	<0.001	1.306 (1.123, 1.520)	0.001	
3	1.650 (1.471, 1.849)	<0.001	1.451 (1.289, 1.632)	<0.001	1.369 (1.193, 1.570)	<0.001	
Sleep disturbance							0.002
0	1		1		1		
1	1.217 (1.069, 1.384)	0.003	1.104 (0.967, 1.259)	0.143	1.022 (0.881, 1.184)	0.777	
2	1.898 (1.595, 2.259)	<0.001	1.686 (1.411, 2.013)	<0.001	1.354 (1.102, 1.664)	0.004	
3	3.022 (1.520, 6.011)	0.002	2.568 (1.283, 5.141)	0.008	2.539 (1.169, 5.518)	0.019	
Use of sleep drugs							0.086
0	1		1		1		
1	1.252 (0.770, 2.035)	0.365	1.216 (0.745, 1.985)	0.435	1.152 (0.648, 2.050)	0.629	

(Continued)

TABLE 3 (Continued)

	Non-adjusted OR (95%CI)	*P*-value	Adjusted I OR (95%CI)	*P*-value	Adjusted II OR (95%CI)	*P*-value	*P* for trend
2	1.477 (0.866, 2.520)	0.152	1.347 (0.784, 2.313)	0.281	1.149 (0.596, 2.218)	0.678	
3	1.706 (1.113, 2.615)	0.014	1.497 (0.972, 2.306)	0.067	1.517 (0.927, 2.481)	0.097	
Daytime dysfunction							<0.001
0	1		1		1		
1	0.970 (0.858, 1.097)	0.631	1.111 (0.979, 1.260)	0.103	1.084 (0.935, 1.256)	0.286	
2	1.033 (0.871, 1.226)	0.710	1.196 (1.003, 1.425)	0.046	1.152 (0.942, 1.408)	0.168	
3	1.603 (1.253, 2.050)	<0.001	1.890 (1.469, 2.431)	<0.001	1.702 (1.278, 2.267)	<0.001	

Non-adjusted: no covariates were adjusted.
Adjusted I: adjusted for age.
Adjusted II: adjusted for age, BMI, educational level, marital status, smoking status, drinking status, coffee intake, tea intake, WHR, PHQ-9, GAD-7, comorbidity index, DM, physical activity, Cr.

in a week to indicate the intake of caffeine (times/week, classification).

Statistical analysis

In this study, continuous variables were presented as mean ± standard deviation, while categorical variables were presented as proportions. Chi-square (or Fisher's exact test) analysis was used to assess categorical variables and *t*-test analysis was used to assess continuous variables. Multivariate logistic regression analysis was used to examine the odds ratio (or) and 95% confidence interval (CI) of the risk of LUTS/BPH. The independent variable in this study is the presence or absence of LUTS/BPH status, and sleep quality (PSQI global score ≤ 7 or >7) and seven components of PSQI were used as independent variables. ORs and 95% CI were calculated. Meanwhile, we regard the PSQI global score as a continuous variable and conduct multiple logistic regression again as a sensitivity analysis. The ORs were adjusted for age in the minimum adjustment model (Model I). In the fully-adjusted model (Model II), the ORs is adjusted for age, BMI, educational level, marital status, smoking status, drinking status, coffee intake, tea intake, WHR, PHQ-9, GAD-7, comorbidity index, DM, physical activity and Cr. We repeated the above analysis in daytime LUTS to reduce the impact of nocturia on the results. In order to explore whether this association was modified by other confounding factors, we conducted subgroup analyses. The statistical software packages R[1] (The R Foundation) and EmpowerStats[2] (X&Y Solutions, Inc., Boston, MA, United States) were used in the above statistical analyses. A p-value < 0.05 was considered statistically significant.

1 http://www.R-project.org

2 http://www.empowerstats.com

Results

Study population

A total of 11,824 participants were included in the study. Table 1 details the baseline characteristics of participants. The LUTS/BPH group was older, with higher WHR, lower education level, higher anxiety index, more obvious depression, easier smoking, more tea intake, higher comorbidity index, and inactive outdoor sports. In addition, there were significant differences in creatinine concentration, marital status, drinking, and coffee intake in the group with LUTS/BPH ($P < 0.005$).

Association between sleep quality and lower urinary tract symptoms/benign prostatic hyperplasia

We compared the distribution of sleep quality between the two groups with and without LUTS/BPH (Figure 2). In the LUTS/BPH group with a global PSQI score higher than 7, and this group showed higher score, which means worse sleep quality in six aspects: objective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance and daytime dysfunction, which were more obvious than those without LUTS/BPH. Among them, the difference of habitual sleep efficiency between the two groups was the most obvious. The above results have significant statistical significance ($P < 0.005$).

TABLE 4 Stratified multiple logistic regression analysis to identify variables that modify the correlation between sleep quality and LUTS/BPH.

Subgroup		Non-adjusted OR (95%CI)	*P*-value	Adjusted I OR (95%CI)	*P*-value	Adjusted II OR (95%CI)	*P*-value
Age-young	Global PSQI score	1.007 (0.976, 1.039)	0.651	0.999 (0.967, 1.032)	0.946	0.996 (0.955, 1.039)	0.863
	Sleep disorder						
	No	1		1		1	
	Yes	1.198 (0.956, 1.500)	0.116	1.166 (0.927, 1.468)	0.189	1.161 (0.876, 1.539)	0.300
Age-middle	Global PSQI score	1.014 (0.988, 1.040)	0.290	1.010 (0.984, 1.036)	0.457	1.005 (0.974, 1.037)	0.778
	Sleep disorder						
	No	1		1		1	
	Yes	1.361 (1.133, 1.635)	<0.001	1.334 (1.108, 1.605)	0.002	1.277 (1.026, 1.589)	0.029
Age-older	Global PSQI score	1.061 (1.042, 1.080)	<0.001	1.061 (1.042, 1.080)	<0.001	1.036 (1.013, 1.059)	0.002
	Sleep disorder						
	No	1		1		1	
	Yes	1.465 (1.282, 1.675)	<0.001	1.456 (1.272, 1.668)	<0.001	1.277 (1.088, 1.499)	0.003
Total	PSQI	1.038 (1.024, 1.052)	<0.001	1.035 (1.022, 1.049)	<0.001	1.022 (1.005, 1.039)	0.009
	PSQI domain scores						
	≤7	1		1		1	
	>7	1.381 (1.253, 1.522)	<0.001	1.362 (1.234, 1.503)	<0.001	1.264 (1.126, 1.419)	<0.001
P interaction		0.002		<0.001		0.005	

Age-young: aged ≤ 51.
Age-middle: 51 < aged < 61.
Age-older: aged ≥ 61.
Non-adjusted: no covariates were adjusted.
Adjusted I: adjusted for age.
Adjusted II: adjusted for age, BMI, educational level, marital status, smoking status, drinking status, coffee intake, tea intake, WHR, PHQ-9, GAD-7, comorbidity index, DM, physical activity, Cr.

In addition, it was found that sleep disorders were associated with an increased prevalence of LUTS/BPH. As a sensitivity analysis, we excluded daytime LUTS/BPH analysis after nocturia, similarly, sleep disorders were positively correlated with increased prevalence of daytime LUTS/BPH (Table 2), further enhancing the robustness of our study.

The results of logistic regression analyses of the correlation between global PSQI and its seven components and LUTS/BPH prevalence among participants were shown in Table 3. In the crude model, the global PSQI score (≤7 or >7) was positively correlated with the prevalence of LUTS/BPH. After adjusting for confounding variables, the global PSQI score (≤7 or >7) was still significantly positively correlated with the prevalence of LUTS/BPH (OR: 1.021; 95% CI: 1.004–1.038; $P = 0.013$). Similar results can be observed in the seven components of PSQI. We found that among the six components other than use of sleep drugs, the higher PSQI components score, the more significant positive correlation between sleep quality and the risk of LUTS/BPH ($P < 0.05$).

Subgroup analysis stratified by the age of participants also found a positive correlation between sleep quality and the prevalence of LUTS/BPH (Table 4). We observed no statistically significant association of sleep quality and LUTS/BPH among young-aged male participants. On the other hand, among the middle and older-aged male participants, higher PSQI score (>7) were significantly associated with the increased prevalence of LUTS/BPH in all models (51 ≤ middle-aged ≤ 61: OR: 1.277; 95%Cl: 1.026–1.589; $P = 0.029$) (older-aged > 61: OR: 1.277; 95%Cl: 1.088–1.499; $P = 0.003$) (Figure 3). Meanwhile, we observed a significant interaction between sleep quality and LUTS/BPH in the correlation of age in the three models (p for interaction = 0.005). In addition, we further carried out subgroup analysis (Figure 4). The results of subgroup analysis showed that our findings were significant in most subgroups ($P < 0.05$), reflecting the stability of the results. Only in the age < 52 group and BMI > 28 group, we did not observe significant results.

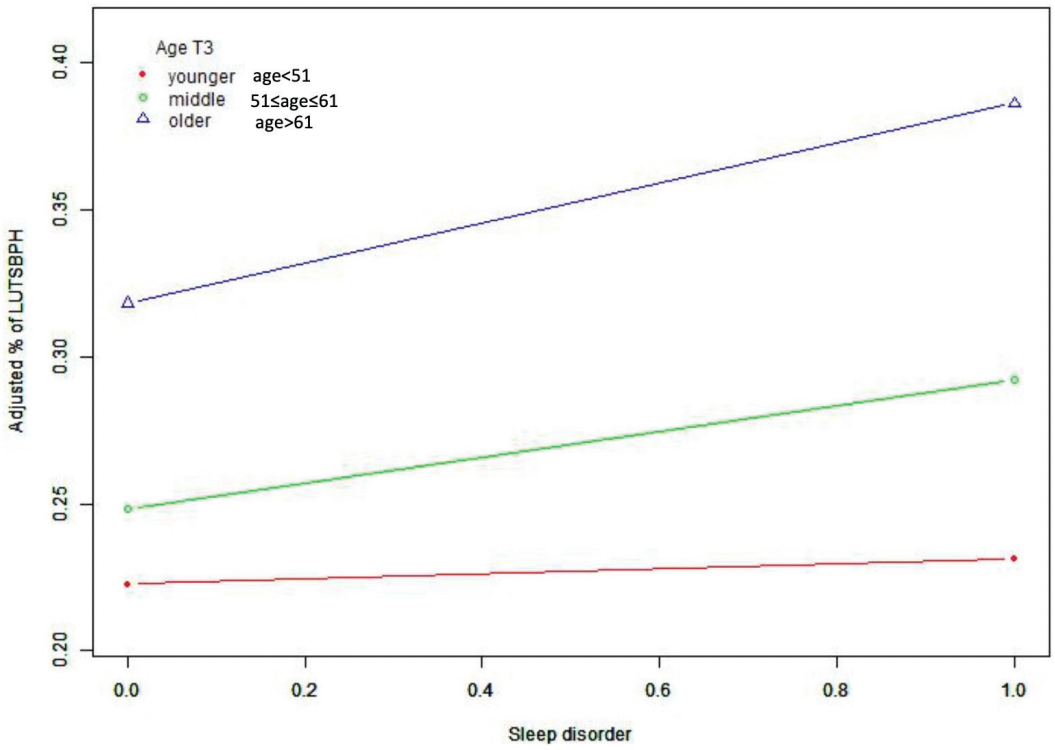

FIGURE 3
Smooth curve fitting of sleep disorder and risk of LUTS/BPH, grouped by age.

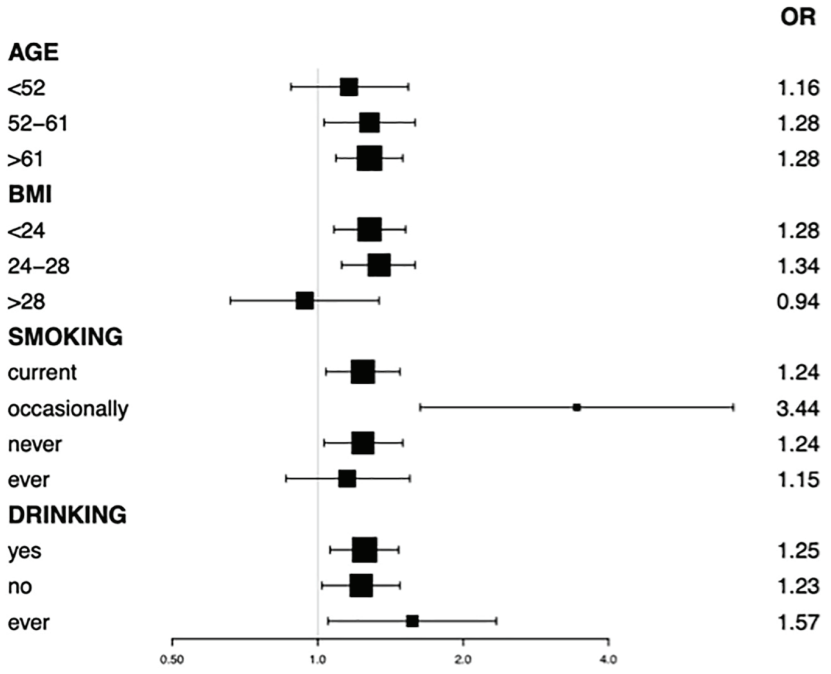

FIGURE 4
Subgroup analysis by age, BMI, smoking, drinking status on association between PSQI with LUTS/BPH.

Discussion

With the continuous development of population aging trend, it is estimated that by 2050, the population over 65 years old will reach 400 million (accounting for 26.9% of the total population), and the population over 80 years old can grow to 150 million (Fang et al., 2015). More evidence shows that LUTS/BPH is the manifestation of urinary system caused by multiple systems (Fantus et al., 2018). The purpose of this study was to further clarify the correlation between sleep disorders and LUTS/BPH in Chinese males. The data come from a large natural population cohort represented by people in Southwest China. This study showed that poor sleep quality was significantly associated with the prevalence of LUTS/BPH in men. In the subgroup analysis, the fully adjusted model results showed that the significant association between poor sleep quality and the prevalence of LUTS/BPH mainly existed in male participants aged ≥ 51. There was a significant interaction between age and sleep quality and the prevalence of LUTS/BPH.

The results of a prospective cohort study conducted by Araujo et al. (2014) showed that the correlation between sleep disorders and urinary symptoms is bi-directional. Similar results were also observed in the study of Scovell et al. (2017), which also found that men with sleep difficulties, difficulty in maintaining sleep and difficulty in returning to sleep reported more serious LUTS than men without similar sleep difficulties. The results of the above articles show that sleep problems are associated to male LUTS/BPH, which is consistent with the results of this study.

There are several hypotheses about the biological basis of sleep and its correlation with LUTS/BPH. One theory involves the role of circadian rhythm in hormone metabolism. Sleep structure is usually affected by the aging process: it becomes more and more fragmented due to the arousals and episodes of awakening, which may lead to poor sleep maintenance and consolidation, as well as the reduction of the overall amount of sleep (Kryger et al., 2004). Now emerging evidence shows that similar partial sleep deprivation patterns can affect many aspects of mammalian endocrine system, including the decline of some anabolic hormones (Everson and Crowley, 2004). Age related sleep fragmentation may disrupt androgen secretion in older men (Luboshitzky et al., 2001). Copinschi and Caufriez (2013) found that circadian rhythm of circulating testosterone seems to be mainly driven by the sleep wake cycle. In fact, daytime and nighttime sleep are associated with strong increases in circulating testosterone levels. However, although it is weakened compared with night sleep, testosterone levels continue to rise when awake at night. Nocturnal sleep time has been shown to be an independent predictor of morning total and free testosterone in the elderly. Therefore, with the duration and length of sleep time, it has a great impact on the fluctuation of hormone level in the body, especially testosterone, so LUTS/BPH dependent on androgen level is more likely to occur. Related studies put forward a possibility that the change of sleep may be one of the mechanisms that transform the physiological aging process of the elderly into the change of neuroendocrine function (Penev, 2007). In addition, reduced sleep duration can cause the disorders of circadian clock genes, such as *Per 2*, *Per 3* (Goel et al., 2013). Meanwhile, in the research results reported by Li et al. (2019), the decreased expression of *Per 2* can inhibit cell apoptosis, leading to BPH. Therefore, good sleep is of great significance to maintain the stability of the circadian rhythm.

Another theory suggested that autonomic nerve activity may be one of the causes of LUTS/BPH in men. Roehrborn (2008) found that autonomic nervous system activity, plasma and urinary catecholamines were positively correlated with symptom scores and other BPH measurements. Meanwhile, previous studies by Spiegel et al. (1999) showed that the activity of sympathetic nervous system increased under the condition of sleep debt ($p < 0.02$). Thus, sleep disorder may affect the pathological changes of prostate by promoting the activity of sympathetic nerve, so as to cause the occurrence of LUTS. In addition, the latest research results suggested that prostatic hyperplasia and pathological progression are related to the level of inflammation, and sleep disorders can increase the level of inflammatory factors in the body (Navaneethakannan et al., 2022).

Subgroup analysis showed that the association between increased sleep disorder incidence and increased prevalence of LUTS/BPH was much more common among older male participants. Poor sleep has many negative effects on quality of life and increases the risk of other comorbidity such as depression, obesity, type 2 diabetes, cardiovascular disease, which may have a negative impact on mood, subjective well-being and overall function (Araujo et al., 2014; Scovell et al., 2017). For the elderly, the risk of comorbidity caused by sleep disorders will increase, which is more likely to lead to the occurrence of LUTS/BPH.

It should be noted that there are several limitations of this study. First, due to the cross-sectional nature of this study, the causal association between sleep quality and LUTS/BPH may not be clarified, and further prospective and intervention studies may provide a better explanation. Secondly, although we adjusted for potential confounding factors, we still can not rule out the impact of other unmeasured correlation factors on the results. What is more, because the diagnostic information of LUTS/BPH is self-reported, there is a risk of bias. Finally, there may be recall bias for some variables (such as smoking, drinking, exercise variables, etc.).

Conclusion

Our findings suggested that sleep disorders are associated with an increased prevalence of LUTS/BPH. Moreover, the significant interaction between age and sleep disorders showed

that older people are more likely to develop LUTS/BPH due to sleep disorders. Our study provides data support for the possible future proposal to prevent LUTS/BPH by improving sleep disorders. Their potential biological mechanisms need to be further studied.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Biomedical Ethics Review Committee of West China Hospital of Sichuan University. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

YL contributed to the design of the study, was responsible for data processing and analysis, and drafted the initial manuscript. SQ, XZ, and KJ decided the methods of data analysis. LC, ZJ, DH, and MW were responsible for determining whether the data should be included in the discharge criteria. SW and BC polished the manuscript. QW, LY, and LM conceptualized and designed the study, supervised all aspects of the study, critically reviewed and revised the manuscript, and approved the final manuscript submitted. All authors read and approved the final manuscript.

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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.

Publisher's note

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Comparative efficacy and acceptability of treatments for depressive symptoms in cognitive impairment: A systematic review and Bayesian network meta-analysis

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Background: Depressive symptoms play an essential role in cognition decline, while the benefit and acceptability of treatments for depressive symptoms in cognitive impairment are still unknown.

Objective: To comprehensively evaluate the comparative efficacy and acceptability of treatments for depressive symptoms in cognitive impairment based on the quantitative Bayesian network meta-analysis method (NMA).

Method: We searched MEDLINE, Embase, the Cochrane Library, CINAHL, and PsycINFO from inception until August 2022 to identify randomized clinical trials (RCTs) evaluating treatments for depressive symptoms in cognitive impairment. Efficacy was evaluated by the Cornell Scale for Depression in Dementia (CSDD), the Hamilton Depression Rating Scale (HDRS), and the Geriatric Depression Scale (GDS) for depression; the Neuropsychiatric Inventory (NPI) and the Cohen–Mansfeld Agitation Inventory (CMAI) for behavior; and the Mini-Mental State Examination (MMSE) for cognition. Safety was evaluated by total adverse events (AEs), serious AEs, diarrhea, headache, and nausea.

Results: In this study, 13,043 participants from 107 RCTs were included, involving 28 treatments and the discontinuation of antidepressants. On CSDD, aerobic exercise (MD −4.51, 95%CrI −8.60 to −0.37), aripiprazole (MD −1.85, 95%CrI −3.66 to −0.02), behavioral training (MD −1.14, 95%CrI −2.04 to −0.34), electrical current stimulation (MD −3.30, 95%CrI −5.94 to −0.73), massage (MD −12.67, 95%CrI −14.71 to −10.59), music therapy (MD −2.63, 95%CrI −4.72 to −0.58), and reminiscence therapy (MD −2.34, 95%CrI −3.51 to −1.25) significantly outperformed the placebo. On MMSE, cognitive stimulation therapy (MD 1.42, 95%CrI 0.49 to 2.39), electrical current stimulation (MD 4.08, 95%CrI 1.07 to 7.11), and reminiscence therapy (MD 1.31, 95%CrI 0.04 to 2.91) significantly outperformed the placebo. Additionally, no treatments showed a significantly higher risk than the placebo.

Conclusion: Our NMAs indicated that non-pharmacological interventions were more efficacious and safe than pharmacological treatments for reducing depressive symptoms as well as improving cognitive impairment. Electrical current stimulation, aerobic exercise, and reminiscence therapy could be first recommended considering their beneficial performance on both depression and cognition. Hence, non-pharmacological treatments deserve more attention and extensive application and should at least be considered as an alternative or assistance in clinical settings.

Systematic review registration: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021239621, identifier: CRD42021239621.

KEYWORDS

depressive symptoms, cognitive impairment, network meta-analysis, non-pharmacological (NON-Mesh), pharmacological

Introduction

Dementia is a common debilitating disorder affecting an estimated population of 55 million worldwide, and there are nearly 10 million new cases every year (World Health Organization, 2021). Apart from cognition decline, depressive symptoms are closely associated with faster progression of the disease and a huge burden on caregivers (Diniz et al., 2013; Vaughan et al., 2015), affecting up to 63% of the patients with cognitive impairment (Solfrizzi et al., 2007).

Depressive symptoms play an essential role in the occurrence and prognosis of cognition impairment. First, it may be an independent risk factor for cognitive impairment (Bennett and Thomas, 2014), which means that the risk of developing dementia is two-fold in elderly people with a history of depression (Saczynski et al., 2010; Byers and Yaffe, 2011). Second, depressive symptoms can accelerate the deterioration of cognitive impairment and behavioral disturbance, resulting in increased morbidity and mortality (Rapp et al., 2011a,b). Third, viewed as a prodrome of dementia, it could be a reaction or a psychological response to the disease (Kessing, 2012; Bennett and Thomas, 2014; Baruch et al., 2019). Moreover, depressed patients with cognitive impairment are more likely to experience recurrent depression compared to those with simple depression (Hall and Reynolds-Iii, 2014). Hence, alleviation of depressive symptoms is of paramount importance to delay the course of the disease and improve the quality of life of patients.

Pharmacological approaches for depression remain the mainstay of treating depressive symptoms in cognitive impairment (Kessing et al., 2007), though they may not be necessarily effective and tolerable (Bingham et al., 2019). Recent reviews provided conflicting results on the benefits of antidepressants and even claimed minimal or no effect on depression symptoms, cognitive function, or activities of daily living (Orgeta et al., 2017; Dudas et al., 2018). Some also indicated that patients on antidepressants were more likely to suffer from side effects (Farina et al., 2017; Dudas et al., 2018; Baruch et al., 2019). Meanwhile, a few meta-analyses claimed the beneficial effects of non-pharmacological treatments for depressive symptoms in cognitive impairment, though most of them only focused on limited interventions and were of unsatisfactory quality (Woods et al., 2018; Bennett et al., 2019; Li H. C. et al., 2019; Li X. et al., 2019; Zafra-Tanaka et al., 2019). There is still a lack of comprehensive assessment of both pharmacological and non-pharmacological treatments for depressive symptoms in cognitive impairment.

The traditional pairwise meta-analysis method can only compare two interventions at a time utilizing direct evidence, which would provide limited insights when there are no head-to-head clinical trials. Given the complexity of this targeted issue, network meta-analysis (NMA) is adopted to face this challenge, as it is capable of fully utilizing both direct and indirect evidence and presenting a comparative hierarchy of efficacy and acceptability. As a powerful and reliable method, NMA has been widely applied to explore the potential evidence (Mutz et al., 2019; Zhou et al., 2020).

Hence, we conducted this systematic review and NMA to evaluate all available treatments for depressive symptoms in cognitive impairment, aiming to provide comparative evidence and quantitative hierarchies on both efficacy and acceptability.

Methods

We performed a series of NMAs using the Bayesian model, which strictly conformed to the principles of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) extension statement for reporting

systematic reviews incorporating NMA of health interventions (Supplementary material 1) (Hutton et al., 2015). We registered our work in the PROSPERO database (https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021239621, identifier: CRD42021239621).

Eligibility criteria

Participants

Participants were diagnosed with mild cognitive impairment (MCI) or various types of dementia according to corresponding criteria. According to the ADNI definition, cognitive impairment is found to be consistent with amnestic MCI (Petersen, 2004; Albert et al., 2011). Dementia was defined by the study authors on the basis of diagnostic criteria such as the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) for dementia (American Psychiatric Association, 2013), the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS–ADRDA) criteria for Alzheimer's disease (AD) (McKhann et al., 1984), etc. There were no restrictions on sex, age, ethnicity, nationality, or duration of disease.

Interventions

All available treatments including both pharmacological and non-pharmacological treatments of depressive symptoms in cognitive impairment were carefully considered. Accordingly, we mainly searched several fields of pharmacological and non-pharmacological therapies, such as antidepressants, antipsychotics, N-Methyl-d-aspartate receptor antagonists (NMDA), analgesics, hormones, cognitive stimulation therapy, non-invasive brain stimulation, psychological treatments, multidomain interventions, and so on. Specific potential pharmacological and non-pharmacological treatments that we searched for are listed in Table 1.

Comparators

Placebo, usual care or therapy, and any other corresponding pharmacological or non-pharmacological interventions were eligible.

Outcomes

After a comprehensive investigation of all the scales evaluating symptoms of cognition impairment, we finally selected the Cornell Scale for Depression in Dementia (CSDD) (Alexopoulos et al., 1988), the Hamilton Depression Rating Scale (HDRS) (Endicott et al., 1981), and the Geriatric Depression Scale (GDS) (Yesavage, 1988) to evaluate the alleviation of depressive symptoms; the Neuropsychiatric Inventory (NPI) (Cummings et al., 1994) and the Cohen–Mansfeld Agitation Inventory (CMAI) (Finkel et al., 1992) to appraise the psychiatric condition; and the Mini-Mental State Examination (MMSE) (Folstein et al., 1983) to access the change of cognition impairment. Among the overall adverse events (AEs), we selected the risk of total AEs, diarrhea, headaches, nausea, and severe AEs as secondary outcomes of acceptability because of their highest occurrence. The data that we extracted were the results of the intent-to-treat population using the last observation carried forward method, but some were unavailable.

Information source and literature search

The systematic literature search was performed utilizing databases of MEDLINE, Embase, Cochrane Central Register of Controlled Trials (CENTRAL), Cumulative Index to Nursing and Allied Health (CINAHL), and PsycINFO. The search strategy was characteristically designed for each of the five databases by combining free text, Medical Subject Heading, and EMTREE terms, among others (Supplementary material 2). The search covered English-language articles from inception until August 2022. Each database and registration platform would be retrieved again before completing the NMAs in case of omitting any newly published works. The unpublished studies were retrieved *via* conference proceedings, clinical trial registries, and author contact. Only potential studies for inclusion were scanned carefully from the reference lists of included studies and related reviews.

Data collection and analysis

Study selection

We only included high-quality randomized controlled trials (RCTs) in English that appraised the efficacy or acceptability of any pharmacological or non-pharmacological intervention treating depressive symptoms in cognitive impairment. Following the eligibility criteria elucidated above, the evaluation and screening of articles were performed by two reviewers independently. When there is any controversy after elaborate discussion, a third reviewer then intervened to make the final decision. After deleting the duplicates, they screened the titles and abstracts of the left literature to select the ones that were worthy of being reviewed in full text. Based on such a rigorous and scientific review, the finally included RCTs were identified.

Data extraction and quality

Baseline characteristics of the included studies and potential effect modifiers were widely abstracted, including age, sex constituent ratio of patients, duration of treatment, the

TABLE 1 Potential pharmacological and non-pharmacological interventions.

Pharmaceutical treatments		
1	Selective serotonin reuptake inhibitors	Citalopram, dapoxetine, escitalopram, fluoxetine, fluvoxamine, indalpine, paroxetine, sertraline, vilazodone, zimelidine, venlafaxine, desvenlafaxine, duloxetine, milnacipran, levomilnacipran, sibutramine, bicifadine, etc.
2	Selective serotonin receptor agonists	Triptans, intranasal sumatriptan, almotriptan, eletriptan, frovatriptan, naratriptan, rizatriptan, sumatriptan, zolmitriptan, etc.
3	Tricyclic antidepressants	Amitriptyline, amoxapine, clomipramine, desipramine, dibenzepin, dothiepin, doxepin, imipramine, lofepramine, nortriptyline, opipramol, protriptyline, trimipramine, etc.
4	Serotonin antagonists	Pizotifen, sandomigran, etc.
5	Cholinesterase inhibitors (ChEIs)	Donepezil, galantamine, rivastigmine
6	N-Methyl-d-aspartate receptor antagonist	Memantine
7	Antipsychotics	Aripiprazole, chlorpromazine, clozapine, haloperidol, levomepromazine, perphenazine, prochlorperazine, olanzapine, quetiapine, risperidone, etc
8	Analgesics	Morphine, tramadol, meperidine, acetaminophen, lysine acetylsalicylic acid (L-ASA), etc.
9	Hormone	Progestin, estradiol, norethisterone, estrogen, etc.
10	others	Chinese medicine, lithium, methylphenidate, melatonin, EGb 761 (ginkgo), etc.
Non-pharmaceutical treatments		
1	Cognitive therapy	Mindfulness-based stress reduction, cognitive behavioral therapy, peaceful mind, individualized cognitive rehabilitation, etc.
2	Non-invasive brain stimulation	Transcranial magnetic stimulation (TMS), Transcranial direct current stimulation (tDCS), transcutaneous electrical nerve stimulation, etc.
3	Psychological treatments	Psychological sleep interventions, recognizable psychotherapeutics, reminiscence therapy
4	Physiotherapy approach	Manual therapy, soft-tissue techniques, strength and endurance training, yoga and tai chi, spinal manipulation, massage, oxygen therapy, etc.
5	Exercise	Aerobic-exercise, strength-exercise, etc.
6	Multidomain interventions	Multi-faceted intervention, collaborative care, multimodal rehabilitative intervention, comprehensive home-based care intervention, etc.
7	Others	Occupational therapy, music therapy, supplements, botanicals and diet alteration, mind-body therapy, robot-assisted therapy, animal-assisted therapy, ultrasound guided nerve pulsed radiofrequency, acupuncture, etc.

sample size of trials, the dosage of treatments, baseline scores of MMSE, CSDD, GDS, and NPI, and completed ratio of participants. Then, primary outcomes of efficacy and secondary outcomes of acceptability were extracted, carefully considering the methods of drug delivery, schedules of drug administration, the context of non-pharmacological treatment, etc.

Risk of bias assessment

The risk of bias of included RCTs was strictly evaluated according to the criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions (Higgins et al., 2019), which appraises six aspects including random sequence generation (selection bias), allocation concealment (selection bias), blinding of participants and personnel (performance bias), blinding of outcome assessment (detection bias), incomplete outcome data (attrition bias), selective reporting (reporting bias), and other bias. This evaluation was done by two reviewers independently to assign a level of risk of bias (high risk, unclear risk, and low risk) for each item, and when there was any controversy, a third reviewer would give the final assessment. Overall, RCTs included in our NMAs had a relatively acceptable low risk of biases across different parameters scored. Additionally, the non-pharmacological treatment could not realize the concealment of patients, thus causing minimal bias which is usually allowed.

Outcome measurement

The primary outcomes were described by the changed scores of the scales, which belong to continuous data and were computed as mean difference (MD) and 95% credibility intervals (CrI). The secondary outcomes of acceptability were described by the number of adverse events that occurred, which belong to discontinuous data, and were computed as the hazard ratio (HR) and 95% CrIs.

Data synthesis and statistical analysis

Our systematic review and NMAs were done across all types of dementia and MCI to derive the overall efficacy and acceptability of comprehensive therapies for depressive symptoms in cognitive impairment. Initially, we summarized and examined baseline data of characteristics of the involved RCTs and patients to access the clinical and methodological heterogeneity. Additionally, the geometry of the network of comparisons across trials was connected to make sure each included RCT would be involved in our NMAs. Then, traditional pairwise meta-analyses were done to anticipate the heterogeneity and publication bias among the RCTs before NMAs. The heterogeneity was assessed by I^2 statistic, and the publication bias was judged by funnel plots.

Next, NMAs were conducted within a Bayesian hierarchical model framework to estimate all the included valuable treatments. We adopted the random-effect model in our NMAs because it could be the most appropriate and advisable methodology in consideration of between-study heterogeneities (Mills et al., 2013). Specifically, models were applied using four chains of Markov Chain Monte Carlo estimation running for 100,000 iterations with thinning of 10, and the first 20,000 iterations were discarded as burn-in after visual inspection of the mixing chains. The convergence was estimated by visually examining the iteration plot and the potential scale reduction factor. Overall, the process above was performed in R version 4.0.4.

Additionally, we carefully considered transitivity and similarity, based on which assumption was made by comparing the distribution of studies and baseline characteristics of participants and by examining potential effect modifiers such as age, the timing of exposure, and the risk of bias. Besides, since a large number of treatments may lead to unavailable cases, the common within-network between-study variance ($\tau 2$) across comparisons was presumed for all comparisons in the entire networks. As for consistency, the design-by-treatment interaction model was adapted to examine the consistency of NMAs. If the inconsistency was tracked without identifying any discrepancy to blame, we then appraised the local inconsistency of each network loop using a loop-specific method (Veroniki et al., 2013). Furthermore, additional analyses were done to enhance the scientificity and preciseness of this NMA, such as sensitivity analysis and subgroup analysis.

Results

Literature search and description of studies

The literature search yielded 129,543 potentially relevant records. After the deletion of duplicates and titles, 11,484 abstracts were screened, and 2,832 articles were left for full-text review. Finally, 107 articles were identified referring to the inclusion criteria (Figure 1). Notably, one study fulfilled all criteria but was excluded from the NMA because of the apparent publication bias displayed in funnel plots and recognized by three reviewers (Rodriguez-Mansilla et al., 2015).

Characteristics of the studies

In this study, 13,043 participants from 107 RCTs were included, involving 13 pharmacological treatments, 15 non-pharmacological treatments, and the discontinuation of antidepressants. The weighted network plots of efficacy and safety are described in Figures 2A,B. The characteristics of these RCTs are displayed in Supplementary material 3.

Risk of bias

The risk of bias within each study included in our NMAs was acceptable (Figure 2C), though the blinding of participants and personnel in non-pharmacological interventions was impossible and appeared to cause a high risk. Funnel plots were also employed to evaluate the publication bias, and they were all visualized as being symmetric generally (Supplementary material 4). The heterogeneity described by I^2 was small, and the overall stability of NMAs revealed from sensitivity analysis was quite well, except for a minimal hesitation on MMSE (Supplementary material 5). Therefore, we further did a subgroup analysis of AD to explore the potential effect of diagnosis and gained generally consistent results with the whole group (Supplementary materials 6–8).

Grading the evidence

We used the Grades of Recommendation, Assessment, Development, and Evaluation (GRADE) approach to grade the quality of underlying evidence and the strength of recommendations in this study. Our GRADE judgments focused on six aspects, including within-study bias, reporting bias, indirectness, imprecision, heterogeneity, and incoherence, and finally led to a good confidence rating. Additionally, we performed this evaluation using CINeMA, which is recommended for assessing confidence in the results of a network meta-analysis (Nikolakopoulou et al., 2020) (Supplementary material 9).

Efficacy

Depression

The NMA on CSDD was performed across 20 treatments and the discontinuation of antidepressants, based on 46 RCTs with 5,143 patients. The results showed that seven

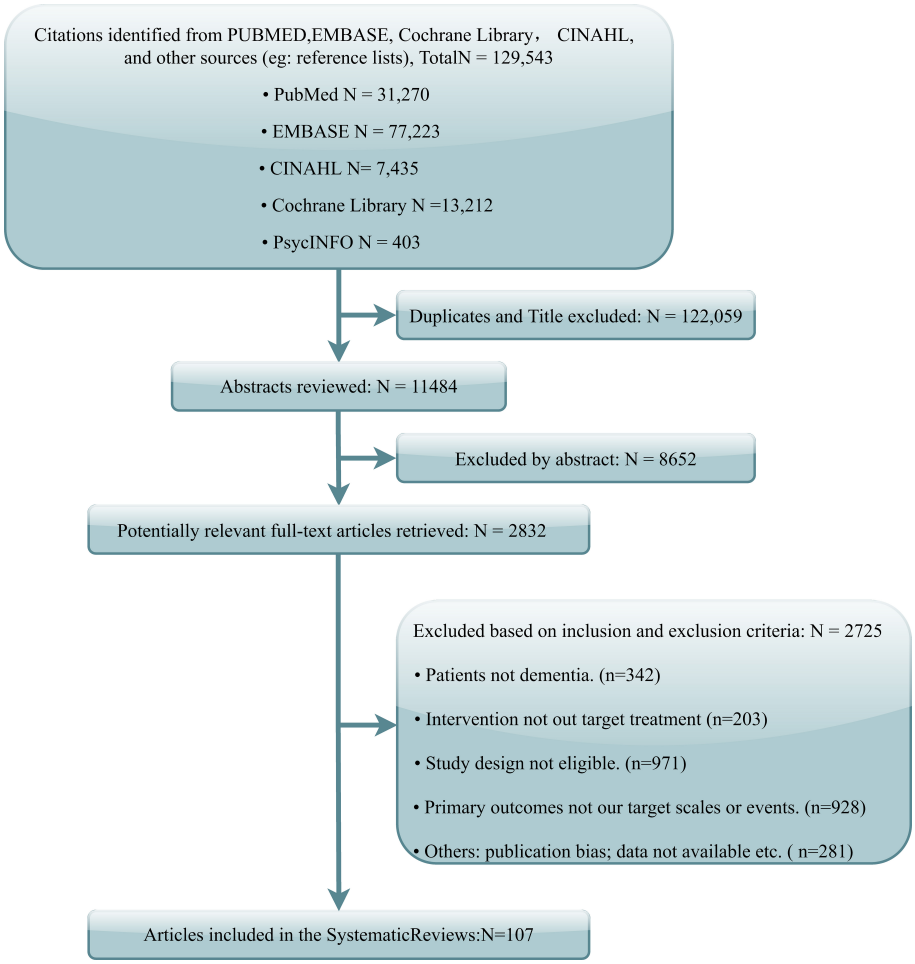

FIGURE 1
A flowchart of the screening process.

treatments showed significant differences superior to placebo including aerobic exercise (MD −4.51, 95%CrI −8.60 to −0.37), aripiprazole (MD −1.86, 95%CrI −3.66 to −0.02), behavioral training (MD −1.14, 95%CrI −2.04 to −0.34), electrical current stimulation (MD −3.30, 95%CrI −5.94 to −0.73), massage (MD −12.67, 95%CrI −14.71 to −10.59), music therapy (MD −2.63, 95%CrI −4.72 to −0.58), and reminiscence therapy (MD −2.34, 95%CrI −3.51 to −1.25). Massage outperformed all the other 21 interventions. Interventions such as aerobic exercise, behavioral training, and electrical current stimulation also demonstrated significant efficacy, while the venlafaxine behaved quite unsatisfactorily. Antidepressants that were discontinued displayed no significant harm. Specific results of efficacy and elaborate rank of the hierarchy are shown in Figure 3A and Supplementary materials 10A, 11A.

The NMA on GDS was performed across 18 treatments based on 31 RCTs with 2,872 patients. The results demonstrated that multidomain interventions (MD −2.93, 95%CrI −5.52 to −0.26), reminiscence therapy (MD −2.20, 95%CrI −4.12 to −0.31), and rivastigmine (MD −4.92, 95%CrI −8.92 to −0.90) significantly outperformed the placebo, and other interventions also demonstrated an effective tendency over placebo, though without significance. Specific results of efficacy and elaborate rank of the hierarchy are shown in Figure 3B and Supplementary materials 10B, 11B.

The NMA on HDRS was performed across 15 treatments based on 19 RCTs with 1,591 patients. The results indicated that behavioral training (MD −4.78, 95%CrI −8.06 to −1.51), cognitive stimulation therapy (MD −5.19, 95%CrI −9.62 to −0.63), music therapy (MD −11.43, 95%CrI −17.66 to −5.12), and sertraline (MD −5.79, 95%CrI −10.73 to −0.89) significantly outperformed the placebo. Music therapy outperformed nine other treatments with significant differences, while amitriptyline performed disappointingly. Specific results of efficacy and elaborate rank of the hierarchy are shown in Figure 3C and Supplementary materials 10C, 11C.

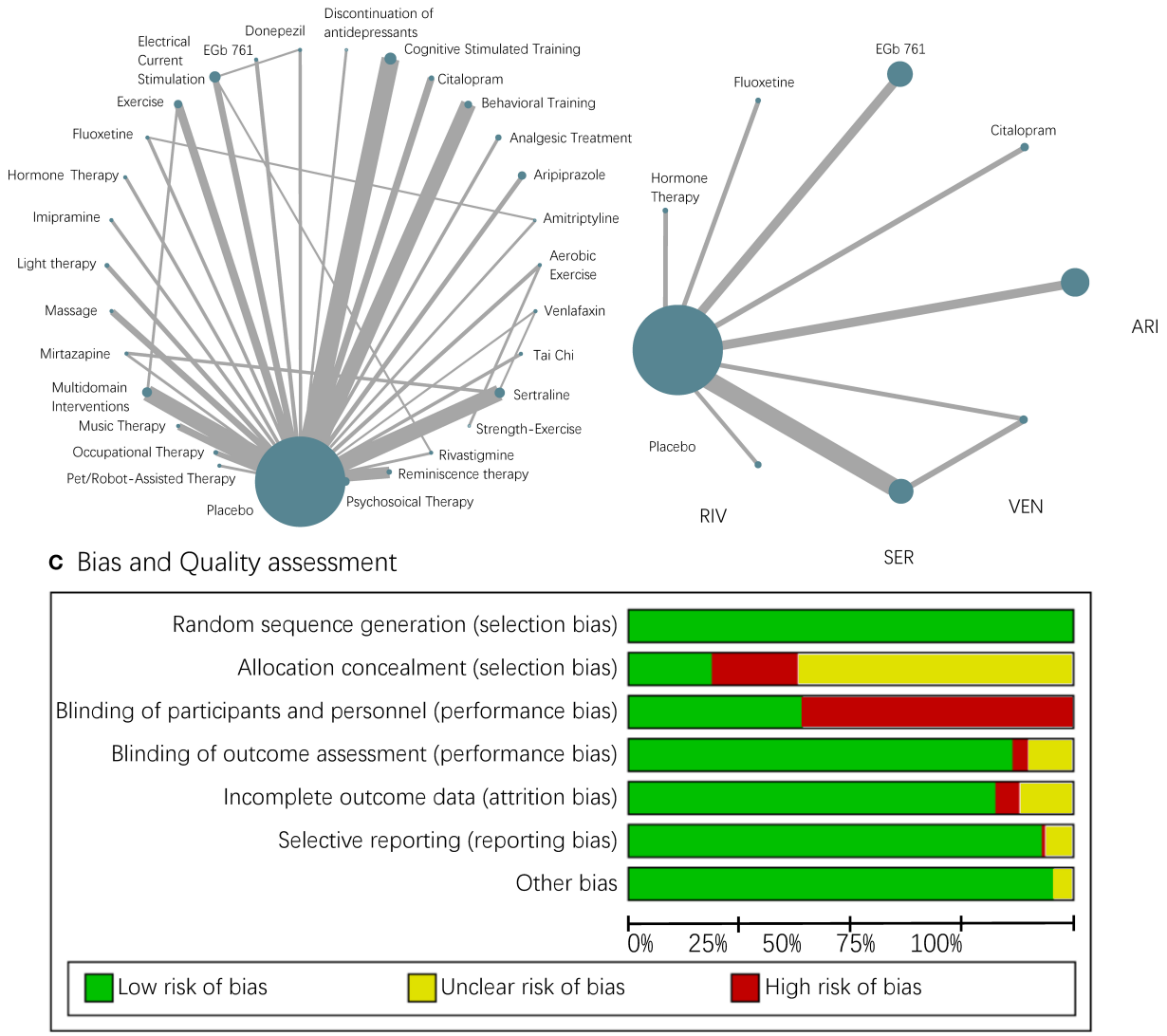

FIGURE 2
A network plot and risk of bias. **(A,B)** Each node in the network geometry represents a kind of intervention. Nodes will be linked by a line when the treatments are directly comparable with "head-to-head trails." The size of the nodes corresponds to the number of participants receiving that intervention, and the width of the lines is proportional to the number of RCTs this comparison included. **(C)** Risk of bias chart of studies included in the quantitative analysis.

Cognition

The NMA on MMSE was performed across 21 treatments based on 53 RCTs with 5,995 patients. The results indicated that cognitive stimulation therapy (MD 1.42, 95%CrI 0.49 to 2.39), electrical current stimulation (MD 4.08, 95%CrI 1.07 to 7.11), and reminiscence therapy (MD 1.31, 95%CrI 0.04 to 2.56) significantly outperformed the placebo. Especially, electrical current stimulation significantly outperformed the other 10 treatments, while sertraline performed inferior to the three treatments. Specific results of efficacy and elaborate rank of the hierarchy are shown in Figure 3D and Supplementary materials 10D, 11D.

Behavior

The NMA on NPI was performed across 17 treatments based on 33 RCTs with 6,524 patients. The results suggested that aerobic exercise (MD −6.69, 95%CrI −9.97 to −0.78), aripiprazole (MD −2.51, 95%CrI −4.78 to −0.61), analgesic treatment (MD −9.68, 95%CrI −14.21 to −5.26), behavioral training (MD −8.03, 95%CrI −10.80 to −4.71), citalopram

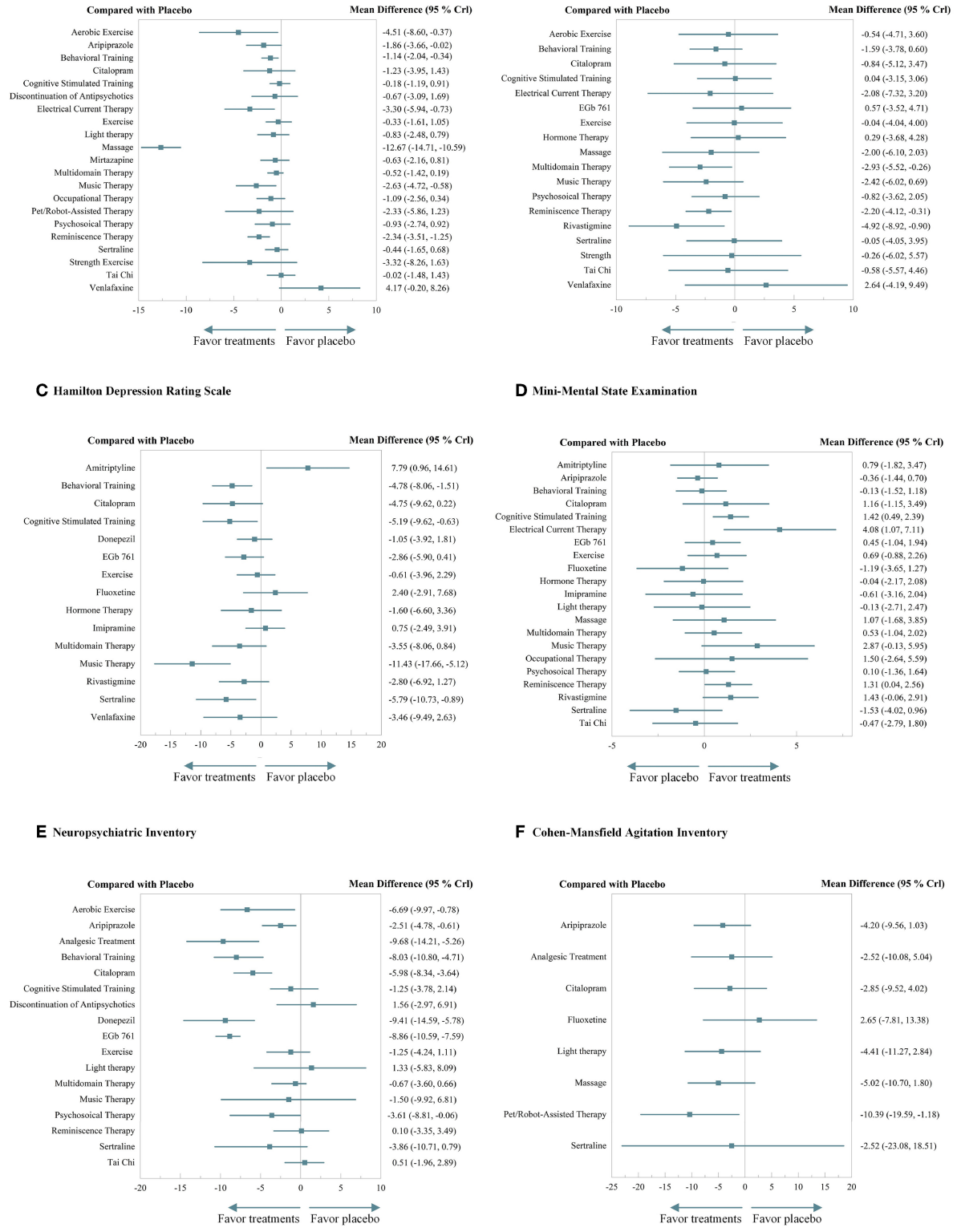

FIGURE 3
A forest plot of efficacy. **(A)** Cornell Scale for Depression. **(B)** Geriatric Depression Scale. **(C)** Hamilton Depression Rating Scale. **(D)** Mini-Mental State Examination. **(E)** Neuropsychiatric Inventory. **(F)** Cohen–Mansfeld Agitation Inventory.

(MD −5.98, 95%CrI −8.34 to −3.64), donepezil (MD −9.41, 95%CrI −14.59 to −5.78), EGb761 (MD −8.86, 95%CrI −10.59 to −7.59), and psychosocial therapy (MD −3.61, 95%CrI −8.81 to −0.06) significantly outperformed the placebo. Citalopram significantly outperformed five treatments. Analgesic treatment, behavioral training, and EGb761 also demonstrated beneficial significance over others. Besides, the discontinuation of antidepressants did not show significant harm. Specific results of efficacy and elaborate rank of the hierarchy are shown in Figure 3E and Supplementary materials 10E, 11E.

The NMA on CMAI was performed across 8 treatments based on 11 RCTs with 1,508 patients. The results demonstrated that pet/robot-assisted therapy (MD −10.39, 95%CrI −19.59 to −1.18) significantly outperformed the placebo. Specific results of efficacy and elaborate rank of the hierarchy are shown in Figure 3F and Supplementary materials 10F, 11F.

Acceptability

The reported data on adverse events from the included RCTs were carefully considered based on eight pharmacological treatments. NMAs were conducted over the incidence of total adverse events and the four most common and important adverse events, including headache, nausea, diarrhea, and serious adverse events. Overall, none of the treatments showed any significance compared to the placebo, while most of them had a riskier tendency than the placebo. In terms of total adverse events, severe AEs, and nausea, venlafaxine seemed to be the most likely to cause adverse events. EGB 761 and citalopram performed well usually, with relatively lower risk. Specific results of efficacy and elaborate rank of the hierarchy are shown in Figure 4 and Supplementary materials 12, 13.

Discussion

Our NMAs are based on 107 RCTs, including 13,043 patients with depressive symptoms in cognitive impairment, who were randomly assigned to 13 pharmacological treatments, 15 non-pharmacological treatments, and the discontinuation of antidepressants. We evaluated all available and high-quality treatments for depressive symptoms in cognitive impairment concerning four aspects of depression, cognition, behavior, and acceptability, providing comparative evidence and quantitative hierarchies using the NMA method. Our objective was to quantitatively determine if all the most common 28 treatments helped patients with depressive symptoms in cognitive impairment and if the discontinuation of antidepressants did significantly harm patients.

Overall, our results suggested that non-pharmacological treatments could be beneficial in reducing symptoms of depression and may be even more efficacious than pharmacological ones. On depression, NMA on CSDD revealed that six treatments could provide more benefits than control care or placebo, including aerobic exercise, behavioral training, electrical current stimulation, massage, music therapy, and reminiscence therapy. However, no pharmacological treatments presented any statistical significance superior to a placebo on depression, some of which even had a tendency of aggravation, such as venlafaxine and amitriptyline. On cognition, non-pharmacological interventions such as cognitive stimulation therapy, electrical current stimulation, and reminiscence therapy outperformed the usual care and pharmacological ones. On behavior, non-pharmacological interventions also behaved quite well, especially for the pet/robot-assisted therapy on agitation and aerobic exercise on total neuropsychiatric symptoms. Moreover, NMAs of acceptability revealed that most pharmacological treatments were associated with a riskier tendency of adverse events, indicating potential poor acceptability. Hence, our analysis suggested that non-pharmacological treatments could provide more benefits and cause less risk, which should be applied more widely and at least considered as an alternative in clinical settings.

Specifically, among the non-pharmacological treatments, electrical current stimulation, aerobic exercise, and reminiscence therapy are quite recommended due to their satisfactory performance in both depression and cognition. When considering the behavioral problem, aerobic exercise may be the first choice due to its beneficial performance in all aspects. Although massage demonstrated significant efficacy over usual care, there should be hesitation in interpretation due to the limited data and asymmetric funnel plot. As for the pharmacological treatments, aripiprazole and citalopram may own the most possibility to have some benefits on depression, which also showed significant improvements on NPI and no harm to MMSE. We also noted that the efficacy of pharmacological treatments on the alleviation of neuropsychiatric symptoms according to NMA on NPI should be claimed, which reminds us that there may be a combined treatment when there are severe neuropsychiatric problems. Besides, no significant harm caused by the discontinuation of antidepressants was observed for both depressive and neuropsychiatric symptoms. A clinical treatment strategy should be designed concerning the specific situation of physical and mental health of patients to figure out the most beneficial simple or combined interventions. In addition, we called for more RCTs on combined interventions to help us better assess and design the clinical treatment strategy.

Pursuing the credibility of evidence, the bias of risk accessed by the Cochrane risk of bias tool implies relatively low bias, though some unclear bias may come from the blinding inadequacy due to the methodological shortcoming that non-pharmacological approaches are unable to realize double-blind. No important discrepancies

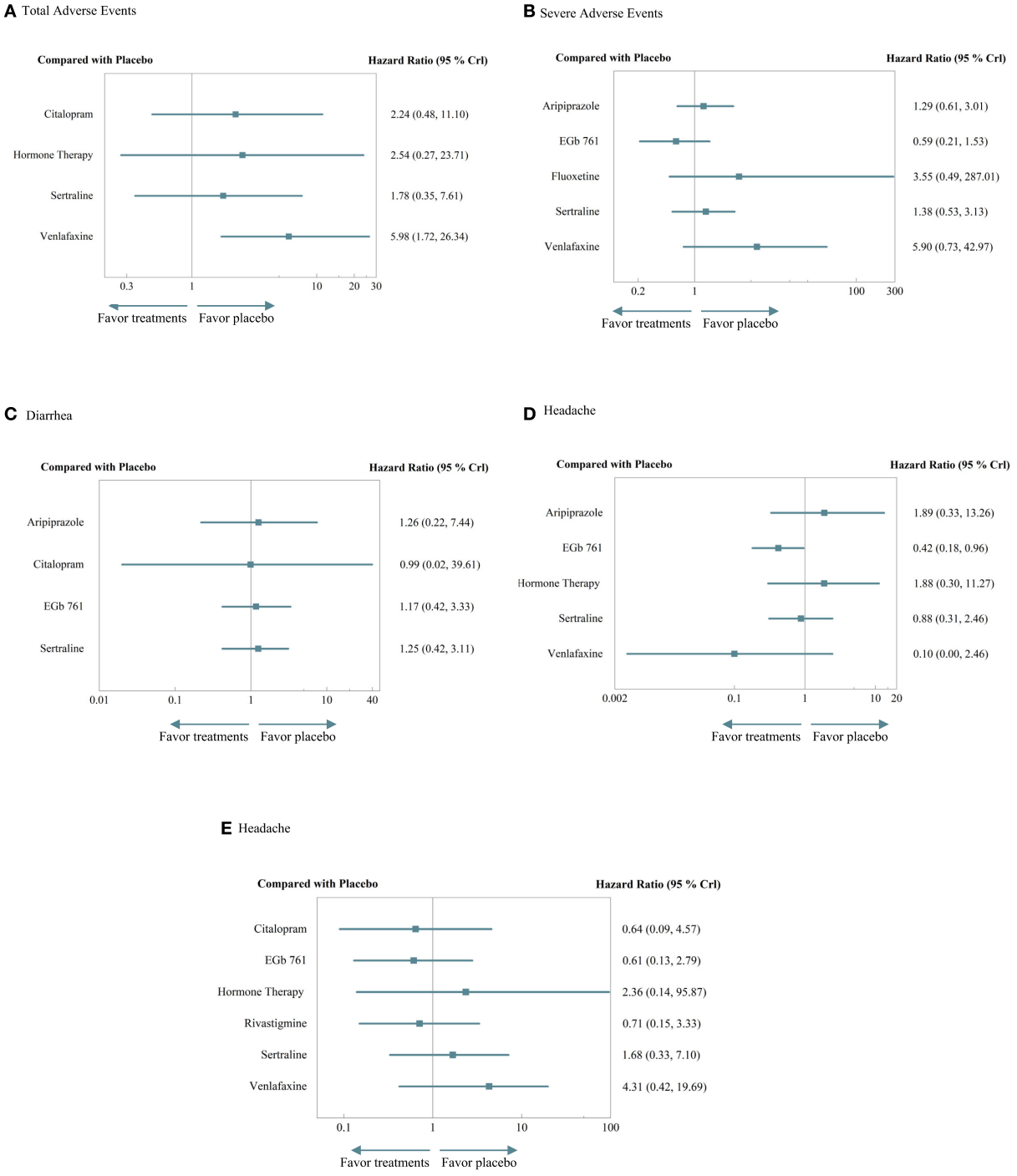

FIGURE 4
A forest plot of acceptability. **(A)** Total adverse events. **(B)** Severe adverse events. **(C)** Diarrhea. **(D)** Headache. **(E)** Nausea.

across the direct comparisons in the distribution of study characteristics were observed after attempting to examine the potential effect modifiers on transitivity. Funnel plots were evaluated visually and believed to be symmetrical after excluding one; thus, publication bias is unlikely with our comprehensive search strategy. Subgroup analysis of AD was done to explore the potential heterogeneity from diagnosis and delivered consistent conclusions. Besides, we appraise the network inconsistency through node-split modeling.

It should be highlighted that we have several strengths. First, as there is a lack of evidence, this study may be the first attempt to quantitatively synthesize the efficacy and safety of treatments for depressive symptoms in cognitive impairment by the NMA method. The included RCTs were concerned with both pharmacological and non-pharmacological approaches, all of which strictly followed the inclusion/exclusion criteria with high quality and low bias. Second, based on the NMA method, not only the head-to-head studies but also the indirect comparisons were comprehensively analyzed, which gave rise to comparative evaluation and derived hierarchies. Third, we performed NMAs on three aspects of efficacy, including depression, cognition, and behavior, utilizing six professional scales, aiming at providing a more specific evaluation and description of the benefits and harms. In addition, acceptability was carefully assessed to rise some attention to clinical prescriptions. Fourth, our conclusions were based on a substantial number of patients and RCTs compared with the previous knowledge syntheses, giving rise to a great guarantee of precision and credibility.

Our analysis also has limitations. First, for some treatments, the paucity of reported RCTs may limit the comprehensiveness and power of this analysis. Second, to maintain the precision of conclusions, the review was restricted to high-quality trials of a single intervention, given that most combined ones were inconsistent and lacked enough data. Third, since the number of patients and comprehensive interventions are quite large, some biases may be inevitable, such as the discrepancies in duration and gender radio, though we have tried our best to appraise and avoid them. Fourth, since different scales may have incompatible abilities in evaluation owing to their intrinsic characteristics, the divergences of their results should be interpreted modestly.

Conclusion

Our NMAs indicated that non-pharmacological interventions were more efficacious and safe than pharmacological treatments for treating depressive symptoms in cognitive impairment. Specifically, electrical current stimulation, aerobic exercise, and reminiscence therapy were quite recommended considering their beneficial performance on both depression and cognition. Additionally, since there were some limitations, we expect to update and revise our NMAs furthermore.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Author contributions

HL, LQ, and BJ developed the concept and design of the study. BJ and BZ conducted systematic literature searches, extracted the data, and rated the risk of bias and methodological quality criteria of included studies, under the supervision of YX. LQ and BJ analyzed and interpreted the data. BJ wrote the first draft of the manuscript. All authors have contributed to the further revision and have approved the final manuscript.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2022.1037414/full#supplementary-material

SUPPLEMENTARY MATERIAL 1
PRISMA NMA checklist.

SUPPLEMENTARY MATERIAL 2
Searching strategies of the current network meta-analysis.

SUPPLEMENTARY MATERIAL 3
Baseline characteristics of the included randomized controlled trails.

SUPPLEMENTARY MATERIAL 4
Comparison-adjusted funnel plot. The red vertical line represents the null hypothesis that independent effect size estimates do not differ from the comparison-specific pooled estimates.

SUPPLEMENTARY MATERIAL 5
Sensitivity and heterogeneity analysis. When the difference in DIC between the random-effect and fixed-effect models is close, the

models could be viewed as stable and reliable. When the I^2% is <28%, we can conclude that the heterogeneity is relatively minor.

SUPPLEMENTARY MATERIAL 6
A subgroup analysis of Alzheimer's disease (forest plot of efficacy).

SUPPLEMENTARY MATERIAL 7
A subgroup analysis of Alzheimer's disease (league tables of efficacy). The diagonal gives the different treatments examined in the network meta-analyses. The efficacy data are given as mean difference (MD) with a 95% credible interval (CrI), in which each cell indicates the values for a specific contrast between the treatments.

SUPPLEMENTARY MATERIAL 8
A subgroup analysis of Alzheimer's disease (relative ranks of efficacy). The derived hierarchies (relative ranks) are described from the most to the least effective and the most effective to the least.

SUPPLEMENTARY MATERIAL 9
Grades of Recommendation, Assessment, Development, and Evaluation (GRADE).

SUPPLEMENTARY MATERIAL 10
League tables of efficacy. The diagonal gives the different treatments examined in the network meta-analyses. The efficacy data are given as mean difference (MD) with a 95% credible interval (CrI), in which each cell indicates the values for a specific contrast between the treatments.

SUPPLEMENTARY MATERIAL 11
Relative ranks of efficacy. The derived hierarchies (relative ranks) are described from the most to the least effective and the most effective to the least.

SUPPLEMENTARY MATERIAL 12
League tables of acceptability. The diagonal gives the different treatments examined in the network meta-analyses. The efficacy data are given as hazard ratio (HR) with a 95% credible interval (CrI), in which each cell indicates the values for a specific contrast between the treatments.

SUPPLEMENTARY MATERIAL 13
Relative ranks of acceptability. The derived hierarchies (relative ranks) are described from the most to the least effective and the most acceptable to the least.

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A three-week in-hospital multidisciplinary body weight reduction program exerts beneficial effects on physical and mental health and fatiguability of elderly patients with obesity

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Introduction: Obesity represents one of the most serious problems of public health affecting elderly populations in an increasingly relevant way. The aim of the current study was to assess the effects of a 3-week in-hospital multidisciplinary body weight reduction program (BWRP) in a sample of elderly patients with obesity on reducing body mass index (BMI), improving fatigue, muscle performance, and psychological well-being.

Methods: Two hundred and thirty-seven consecutive elderly in-patients with obesity (males = 84; females = 153; age range = 65–86 yrs.; mean BMI = 43.7) undergoing a three-week multidisciplinary BWRP participated in the study. Data on BMI, fatiguability (measured with the Fatigue Severity Scale, FSS), muscle performance (evaluated with the Stair Climbing Test, SCT), and psychological well-being (assessed with the Psychological General Well-Being Index, PGWBI) were collected before and after the intervention.

Results: Results showed that BWRP was capable to reduce BMI [$F(1.00, 235.00) = 1226.8$; $p < 0.001$; $\eta^2 = 0.024$], improve perceived fatigue [$F(1,234) = 296.80125$; $p < 0.001$; $\eta^2 = 0.129$], physical performance [$F(1.00,158.00) = 119.26$; $p < 0.001$; $\eta^2 = 0.026$], and enhance psychological well-being [$F(1,235) = 169.0$; $p < 0.001$; $\eta^2 = 0.103$] in both males and females.

Discussion: Although it will be necessary to demonstrate with further longitudinal studies whether the reported beneficial effects will be maintained over time, the effectiveness of a 3-week BWRP on different aspects involved in determining a level of autonomy and good quality of life of elderly obese patients appears to represent a valid attempt to counteract – at least in part – the unavoidable and progressive disability of these patients.

KEYWORDS

body weight reduction program, obesity, elderly patients, fatigue, psychological well-being

1. Introduction

Obesity represents one of the most serious problems of public health affecting over 600 million adults worldwide (Gutiérrez-Fisac and Rodríguez-Artalejo, 2006; World Health Organization, 2006; Davin and Taylor, 2009; Ayensa and Calderon, 2011; Castelnuovo et al., 2015; DerSarkissian et al., 2017). The World Health Organization European Regional Obesity Report 2022 (World Health Organization, 2022) pointed out that 59% of European adults are living with overweight or obesity. In addition, more than one-fifth of adults in 49 out of 53 member states are living with obesity, with levels reaching one-third in several countries. While overweight is higher among males (63%) than females (54%) across the European countries, on the contrary, obesity is more prevalent in females (24%) than males (22%) in about half of the European countries. Importantly, the prevalence of overweight and obesity is still rising with justified concerns about the impact on public health and economies.

Obesity is associated with many medical conditions, such as s type 2 diabetes, cardiovascular diseases, chronic back pain, obstructive sleep apnoea syndrome, obesity hypoventilation syndrome, gallbladder disease, liver diseases, gout, and several common forms of cancer. In addition, individuals with obesity are at risk for many mental conditions, such as depression and anxiety disorders (Wadden et al., 2002; Byrne et al., 2004; Klein et al., 2004; Flegal et al., 2005; Dong et al., 2006; Castelnuovo et al., 2014; Boles et al., 2017).

The condition of obesity is strongly related to many forms of disability. Excess of adiposity negatively influences postural control and reduces functional mobility, causing impairment in stability and walking speed. In addition, obesity is often associated with a higher perception of fatigue, a subjective difficulty to carry out voluntary activities often accompanied by a lack of energy, apathy, and tiredness. As far as psychological health is concerned, many individuals with obesity are more likely to deal with psychological issues, such as depression, anxiety, low self-esteem, and reduced quality of life. Several studies found that people suffering from obesity were almost five times more likely to have experienced an episode of major depression in the past years, as compared with their healthy counterparts (Onyike et al., 2003). In addition, the relationship between obesity and depression seems to be stronger for women than men (Carpenter et al., 2000). People with obesity are also more likely to suffer from anxiety disorders, specifically social anxiety (Kalarchian et al., 2007; Sarwer et al., 2012), and their quality of life is generally impaired. Frequently, individuals with obesity are stigmatized and discriminated, with negative effects on their general life functioning (Friedman et al., 2008).

Obesity is a complex chronic disease, with multifaceted causes and several health consequences. This means that no single intervention alone can flat the constant rise of the obesity epidemic, but rehabilitation programs are required to address the clinical needs of individuals with obesity. Existing clinical guidelines for the treatment and management of obesity recommend comprehensive multidisciplinary and multiprofessional lifestyle interventions for weight loss. Such interventions should consist of nutritional, physical, and psychological components (Giusti et al., 2020).

The potential benefits associated with weight loss in patients with obesity should be not only established but also constantly updated. While the short and long-term effects of BWRP on adult and pediatric populations have been well-established by previous studies performed by our group (Lazzer et al., 2020; Rigamonti et al., 2020a,b) little attention has been paid on to elderly obese people so far (Flegal et al., 2016; Rigamonti et al., 2019).

The aim of the current study, therefore, was to assess the effects of a 3-week in-hospital multidisciplinary body weight reduction program (BWRP) consisting of diet, physical activity, psychological support and nutritional counseling in a sample of elderly people with obesity. Outcomes were BMI, fatigue, muscle performance, and psychological well-being in both sexes, which were compared. Our hypothesis was that BWRP produced improvements in BMI, fatigue, muscle performance, and psychological well-being.

2. Materials and methods

2.1. Participants and procedures

Two hundred and thirty-seven consecutive elderly inpatients with obesity (mean BMI: Kg/m^2: 43.7) aged between 65 and 86 yrs., without any physical or psychological condition that could compromise their participation in the study were recruited for the study. Participants were included if they were Italian, older than 64 yrs., with a BMI greater than 35 (World Health Organization, 2006). Exclusion criteria included any form of physical or psychological impairment that could have compromised the participation in the study. Participants were referred to the Division of Metabolic Diseases, IRCCS Istituto Auxologico Italiano, Piancavallo-Verbania, Italy, a specialized clinical center (i.e., third level) offering a 3-week in-hospital multidisciplinary BWRP. At the admission to the hospital, patients who met the inclusion/exclusion criteria were informed about the study and were asked to provide written informed consent to participate.

Before (baseline, T0) and after 3-week BWRP (post BWRP, T1), participants were asked to fill in a battery of self-report questionnaires used to assess the variables of interest for the study. To calculate BMI weight and height were assessed by the medical team.

All the variables have been measured in the total sample (237), except for the SCT (muscle performance) which has been assessed only in those who had the necessary physical capabilities to perform this test (160). For this reason, 77 patients were excluded for their inability to do SCT. The eventual differences between subgroups (physically healthy to do SCT versus. physically impaired to do SCT) were out of interest for this paper. This is the reason why we analyzed all the other variables in the total sample (237).

The study was approved by the Ethical Committee of Istituto Auxologico Italiano (registration code: 2013_06_27; project code:

18A301) and followed the Helsinki Declaration and its later advancements.

2.1.1. Body weight reduction program

The BWRP lasted 3 weeks. During this period, patients were placed on a hypocaloric diet. The amount of energy to be given with diet was calculated by subtracting approximately 25% from the total energy expenditure, which is obtained by multiplying the estimated daily resting energy expenditure (eREE) by the physical activity level during the BWRP, as previously described (Tamini et al., 2021). REE was estimated using the Mifflin equation (Mifflin, 1990) as follows: eREE = 9.99 × weight (kg) + 6.25 × height (cm) − 4.92 × age (years) + 166 × sex − 161, where sex values are = 1 for males and = 0 for females. In terms of macronutrients, the diet contained 21% proteins, 53% carbohydrates, and 26% lipids. The diet composition was formulated according to the Italian recommended daily allowance (ISo Nutrition, 1996). Each patient was free to choose foods from a heterogeneous daily menu, although five daily servings of fruits and vegetables were mandatory. Foods to which the patient reported allergic reactions were eliminated from the menu. A fluid intake of at least 1.5 l/day was encouraged. In addition, the dietitian team checked that each subject had eaten every meal. On each day of the BWRP, the patients had dietetics classes consisting of lectures, demonstrations, and group discussions with and without a supervisor. They also followed a daily program of adapted physical activity at moderate intensity (1 h) with indoor light jogging, dynamic exercises of the upper and lower limbs, 20–30 min of aerobic activity, postural gymnastics, and walking (3–4 km). Finally, they received psychological counseling based on Cognitive Behavioral Therapy with individual and group sessions provided once a week. The psychological intervention is aimed at helping patients to develop coping strategies and problem-solving skills, enhance self-efficacy, improve stimulus control and foster social activation measures (Giusti et al., 2020).

2.2. Measures

2.2.1. Body mass index

Body Mass Index (BMI) was calculated by dividing the person's weight in kilograms by the height in meters squared according to the proper formula: BMI = (kg/m^2).

2.2.2. Fatigue

The Fatigue Severity Scale (FSS) (Hjollund et al., 2007; Impellizzeri et al., 2013) is one of the most commonly used self-report questionnaires to assess the impact of fatigue on motivation, exercise, physical functioning, carrying out duties, interfering with work, family, or social life. The FSS consists of 9 statements about the impact of fatigue on functioning (e.g., "My motivation is lower when I am fatigued") each rated on a scale from 1 "strongly disagree" to 7 "strongly agree." The total score is obtained by the mean of item scores.

2.2.3. Psychological well-being

The Psychological General Well-Being Index (PGWBI) (Dupuy, 1984; Grossi et al., 2006) is a well-validated questionnaire used in different contexts, including clinical trials and research to assess health-related quality of life (Grossi et al., 2006). It is composed of 22 items rated on a 6-point Likert scale which address six dimensions: anxiety (PGWBI-A), depression (PGWBI-D), positive well-being (PGWBI-PWB), self-control (PGWBI-SC), general health (PGWBI-GH), and vitality (PGWBI-V). The sub-scales scores and the total score (PGWBI-TOT) are obtained by the sum of item scores. Higher total scores indicated greater well-being. Higher scores in Anxiety and Depression subscales indicated less anxiety and depression, while lower scores in those subscales suggest greater anxiety and depression.

2.2.4. Muscle performance

The Stair climbing test (SCT) (Margaria et al., 1966; Sartorio et al., 2001; Lafortuna et al., 2002) was used to measure the maximal anaerobic power muscles. Participants were asked to climb up ordinary stairs (13 steps of 15.3 cm each, with a total vertical distance of 1.99 m) at the highest possible speed, according to their capabilities. An experimenter measured the time employed to cover the test with a digital stopwatch. In line with Margaria assumptions, anaerobic power (in W) was calculated by using the following formula: [(Kg × 9:81 × 1.99) /s] where kg was body mass, 9.81 m/s^2 was the acceleration of gravity, 1.99 m was vertical distance and s was time.

2.3. Statistical analysis

Descriptive statistics were computed to assess the baseline characteristics of the patients participating in the study. To assess changes from pre-to-post intervention in all the study variables, several mixed between within 2 (groups: males versus. females) × 2 (times: baseline versus. post BWRP) repeated measures analysis of variance (ANOVAs) were conducted. Effect size (η^2) was used to quantify the global difference of the two groups across times and was interpreted with the following benchmarks: null ($\eta^2 < 0.003$); small ($0.003 < \eta^2 > 0.003$ to 0.039); moderate ($0.110 < \eta^2 > 0.40$); and large ($\eta^2 > 0.110$; Cohen, 2013). In addition, Spearman's rho correlation between pre–post BWRP difference (Δ %) of FSS (total score) and SCT time (s) have been assessed.

Analyzes were performed using Jamovi (The jamovi project 2021). Jamovi (Version 1.6) [Computer Software] retrieved from https://www.jamovi.org.

3. Results

The baseline characteristics of the sample are shown in Table 1. The sample was composed of 153 (64.6%) females and 84 (35.4%) males. The mean age of males was 69.9 (SD = 4.11) and the mean age of females was 71 (SD = 4.46), these values being not

significantly different. By exploring pre-test conditions, females had a higher BMI ($p < 0.001$), greater levels of FSS ($p = 0.015$), worse SCT ($p < 0.001$), and lower levels of PGWBI-TOT ($p < 0.001$) than males of comparable age.

3.1. Effects of BWRP on BMI

Results showed a significant main effect of time suggesting that BMI significantly decreased from baseline to post BWRP [$F(1.00, 235.00) = 1226.8$; $p < 0.001$; $\eta^2 = 0.024$] independently from sex, and a significant main effect of group [$F(1,235) = 18.0$; $p < 0.001$; $\eta^2 = 0.069$]. This means that there was a significant difference in the BWRP-induced effects on BMI between males and females. The interaction effect of time x group was significant [$F(1.00, 235.00) = 10.9$; $p = 0.001$; $\eta^2 = 0.000$], indicating that the change from baseline to post BWRP was different depending upon the two groups (males versus. females). In particular, independent sample t-tests showed that males reported lower BMI than females both at baseline and post BWRP ($p < 0.001$ respectively). However, the two groups decreased significantly BMI from baseline to post BWRP ($p < 0.001$ respectively).

3.2. Effects of BWRP on fatigue

Results showed that fatigue significantly decreased from baseline to post BWRP [$F(1,234) = 296.80125$; $p < 0.001$; $\eta^2 = 0.129$] independently from sex. In addition, there was a significant difference in the BWRP-induced effects on fatigue between males and females [$F(1,234) = 8.07$; $p = 0.005$; $\eta^2 = 0.026$]. The interaction effect of time x group was not significant [$F(1, 234) = 0.00493$; $p = 0.944$; $\eta^2 = 0.000$].

3.3. Effects of BWRP on psychological well-being

Results showed that psychological general well-being (PGWBI-TOT) significantly decreased from baseline to post BWRP [$F(1,235) = 169.0$; $p < 0.001$; $\eta^2 = 0.103$] independently from sex. In addition, there was a significant difference in the BWRP-induced effects on PGWBI-TOT between males and females [$F(1,235) = 20.04$; $p < 0.001$; $\eta^2 = 0.060$]. The interaction effect of time x group was not significant [$F(1,235) = 11.9$; $p < 0.001$; $\eta^2 = 0.060$].

As far as anxiety (PGWBI-A) is concerned, results showed that anxiety (PGWBI-A) significantly decreased from baseline to post BWRP [$F(1,235) = 104.42$; $p < 0.001$; $\eta^2 = 0.145$] independently from sex. In addition, we found a significant main effect of group [$F(1,235) = 14.8.$; $p < 0.001$; $\eta^2 = 0.060$] suggesting that there was a significant difference in the BWRP-induced effects on PGWBI-A between males and females. The interaction effect of time x group was significant [$F(1,235) = 6.42$; $p = 0.012$; $\eta^2 = 0.009$], indicating that the change from baseline to post BWRP was different depending upon the two groups (males versus. females). In

TABLE 1 Descriptive characteristics of the study group.

	Males (N=84)			Females (N=153)		
Variables	T0	T1	Value of p	T0	T1	Value of p
BMI	42.2 ± (4.05)	40.03 ± (3.91)	<0.001	44.6 ± (5.18)	43.02 ± (5.05)	<0.001
Δ BMI	−1,77 ± (0.74)			−1.46 ± (0.63)		
FSS	39.06 ± (13.5)	29.9 ± (11.5)	<0.001	43.8 ± (12.5)	34.2 ± (10.4)	<0.001
Δ FSS	−9.69 ± (8.71)			−9.61 ± (7.97)		
PGWBI-A	17.9 ± (4.82)	20.5 ± (2.08)	<0.001	15.5 ± (5.17)	19.8 ± (2.37)	<0.001
PGWBI-D	12.0 ± (2.91)	13.0 ± (2.27)	<0.001	10.6 ± (3.26)	12.05 ± (2.50)	<0.001
PGWBI-PWB	11.8 ± (3.84)	13.8 ± (3.43)	<0.001	9.19 ± (3.90)	12.3 ± (3.63)	<0.001
PGWBI-SC	11.4 ± (3.10)	12.5 ± (2.80)	<0.001	9.86 ± (3.28)	11.3 ± (2.97)	<0.001
PGWBI-GH	9.23 ± (3.13)	9.81 ± (2.74)	<0.001	7.29 ± (2.84)	8.73 ± (2.82)	<0.001
PGWBI-V	11.6 ± (4.23)	13.4 ± (3.50)	<0.001	9.29 ± (3.92)	12.4 ± (3.82)	<0.001
PGWBI-TOT	74.1 ± (18.4)	83.0 ± (13.5)	<0.001	61.7 ± (18.9)	77.0 ± (14.0)	<0.001
	Males (N = 64)			Females (N = 96)		
	T0	T1		T0	T1	
SCT	5.88 ± (1.82)	5.25 ± (1.66)	<0.001	8.64 ± (2.83)	7.56 ± (2.26)	<0.001
Δ SCT	−0.625 ± (0.557)			−1.08 ± (1.16)		

T0, baseline; T1, after 3-week BWRP; BMI, Body Mass Index; FSS, Fatigue Severity Scale; SCT, Stair Climbing Test; PGWBI-A, Psychological General Well-Being Index-Anxiety; PGWBI-D, Psychological General Well-Being Index-Depression; PGWBI-PWB, Psychological General Well-Being Index-Positive Well-Being; PGWBI-SC, Psychological General Well-Being Index-Self-Control; PGWBI-GH, Psychological General Well-Being Index-General Health; PGWBI-V, Psychological General Well-Being Index-Vitality; PGWBI-TOT, Psychological General Well-Being Index-Total Score. Data are expressed as means and standard deviations.

particular, independent sample t-tests showed that males reported lower PGWBI-A than females both at baseline ($p < 0.001$), and post BWRP ($p = 0.018$). However, the two groups decreased significantly in PGWBI-A from baseline to post BWRP ($p < 0.001$ respectively).

Results pointed out that depression (PGWBI-D) significantly decreased from baseline to post BWRP [$F(1,235) = 68.69$; $p < 0.001$; $\eta^2 = 0.055$] independently from sex. In addition, there was a significant difference in the BWRP-induced effects on PGWBI-D between males and females [$F(1,235) = 8.30$; $p = 0.004$; $\eta^2 = 0.026$]. The interaction effect of time x group was significant [$F(1,235) = 6.60$; $p = 0.011$; $\eta^2 = 0.005$], indicating that the change from baseline to post BWRP was different depending upon the two groups (males versus. females). In particular, independent sample t-tests showed that males reported lower PGWBI-D than females at baseline ($p < 0.001$), while at post BWRP the difference was not significant ($p = 0.106$). However, the two groups decreased significantly in PGWBI-D from baseline to post BWRP ($p < 0.001$ respectively).

As far as positive well-being (PGWBI-PWB) is concerned, results showed a significant main effect of time suggesting that positive well-being (PGWBI-PWB) significantly increased from baseline to post BWRP [$F(1,235) = 151.82$; $p < 0.001$; $\eta^2 = 0.093$] independently from sex, and a significant main effect of group [$F(1,235) = 20.0$; $p < 0.001$; $\eta^2 = 0.059$]. This means that there was a significant difference in the BWRP-induced effects on PGWBI-PWB between males and females. The interaction effect of time x group was significant [$F(1,235) = 6.39$; $p = 0.012$; $\eta^2 = 0.004$], indicating that the change from baseline to post BWRP was different depending upon the two groups (males versus. females). In particular, independent sample t-tests showed that males reported higher PGWBI-PWB than females both at baseline ($p < 0.001$) and post BWRP ($p = 0.002$). However, the two groups improved significantly in PGWBI-PWB from baseline to post BWRP ($p < 0.001$ respectively).

Self-control (PGWBI-SC) significantly increased from baseline to post BWRP [$F(1,235) = 48.34$; $p < 0.001$; $\eta^2 = 0.036$] independently from sex. In addition, we found a significant difference in the BWRP-induced effects on PGWBI-SC between males and females [$F(1,235) = 13.0$; $p < 0.001$; $\eta^2 = 0.041$]. The interaction effect of time x group was not significant [$F(1,235) = 1.66$; $p = 0.198$; $\eta^2 = 0.001$].

Results about general health (PGWBI-GH) showed a significant main effect of time suggesting that PGWBI-GH significantly increased from baseline to post BWRP [$F(1,235) = 33.20$; $p < 0.001$; $\eta^2 = 0.026$] independently from sex. In addition, there was a significant difference in the BWRP-induced effects on PGWBI-GH between males and females [$F(1,235) = 18.7$; $p < 0.001$; $\eta^2 = 0.058$]. The interaction effect of time x group was significant [$F(1,235) = 5.94$; $p = 0.016$; $\eta^2 = 0.005$], indicating that the change from baseline to post BWRP was different depending upon the two groups (males versus. females). In particular, independent sample t-tests showed that males reported higher PGWBI-GH than females both at pre-test ($p < 0.001$) and post-test ($p = 0.005$). However, the two groups improved significantly in PGWBI-GH from baseline to post BWRP ($p < 0.001$ respectively).

Finally, vitality (PGWBI-V) significantly increased from baseline to post BWRP [$F(1,235) = 116.25$; $p < 0.001$; $\eta^2 = 0.081$] independently from sex. In addition, we found a significant main effect of group [$F(1,235) = 12.3$; $p < 0.001$; $\eta^2 = 0.037$] suggesting that that there was a significant difference in the BWRP-induced effects on PGWBI-V between males and females. The interaction effect of time x group was significant [$F(1,235) = 8.07$; $p = 0.005$; $\eta^2 = 0.006$], indicating that the change from baseline to post BWRP was different depending upon the two groups (males versus. females). In particular, independent sample t-tests showed that males reported higher PGWBI-V than females both at baseline ($p < 0.001$) and post BWRP ($p = 0.045$). However, the two groups improved significantly in PGWBI-V from baseline to post BWRP ($p < 0.001$ respectively).

3.4. Effects of BWRP on muscle performance

Among participants, a subgroup of 160 patients were assessed for their physical capability to go up a flight of stairs, a simple, repeatable, and safe test used to evaluate lower limb muscle capacity (by SCT) before and after the BWRP, according to their own capabilities, in order to assess changes from pre-to-post intervention. Seventy-seven patients were excluded to perform this test due to their physical inability at the admission to the hospital.

Results showed a significant main effect of time suggesting that time requested to perform SCT significantly reduced from baseline to post BWRP [$F(1.00,158.00) = 119.26$; $p < 0.001$; $\eta^2 = 0.026$] independently from sex, and a significant main effect of group [$F(1,158) = 50.0$; $p < 0.001$; $\eta 2 = 0.226$]. This means that there was a significant difference in the BWRP-induced effects on SCT between males and females. The interaction effect of time x group was significant [$F(1.00, 158.00) = 8.47$; $p = 0.004$; $\eta^2 = 0.002$], indicating that the change from baseline to post BWRP was different depending upon the two groups (males versus. females). In particular, independent sample t-tests showed that males reported better SCT (time) than females both at baseline and post BWRP ($p < 0.001$ respectively). However, the two groups improved significantly in SCT (time) from baseline to post BWRP ($p < 0.001$ respectively).

3.5. Interaction between fatigue and muscle performance

Analyzing data from all participants who were tested with SCT, we assessed the relationship between the BWRP-induced effects on fatigue (FSS) and SCT time, hypothesizing that the reduction in fatigue due to the BWRP was correlated to an improvement of SCT (i.e., less time to climb the stair). No

correlation was found between pre–post BWRP difference (Δ %) of FSS (total score) and SCT time (s) ($\rho = -0.016$; $p = 0.804$). See Supplementary Figure S1.

4. Discussion

According to the main findings of the present study, 3-week in-hospital multidisciplinary BWRP was capable to reduce BMI, reduce fatigue (FSS), improve muscle performances (SCT), and enhance psychological well-being (PGWBI) in a sample of elderly people with obesity. In particular, anxiety and depression (PGWBI-A and PGWBI-D) significantly decreased, while positive well-being, self-control, general health, and vitality (PGWBI-PWB, PGWBI-SC; PGWBI-GH, and PGWBI-V respectively) increased. These results were achieved both in males and females with positive effects in all the study variables in both genders, even if the baseline and post-intervention conditions of females were worse than those of male participants (i.e., greater BMI, higher FSS, lower SCT, and lower PGWBI). According to our results, at the end of the intervention males reported greater psychological conditions than females as indicated by lower levels of PGWBI-A and PGWBI-D, as well as greater rates of PGWBI-PWB, PGWBI-GH, and PGWBI-V.

The better starting condition of elderly males was associated with a better final condition than in females, thus indicating that the positive effects exerted by 3-week BWRP were obtained irrespective of the baseline conditions (greater or lower general impairment).

The baseline and final worse psychological conditions of females, as compared to males are not to be considered surprising, since the psychological conditions associated with obesity (such as depression and anxiety) were reported to be more remarkable in females than males (Cooper et al., 2021) and the relationships between perceived stress and stress-related eating and weight gain were stronger in females when compared with males (Udo et al., 2014; Cotter and Kelly, 2018). Similarly, females showed baseline and final higher FSS and SCT time than age-matched males, suggesting the presence of a greater difficulty level in performing a common physical task, tentatively attributable to the gender-related lower (limb) muscle mass and strength, as already found in a previous study by our group (Rigamonti et al., 2019).

In contrast to our expectations, no correlation was found between Δ % FSS (total score) and Δ % SCT time, suggesting that a reduction of perceived fatigue was not directly accompanied by an improvement in muscle performance. The lack of correlation between the decrease in fatigue and the improvement of muscle performance might be due to the different measurements involved. In fact, while FSS is a self-report instrument that reflects a subjective perception of fatigue, SCT is an objective measure of muscle performance based on the measure of time spent doing a physical task (climb upstairs).

However, the improvement of SCT time in people who suffer from obesity reflects a functional improvement in performing the common actions of daily life, which are fundamental to increase the degree of autonomy of these individuals, amplified by the reduction in the subjective feeling of fatigue.

In addition, according to our results, such significant improvements are associated with a substantial improvement of the psychological state, as resulted in the PGWBI. In particular, levels of anxiety and depression decreased, while positive well-being, self-control, general health, and vitality significantly increased.

The above-reported positive results confirm and extend those obtained in previous works, where the same BWRP had been evaluated in adult and pediatric populations with obesity-related comorbidities (Rigamonti et al., 2020a,b). However, the novelty of the present study is having extended our knowledge on the beneficial effects of BWRP in elderly people with obesity and introduced a specific psychological measure capable to describe psychological well-being (PGWBI).

The current study presents some strengths that need to be pointed out, such as the relative large sample of elderly patients with a high rate of obesity, which made the sample of particular interest, the recruitment in a single clinical center delivering a high-quality in-hospital multidisciplinary BWRP, and the use of well-validated and widely-adopted objective and subjective measures (FSS, SCT; PGWBI).

As far as limitations of the study is concerned, the high specificity of the context where the study took place as well as the above-mentioned particularity of the sample (elderly people with a high rate of obesity) with an imbalance between males and females may limit the generalizability of the results and require caution before doing any extrapolation of the present results in a different context. However, we enrolled consecutively our population at the admission to the hospital, thus the study group actually reflects the natural composition of the adult obese populationafferent to our Institution (females approx. 2-fold more than males). In addition, the use of self-report measures of fatigue and psychological well-being should be limited by biases. Finally, the lack of follow-up measures prevents us to drive conclusions about the long-term effects of the intervention.

In conclusion, according to our results, BWRP provides encouraging results in terms of decreased BMI and fatigue, increased muscle performance, and enhanced psychological conditions in elderly people with obesity. Along with similar results obtained in previous studies, the present work provides additional support to the evidence that short-term BWRP entailing diet, physical activity, and psychological support is able to favorably modify and improve the physical and psychological conditions of elderly patients with obesity. This makes the BWRP capable to adhere to the recent recommendations by the most important scientific societies in clinical nutrition, geriatrics, and obesity (Villareal et al., 2005), according to which any BWRP administered in elderly obese patients, in addition to reduce obesity-associated medical complications, might focus on improving physical function and quality of life.

The novelty of this work is provided by the use of a varied pool of evaluation measures addressing both physical (BMI, FSS, SCT) and psychological outcomes (PGWBI) of a BWRP in a sample of particular interest from a clinical point of view, such as elderly patients with a high rate of obesity,

Further additional studies are required to address the main limitations of the current research (i.e., imbalance between males and females, possible confounding factors) and to evaluate the effective long-term maintenance of the positive effects with follow-up measures.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by Ethical Committee of Istituto Auxologico Italiano (registration code: 2013_06_27; project code: 18A301). The patients/participants provided their written informed consent to participate in this study.

Author contributions

AG and AS designed the study. MB, AB, and DC helped to acquire the clinical data. AG analyzed the data and drafted this manuscript for the work. GC and AS reviewed the manuscript and provided final approval for the manuscript to be published. All authors read and approved the final manuscript.

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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.

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Supplementary material

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Effects of "Taking the Waist as the Axis" Therapy on trunk postural control disorder after stroke: A randomized controlled trial

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Background: Sufficient attention to trunk rehabilitation after stroke is still lacking. Loss of trunk selective activity is considered to be the leading cause of trunk postural control disorder after stroke. "Taking the Waist as the Axis" Therapy (WAT) was developed as a combination of the concept of "Taking the Waist as the Axis" from Tai Chi and the rehabilitation of trunk dysfunction after stroke. The present clinical trial examined and assessed the effects of WAT on stroke patients.

Methods: A total of 43 stroke hemiplegic patients with trunk postural control disorder, whose Trunk Impairment Scale (TIS) scoring between 8 and 18, participated in the present study and were allocated randomly to the experimental (n=23) or control groups (n=20). The experimental group received WAT plus conventional therapy, and the control group received "Trunk Selective Activity" Therapy (TSAT) plus conventional therapy. Both groups received treatment once daily and 5 times per week for 3weeks. The Trunk Impairment Scale (TIS), Fugl-Meyer Assessment (FMA), Berg Balance Scale (BBS), change of Intra-abdominal Pressure (IAP), static balance ability assessment, rapid ventilation lung function test and the Modified Barthel Index (MBI) were evaluated before and after intervention for both groups.

Results: The experimental group was superior to the control group in TIS [4 (2, 5) vs. 3 (1.25, 4), p=0.030], change of IAP [−3 (−8, −1.33) vs. −0.02 (−3.08, 6), p=0.011], FMA-upper extremity [10 (6, 18) vs. 1 (0, 3), p=0.002], FMA-lower extremity [2 (1, 4) vs. 1 (0, 2), p=0.009] and FMA [14 (7, 21) vs. 2 (0.25, 3.75), p=0.001]. Within experimental group, forced vital capacity (FVC) [81.35 (63.30, 94.88) vs. 91.75 (79.40, 97.90), p=0.02] was significantly improved.

Conclusion: WAT was an effective trunk treatment after stroke, which significantly improved the patients' trunk posture control ability, motor function and forced vital capacity. However, the results still need to be interpreted with caution for the intervention only lasted for 3weeks.

KEYWORDS

Tai Chi, "Taking the Waist as the Axis" Therapy (WAT), trunk postural control disorder, selective activity, stroke

Introduction

Trunk postural control disorder is a consequence of hemiplegia after stroke, and has been closely associated with impaired balance, mobility and functional independence (Haruyama et al., 2017). It is characterized by problems with rigid movement, abnormal muscle tone and weight-bearing asymmetry (Brown et al., 1997; Huang et al., 2013; Jamal et al., 2018). Compared with healthy people, stroke patients usually show greater trunk postural oscillations and altered muscular activation, which would increase the risk of falling during walking and standing (De Luca et al., 2020). In addition, abnormal elevation of the thorax and the decreased activity of diaphragm due to impaired trunk postural control, which in turn impairs lung function (Laroche et al., 1988). The main factor contributing to posture disorder is the loss of trunk selective activity, especially the loss of trunk flexion, lateral flexion and rotation. For example, when patients bent their trunk laterally, they could not keep the trunk extended synchronously. In addition, the trunk and limbs could not move independently, such as sitting from the supine position with lower limb flexion, standing with the trunk tilted back and the hips extended, walking through pelvic lifting to complete a lower limb stride, moving the hemiplegic upper limb with hyperextension of the spine, etc. (Davies, 1990). Trunk performance could also predict the functional status and prognosis after stroke (Souza et al., 2019). It was proved that the initial ability of trunk postural control after stroke could predict the performance of activity of daily living (ADL) after 6 months (Hsieh et al., 2002). To date, more and more attention has been paid to the rehabilitation of hemiplegic limbs, while the ability of trunk selective movement has been largely ignored, yet it is critical to the recovery of motor functions in hemiplegic patients after stroke (Saeys et al., 2012).

At the present time, the main intervention for selective trunk activity dysfunction after stroke, such as "Trunk Selective Activity" therapy (TSAT), was designed based on the Bobath concept and emphasizes the regulation of trunk selective activity and the integration of postural control, as well as the task performance for developing coordinated movement (Huseyinsinoglu et al., 2012). Trunk treatments that focus on the intensive training of trunk flexion, extension and lateral flexion can positively influent trunk performance, balance and the walking ability of a stroke patient (Brock et al., 2011; Kılınç et al., 2016). Besides, our daily activities contain a variety of trunk rotation, which means that training focused on trunk rotation is warranted in trunk rehabilitation. However, it has not received enough attention in TSAT. Intensive trunk rotation exercises can activate the abdominal muscles, relieve trunk spasticity and improve trunk stability and flexibility (Ng et al., 2001; Niewiadomy et al., 2021). Thus, training focusing on trunk rotation will likely be a promising strategy to restore trunk capacity in hemiplegic patients after stroke.

Tai Chi, a traditional Chinese fitness regimen, has long been employed in translation medicine. Significant improvements have been demonstrated in balance, motor functions and the gait ability of stroke patients after Tai Chi practice (Au-Yeung et al., 2009; Chen et al., 2015; Kim et al., 2015; Li et al., 2018). Most patients with mild to moderate motor dysfunctions were recruited and taught in a group mode under the guidance of experienced Tai Chi coaches in previous studies (Li et al., 2012; Bhalsing et al., 2018). However, it is difficult for patients with moderate to severe motor dysfunctions to practice Tai Chi movements as normal practitioners, for example due to the increased risk of falls or other injuries (Zhao et al., 2021, 2022). Accordingly, Tai Chi practice should be refined and documented to form a set of practical and scientific rehabilitation programs according to the specific dysfunctions of individual stroke survivor.

The "Taking the Waist as the Axis" is an essential concept of Tai Chi and is firmly ingrained throughout its entire practice. The waist is a vital part of the trunk, which helps with limb movements (Fu and Swaim, 1999). Tai Chi develops flexibility through various circular or arc-shaped movements of the waist, promoting motion of the limbs. Tai Chi theory often mentions: "Dominate in the waist" and "Always pay attention to the waist" (Ma, 2006). We applied these principles to stroke patients, essentially "teaching them how to use the trunk flexibly." With repeated intensive training, the patient learns to use the trunk properly, finally getting rid of themselves of arduous movement patterns. From extensive clinical practice, the investigators chose 8 postures from the 24-form Tai Chi and reformed and summarized them into "Taking the Waist as the Axis" Therapy (WAT). The therapy emphasizes strengthening axial rotation, compound rotation and diagonal rotation of the trunk, and facilitates a one-to-one training mode in sitting or standing positions. Considering that training at least 3 days a week and a training duration of 20–60 min per session are the recommended training intensity, we took a training intensity of 50 min 5 times per week in our study (Billinger et al., 2014).

The purpose of the present trial was to compare the clinical effects of WAT based on Tai Chi, and TSAT based on the Bobath concept in the hemiplegic patient with trunk postural control disorder. We hypothesized that WAT would show enhanced clinical outcomes compared with TSAT on trunk postural control ability, motor functions, balance, lung function and the ability of ADL in hemiplegic stroke patients.

Methods

Study design

A single-center, parallel-group, randomized control trial was designed to explore the efficacy of WAT for the treatment of trunk postural control disorders in stroke patients with hemiplegia. The trial design was approved by Shanghai Xuhui Central Hospital Ethics Committee (Approval No. 2021-013) and was registered with the Chinese Clinical Trials Registry Platform (ChiCTR2100043760).

Participants

All patients were recruited from February 2021 to March 2022 from a cohort of inpatients admitted to the Department of Rehabilitation of Shanghai Xuhui Central Hospital, Shanghai as a result of stroke. Patients were recruited to the trial according to the following criteria:

(1) Physicians screened potential patients and contacted the lead trial researcher;

(2) The researcher introduced the trial concept to potential patients and asked them about their willingness to participate;

(3) The eligibility of patients was assessed;

Abbreviations: WAT, "Taking the Waist as the Axis" Therapy; TSAT, "Trunk Selective Activity" Therapy; TIS, Trunk Impairment Scale; FMA, Fugl-Meyer Assessment; BBS, Berg Balance Scale; MBI, Modified Barthel Index; ADL, activities of daily living; MMSE, A Mini-Mental State Examination; IAP, intra-abdominal pressure; IQR, interquartile range; COP, central of pressure; MIP, maximum inspiratory pressure; MEP, maximum expiratory pressure; FEV1, forced expiratory volume in the first second; FVC, forced vital capacity; PEF, peak expiratory flow; MMEF, maximum expiratory mid-flow; %pred, percentage of the predicted values; TrA, transverse abdominis; COG, center of gravity..

(4) The enrolled patients provided written informed consent before commencement of the trial.

The inclusion criteria

(1) First-time stroke
(2) 2 weeks to 6 months after stroke;
(3) Age of 60 to 80 years;
(4) The Trunk Impairment Scale (TIS) scoring 8–18;
(5) The level of standing balance ≥ I;
(6) Unilateral limb dysfunction;
(7) Ability to tolerate at least 40 min exercise and agree to sign the written informed consents.

The exclusion criteria

(1) An inability of patients to finish a 40 min course of exercise;
(2) A Mini-Mental State Examination (MMSE) score ≤ 23;
(3) Acute diseases of the heart, brain, kidney and other organs.

Patients were randomly allocated to the experimental group or control group according to a computer-based randomized sequence. Before the experiment, sealed opaque envelopes were sent to the patients to determine which group they would be assigned. The specified researcher was responsible for the data collection and group allocation. All patients were evaluated by the specific evaluators who were not involved in the randomization or implementation of interventions. Information exchange was not permitted among researchers during the progress of the research, nor was information collected from the involved patients.

Intervention

Both groups received conventional rehabilitation therapy which was conducted according to well defined patient daily rehabilitation therapy regimes, including dynamic sitting and standing transition training, proprioceptive training, occupational therapy and balance bar feedback training. The experimental group received WAT based on Tai Chi and the control group received TSAT based on the Bobath concept.

The intervention time in each group was 50 min for each session, with either WAT or TSAT lasting 30 min, followed by conventional rehabilitation therapy for 20 min, conducted 5 times per week for 3 weeks.

Experimental group

The therapy was carried out by two therapists with formal training in Tai Chi, who had also received 6-month training sessions of WAT. The therapy, involving eight movements, was based on the 24-form Tai Chi that published by the General Administration of Sport of China and the book *Taijiquan "Taking the Waist as the Axis" Hemiplegia Trunk Rehabilitation Manual* (Fu and Swaim, 1999; Liu et al., 2022). The details of the WAT are as follows:

(1) **Qi Shi** (起势): The patient bends the knees slowly, presses the ball with palms, then pulls it back beside the hips, and inhales simultaneously. Next, the patient stands up slowly, raises arms to shoulder level, and inhales. The therapist locates on the effected side of the patient and assists the patient by controlling the belt and hemiplegic upper limb. The therapist controls the flexion and extension of the patient's hemiplegic knee with his own knee (Figure 1A).

(2) **Ye Ma Fen Zong** (野马分鬃): The therapist stands behind the patient with his hands control the patient's hemiplegic arm and waist belt. Under the guidance of the therapist, the patient rotates the trunk (45°–20°–20°–45°) with the weight shifting between two legs. On this basis, the therapist can assist the patient with arm movement (Fen Shou) by controlling the patient's proximal or distal upper extremity (Figure 1B).

(3) **Lou Xi Ao Bu** (搂膝拗步): The patient rotates the right arm to the ear, rotates the trunk axially and pushs the palm forward, then turns the left arm inward and rotates it internally. Repeated "Shang Tui Zhang" and "Xia Lou Xi" can be practiced alone (Figure 1C).

(4) **Lan Que Wei** (揽雀尾): For patients with poor limb function, their arms can be wrapped around the chest. Then, the patient rotates the trunk 45° to the left (Peng), continues to rotate the left 20° more, and then pulls the trunk back with composite rotation (Lv). While for those with better upper extremity function, this can be accomplished with the help provided by the therapist for their upper extremity (Figure 1D).

(5) **Dao Juan Gong** (倒卷肱): The therapist stands behind the patient and controls the patient's iliac spine with both hands. The patient rotates his trunk to the right then shifts the weight to the right leg. The therapist applied slight pressure on the patient's left iliac spine to guide backward extension of the ipsilateral lower limb. Pause for several seconds to stretch the trunk, then continue the contralateral movement (Figure 1E).

(6) **Zuo You Xia Shi** (左右下势): The therapist stands behind the patient with his hands controlling the patient's shoulder or controlling patient's manubrium sternum and thoracic vertebrae. The therapist assists the patient to bend the trunk laterally then to rotate the trunk from left to right, and to stretch the trunk for several seconds. Afterwards, rotate the trunk from right to the starting position and then stretch briefly (Figure 1F).

(7) **Hai Di Zhen** (海底针): The therapist stands in front of the patient with his hands control the patient's hemiplegic scapula and arm. With the help of the therapist, the patient bends the trunk and pulls it toward the contralateral toes to accomplish the diagonal rotation movement of the trunk (Figure 1G).

(8) **Zhuan Shen Ban Lan Chui** (转身搬拦捶): The therapist stands behind the patient with his hands control the hemiplegic hand and waist belt. The patient stands with feet separated and abducent, and the hemiplegic side arm supporting the wall. Rotate the trunk to the contralateral side, the non-hemiplegic arm synchronizes with an arc motion overhead and then extends horizontally to the contralateral side (Figure 1H).

(9) **Shou Shi** (收势): Feet apart, palms up and out in an arcing motion and press down the hands (Figure 1I).

The control of the trunk rotation was realized by controlling the patient's thoracic and manubrium sternum and shoulders. Patients gradually superimpose limb movements based on the completion of trunk movements, which can be done with the assistance of a therapist, in addition, special attention needs to be paid to the control of the proximal upper limb and scapular girdle. All the movements above follow slow, continuous, relaxed and repetitive modes and the eyes follow the main hand. The sitting or standing position training is carried out according to a patient's balance function, with the therapist's one-to-one instruction or

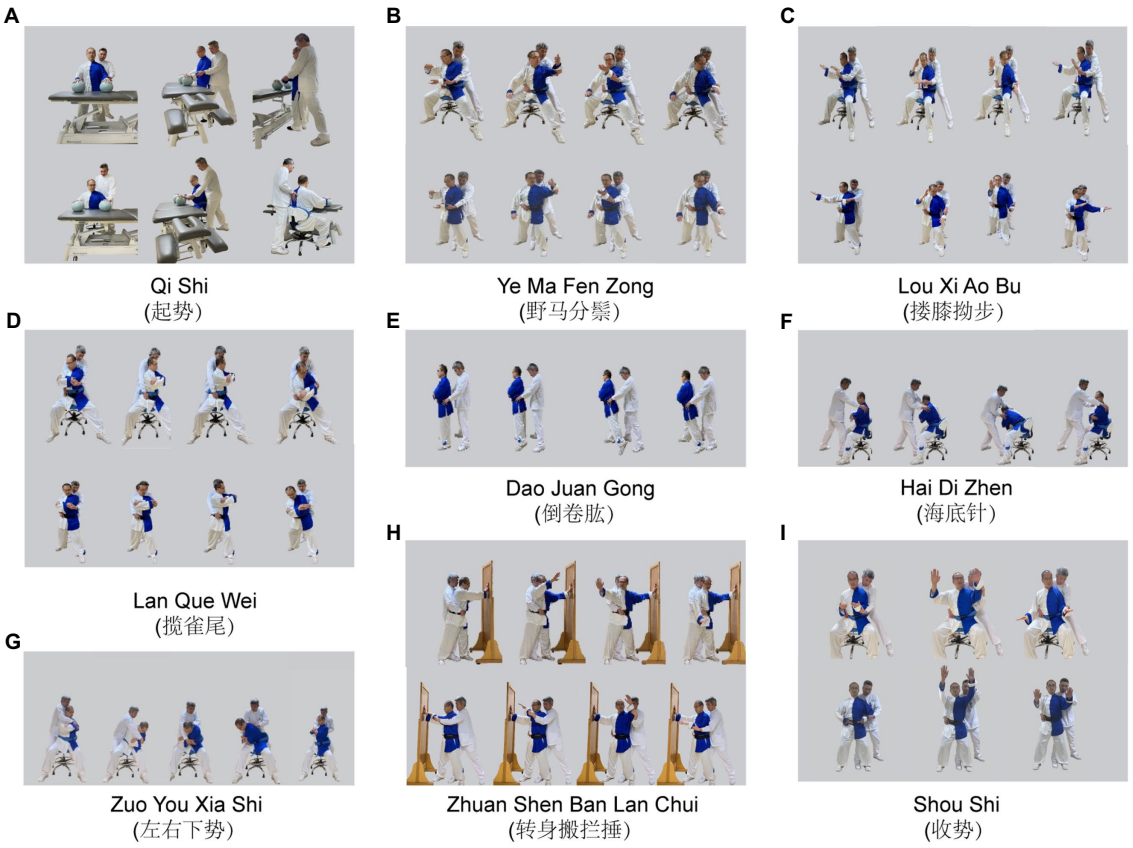

FIGURE 1
Experimental group (WAT). Two position are applied in the WAT. (1) Sitting position: The patient sits on the swivel chair with fixed chassis with a protective belt or regular chair. Legs slightly wider than shoulders apart. The toe is in line with knee joint. Hold body erect and gaze attending to the main hand. (2) Standing position: lunge position (left): The patient stands with two feet shoulder-width apart, then the right foot externally rotates at 45^0, weight shift to the right, left foot takes a step forward with the knee facing forward. The therapist stands behind the patient, placing his knee against the popliteal fossa on the affected side (left) and assisting the patient to stand in a lunge with a slight bent knee.

assistance. And a custom-made swivel chair with a fixed chassis or regular chair is used for sitting position training. It is important to note that the trunk and limbs on the non-hemiplegic side should also be exercised accordingly and efforts should be made to alternate the movements of the limbs bilaterally.

Control group

This therapy consists of training in four different positions and two professional therapists who are familiar with TSAT were in charge of this group. The TSAT is based on the book named *Right in the middle—selective trunk activity in the treatment of adult hemiplegia*, which is based on the Bobath concept (Davies, 1990).

1. Sitting activities

(1) Selective flexion and extension movements of the lower trunk.

(2) Rotation of trunk accompanied by flexion movement.

(3) Rotation of trunk with both arms supported on the same side (Figure 2A).

(4) Flexion and extension of the upper trunk which inhibits spasticity of the distal arm (Figure 2B).

(5) Lateral movement about the center of gravity (Figure 2C).

(6) Active lateral flexion of the trunk against gravity (Figure 2D).

2. From sitting to standing

(1) Therapist helps patient stand up from a seat with hands supported on a stool or with the affected leg alone (Figure 2E).

(2) Selective alternating extensor and flexor activity of the hip and trunk (Figure 2F).

3. Standing activities

(1) Anterior and posterior tilt of the pelvis.

(2) Standing with the hemiplegic leg accompanying adduction and abduction of the healthy hip.

(3) Bending trunk forwards and backwards to upright (Figure 2G).

(4) Standing with the hemiplegic leg accompanied by flexion and extension movements of the hemiplegic knee and abduction of the healthy leg (Figure 2H).

(5) Hip extension accompanied by abduction and external rotation (Figure 2I).

(6) Active upper limb movements in the standing position (Figure 2J).

4. Walking

(1) Stabilizing patient's trunk or helping stretch the hip or supporting the hemiplegic upper limb when walking forward.

(2) Walking backwards, walking to the affected side and walking to the sound side (Figure 2K).

The intervention time in each group was 50 min for each session, with either WAT or TSAT lasting 30 min, followed by conventional rehabilitation training for 20 min, conducted 5 times per week for 3 weeks.

Primary outcome measures

The trunk impairment scale

The TIS consists of 3 components, namely sit-static, sit-dynamic and coordinated assessment. TIS was used to evaluate the ability to keep the trunk stable and to conduct selective activity, including maintaining trunk balance with two legs crossed in the sitting position, trunk lateral bending and rotation of the upper and lower trunk. The scale was scored out of 23, with higher scores indicating better trunk control (Verheyden et al., 2004). The test–retest (ICC = 0.87–0.96) and inter-rater reliability (ICC = 0.87–0.96) of TIS were found to be good (Sorrentino et al., 2018).

Secondary outcome measures

Fugl-Meyer assessment

The FMA, which is a tool to evaluate motor recovery after stroke, is comprised of upper extremity and lower extremity subscales. The maximum score is 100 points, of which 66 points are assigned to the upper extremity (UE) and 34 points to the lower extremity (LE) subscales. A higher score indicates better motor function recovery. Previous studies have shown that the inter-rater reliability of the FMA's total score (ICC = 0.96), upper extremity motor sub-score (ICC = 0.97) and lower extremity motor sub-score (ICC = 0.92) were high (Gladstone et al., 2002).

Berg balance scale

The BBS is a measurement scale of functional balance for stroke population and consists of 14 items. Each item is scored on a scale of 0 to 4 out of 56, with higher scores indicating a better balance function (Downs et al., 2013). The scale has great reliability (ICC = 0.95–0.98) (Blum and Korner-Bitensky, 2008).

Change of intra-abdominal pressure

Change of intra-abdominal pressure (IAP), measured by a pressure feedback unit (STABILIZER™ Pressure Bio-Feedback, America), was used to evaluate transversus abdominis muscle (TrA) recruitment. The methods have been previously described (Zheng et al., 2021). The smaller change in IAP indicate better control ability of the trunk. A moderate to excellent intra-rater reliability (ICC = 0.5–0.81), inter-rater reliability (ICC = 0.47–0.82) and the correlation values assessing validity have been examined by previous studies (ICC = 0.48–0.90) (Hodges et al., 1996; von Garnier et al., 2009; de Paula Lima et al., 2011).

Static balance ability assessment

The static balance abilities of sitting and standing were evaluated using the Prokin proprioception evaluation and training system (PK254P; TecnoBody, Italy). The operational details have been previously published (Wang et al., 2020). The central of pressure (COP) trajectory (mm) and COP area (mm^2) in sitting and standing position were collected with eyes open and closed, with lower values indicating better balance.

Rapid ventilation lung function test

A spirometer was used to perform the rapid ventilation lung function test (X1; XEEK, China). The maximum inspiratory pressure (MIP) and maximum expiratory pressure (MEP) were employed to

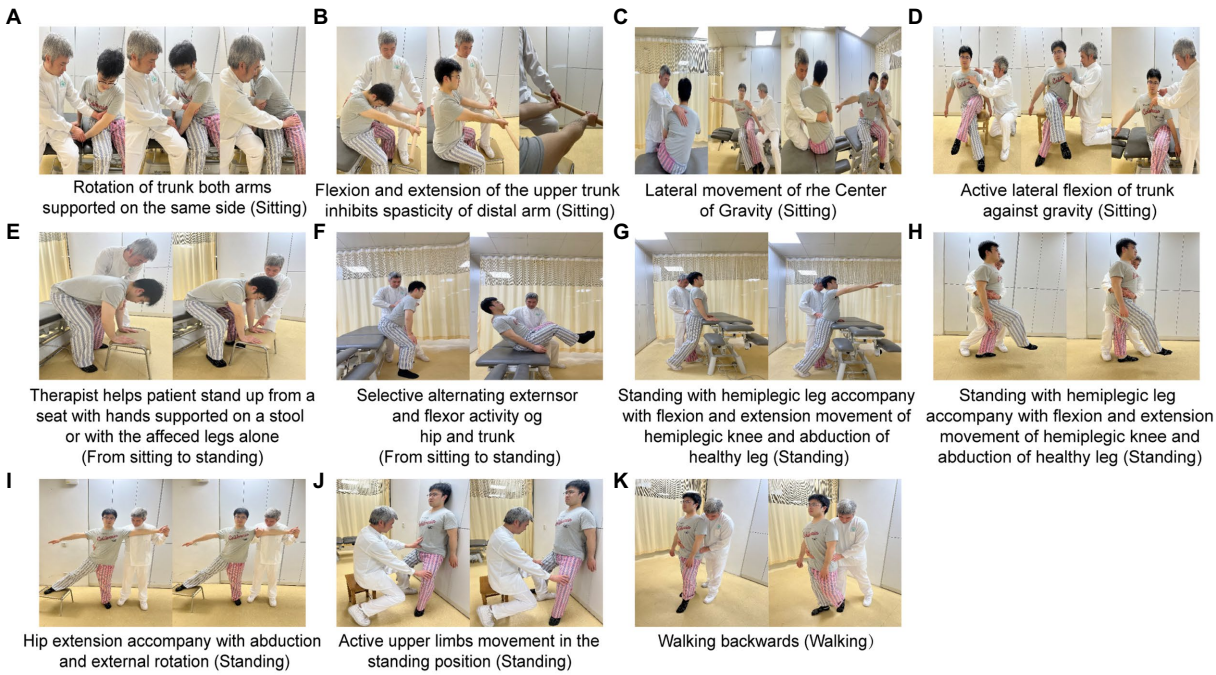

FIGURE 2
Control group (TSAT). Bobath training consists of four activities: sitting **(A–D)**; sitting to standing **(E,F)**; standing **(G–I)**: and walking **(K)**. Each activity includes selectively exercises the truck and limbs.

measure respiratory muscle strength. The forced expiratory volume in the first second (FEV1), forced vital capacity (FVC), peak expiratory flow (PEF), and maximum expiratory mid-flow (MMEF) were used to assess lung ventilation performance. Patients were requested to complete a maximal inspiration and expiration using a mouthpiece linked to a spirometer while seated, and the parameters (% pred) were collected (Zheng et al., 2021).

Modified Barthel Index

The Modified Barthel Index (MBI) was developed to assess ADL performance. The index is comprised of 10 items with a maximum score of 100 and is evaluated according to a patient's self-reporting. A higher score indicates a better ability to perform ADL (Yang et al., 2022).

Statistical analysis

IBM SPSS Statistics ver. 26.0 software was used for all analyses. General descriptions were used for demographic and clinical characteristics, such as the number of cases and median (IQR). TIS scores are discontinuous variables and presented as medians (IQR). FMA, BBS, MBI, change of IAP, static balance ability, MIP, MEP, FVC, FEV1, PEF and MMEF did not comply with a normal distribution or homogeneity of variance and are presented as medians (IQR). Data were analyzed according to intention-to-treat. The Wilcoxon sign-rank test was used to compare pre- and post-treatment within a group and the Mann–Whitney test to compare pre- and post-treatment differences between groups. A value of $p < 0.05$ was deemed to be statistically significant.

Results

Baseline characteristics

Total 43 patients met the inclusion criteria and all completed the study (experimental group, $n = 23$; control group, $n = 20$). Figure 3 shows the flowchart of the trail. No between-group differences in baseline characteristics were found (Table 1) and no serious adverse events occurred.

Primary outcome measure

The TIS, as the primary outcome measure, was showed a significant improvement in the experimental group compared with the control group after 3 weeks treatment [4 (2, 5) vs. 3 (1.25, 4), $p = 0.030$] (Table 2).

Secondary outcome measures

Comparisons of some secondary outcome measures between two groups showed a significant improvement after 3-weeks treatment (Table 2). The experimental group outperformed the control group in terms of the change in IAP [−3 (−8, −1.33) vs. −0.02 (−3.08, 6), $p = 0.011$], FMA-UE [10 (6, 18) vs. 1 (0, 3), $p = 0.002$], FMA-LE [2 (1, 4) vs. 1 (0, 2), $p = 0.009$] and FMA [14 (7, 21) vs. 2 (0.25, 3.75), $p = 0.001$]. There was no significant difference between BBS [5 (3, 14) vs. 4 (1.25, 6), $p = 0.221$] and MBI [10 (0, 25) vs. 10 (5, 18.75), $p = 0.814$] between the two groups.

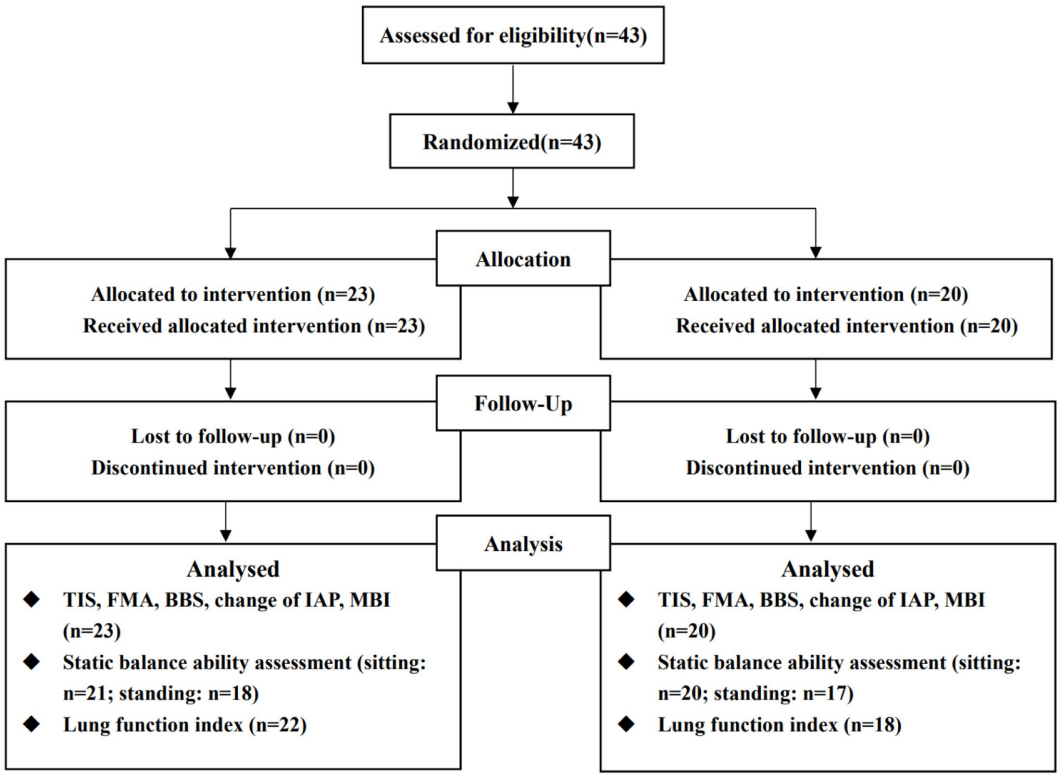

FIGURE 3
Flow chart. The reason for the missing number of measurement indexes is that some subject could not complete the corresponding evaluation content, according to the unified evaluation standards, due to their functional limitation.

Comparisons of static balance ability were shown in Table 3. No significant differences were found in COP trajectory [−6 (−37.17, 1.17) vs. −12.67 (−48.17, 0.38), $p = 0.297$] or area [−3 (−22.67, 1.67) vs. −7.67 (−35.75, −1.58), $p = 0.183$] in the sitting posture with eyes open and no substantial changes in COP trajectory [−7.67 (−22.33, 2) vs. −10.67 (−38.33, 0.67), $p = 0.481$] or area [−1.33 (−9.83, 4.5) vs. −5.67 (−23.75, 0.08), $p = 0.062$] with eyes closed. With the patient's eyes open in the upright posture, neither COP trajectory [−70.67 (−151, 38.71) vs. −42.33 (−153.67, 17.33), $p = 0.817$] nor area [−93.17 (−375.38, 27.08) vs. −38 (−200.83, 276), $p = 0.198$] exhibited a significant difference. Similarly, with closed eyes, neither COP trajectory [−96.67 (−180.50, −27.83) vs. −51.33 (−193.67, 55), $p = 0.424$] nor area [−153.84 (−593.80, −55.5) vs. −160 (−297.67, 86.17), $p = 0.355$] were found to be significantly different.

Comparisons of lung function indexes were shown in Table 4. No significant differences were found in changes of MIP [5.4 (−2, 12.13) vs. −2 (−9.18, 10), $p = 0.211$], MEP [−0.25 (−11.13, 7.6) vs. 0.5 (−13.18, 11.08), $p = 0.910$], FVC [6.85 (−3.53, 20.48) vs. 1.9 (−6.85, 12.4), $p = 0.192$], FEV1 [3.95 (−4.9, 15.33) vs. −4.15 (−9.63, 5.38), $p = 0.146$], PEF [4.7 (−5.05, 10.43) vs. 0.15 (−8.93, 7.08), $p = 0.446$] or MMEF [−6.3 (−18.38, 0.95) vs. 0.95 (−11.55, 25.78), $p = 0.211$] between two groups. The FVC in the experimental group was significantly improved compared to baseline [81.35 (63.30, 94.88) vs. 91.75 (79.40, 97.90), $p = 0.02$].

TABLE 1 Participant characteristics at baseline.

Variables	Experimental group (n=23)	Control group (n=20)	value of p
Age (y), Median (IQR)	66 (60, 72)	65.5 (63, 71)	0.922[a]
Course disease (d), Median (IQR)	39 (21, 86)	74.50 (45, 128.75)	0.111[a]
Sex, n (%)			
Male	18 (78)	17 (85)	0.862[b]
Female	5 (22)	3 (15)	
Affected body side, n (%)			
Left	12 (52)	13 (65)	0.395[b]
Right	11 (48)	7 (35)	
Classification, n(%)Cerebral infarction	21 (91)	20 (100)	0.532[b]
Cerebral hemorrhage	2 (9)	0 (0)	

n, sample size; y, years; d, day; IQR, Interquartile Range; Values shown are n (%) for categorical variables and median (IQR) for continuous variables that were not comply with a normal distribution; [a] Performed by Mann–Whitney test; [b] Performed by χ^2 test.

Discussion

In the current study, we compared two different therapies, WAT and TSAT, to assess their clinical effects of the trunk postural control disorder. The results showed that patients with trunk deficits treated with WAT achieved better trunk postural control, motor function and the FVC than TSAT, which supported our hypothesis.

Trunk postural control disorder of stroke

Most of the published literature pays most attention to hemiplegic limb rehabilitation after stroke while neglecting the importance of trunk recovery (Karthikbabu et al., 2011). According to Devis, postural control disorder is related to a deterioration in upper and lower limb functions, balance, position shift, walking, breath and speech (Jamal et al., 2018). The weakness of the abdominal muscles and the spasticity of the extensor of the trunk make the hemiplegic side trunk adopt an abnormal and frozen mode of movement. This movement is known as the synergic movement pattern and

TABLE 2 Comparison of TIS, FMA, FMA-UE, FMA-LE, BBS, MBI.

Outcome measure	T_0		T_1		T_1-T_0		value of p
	Experimental group (n=23)	Control group (n=20)	Experimental group (n=23)	Control group (n=20)	Experimental group (n=23)	Control Group (n=20)	
TIS	14 (13, 16)	13.5 (12.25, 15.75)	18 (17, 20)	16.5 (15.25, 19)	4 (2, 5)	3 (1.25, 4)	0.030
FMA	50 (39, 65)	45.5 (24.25, 80.25)	63 (49, 81)	51 (25.75, 81)	14 (7, 21)	2 (0.25, 3.75)	0.001
FMA-UE	27 (18, 38)	23 (6.25, 54.75)	40 (28, 52)	27.5 (6.75, 56)	10 (6, 18)	1 (0, 3)	0.002
FMA-LE	23 (22, 27)	22 (18, 26)	26 (23, 30)	24 (18.50, 27.75)	2 (1, 4)	1 (0, 2)	0.009
BBS	36 (30, 46)	37 (25, 42.75)	47 (42, 51)	42.5 (30.50, 48.75)	5 (3, 14)	4 (1.25, 6)	0.221
MBI	60 (55, 70)	55 (46.25, 60)	75 (60, 80)	60 (55, 78.75)	10 (0, 25)	10 (5, 18.75)	0.814
Change of IAP (kPa)	16.33 (10.33, 20.67)	12.33 (9, 17.08)	11.33 (7.60, 14.67)	15.5 (8.83, 18.25)	−3 (−8, −1.33)	−0.02 (−3.08, 6)	0.011

T_0, baseline data; T_1, data of 3th weeks after baseline; T_1-T_0, the difference before and after intervention; TIS, The trunk impairment scale; FMA, The Fugl-Meyer assessment; FMA-UE, The Fugl-Meyer assessment- upper extremity; FMA-LE, The Fugl-Meyer assessment- lower extremity; BBS, Berg Balance Scale; MBI, The modified Barthel Index; IAP, Intra-abdominal pressure; P, comparison of difference between groups before and after intervention.

TABLE 3 Comparison of static balance ability.

Outcome measure	T_0		T_1		T_1-T_0		*p*-value
	Experimental group (*n*=21)	Control group (*n*=20)	Experimental group (*n*=21)	Control group (*n*=20)	Experimental group (*n*=21)	Control group (*n*=20)	
Static open eye sitting balance test							
COP area (mm^2)	12 (6.67, 36.83)	18.33 (8, 65.17)	9.67 (4.67, 15.33)	7.17 (4.84, 13.17)	−3 (−22.67, 1.67)	−7.67 (−35.75, −1.58)	0.183
COP trajectory (mm)	85.33 (61.67, 103.67)	102.33 (71.67, 129.58)	71 (61.33, 79)	77.17 (61.17, 107)	−6 (−37.17, 1.17)	−12.67 (−48.17, 0.38)	0.297
Static closed eye sitting balance test							
COP area (mm^2)	7.33 (5.17, 16)	12.67 (6.50, 41.67)	7.33 (4.33, 14.67)	7 (5.17, 18.67)	−1.33 (−9.83, 4.5)	−5.67 (−23.75, 0.08)	0.062
COP trajectory(mm)	81 (64.17, 94.33)	94.17 (75.42, 128.33)	69 (63.17, 77)	76.67 (67.17, 95.25)	−7.67 (−22.33, 2)	−10.67 (−38.33, 0.67)	0.481
	Experimental group (*n* = 18)	Control group (*n* = 17)	Experimental group (*n* = 18)	Control group (*n* = 17)	Control group (*n* = 18)	Control group (*n* = 17)	
Static open eye standing balance test							
COP area (mm^2)	259.42 (123.50, 625.88)	256 (111, 467)	165.67 (67.92, 282.83)	250 (127.83, 550.83)	−93.17 (−375.38, 27.08)	−38 (−200.83, 276)	0.198
COP trajectory(mm)	276.67 (217.25, 363)	374 (256.17, 476.33)	252.33 (156.33, 381.08)	308 (234.33, 445.67)	−70.67 (−151, 38.71)	−42.33 (−153.67, 17.33)	0.817
Static closed eye standing balance test							
COP area (mm^2)	711 (351.33, 1468.08)	418.33 (293.17, 810.17)	463.83 (173.83, 1030.92)	416 (212.17, 776.33)	−153.84 (−593.80, −55.5)	−160 (−297.67, 86.17)	0.355
COP trajectory (mm)	473.50 (398.50, 602.75)	428 (337.67, 628.33)	413.50 (263.92, 699.92)	399 (357.50, 486)	−96.67 (−180.50, −27.83)	−51.33 (−193.67, 55)	0.424

T_0, baseline data; T_1, data of 3th weeks after baseline; T_1-T_0, the difference before and after intervention; COP, center of pressure; P, comparison of difference between groups before and after intervention.

TABLE 4 Comparison of lung function indexes.

Outcome measure	T_0		T_1		T_1-T_0		*p*-value
	Experimental group (*n*=22)	Control group (*n*=18)	Experimental group (*n*=22)	Control group (*n*=18)	Experimental group (*n*=22)	Control group (*n*=18)	
MIP (%pred)	36.80 (29.23, 49.08)	44.80 (27.85, 63.58)	41.50 (30.85, 55.43)	42.10 (31.63, 61.78)	5.4 (−2, 12.13)	−2 (−9.18, 10)	0.211
MEP (%pred)	57.10 (38.33, 66.15)	41.65 (33.50, 66.70)	51.55 (35.18, 73.48)	49.20 (34.65, 60.73)	−0.25 (−11.13, 7.6)	0.5 (−13.18, 11.08)	0.910
FVC (%pred)	81.35 (63.30, 94.88)	79.25 (48.48, 104.53)	91.75 (79.40, 97.90) *	85.55 (54.45, 101.83)	6.85 (−3.53, 20.48)	1.9 (−6.85, 12.4)	0.192
FEV1 (%pred)	78.05 (62, 89.23)	79.50 (59.73, 102.50)	83.70 (72.80, 98.55)	81.20 (66.76, 103.88)	3.95 (−4.9, 15.33)	−4.15 (−9.63, 5.38)	0.146
PEF (%pred)	48.20 (32.33, 60)	44.50 (28.78, 58.30)	48.25 (37.05, 66.03)	44.25 (33.05, 56.58)	4.7 (−5.05, 10.43)	0.15 (−8.93, 7.08)	0.446
MMEF (%pred)	59.05 (48.95, 75.80)	69.40 (39.90, 87.13)	53.25 (37.85, 73.85)	70.85 (44.33, 89.53)	−6.3 (−18.38, 0.95)	0.95 (−11.55, 25.78)	0.211

T_0, baseline data; T_1, data of 3th weeks after baseline; T_1-T_0, the difference before and after intervention; MIP, maximum inspiratory pressure; MEP, maximum expiratory pressure; FVC, forced vital capacity; FEV1, forced expiratory volume in the first second; PEF, peak expiratory flow; MMEF, maximum expiratory mid-flow; P, comparison of difference between groups before and after intervention; *p-value < 0.05, intra-group comparison before and after intervention, $p = 0.02$.

is characterized by the loss of selective movement. Regardless of the position of the hemiplegic patient, the loss of selective activities among trunk muscles or between the trunk and the limb exists, which hinders a patient's ability to bend forward, bend laterally, extend against gravity, rotate in multiple dimensions, and maintain or transfer their center of gravity (COG). Therefore, strengthening weak abdominal muscles and alleviating trunk spasticity are the key to restoring trunk stability and flexibility, which will ultimately promote selective trunk activities.

WAT improves trunk postural control

The front and center line of the human body is Ren channel, the sea of Yin pulse, meaning blood; the posterior median line is the Du channel, which acts as the Yang vein of the whole body and mains qi. Therefore, Tai Chi attaches great importance to trunk movement, which is conducive to keep the two veins of Ren and Du open and the balance of Yin and Yang. Moreover, Tai Chi emphasizes the spiral circular motion track of the trunk and limbs, so that the joints, muscles and ligaments of the whole body can achieve the effect of Qi and blood running and activating collaterals through the uniform and coherent repeated activities, which is beneficial to the functional recovery of stroke patients with hemiplegia.

The concept of "Taking the Waist as the Axis" originates from Tai Chi, which emphasizes various arc-shaped track movements of the waist, e.g., axial rotation, composite rotation and diagonal rotation, from a small to a wide range, thus, allowing the waist to achieve flexibility. It should be noted that various trunk rotations are involved in daily activities, implying that the treatment based on trunk rotation may be more in keeping with the demands of a stroke patient's daily life.

WAT that involves multidimensional trunk rotations is mainly performed by the bilateral obliquus internus abdominis (OI) and obliquus externus abdominis (OE) muscles (Urquhart and Hodges, 2005). The ipsilateral OI and contralateral OE act as dynamic muscles for centripetal contraction, whereas ipsilateral OE and contralateral OI function as fixed anchors for centrifugal contraction (Ng et al., 2001). "Ye Ma Fen Zong" (野马分鬃, Figure 1B) comprises axial rotations at 45° and 20° degrees alternately and repeatedly to activate the bilateral OE and OI, thus increasing trunk flexibility. Furthermore, trunk rotation activates the transversus abdominis, which is associated with trunk stability (Urquhart and Hodges, 2005), and IAP is a sensitive indicator of trunk stability. Therefore, WAT exhibited a more stable IAP than TSAT.

The abdominal muscles show much higher co-activation than the back muscles during lateral flexion (Huang et al., 2001). Due to the lack of efficient abdominal contractions, the trunk lateral flexion is substituted by overall trunk flexion with excessive abduction of the non-hemiplegic upper limb. During TSAT, the therapist provides considerable assistance to help the patient complete the movements of lateral flexion. In contrast, the composite trunk rotation of the "Zuo You Xia Shi" (左右下势, Figure 1G) and the diagonal rotation of the "Hai Di Zhen" (海底针, Figure 1F), whose processes involve combinations of lateral flexion, forward flexion and anti-gravity stretching, can be easily performed by controlling the patient's shoulders, or managing the manubrium and thoracic vertebrae. Consequently, the buckling capacity of the lower trunk is improved. The synergistic movement patterns of the trunk and lower limbs are effectively inhibited by trunk multidimensional rotation, with the abduction of the lower limbs in the sitting position. During "Lan Que. Wei" (揽雀尾, Figure 1D), the trunk rotates from the front to the posterolateral space, and then the upper trunk pulls the COG to the opposite side, challenging the patient's dynamic control of upper trunk.

It is well-known that trunk rotation is the pivotal factor in reducing hypertonicity (Davies, 1990). WAT stretches the spastic muscles of the trunk through various trunk rotation training, especially in a slow, continuous, relaxed and repetitive movement rhythm that is unique to Tai Chi. From the initial passive trunk rotation to the active trunk rotation guided by controlling the scapula, thoracic vertebra or pelvis, the compensatory posture and spasms of the trunk have been progressively suppressed. For instance, "Dao Juan Gong" (倒卷肱, Figure 1E) maybe relieve trunk spasticity by assisting patient to rotate the trunk to the one side and then extend the contralateral leg to extend backward, and pause for several seconds. "Hai Di Zhen" (海底针, Figure 1F) maybe relieve trunk spasticity by guiding the hemiplegic trunk and arm to extend in the direction of the contralateral toe. Finally, the patient is able to complete various trunk rotation independently in sitting or standing position.

TIS employed in this study was to evaluate the capacity of maintaining trunk stability and performing selective trunk movements (Verheyden et al., 2009; Alhwoaimel et al., 2018). WAT relieves trunk spasm by slowly rotating the trunk, promotes trunk stability by activating abdominal muscles, and improves selective trunk control by multi-dimensional rotation, thus improving TIS scores. In the present trial, it was found that WAT had a superior impact on improving trunk postural control compared to TSAT for hemiplegic patients.

Improvement of limb functions

Upper limb mobility relies on the shoulder girdle's proximal fixation, which in turn depends on thorax stability, which is achieved by contraction of the abdominal muscles to keep the ribs in a descending position. WAT, based on the trunk rotation, strengthens the rectus abdominis and external abdominal oblique muscles, counteracting excessive upward and lateral movement of the thorax, thus stabilizing the shoulder and improving upper limb performance. Surprisingly, the present trail results found superior limb function recovery after WAT compared to TSAT. Hemiplegic upper limbs often abduct with an abnormal flexion synergy pattern which makes them unable to engage in a reaching function (Ellis et al., 2017). Additionally, hemiplegic patients have difficulty with flexion and abduction of the shoulder, which is accompanied by elevation of the hemiplegic shoulder girdle, leading to subacromial impingement and shoulder pain.

Previous studies have demonstrated the "Loud Hand" of Tai Chi significantly improved the limb motor function, with strengthening the internal and external rotation of the shoulder, promoting forward flexion and abduction of the shoulder and alleviating shoulder pain (Lyu et al., 2018; Luo et al., 2020; You et al., 2021). WAT applies round rotation motion for both trunk and limb training. In the "Lou Xi Ao Bu" (搂膝拗步, Figure 1C), the unilateral shoulder is rotated outward and which is then extended forward with the palm pushed forward. Simultaneously, the contralateral shoulder is rotated inward and then returned to the neutral position with the palm pressed down, which can effectively prevent shoulder injury and also promote shoulder function. The slow rotation of the shoulder relieves the high tension of the internal rotators induced by hemiplegia, whilst the up-down movement of the upper limbs prevents spinal overextension and facilitates isolated movement between the upper limbs. Anterior–posterior lunge training and trunk rotations are performed simultaneously in a standing position, which may promote isolated movement between the trunk and lower limbs.

Thus, satisfactory trunk function plays an essential role in accelerating limb functions. The isolated movement of the affected limbs is driven by the trunk rotation movement, which is consistent with the concept of "Taking the Waist as the Axis," that is, the trunk leads the entire body.

Balance function

WAT involves multi-directional weight shifts, such as the "Zuo You Xia Shi" (左右下势, Figure 1G) with the COG moving left, right, up and down. Also, the "Hai Di Zhen" (海底针, Figure 1F) with COG moving back and forth in a diagonal orientation and "Zhuan Shen Ban Lan Chui" (转身搬拦捶, Figure 1H) with COG shifting between left and right. Furthermore, the slow, continuous, relaxed and repetitive movement patterns enhance proprioceptive input. Therefore, after the WAT intervention, the experimental group obtained a trend of improvement in the COP trajectory with eyes closed in sitting position. Unfortunately, given the short intervention period, we could not detect differences in measures related to balance between the two groups. As daily living ability was strongly associated with balance, there was no significant difference in MBI scores between the two groups accordingly (Kim and Park, 2014).

Lung function

A reduced respiratory function is frequently found in hemiplegic patients, usually characterized by respiratory muscle weakness and altered chest wall kinematics (Lima et al., 2014; Menezes et al., 2016). During WAT, the thorax is repeatedly deformed and restored with repetitive trunk rotation training, which benefits flexibility of the thorax. Thus, the improved abdominal muscles help draw the rib cage down, promoting the expansion of the thorax symmetrically, thus improving lung ventilation. The within-group differences in FVC following WAT might be attributed to the above findings. However, there was no difference in FVC between the two groups that might relate to a 3-week intervention that was insufficient.

Limitations

This study had a number of limitations. The intervention only lasted for 3 weeks, and no follow-ups were conducted, making it impossible to assess the long-term influence of the therapy regiments. Besides, the results still need to be interpreted with caution for the measurement we used mostly are the scales. Further randomized studies with a larger cohort size and longer duration of study will be required to confirm its clinically worthwhile benefits. In addition, more quantitative measures should be emphasized in the future research. What's more, additional studies should be well-designed to implement WAT in stroke patients with varying degrees of trunk dysfunction to explore the correlation between the trunk and limb functions.

Conclusion

Compared to "Trunk Selective Activity" therapy (TSAT), it was found that "Taking the Waist as Axis" therapy (WAT) not only improved trunk postural control in stroke patients with hemiplegia but also produced significant improvements in their motor functions. Besides, within group, WAT showed an improvement in forced vital capacity (FVC). The present clinical trial supports the feasibility and effectiveness of WAT derived from Tai Chi for the treatment of stroke patients and provides an important reference for employing traditional fitness regimes in stroke rehabilitation.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by the Shanghai Xuhui Central Hospital Ethics Committee. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

RC and JW: formal analysis. RC, YingZ, and ML: writing – original draft. YingZ and WF: writing – review, revise and finalize the manuscript. LY, YanW, YanmW, and YiZ: patient recruitment and enrollment. JM, GL, and PH: assessment. JY and HL: methodology – "Taking the Waist as Axis" therapy. ML and WN: methodology – "Trunk Selective Activity" therapy. FW and JP: methodology – conventional therapy. CC and ZP: data curation. NZ: financial management. All authors contributed to the article and approved the submitted version.

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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.

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Whole-body vibration as a passive alternative to exercise after myocardial damage in middle-aged female rats: Effects on the heart, the brain, and behavior

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Background: Females with cardiovascular disease seem more vulnerable to develop concomitant mental problems, such as depression and cognitive decline. Although exercise is shown beneficial in cardiovascular disease as well as in mental functions, these patients may be incapable or unmotivated to perform exercise. Whole body vibration (WBV) could provide a passive alternative to exercise. Aim of the present study was to compare WBV to exercise after isoproterenol (ISO)-induced myocardial damage in female rats, regarding effects on heart, brain and behavior.

Methods: One week after ISO (70 mg/kg s.c., on 2 consecutive days) or saline injections, 12 months old female rats were assigned to WBV (10 minutes daily), treadmill running (30 minutes daily) or pseudo intervention for 5 weeks. During the last 10 days, behavioral tests were performed regarding depressive-like behavior, cognitive function, and motor performance. Rats were sacrificed, brains and hearts were dissected for (immuno)histochemistry.

Results: Significant ISO-induced cardiac collagen deposition (0.67 ± 0.10 vs 0.18 ± 0.03%) was absent after running (0.45 ± 0.26 vs 0.46 ± 0.08%), but not after WBV (0.83 ± 0.12 vs 0.41 ± 0.05%). However, WBV as well as running significantly reduced hippocampal (CA3) collagen content in ISO-treated rats. Significant regional differences in hippocampal microglia activity and brain derived neurotrophic factor (BDNF) expression were observed. Significant ISO-induced CA1 microglia activation was reduced after WBV as well as running, while opposite effects were observed in the CA3; significant reduction after ISO that was restored by WBV and running. Both WBV and running reversed the ISO-induced increased BDNF expression in the CA1, Dentate gyrus and Hilus, but not in the CA3 area. Whereas running had no significant effect on behavior in the ISO-treated rats, WBV may be associated with short-term spatial memory in the novel location recognition test.

Conclusion: Although the female rats did not show the anticipated depressive-like behavior or cognitive decline after ISO, our data indicated regional effects on neuroinflammation and BDNF expression in the hippocampus, that were merely normalized by both WBV and exercise. Therefore, apart from the potential concern about the lack of cardiac collagen reduction, WBV may provide a relevant alternative for physical exercise.

KEYWORDS

isoproterenol-induced cardiac damage, exercise, whole body vibration (WBV), depressive-like behavior, cognition, neuroinflammation, brain collagen

1. Introduction

Many patients with cardiovascular disease may also experience mental disorders, including depression and cognitive impairment. These mental disorders are often overlooked or regarded as "natural" responses to a life-threatening condition. However, mental disorders can be associated with increased morbidity and mortality (Gharacholou et al., 2011; Meijer et al., 2013). Moreover, female patients seemed more vulnerable to developing heart failure-associated depression (Gottlieb et al., 2004; Eastwood et al., 2012) and cognitive decline (Ghanbari et al., 2013) than male patients. Although the worsening of cardiovascular prognosis by comorbid depression is well recognized (Nabi et al., 2010), anti-depressant treatment may alleviate depressive symptoms but does not improve cardiovascular prognosis (Thombs et al., 2008). In female patients, it may even deteriorate cardiovascular prognosis (Krantz et al., 2009). A rationale for therapy of this comorbidity is hampered by the lack of understanding of the heart–brain interaction and the potential difference in male and female patients.

Extensive evidence points to a role of a derailed (neuro)inflammatory response to cardiac damage, as a mechanism underlying the cardiovascular disease-depression association (Liu et al., 2013; Angermann and Ertl, 2018). However, the efficacy of anti-inflammatory therapy (Kosmas et al., 2019) seems rather poor. A lot of knowledge about the heart–brain interaction comes from animal studies. Heart failure induced by coronary artery ligation was associated with cognitive impairment (Hovens et al., 2016), as well as depressive-like behavior in rodents (Schoemaker and Smits, 1994; Wang et al., 2013; Frey et al., 2014). This behavior could be affected by cardiovascular-directed treatment (Schoemaker et al., 1996) and by treatment targeted at the brain and behavior (Grippo et al., 2003, 2006; Bah et al., 2011a,b; Ito et al., 2013). The isoproterenol (ISO)-induced cardiac damage model is often used as a way to induce focal cardiac damage and could be preferred above the coronary artery ligation model, as the former does not require brain-changing thoracic surgery (Hovens et al., 2016; Toth et al., 2021). In the ISO model, the effects of treatment on cardiovascular aspects are extensively studied (Nichtova et al., 2012; Ma et al., 2015; Alemasi et al., 2019), but studies on behavioral consequences are limited. Reduced exploratory behavior (Tkachenko et al., 2018) and declined sucrose preference after ISO (Hu et al., 2020) suggest depressive-like behavior, while the cognitive decline was also observed (Ravindran et al., 2020). Concomitant cardio and neuroprotective effects have been obtained in this model with Corvitin (Tkachenko et al., 2018), sodium thiosulfate (Ravindran et al., 2020), and traditional Chinese medicine Kai-Xin-San (Hu et al., 2020). However, these effects were only studied in young male rats, leaving potentially different effects in female rats unrevealed. Sex dimorphism in the response to ISO was already recognized in the 70s (Wexler et al., 1974; Wexler and Greenberg, 1979), and supported by our group (Toth et al., 2022b).

Exercise is generally acknowledged for its beneficial effects on physical as well as mental conditions in health and disease (Pedersen and Saltin, 2015). Recently, we showed that exercise training after ISO-induced cardiac damage could reverse the anxiety-like behavior in male rats (Toth et al., 2021), but not in female rats (Toth et al., 2022a). Physical exercise before ISO prevented cardiac fibrosis and the upregulation of pro-inflammatory cytokines (Ma et al., 2015; Alemasi et al., 2019), but exercise after ISO seemed to deteriorate the cardiac damage (Azamian Jazi et al., 2017).

However, not all patients are capable and/or motivated to perform physical exercise. For them, a passive form of exercise, such as whole-body vibration, could provide an alternative (Runge et al., 2000; Zhang et al., 2014). Whole-body vibration (WBV) is a passive mechanical stimulation on a vibrating platform (van Heuvelen et al., 2021). In addition to increased muscle strength (Annino et al., 2017) and aerobic fitness (Zhang et al., 2014), WBV was associated with improved wound healing (Wano et al., 2021) and a reduced inflammatory phenotype (Weinheimer-Haus et al., 2014). Moreover, we recently showed that WBV improved motor performance, spatial memory, and anxiety-like behavior in aged rats (Oroszi et al., 2022a). However, these effects seemed more pronounced in male than in female rats. In a study of middle-aged female rats, treated with WBV from 1 to 30 days after mid-cerebral ischemia-reperfusion, decreased inflammasome activation (caspase-1 and IL1-beta) and increased brain-derived neurotrophic factor (BDNF) expression were observed, concomitant with infarct reduction (Raval et al., 2018).

Taken together, female patients with cardiovascular disease seemed more prone to develop mental disorders; beneficial effects of exercise training on behavior were observed in male rats with ISO-induced myocardial infarction (Toth et al., 2021), but not in female rats; WBV has indicated a passive alternative to exercise. Therefore, the present study aimed to explore WBV as an alternative to physical exercise in female rats after ISO-induced myocardial infarction, regarding its effects on the heart, the brain, and behavior.

2. Methods

2.1. Animals and experimental design

2.1.1. Animals

Animals were housed in groups of two or three in cages of 30*42*20 cm with sawdust as bedding in the conventional animal facility of the University of Physical Education, Hungary, in a room with 22 ± 2°C and humidity of 50 ± 10%. The light was provided from 6 a.m. to 6 p.m. CEST. Experiments were performed approximately between 9 a.m. and 5 p.m. Standard rodent chow (LT/R, Innovo Ltd., Gödöllo, Hungary) and tap water were provided *ad libitum*. All methods were performed in accordance with the ARRIVE guidelines. Experimental animals and procedures were approved by the local animal committee of the University of Physical Education, Budapest, Hungary.

2.1.2. Pilot

Exposure time–effect relation of WBV Before starting the main study on WBV as an alternative to exercise, a pilot exposure time–effect study was performed in order to find the optimal WBV exposure time per session for our female rats. For that, 28 female Wistar rats were collected from the breeding colony of the University of Sports Science, Hungary. Rats were randomly divided into four experimental groups, receiving 5 weeks (one time a day and 5 days a week) of treatment with either pseudo-WBV (0 min), or 5, 10, or 20 min of WBV per day. At the end of these 5 weeks, effects on behavior were evaluated, regarding open field (OF) exploration, short-term memory in the novel object/novel location recognition tests (NOR/NLR), and motor performance in the balance beam and grip hanging tests. For details about the WBV and behavioral testing, refer to Section 2 of the main study.

2.1.3. Main study

Sixty-four middle-aged (on average 12 months old) female Wistar rats were obtained from the breeding colony of the University of Sports Science, Hungary. Rats were randomized to six experimental groups: running ISO/saline, WBV ISO/saline, and as control pseudo-ISO/saline, and were subjected to the protocol presented in Figure 1. For that, rats were treated with ISO to induce heart lesions or received saline injections. After 1 week of recovery, rats were either subjected to 5 weeks of WBV or treadmill running or received pseudo-treatment (sedentary). Exploratory behavior, cognitive performance, and motor function were assessed during the last 10 days of the training period. After completion of all tests, animals were killed, and heart and brain tissues were collected for further analyses of cardiac collagen, brain collagen, neuroinflammation, and neuronal function. This experimental protocol is similar to the one described in detail in our previous studies (Toth et al., 2021; Oroszi et al., 2022a).

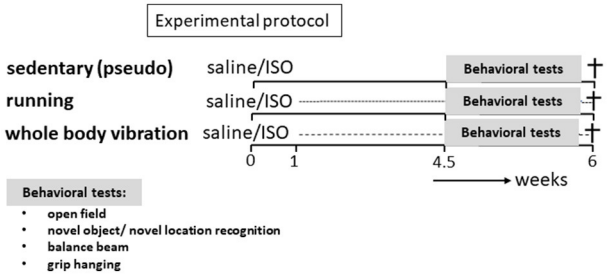

FIGURE 1
Experimental protocol. Rats ($n = 64$) were subdivided into six experimental groups. Part of the rats ($n = 25$) received two saline injections and the other part ($n = 39$) received two ISO injections, both 24 h apart. One week later, rats were subdivided into a sedentary group, receiving the pseudo-intervention, an exercise intervention group (treadmill running), and a whole-body vibration intervention group, for 5 weeks. In the last 10 days of this period, behavioral tests were performed, and rats were subsequently killed.

2.2. Interventions

2.2.1. Cardiac damage

Acute cardiac damage was induced by isoproterenol hydrochloride (ISO; $C_{11}H_7NO_3 \cdot HCl$: ISO), a non-selective β-adrenoceptor agonist that mimics the histological, physical, and endocrinological events of human myocardial infarction presumably by myocardial hyperactivity-induced ischemia and energy depletion (Wexler et al., 1968). Rats were injected subcutaneously with ISO (Sigma Aldrich) in a dose of 70 mg/kg dissolved in 1 ml/kg saline (Toth et al., 2021, 2022a,b). Control animals received 1 ml/kg saline. Both groups received two injections with 24 h in between, according to the protocol described by Ravindran et al. (2020).

2.2.2. Whole-body vibration

Rats received a single vibration session of 10 min, five times per week, for 5 consecutive weeks, using a vibration platform (MarodyneLiV—low-intensity vibration; BTT 129 Health GmbH, Germany), as described in detail elsewhere (Oroszi et al., 2022a). The platform ensures constant vibration exposure with a frequency of 30 Hz and an amplitude of 50–200 microns. Rats were placed in empty individual cages on the platform (the same shape as the individual home cage, but without bedding). WBV took place in an adjacent room with the same climate conditions as the housing room.

2.2.3. Treadmill running

Saline- and ISO-treated groups were assigned to a treadmill running protocol on a six-lane rat treadmill (Tartonik Elektronika, Italy) with individual lanes of 12*54*13 cm, as described in detail previously (Toth et al., 2021). The training program lasted for 5 weeks, five times per week on each weekday. On the 1st week of the training program, rats were habituated to running: on the 1st day, rats started with 10 min of running with a maximal speed of 10 m/min, which was gradually increased to 30 min and a maximal speed of 18 m/min (moderate intensity; ~65% of VO_2max; Hoydal et al., 2007) by the 5th day. For the following 4 weeks, each running session lasted 30 min at 18 m/min.

2.2.4. Pseudo-WBV/running

Rats that received pseudo-treatment served as sedentary controls for both WBV and exercise. Pseudo-treatment consisted of either 10 min on the turned-off vibrating platform or 30 min on the turned-off treadmill, on alternate days, for 5 weeks, 5 days a week.

2.3. Behavior

2.3.1. General

Effects on behavior were tested during the last 10 days of the protocol (refer to Figure 1). Open field behavior (OF) was used to assess anxiety/depressive-like behavior (Schoemaker and Smits, 1994). Short-term memory was tested in the novel object recognition (NOR) and the novel location recognition test (NLR), as a measure for cognitive effects (Hovens et al., 2014b). Effects on motor performance were obtained in the balance beam test for motor coordination (Song et al., 2006) and in the grip strength test (Shear et al., 1998) for effects on muscle strength. Tests have been described in detail in our previous study (Oroszi et al., 2022a). All tests were recorded with a digital video camera (Canon Legria HFR106, Canon Inc., Tokyo, Japan) and stored on a memory card for later offline analyses.

2.3.2. Open field

An open-field exploration test was performed to assess exploratory and anxiety-related behavior, as we described earlier (Toth et al., 2021). A round-shaped arena (diameter of 80 cm) was divided into an inner circle (diameter of 48 cm; center area) and an outer annulus (wall area). Initially, the area was divided into three concentric circles: wall, middle, and center (each 16 cm wide). However, as the rats hardly moved into the so-defined center area, this center area plus the middle area were taken together as "çenter" in our measurements. Animals were placed in the arena and allowed to explore for 5 min. After each animal, the arena was cleaned with 70% ethanol to remove smell cues. Time spent in the center and the wall area, as well as the number of visits to the center, were obtained using Eline software (University Groningen, the Netherlands). The total number of crossings between the initially defined three areas was used as an estimate for locomotion activity and exploration.

2.3.3. Novel object and novel location recognition

To assess short-term visual memory, which depends primarily on prefrontal cortex function, a novel object recognition test (NOR) was performed, while short-term spatial memory, associated with hippocampal function, was assessed in the novel location recognition test (NLR), as described previously (Hovens et al., 2014b). After habituation to the test environment, the test battery consisted of three phases, each lasting 3 min, with 1 min in-between: For habituation, the animal was placed in the test box and allowed 3 min to explore the set-up; then the rat was presented two identical objects and allowed to explore those for 3 min. Subsequently, objects were removed and after 1 min placed back, but one of them in a different location than in the previous phase (NLR), followed by exploration for 3 min. Finally, after being removed for 1 min, the objects were presented again, but now one of the two identical objects was replaced by a different object and put in the same location as in the preceding phase (NOR). Between the phases, the objects were removed and cleaned with 70% ethanol to remove smell cues. After each animal, the test box and objects were also cleaned with 70% ethanol. Time spent exploring the objects was measured using Eline software (the University of Groningen, the Netherlands). Preference for the novel location or the novel object was calculated by dividing the time spent exploring the novel location or novel object by the time spent exploring both objects. Preference of 50% indicated chance level = no recognition. Results from rats that did not explore the objects or only one of them were excluded from further analyses.

2.3.4. Balance beam test

A balance beam test was conducted two times on separate days to analyze motor coordination (Oroszi et al., 2022a). A 150-cm long and 2-cm wide wooden slat was positioned horizontally at 1 m above the floor, and on one end connected to the home cage of the rat, as a target. On the first day, the rats were trained to walk across the suspended beam, at which the rats performed three trials [50 cm, 100 cm, and one full test (150 cm)]. After these three training trials, the rats performed three full test trials. On the second day, the rats performed also full three test trials. The average latency to reach the home cage was used as a measure of performance on the balance beam. Animals who were unable and/or unwilling to perform the test procedure were excluded from the final statistical analysis.

2.3.5. Grip strength test

The grip strength test was performed two times on separate days and included three trials per day (Oroszi et al., 2022a). Animals were held by their trunk and were guided to get grip on a suspended wood beam (2 mm in diameter, 35 cm in length, and 50 cm above the surface of the table) by their forepaws. Time until drop-off was recorded. During the three trials, the animals were rotated to offer time for recovery between the trials. The average of the six trials over the 2 days was utilized for statistical analysis.

2.4. Tissue collection and processing

At the end of the experiment, rats were anesthetized with 6% sodium pentobarbital solution and injected intraperitoneally (2 ml/kg). Rats were transcardially perfused with heparinized (1 ml/l) 0.9% saline until the liver turned pale. The right gastrocnemius and soleus muscles as well as the heart and the brain were dissected and weighed. The brain and the heart tissues were immersion fixated in 4% buffered formaldehyde freshly depolymerized with paraformaldehyde. After 4 days, tissues were washed in 0.01 M phosphate-buffered saline (PBS), dehydrated using a 30% sucrose solution, and subsequently quickly frozen in liquid nitrogen and stored at −80°C until 25 μm coronal sections were cut using a microtome. Heart sections and three brain sections were placed on glass immediately after cutting and processed for histochemical staining. The remaining brain sections were stored free-floating

in 0.01 M (PBS) containing 0.1% sodium azide at 4°C till further processing for (immuno)histochemistry.

2.5. (Immuno)histochemistry

2.5.1. Microglia

To visualize microglia, immunohistochemical staining of ionized calcium binding adaptor molecule 1 (IBA-1) was performed, as described in detail previously (Hovens et al., 2014a). In brief, after incubation for 3 days with 1:2,500 rabbit-anti IBA-1 (Wako, Neuss, Germany) in 2% bovine serum albumin, 0.1% triton X-100 at 4°C, followed by a 1 h incubation with 1:500 goat-anti-rabbit secondary antibody (Jackson, Wet Grove, USA) at room temperature, sections were then incubated for 2 h with avidin–biotin–peroxidase complex (Vectastain ABC kit, Vector, Burlingame, USA) at room temperature. Labeling was visualized by using a 0.075 mg/mL diaminobenzidine (DAB) solution activated with 0.1% H_2O_2. Sections were mounted onto glass slides and photographs were taken from the dorsal hippocampus (hippocampus) at 200 times magnification (Toth et al., 2022b). Microglia morphology was analyzed in the different areas of the dorsal hippocampus: CA1, CA3, dentate gyrus (DG), and hilus, according to our previous publication (Hovens et al., 2014a), regarding coverage, density, cell size, cell body area, and processes area. Microglia activity was calculated as cell body area/total cell size (Hovens et al., 2014a).

2.5.2. Brain-derived neurotrophic factor

For brain function, brain slices were stained for brain-derived neurotrophic factor (BDNF), as described previously (Hovens et al., 2014b). In brief, sections were blocked for 1 h with 5% normal goat serum, then incubated with 1:1,000 rabbit-anti BDNF antibody (Alomone Labs, Israel) in 1% BSA, followed by incubation with 1:5,000 goat-antirabbit secondary antibody (Jackson, Wet Grove, USA). Photographs were taken at 50× magnification (Toth et al., 2022b) from the different areas of the dorsal hippocampus, CA1, CA3, DG, and hilus, and BDNF expression was obtained as corrected optical density (Image-J) compared to an underlying reference area (Hovens et al., 2014b).

2.5.3. Collagen

In the heart, the percentage of collagen was used to measure cardiac damage. For that, 25 μm thick transverse slices at mid-ventricular and apex levels of the heart were stained with Sirius red (Sigma, Aldrich) and Fast green as counterstaining (Hovens et al., 2016). Color pictures were taken and enlarged to cover the complete left ventricle in the image analyses screen. Image analysis (Image Pro Plus, USA) was used to measure the collagen-positive (red) area and was expressed as a percentage of the total left ventricular tissue area. Since WBV may also affect collagen in other organs than the heart, a similar Sirius red/Fast green stain was performed on three brain sections that were immediately placed on glass, using the same procedure as was used for the cardiac sections.

Because of a positive collagen signal observed in the hippocampus in this pilot, for a subgroup of rats randomly selected from the experimental groups, free-floating sections were stained with Sirius red (without Fast green), after thoroughly washing (two times daily for 4–5 days) to remove the azide. Photographs were taken from the granular layers of the dorsal hippocampus (CA1, CA2, CA3, DG, and hilus; 100× magnification), and collagen expression was obtained as corrected optical density, compared to the underlying reference area (Image-J).

2.6. Data analyses

Data are presented as mean ± 95% confidence interval (CI; figures) and standard error of the mean (SEM; tables). Results more than two times the standard deviation of its group were considered outliers and were excluded before analyses (maximally one per experimental group). Results were compared using a two-way analysis of variance (ANOVA) with a least square difference (LSD) *post-hoc* test, with saline/ISO and sedentary/runner/whole-body vibration as factors. Association between selected parameters was measured with Pearson linear correlation. For the novel object/novel location recognition tests, outcomes were also tested against chance level (=50%), using a single sample *t*-test. A *p*-value of <0.05 was considered statistically significant and is denoted as *. Potentially relevant tendencies ($p < 0.1$) were mentioned as well.

3. Results

3.1. WBV exposure time–effect pilot

To obtain insight into the effects of different exposure times of WBV in order to find an effective protocol to compare with the active exercise in our study, a pilot exposure time—effect study on behavior was performed. The results are presented in Figure 2. We tested daily for 5, 10, or 20 min against pseudo-treatment (=0 min). Five and ten minutes of WBV per day had similar effects, whereas the effects of 20 min per day appeared deviant. Daily 5 or 10 min of WBV significantly reduced anxiety-like behavior in the OF, without effects on short-term memory and balance beam performance, while muscle strength measured as grip hanging was slightly improved. From these results, we chose 10 min of WBV per day to use in our main study.

3.2. Main study

3.2.1. General

Mortality in the ISO group was 26% (10 out of 39 rats), whereas none of the saline-treated rats died. At the start of the experiment, rats weighed on average 249 ± 3 g, with no differences between the experimental groups. Before killing, the rat's body weight was, on average, 251 ± 3 g. Body weights and organ weights corrected for body weight are presented in Table 1. Neither body weight nor relative organ weights were different between the experimental groups.

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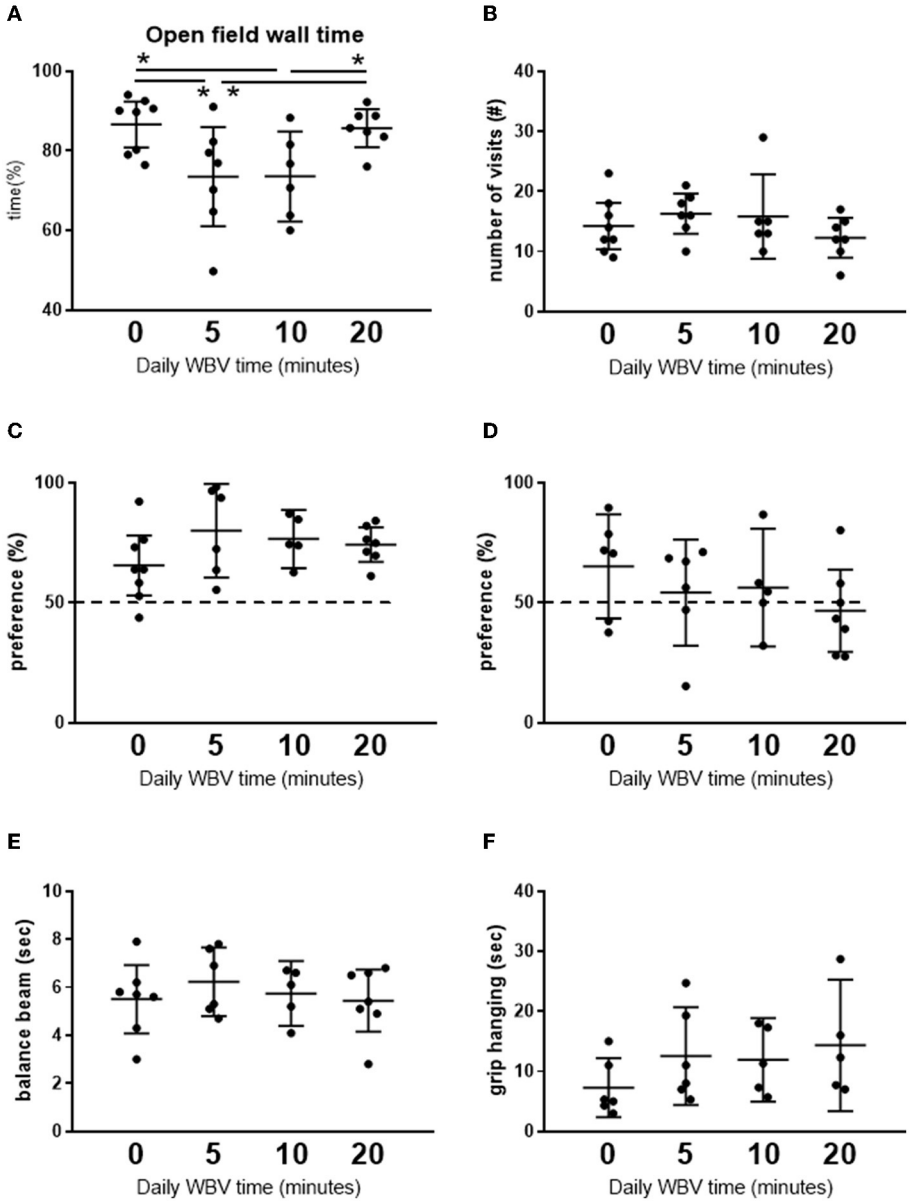

FIGURE 2
(A, B) Results from the open field test. **(C, D)** Short-term memory in the NOR and NLR test, respectively. **(E, F)** Effects on motor function. Results from the pilot exposure time-effect study on the different whole-body vibration (WBV) session times ($n = 5–8$ per group). Rats were subjected to 5 weeks daily (5 days a week) WBV for 0 (pseudo), 5, 10, or 20 min. Behavioral responses were obtained according to descriptions in the methods section. *Significant effect ($p < 0.05$).

3.2.2. Cardiac collagen

The percentage of collagen in the left ventricle measured at mid-ventricular and apex levels was used as a measure of cardiac damage (Figure 3). Two-way ANOVA revealed a significantly elevated collagen percentage after ISO compared to saline-treated rats at both ventricular levels (middle: $p = 0.003$; apex: $p = 0.002$), and a significant intervention effect ($p = 0.040$) and interaction effect ($p = 0.024$) only at the mid-ventricular level. *Post-hoc* analyses indicated that the fibrotic effect of ISO, seen in the sedentary rats (middle: $p = 0.008$; apex: $p = 0.001$), was absent after running, but not after WBV. In fact, WBV in ISO rats tended to even further increase collagen (mid-ventricular level; $p = 0.052$). However, the effect of running may at least in part be attributed to increased collagen in the control saline-treated rats, since running ($p = 0.030$), and to a lower extent WBV (ns), by itself already increased cardiac collagen.

3.2.3. Hippocampal collagen

Figure 4A shows photographs of the dorsal hippocampus (dentate gyrus), stained according to the same protocol as had been used for cardiac sections; Sirius red/Fast green. The

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TABLE 1 Body weight (bw) and relative organ weights before killing in the different experimental groups.

	Sed saline	Sed ISO	Run saline	Run ISO	WBV saline	WBV ISO
n	8	9	8	10	9	10
Body weight (g)	253 ± 7	247 ± 4	258 ± 12	254 ± 7	250 ± 10	245 ± 7
Heart weight (%bw)	0.38 ± 0.02	0.40 ± 0.01	0.40 ± 0.02	0.39 ± 0.02	0.38 ± 0.01	0.40 ± 0.01
Brain weight (%bw)	0.78 ± 0.02	0.79 ± 0.02	0.75 ± 0.03	0.77 ± 0.02	0.79 ± 0.03	0.79 ± 0.01
Soleus weight (%bw)	0.084 ± 0.004	0.081 ± 0.003	0.080 ± 0.003	0.076 ± 0.003	0.081 ± 0.002	0.083 ± 0.005
Right gastronemicus weight (%bw)	0.58 ± 0.01	0.60 ± 0.01	0.59 ± 0.02	0.60 ± 0.01	0.60 ± 0.01	0.58 ± 0.02

Sed, sedentary; ISO, isoproterenol; Run, runner; WBV, whole-body vibration.
No significant effects of ISO vs. saline, nor interventions were observed.

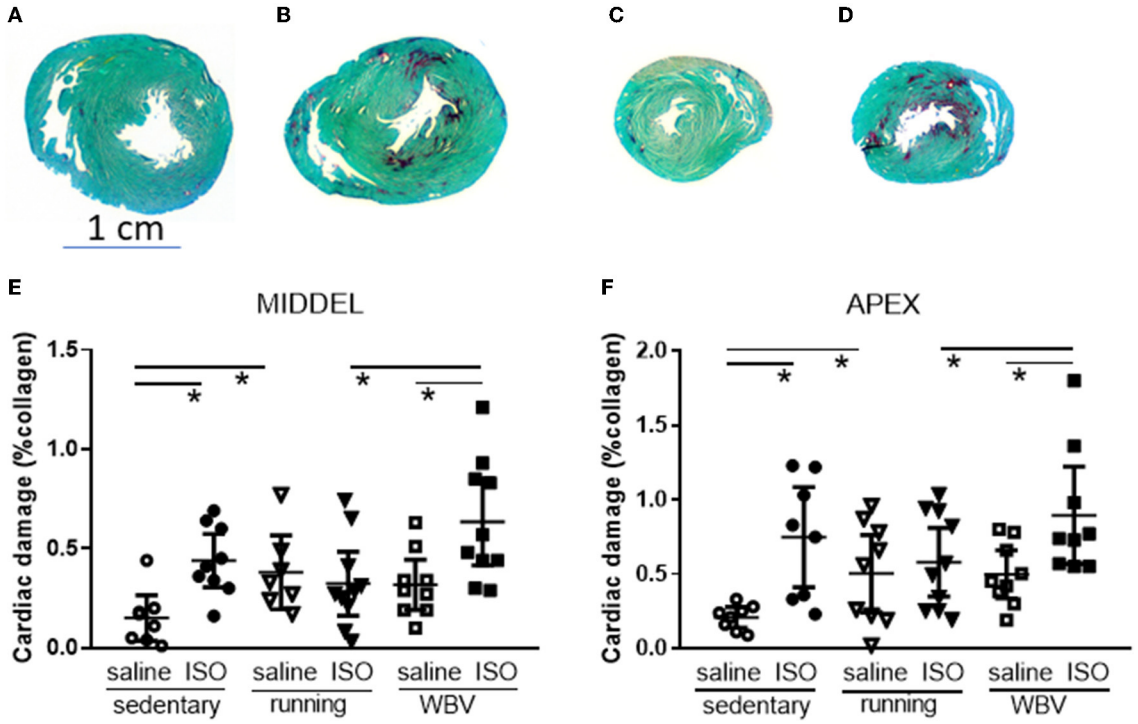

FIGURE 3
Presentation of midventricular and apex sections stained for Sirius red (red) and Fast green (green), from sedentary saline [**(A, C)**, respectively] and isoproterenol whole-body vibration-treated rats [**(B, D)**, respectively], showing increased Sirius red positive areas after ISO treatment. Lower panels: actual measurements of the percentage of collagen in the left ventricle as Sirius Red positive area at mid-ventricular **(E)** and apex level **(F)**, in saline or isoproterenol (ISO)-treated rats, under sedentary conditions, or after 5 weeks of exercise training (running) or whole-body vibration (vibration). *Significant difference between indicated groups ($p < 0.05$).

photograph showed that in addition to the expected positive staining (red) of blood vessel walls and the fibrous tissue of the choroid plexus, the granular layers (containing mostly the neuronal soma) in the hippocampus also stained positive for collagen. The Sirius red/Fast green staining protocol for sections collected directly on the glass, however, could not be used for free-floating sored sections, potentially due to the presence of azide in the storage solution. Therefore, in order to visualize collagen in the hippocampus in the sections from our experimental groups, 4–5 days of daily washing appeared to be necessary to obtain a sufficient signal-to-noise ratio, and quantification was obtained from optical density (Figures 4B, C). The results of the measurement were shown in the lower part of the figure. While the hippocampus (Figure 4I) overall did not show statistically significant differences between the experimental groups regarding collagen, effects seemed to differ locally. In the CA1 (Figure 4D), DG (Figure 4G), and hilus areas (Figure 3H), no differences were observed between groups. However, in the CA2 area (Figure 4E), the saline-treated WBV rats appeared to show consistently increased collagen expression,

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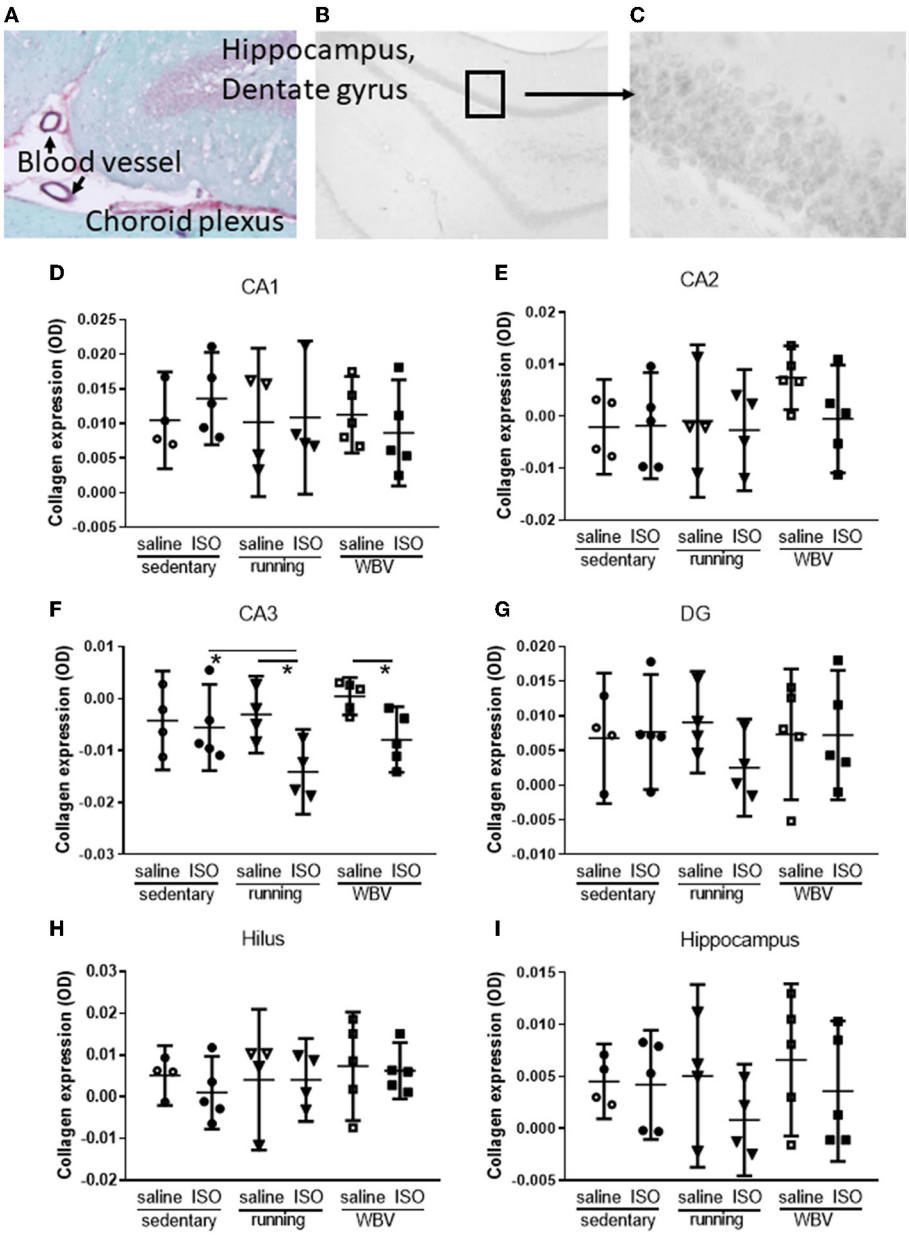

FIGURE 4
(A) Example of hippocampal dentate gyrus stained with Sirius red (red) and Fast green (green), showing positive staining for collagen (red) in blood vessel walls, fibrous tissue of the choroid plexus, and the granular layer of the dentate gyrus, indicating the presence of collagen in brain tissue (50× magnification). **(B)** A typical example of a black and white photograph of a section stained with Sirius red only (magnification 20×). **(C)** Detail of **(B)** as was used to quantify gray values (100 × magnification). **(D–H)** Actual measurements of optical density in the different areas of the hippocampus, as well as for the whole hippocampus **(I)** in saline- or isoproterenol (ISO)-treated rats, under sedentary conditions ($n = 4$ and $n = 5$, respectively), or after 5 weeks of exercise training (running; $n = 4$ each) or whole-body vibration (vibration; $n = 5$ each). *Significant difference between indicated groups ($p < 0.05$).

whereas, in all other groups, virtually no expression was observed. Although this did not result in significant differences between groups in the CA2, differences reached statistical significance ($p = 0.011$) in the CA3 area (Figure 4F). Surprisingly, running ($p = 0.007$) as well as WBV ($p = 0.019$) caused a significant decline of collagen in ISO-treated, compared to saline-treated, rats. Moreover, running after ISO significantly declined collagen expression ($p = 0.023$) compared to sedentary ISO rats.

3.2.4. Hippocampal microglia activity and BDNF expression

Effects on neuroinflammation were obtained from morphological changes in microglia represented by increased cell body-to-cell size ratio and microglia activity (Figures 5A, C). No significant differences due to either ISO treatment or running/WBV interventions were observed in the overall hippocampal microglia activity score. Similarly, no effects of ISO or intervention were observed on average hippocampal BDNF

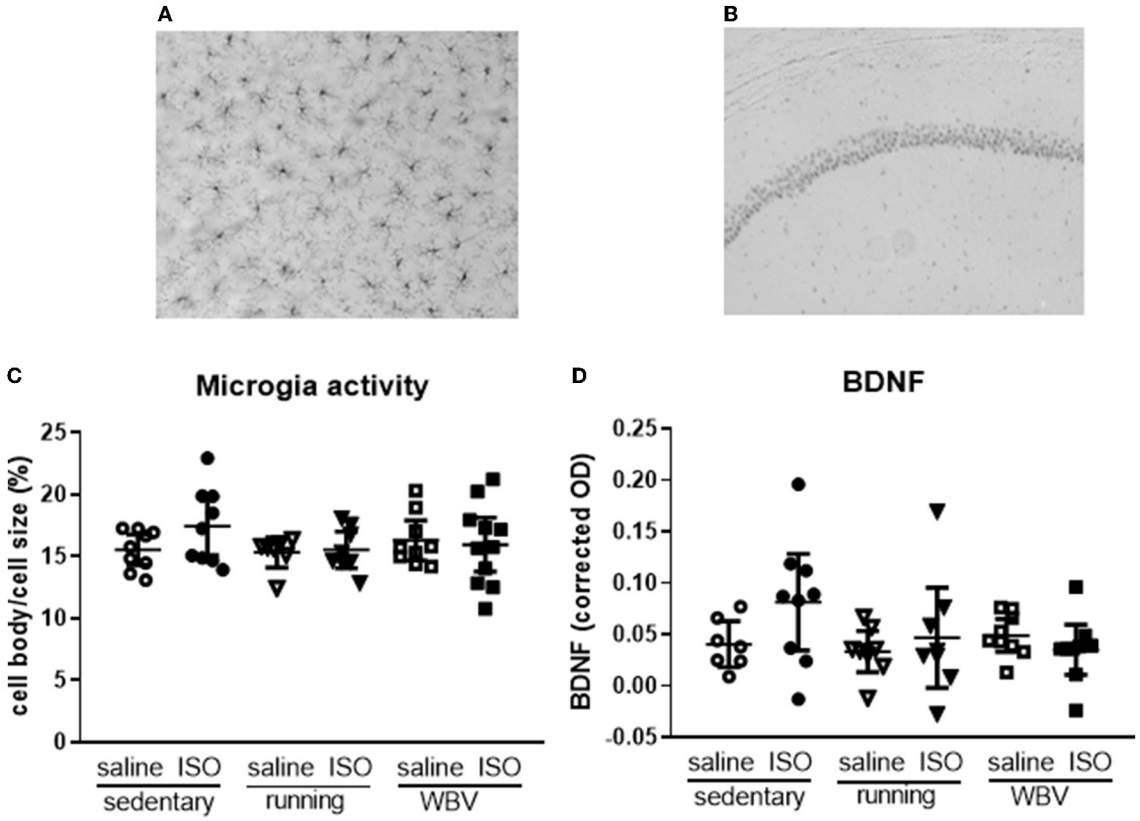

FIGURE 5
Typical pictures of IBA-1—**(A)** (200× magnification) and BDNF staining **(B)** (50× magnification) from the CA1 region of the hippocampus. Mean hippocampal microglia activity **(C)** and BDNF expression **(D)** in saline- or isoproterenol (ISO)-treated rats, under sedentary conditions ($n = 8$ and $n = 9$, respectively), or after 5 weeks of exercise training (running; $n = 6–8$ and $n = 8$, respectively) or whole-body vibration (vibration; $n = 8–9$ and $n = 9$, respectively).

expression (Figure 5D). However, within the hippocampus, the different regions responded differently as described in Sections 3.2.5 and 3.2.6, respectively, and illustrated in Figures 6, 7.

3.2.5. Hippocampal neuroinflammation

Analyzing the different areas within the hippocampus (Figure 6), two-way ANOVA revealed significant differences between groups in the CA1 ($p = 0.010$), CA3 ($p = 0.013$), and DG ($p = 0.003$), but not in the hilus area. In the CA1 ($p = 0.022$), CA3 ($p = 0.011$), and DG ($p = 0.001$), differences could be attributed to a significant effect of the intervention (sedentary, running, or WBV); in the CA3 area, this effect appeared on top of a significant ISO/saline effect ($p = 0.023$). *Post-hoc* testing revealed a significant increase in microglia activity due to ISO in the CA1 ($p = 0.011$), no effect in the DG, and a significant decline in activity in the CA3 ($p = 0.010$). In both the CA1 and CA3 areas, effects of ISO were normalized by WBV as well as running exercise (CA1: $p = 0.001$ and $p = 0.007$, for WBV and running exercise, respectively; CA3: $p = 0.050$ and $p = 0.007$, for WBV and running exercise, respectively). In the DG, WBV seemed to activate microglia in saline ($p = 0.050$) as well as ISO-treated rats ($p = 0.014$). Since the microglia activity (cell body to total cell size ratio) resulted from a calculation based on measured morphological features, effects on the underlying parameters are presented in Table 2. The opposite responses of the CA1 and CA3 microglia activity to ISO, as described in Figure 6, seemed reflected in the underlying morphological parameters. In the CA1, total cell size declined after ISO, due to loss of processes area with a (ns) increase in cell body size, while running, and to a lesser extent WBV, increased cell size by increasing processes size. In the CA3, cell size as well as processes tended to increase ($p < 0.1$), while cell bodies declined in size, resulting in reduced microglia activity after ISO in this area. WBV and exercise after ISO seemed to normalize these parameters. The DG and hilus microglia were not so much altered after ISO. WBV, but not exercise, seemed to reduce cell size and process size, resulting in higher microglia activity (Figure 6).

3.2.6. Hippocampal BDNF

Brain-derived neurotrophic factor expression was used as a measure of neuronal function (Figures 5B, D, 7). Although overall hippocampal BDNF expression appeared not significantly different between groups (Figure 5D), results presented in Figure 7 suggested regional differences within the hippocampus. Two-way ANOVA showed significant differences in the CA1 area ($p = 0.030$), reflected in a significant effect of interventions ($p = 0.025$), which

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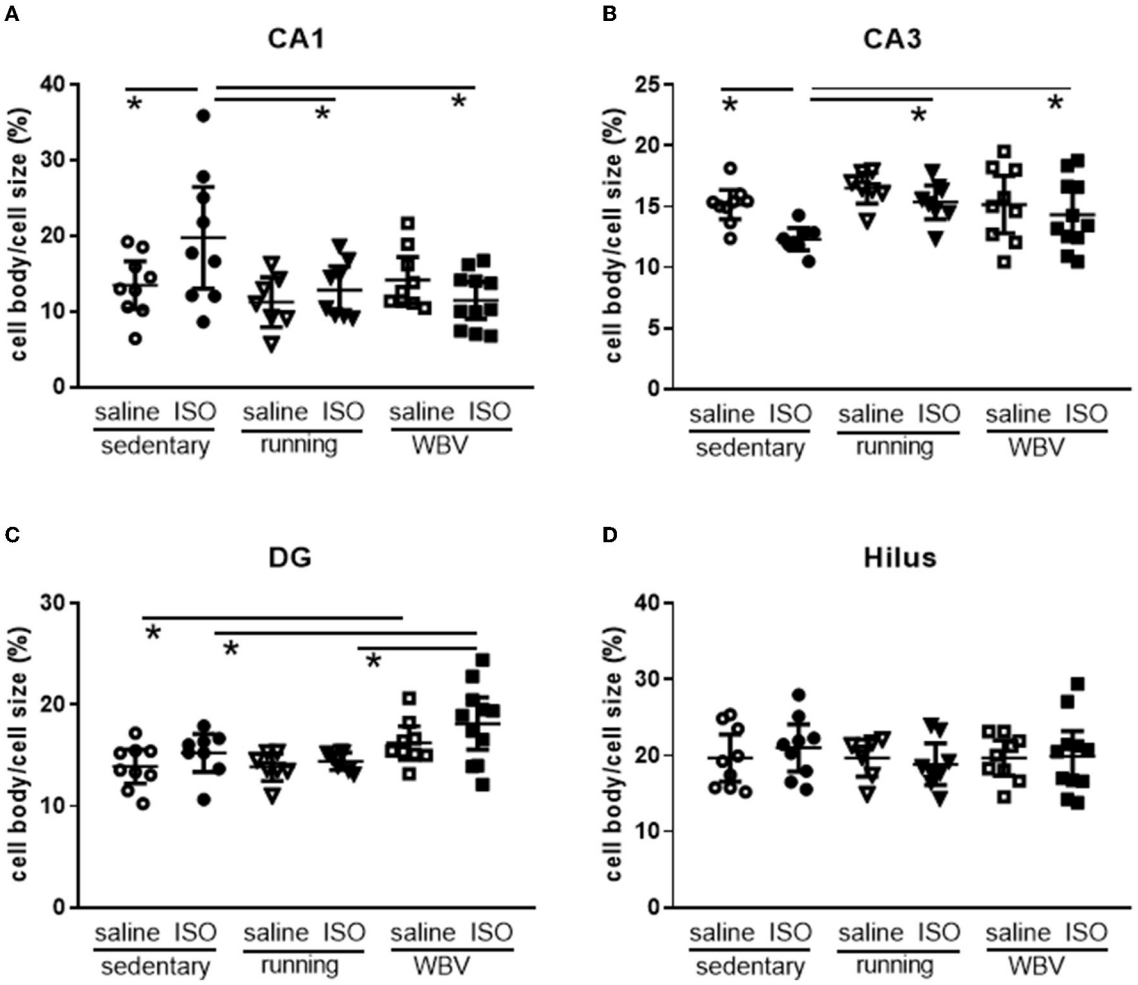

FIGURE 6
(A) CA1, **(B)** CA3, **(C)** DG, and **(D)** Hilus. Measurement of microglia activity as cell body/cell size in the different areas of the hippocampus in saline- or isoproterenol (ISO)-treated rats, under sedentary conditions ($n = 8$ and $n = 9$, respectively), or after 5 weeks of exercise training (running; $n = 6$ and $n = 8$, respectively) or whole-body vibration (vibration; $n = 8$ and $n = 10$, respectively). *Significant difference between indicated groups ($p < 0.05$).

resulted in ISO causing a significantly increased BDNF expression ($p = 0.043$), that was reversed by running exercise ($p = 0.003$) as well as by WBV ($p = 0.013$) in *post-hoc* testing (Figure 7). Similar effects were observed in the DG and hilus areas; DG: a trend toward differences between groups ($p = 0.071$), resulting in significantly increased expression after ISO ($p = 0.050$), which was reversed by both running exercise ($p = 0.043$) as well as WBV ($p = 0.005$); hilus: significant differences between groups (two-way ANOVA: $p = 0.020$), with a trend toward effects of interventions ($p = 0.055$) and significant interactions between effects of ISO and interventions ($p = 0.034$). ISO significantly increased BDNF expression ($p = 0.018$), which was reversed by running exercise ($p = 0.003$) as well as WBV ($p = 0.002$). In the CA3 area, no significant differences were observed.

No correlations were observed between microglia activity and BDNF expression in the overall hippocampal data, nor in any of the hippocampal areas. Neither microglia activity nor BDNF expressions were significantly associated with collagen expression in the specific hippocampal areas.

3.2.7. Behavior

Levels of exploration and anxiety were obtained from behavior in the OF. Regarding exploration, a tendency for differences in locomotion activity between the groups was observed ($p = 0.07$), resulting from a significant effect of interventions ($p = 0.012$). *Post-hoc* analyses indicated that WBV rats displayed reduced locomotion (sedentary saline: 26 ± 2; sedentary ISO: 28 ± 2; sedentary running: 25 ± 4; running ISO: 27 ± 2; sedentary WBV: 19 ± 2; WBV ISO: 21 ± 3 crossings per 5 min). No differences between groups, regarding ISO/saline, interventions, or interactions, were observed for the time rats spent in the relatively safe area and the wall. For the number of center visits, ANOVA revealed a tendency for differences between groups ($p = 0.10$), which could be attributed to a significant effect of the intervention ($p = 0.020$), as no effect of ISO vs. saline nor interactions were observed. ISO rats with WBV showed a reduction in center visits ($p = 0.025$; Figures 8A, B). Effects on cognition were measured as short-term memory in the NOR and NLR tests (Figures 8C, D). All rat groups could recognize

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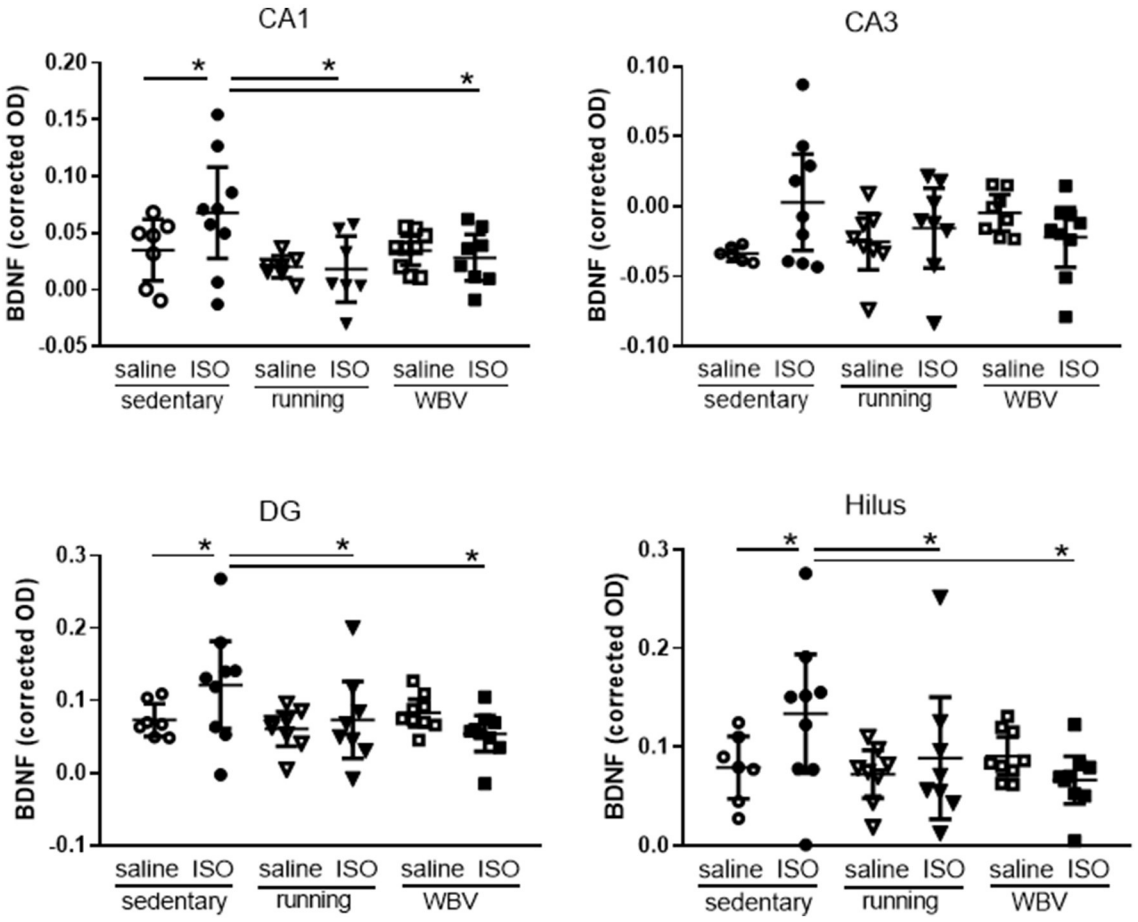

FIGURE 7
Expression of brain-derived neurotrophic factor (BDNF) in the different areas of the hippocampus in saline- or isoproterenol (ISO)-treated rats, under sedentary conditions ($n = 7$ and $n = 9$, respectively), or after 5 weeks of exercise training (running; $n = 8$ each) or whole-body vibration (vibration; $n = 9$ each). *Significant difference between indicated groups in *post-hoc* analyses ($p < 0.05$).

the novel object in the NOR, as they all performed significantly above the chance level. However, no difference in performance between the groups was observed. Similarly, in the NLR, no differences between groups were seen, but here only saline runners and WBV ISO rats performed above the chance level. Motor performance was obtained from the balance beam test and the grip hanging test, presented in Figures 8E, F. Balance beam performance appeared similar in all groups. In the grip hanging test, the saline-treated runners stood out, as they seem to perform better than all other groups. No significant associations between behavioral parameters and microglia activity or BDNF expression were observed.

4. Discussion

4.1. General

Myocardial infarction is often associated with mental disorders, including depression and cognitive decline, with female patients being more susceptible than male patients. Exercise training may provide a rational therapeutic approach for this comorbidity, due to its known beneficial effects on physical as well as mental conditions. However, physical exercise may not be appreciated shortly after myocardial infarction. WBV could provide a passive alternative to exercise in this condition (Alam et al., 2018). The present study aimed to explore WBV compared to physical exercise in female rats after ISO-induced myocardial damage. Exercise, but not WBV, reversed cardiac damage. Surprisingly, collagen was also expressed in the hippocampus and a reduced expression was found after exercise as well as after WBV in the hippocampal CA3 of ISO-treated rats. Effects of ISO on microglia activity varied from increased (CA1), no difference (DG and hilus), to decreased (CA3). Both exercise and WBV normalized the effects of ISO. The ISO-induced elevated BDNF expression was no longer present after exercise or WBV. These WBV effects were associated with less locomotion and lower interest in the center area of the OF, but preserved short-term spatial memory in ISO-treated rats; an effect that was not observed after exercise. On the contrary, exercise, but not WBV, seemed to improve grip strength. Results indicate that apart from the lack of effect on cardiac collagen, the effects of WBV appeared quite comparable to exercise, indicating that WBV may provide a valuable alternative for patients who cannot perform physical

TABLE 2 Morphological parameters of microglia obtained in different hippocampal areas: CA1, CA3, dentate gyrus (DG), and hilus.

Experimental group/hippocampal area	Sedentary saline	Sedentary ISO	Runner saline	Runner ISO	Vibration saline	Vibration ISO
n	8	9	6	8	8	10
CA1						
Density (#/area)	63.6 ± 3.1	57.9 ± 4.1	65.0 ± 3.5	67.4 ± 5.4	58.1 ± 5.3	55.6 ± 4.2
Coverage (%)	4.4 ± 0.5	3.4 ± 0.2	5.1 ± 0.6	3.9 ± 0.3	4.2 ± 0.6	3.5 ± 0.3
Cell size (px)	760 ± 59	**605 ± 41$^{\#}$**	**899 ± 81***	**702 ± 35$^{\#}$**	**717 ± 28^{+}**	686 ± 24
Cell body size (px)	88 ± 7	97 ± 9	86 ± 4	78 ± 6	88 ± 6	**68 ± 5$^{*\#}$**
Processes size (px)	685 ± 61	**530 ± 42$^{\#}$**	822 ± 85	**630 ± 35$^{\#}$**	**636 ± 27^{+}**	607 ± 25
CA3						
Density (#/area)	68.9 ± 3.0	69.2 ± 4.1	**56.3 ± 3.2***	**67.3 ± 2.7$^{\#}$**	59.7 ± 3.0	**59.0 ± 3.6***
Coverage (%)	3.7 ± 0.2	3.8 ± 0.3	3.2 ± 0.2	**4.0 ± 0.2$^{\#}$**	3.2 ± 0.2	**3.7 ± 0.2$^{\#}$**
Cell size (px)	629 ± 16	688 ± 28	596 ± 23	**592 ± 23***	627 ± 26	**570 ± 17***
Cell body size (px)	95 ± 4	**82 ± 2$^{\#}$**	98 ± 4	91 ± 4	93 ± 4	**81 ± 4$^{\#}$**
Processes size (px)	542 ± 15	602 ± 23	516 ± 23	**523 ± 21***	541 ± 29	**513 ± 19***
DG						
Density (#/area)	70.3 ± 3.5	68.8 ± 4.9	66.3 ± 3.2	66.4 ± 3.0	64.7 ± 4.5	61.3 ± 4.1
Coverage (%)	3.9 ± 0.1	3.8 ± 0.2	**3.2 ± 0.3***	3.6 ± 0.2	**3.0 ± 0.2***	3.3 ± 0.2
Cell size (px)	629 ± 17	598 ± 14	627 ± 14	650 ± 7	599 ± 26	**548 ± 20$^{\#+}$**
Cell body size (px)	87 ± 4	100 ± 7	87 ± 5	93 ± 2	97 ± 5	98 ± 5
Processes size (px)	554 ± 17	532 ± 22	540 ± 13	541 ± 26	504 ± 26	**470 ± 20^{*+}**
Hilus						
Density (#/area)	56.8 ± 3.1	56.0 ± 4.4	46.6 ± 2.8	57.1 ± 4.1	**37.9 ± 3.3***	**48.6 ± 3.1$^{\#}$**
Coverage (%)	4.3 ± 0.3	4.6 ± 0.3	3.9 ± 0.3	4.3 ± 0.3	4.5 ± 0.4	4.6 ± 0.3
Cell size (px)	512 ± 21	491 ± 28	545 ± 29	483 ± 26	506 ± 27	482 ± 20
Cell body size (px)	99 ± 5	94 ± 6	106 ± 6	90 ± 5	99 ± 6	94 ± 5
Processes size (px)	420 ± 23	378 ± 13	425 ± 35	388 ± 27	411 ± 24	400 ± 23

ISO, isoproterenol.
Significant effects ($p < 0.05$) are presented in bold.
*Significant effect of the intervention (WBV or running) compared to sedentary within the same pre-treatment (ISO or saline).
$^{\#}$Significant effect of ISO compared to saline within the same intervention.
$^{+}$Significant differences between WBV and running within the same pre-treatment.

exercise. However, more research on the optimal WBV protocol is necessary.

4.2. WBV vs. exercise

4.2.1. General

In the present study, WBV was evaluated as an alternative to physical exercise after myocardial damage, as WBV and physical exercise share effects on the body, such as improved muscle strength (Annino et al., 2017, 2021; Beaudart et al., 2017), bone density (Slatkovska et al., 2010; Benedetti et al., 2018), and wound healing (Zhou et al., 2016; Wano et al., 2021), as well as on the brain, including neurotrophic factors (Raval et al., 2018; Mee-Inta et al., 2019) and cognitive improvement (Keijser et al., 2017; Boerema et al., 2018; Cardoso et al., 2022). As reviewed by Alam et al. (2018), WBV is regarded as a neuromuscular training method, that could be used as an alternative to conventional training. Moreover, WBV was shown to reduce brain damage and brain inflammatory markers, with increased BDNF and improved functional activity after transient brain ischemia in middle-aged female rats (Raval et al., 2018).

4.2.2. Effects on the heart

As anticipated from our previous study in female rats (Toth et al., 2022a), ISO increased cardiac collagen levels. Although

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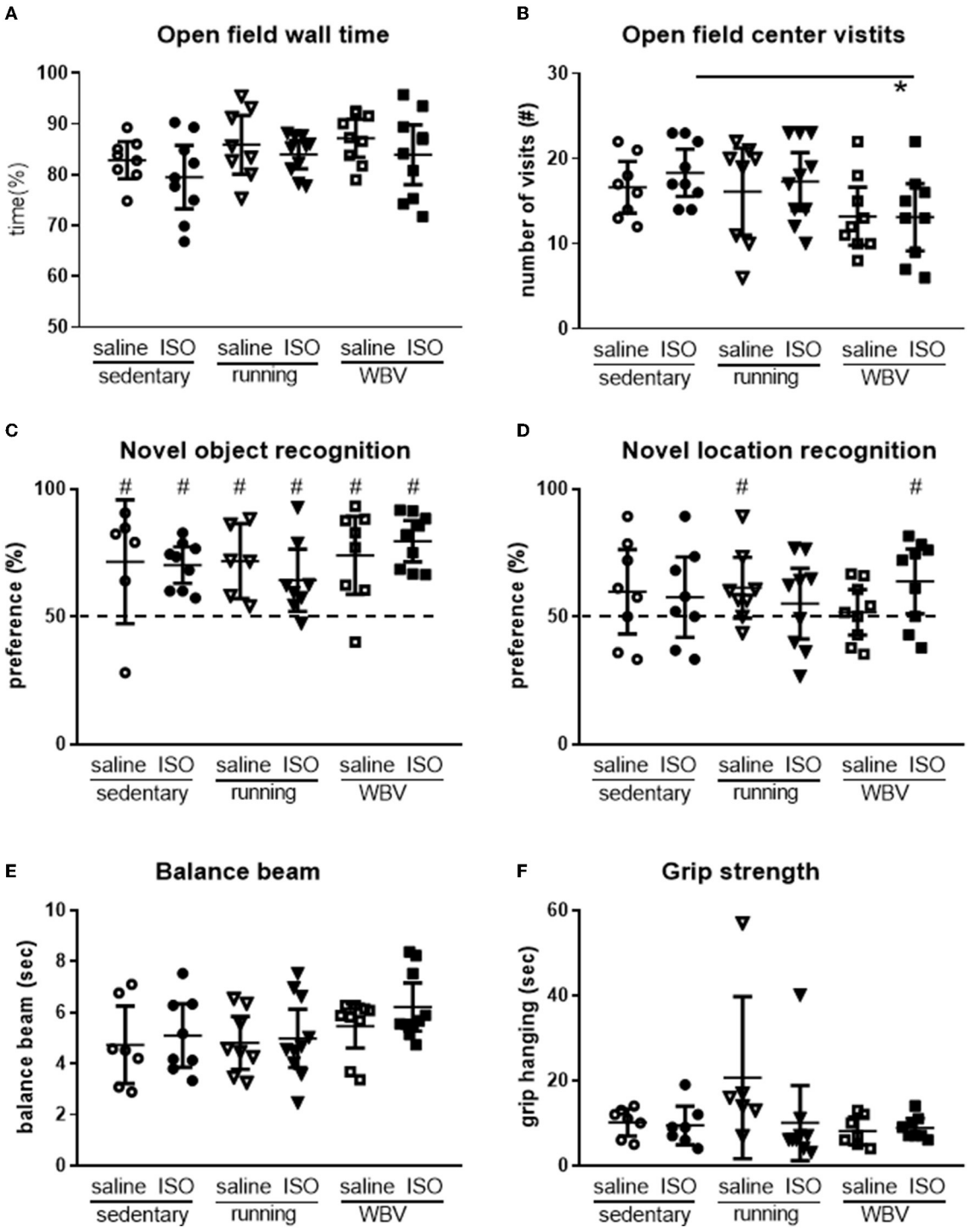

FIGURE 8
Exploration in the open field ($n = 8–10$ per group) was measured as time spent along the wall **(A)** and the number of center visits **(B)**, cognitive performance measured in the novel object- [**(C)**; $n = 6–10$ per group] and the novel location recognition [**(D)**; $n = 8–10$ per group] tests, and motor performance measured by the time rats needed to cross the balance beam [**(E)**; $n = 7–10$ per group] and the time they could keep their grip on the hanging bar (**F**; $n = 6–9$ per group) in saline- or isoproterenol (ISO)-treated rats, under sedentary conditions, or after 5 weeks of exercise training (running) or whole-body vibration (vibration). *Significant difference between indicated groups ($p < 0.05$); $^{\#}$significantly above chance level (dashed line = 50% = chance level).

exercise by itself may slightly increase cardiac collagen in saline-treated rats, potentially by increasing fibroblast growth factor 21 (Ma et al., 2021), the ISO-induced cardiac fibrosis was completely reversed. WBV, however, seemed to even exaggerate ISO-induced cardiac fibrosis. In contrast, WBV was reported to increase tolerance to ischemia-reperfusion injury by reducing

infarct size in rats (Shekarforoush and Naghii, 2019). On the contrary, in patients, although the significant improvement was seen after the standard exercise rehabilitation program, no extra effects of WBV were observed (Nowak-Lis et al., 2022). Both studies, however, were performed only on male subjects. In our previous studies, we showed different responses to ISO in male and female rats, whereas in male rats, ISO increased cardiac collagen, but no effect of exercise was observed (Toth et al., 2021), in female rats of the same age, exercise significantly reduced the ISO-induced cardiac fibrosis (Toth et al., 2022a). Hence, in our middle-aged female rats, exercise, but not WBV, was capable of reversing cardiac damage due to ISO treatment.

4.2.3. Effects on the brain

4.2.3.1. Hippocampal collagen

In addition to the expected expression in brain blood vessel walls and meninges, collagen expression was observed in the granular layers of the hippocampus, where it may reflect extracellular matrix components. The presence of a neuronal cell surface feature, called perineuronal net (PNN), is consistent with a brain extracellular matrix (Bonneh-Barkay and Wiley, 2009). This PNN mainly covers the cell body and dendrites, is usually associated with neuroprotection, and plays an important role in learning, memory, and information processing in health and disease (Krishnawamy et al., 2021). More specifically, it may affect synaptic morphology and function. Loss of PNN is often observed in neurodegenerative diseases, as reviewed by Bonneh-Barkay and Wiley (Bonneh-Barkay and Wiley, 2009). Moreover, PNNs around hippocampal interneurons can resist destruction by activated microglia (Schuppel et al., 2002).

Although WBV often has been associated with increased collagen in the peripheral body, to the best of our knowledge, no literature is available for effects on brain (hippocampal) collagen. In the present study, no effects of ISO treatment or subsequent intervention with running or WBV were observed in the hippocampal CA1, DG, and hilus areas. However, although not statistically significant, WBV caused a consistent increase in collagen expression in the CA2 in saline-treated rats, but not in ISO-treated rats. In the hippocampal CA2 area, the PNN is known to play a role in restricting synaptic plasticity (Carstens et al., 2016). In the CA3 area, running as well as WBV decreased collagen expression in ISO treated rats. If indeed collagen levels might reflect PNN and restrict synaptic plasticity, as described for the CA2 (Carstens et al., 2016), the reduction of collagen by exercise and WBV in ISO-treated rats may then be speculated as an improvement of synaptic plasticity. Alternatively, it may still point to the loss of neuroprotection (Krishnawamy et al., 2021).

4.2.3.2. Neuroinflammation

Exercise (Petersen and Pedersen, 2005) and WBV (Jawed et al., 2020; Sanni et al., 2022) are associated with anti-inflammatory effects. This anti-inflammatory property may extend to neuroinflammation (Mee-Inta et al., 2019; Chen et al., 2022; Oroszi et al., 2022a). Based on the literature, WBV may affect neuroinflammation in female rats (Raval et al., 2018) as well as in male rats (Oroszi et al., 2022a). However, neither in male (Toth et al., 2021) nor in female rats (Toth et al., 2022a), exercise affected microglia activity after ISO. In the present study, overall hippocampal microglia activity was neither affected by ISO, nor by the interventions. However, the areas within the hippocampus showed regional differences; whereas in the CA1, indeed microglia activation was observed after ISO, and was completely reversed by exercise as well as WBV, in the CA3 area, ISO caused a decline of microglia activity, which was also completely reversed by exercise and WBV. While both the CA1 and CA3 areas are involved in learning and memory (Stevenson et al., 2020), the CA3 area is rather specifically involved in pattern completion (Stevenson et al., 2020). We did not perform behavioral testing to specifically explore the potential role of the CA3. For the CA1, the outcome of the NLR was not found to correlate with microglia activity. Therefore, a direct relationship between neuroinflammation and behavior could not be established here. Alternatively, different time courses for the different parameters, as seen before (Hovens et al., 2014b), may provide a potential explanation for the observed differences in effects in CA1 and CA3.

4.2.3.3. Neuronal function (BDNF)

Exercise training is usually associated with increased brain BDNF expression (Sleiman et al., 2016; El Hayek et al., 2019). Exercise-induced increased expression of BDNF and double cortin positive cells were observed in the ischemic hippocampus after stroke in rats (Luo et al., 2019; Cheng et al., 2020). WBV was shown to increase BDNF levels in the peri-infarct regions after brain ischemia-reperfusion in middle-aged female rats (Raval et al., 2018). Furthermore, WBV was demonstrated to reverse the decreased level of BDNF in the CA1 area of the hippocampus induced by chronic restrain stress in male rats (Peng et al., 2021). In male rats, ISO had no effects on hippocampal BDNF expression 6 weeks later, but running exercise significantly increased BDNF expression in the CA1 and hilus areas of the hippocampus (Toth et al., 2021). In contrast, in female rats, exercise after ISO seemed to decline BDNF expression in the CA1 area (Toth et al., 2022a). Accordingly, in the present study, the ISO-induced increases in BDNF in the sedentary rats were completely reversed by exercise as well as by WBV. Since most of the results of exercise-induced increases in BDNF expression were obtained in male subjects, the deviant results in female rats in the present study may well be attributed to sex dimorphism (Toth et al., 2022b).

4.2.4. Effects on behavior

4.2.4.1. Depression/anxiety

Although we anticipated cardiac damage-induced anxiety/depressive-like behavior in the ISO-treated rats (Tkachenko et al., 2018; Hu et al., 2020), in agreement with our previous study on middle-aged female rats (Toth et al., 2022a), ISO with and without exercise training had no effect on behavior in the OF. Similarly, at 24 months of age, no effect of ISO was observed in OF behavior in female rats, but anxiety-associated effects were seen in male rats (Toth et al., 2022b). However, although the lower number of center visits of the ISO-treated WBV rats in the present study may point to a higher level of

anxiety after WBV, no effect on wall time, as a measure for depressive-like behavior, was observed. The isolated reduction of center visits may therefore rather reflect the previously described reduction of arousal after WBV (Boerema et al., 2018), which would be further supported by the reduced OF locomotion of these rats (Oroszi et al., 2022a). Taken together, the results of the different studies indicated that neither ISO nor exercise affected OF behavior in female rats, but the effects of WBV on OF behavior remained inconclusive.

4.2.4.2. Cognition

The pilot exposure time–effect study did not show significant effects of either WBV schedule on short-term memory in the NOR and NLR tests. Similarly, in the main study, no effect of WBV (nor exercise) was observed in the results of the NOR. However, similar to our previous study on middle-aged female rats (Toth et al., 2022a), saline-treated exercise rats performed above chance level in the NLR, an effect that was not seen in the saline WBV rats. Whereas, exercise could not overcome poor performance in rats after ISO, WBV after ISO seemed to improve performance to above chance level.

4.2.4.3. Motor performance

No effects of ISO were observed on motor performance, tested either on the balance beam or in the grip hanging test. Exercise is generally accepted to improve motor performance (Hubner and Voelcker-Rehage, 2017) but actual effects may depend on the exercise protocol. Exercise by itself seemed to improve grip hanging, with large variation, however, but had no effect after ISO. Muscle weight was not increased by either exercise or WBV, indicating no significant effect of training. Several literature studies indicate a positive effect of WBV on muscle strength and motor coordination, although often in combination with regular physical training (Kawanabe et al., 2007; Annino et al., 2017, 2021). In the present study, no significant effect of WBV was seen on balance beam performance. Accordingly, in our pilot exposure time–effect study, no significant effects were observed on motor performance. However, 5 min of WBV already seemed to double grip hanging, without further increase with longer WBV exposure times. Studies in male mice showed that 5 min, but not 30 min, daily WBV improved motor performance (Keijser et al., 2017), and 5 min, but not 20 min, WBV improved rearing in the OF and grip hanging in male rats (Oroszi et al., 2022a). Moreover, in a recent study of our group in aged male and female rats (Oroszi et al., 2022b), behavioral effects of 10 min daily WBV appeared rather mild in female rats compared to male rats. Therefore, apart from male mice vs. female rats, the effects of WBV on motor performance may largely depend on the treatment protocol.

4.3. Limitations

The ISO model was used to avoid surgery-induced brain and behavioral changes that were observed after coronary artery ligation (Hovens et al., 2016). However, we are well aware that this provides a model for the consequences of cardiac damage, rather than its etiology of it. To compare the effects of WBV to exercise after ISO, the exercise protocol was based on our previous studies in male and female rats (Toth et al., 2021, 2022a) and the WBV protocol on our exposure time response pilot. Cardiac function measurements in the female rat study suggested a reduced cardiac performance after exercise, which may suggest a too-severe exercise protocol for these females. It raises the question of what parameter(s) should be optimal for the comparison of the two rather different interventions. As discussed in our previous study in female rats (Toth et al., 2022a), interventions started 1 week after ISO treatment, when inflammatory processes are merely complete (Alemasi et al., 2019). Accordingly, ISO induced cardiac damage, but that did not result in major effects on behavior 6 weeks later, leaving limited scope for improvement by either exercise or WBV. Exercise, but not WBV, reversed cardiac damage, but indeed neither did affect behavior. Nevertheless, in the present study, clear effects on local brain parameters were observed, providing a new potential entrée for treatment, specifically regarding the CA3 area and its associated functions, as this area stood out in the measured brain parameters.

5. Conclusion

The study aimed to explore WBV after ISO as an alternative to exercise, on the heart, the brain, and behavior in female rats. Although the female rats did not show the anticipated depressive-like behavior or cognitive decline after ISO, our data indicated regional effects on neuroinflammation and BDNF expression in the hippocampus, which were merely normalized by both WBV and exercise. Furthermore, collagen expression was observed in the granular layers of the hippocampus and appeared regionally specific and sensitive to exercise as well as WBV in ISO-treated rats. Therefore, apart from the potential concern about the lack of cardiac collagen reduction, WBV may provide a relevant alternative to physical exercise and be of help to (female) subjects that cannot or are not motivated to perform the exercise.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The experiments were conducted under the general license for animal experiments of the Laboratory of Physical Education, University of Budapest, Hungary.

Author contributions

KT: design of the study, acquisition of data, analyses and interpretation of data, and substantively revised the

manuscript. TO: performing behavioral studies, acquisition of data, and substantive revision of the manuscript. CN: conception, design of the study, and interpretation of data. EZ: interpretation of data and substantively revised the manuscript. RS: conception, design of the study, analyses and interpretation of data, drafting of the manuscript, and substantive revision of the manuscript. All authors contributed to the article and approved the submitted version.

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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.

The handling editor RT is currently organizing a Research Topic with the author EZ.

Publisher's note

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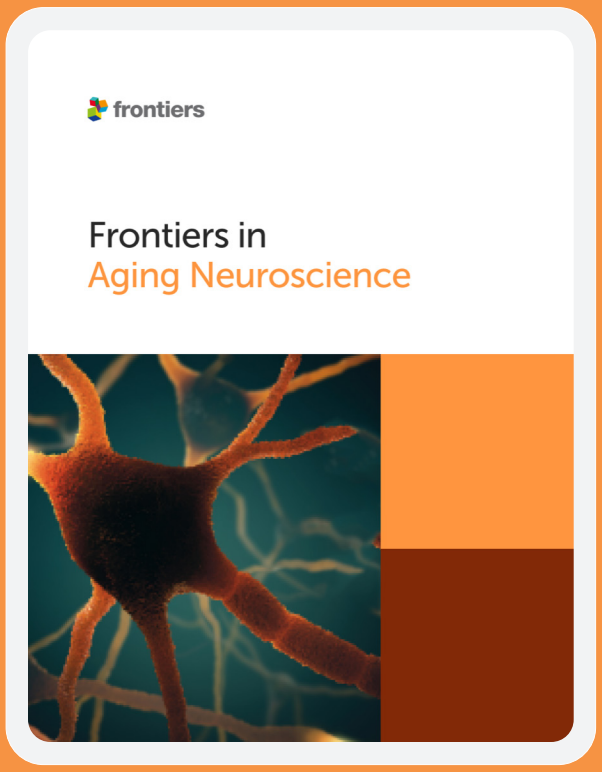

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