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Insights in aging interventions: 2022

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Healthy Eating for Successful Living in Older Adults™ community education program—evaluation of lifestyle behaviors: A randomized controlled trial

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Objective: Older adults face many chronic health issues including heart disease and osteoporosis, which are preventable through changes in lifestyle behaviors. The Healthy Eating for Successful Living in Older Adults™ (HESL) is a 6-week community education program designed specifically for persons aged ≥60 years, to promote behavioral changes toward a healthy lifestyle. Our objective is to evaluate the HESL. This is the first official evaluation of the HESL since its initiation in 2005.

Study Design: A cluster randomized controlled trial.

Method: Program implementation and evaluation took place between July 2018 and January 2020. Twenty-nine sites, with 292 participants aged ≥60 years from across five states (mostly from Massachusetts), were randomized into the intervention group (IG) (16 sites; n = 150 participants) and control group (CG) (13 sites; n = 142 participants). The HESL workshops followed a scripted curriculum including information from the USDA's MyPlate™ and the USDA 2015–2020 dietary guidelines. Intervention elements included goal setting, self-assessment, group support, and problem solving through brainstorming. The CG received no intervention. Outcome measures were collected in both groups at baseline, 2 weeks postintervention (week 8), and 6 months postintervention. These included self-reported lifestyle behaviors, a composite healthy behavior index (HBI), body mass index [weight (kg)/height (m^2)], and waist-to-hip circumference ratio (WHR). Mixed-effects regression models were used to examine the impact of the intervention.

Results: The IG showed significantly improved responses to most healthy lifestyle behavior questions at week 8 compared to the CG. However, not all improved responses were sustained at month 6. Significant improvements

detected at month 6 included responses to the question on making food choices that are healthy for the heart, using MyPlate™ tools for food choices, reading nutrition labels when shopping/planning meals, and confidence in managing own health ($p < 0.001$ in most cases). HBI was significantly improved at week 8 and month 6 ($p < 0.001$). WHR decreased significantly ($p < 0.05$) at month 6.

Conclusion: Positive changes in lifestyle behaviors and WHR were observed in older adults due to the HESL intervention.

Clinical Trial Registration: clinicaltrial.gov, Identifier: NCT04991844; https://clinicaltrials.gov/ct2/show/NCT04991844

KEYWORDS

Healthy Eating for Successful Living in Older Adults™, HESl, older adults, lifestyle behaviors, evaluation, HESL community education program

Introduction

Greater than 60% of adults over the age of 65 have been reported to have more than one chronic condition (Ward and Schiller, 2013), including heart disease, cancer, stroke, diabetes, osteoporosis, obesity, and Alzheimer's disease (Kirkman et al., 2012; Halter et al., 2014; Mark Mather and KelvinPollard, 2015; Arauco Lozada et al., 2021; Fahimfar et al., 2021; Falaschi et al., 2021); these diseases have devastating effects on their functional capability and quality of life. By 2049, functional disability, such as hip fractures and stroke, due to chronic diseases in older persons, is expected to increase at least 300 percent (Boult et al., 1996). Severe, immediate, and progressive disabilities lead to the inability of older adults to care for themselves (Fried and Guralnik, 1997). In addition, health care costs for older adults with chronic diseases and functional impairments, including home care expenses, are high and continue to grow (Stuck et al., 2004; Medical Spending of the Elderly, 2015). The burden of various chronic diseases such as heart disease and osteoporosis can be reduced with changes in lifestyle behaviors. Changes in lifestyle behaviors such as making healthy food choices, increasing physical activity, improving sleep quality, smoking cessation, and maintaining a healthy body weight have been shown to help prevent, slow, stop, or even reverse various chronic diseases (Barnard et al., 1994; Ornish et al., 1998; Hu and Willett, 2002; Roberts and Barnard, 2005; Ornish et al., 2008; Barnard et al., 2009; Frates, 2016; Orenstein et al., 2016; Benjamin et al., 2017) and to increase life expectancy (Loef and Walach, 2012), with great potential to improve quality of life and reduce health care costs. It is therefore important to help empower older adults toward self-care and well-being. This can be accomplished with the development of effective evidence-based interventions that are safe, relatively low-cost, and scalable and have the most potential for the largest impact (Frieden, 2014).

To address this need, in 2005, Hebrew SeniorLife and its associated partners designed and piloted a new program for older adults focused on nutrition and healthy lifestyle behaviors. The program—Healthy Eating for Successful Living in Older Adults™ (HESL)—provides older adults with needed knowledge on healthy food choices and lifestyle behaviors and tools that support behavioral changes (details of the HESL intervention are described below). At present, HESL serves more than 1,000 older persons yearly. The program is available in English, Spanish, and Russian, with plans to translate the intervention for Chinese and Portuguese speakers. The program is now disseminated through its training center at AgeSpan, previously known as the Elder Services of the Merrimack Valley and North Shore, located in Lawrence, Massachusetts. AgeSpan licenses and trains councils on aging, senior centers, congregate housing sites, neighborhood health centers, community centers, faith-based organizations, and others to deliver the program to the older persons in their communities. The program, available in both in-person and remote delivery models, is currently being offered in all 14 counties including 50% of cities and towns in Massachusetts, as well as in 13 additional states.

This study presents data on an evaluation of the HESL and examines the impact of the intervention on factors such as healthy behaviors, food choices, and quality of life at 2 weeks post-intervention (week 8) and 6 months post-intervention. This is the first official evaluation of the HESL since its initiation in 2005.

Methods

Study design

A cluster unblinded randomized controlled trial in persons aged ≥60 years, recruited from community-based settings, was conducted between July 2018 and January 2020. The intervention group (IG) was compared with the control group (CG) receiving no intervention to evaluate the effects of the 6-week HESL

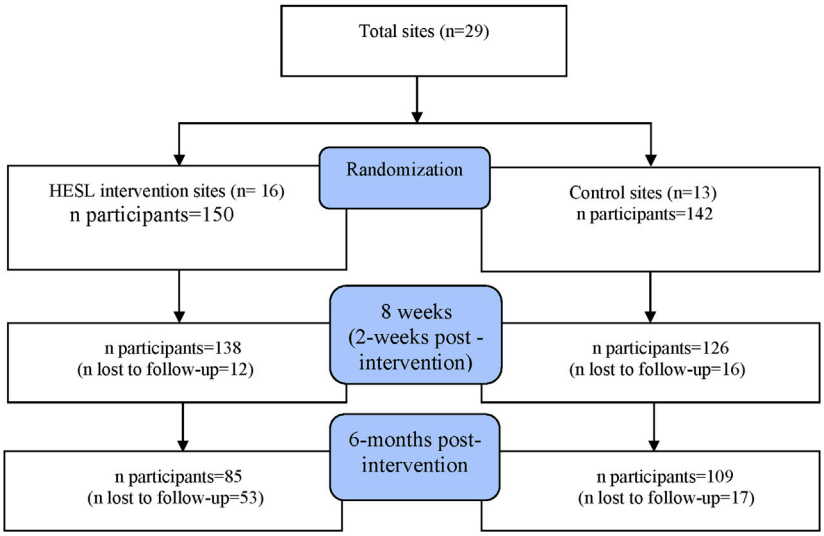

FIGURE 1
Study Consort Diagram.

intervention on outcome measures of interest at week 8 (i.e., at 2 weeks post-intervention), and at 6 months post-intervention. These time points were selected because of interest to determine the short-term or more immediate impact of the intervention, as well as the longer-term impact of 6 months post-intervention.

A biostatistician who was independent of the program implementers conducted the randomization at the site level [i.e., senior centers (n = 14), housing authorities (n = 4), churches (n = 3), assisted living facilities (n = 6), and library (n = 2)]. A total of 29 sites across five states (Massachusetts, Maryland, Florida, Rhode Island, and Michigan) were randomly assigned to the HESL IG and CG, using a single randomization approach with computerized random numbers. Sixteen sites were assigned to the IG, and 13, to the CG. All participants recruited in the IG sites were assigned as IG participants, and those recruited in the CG sites were assigned CG participants by the program implementers. Figure 1 shows the consort diagram of the study. The sample size was determined based on the assumption that the intervention would increase the mean fiber intake (g/day) by 30%, an indicator for a parallel study. With a type I error of 5% and a type II error of 80%, as well as a 25% loss of follow-up, we estimated a sample size of 125 per group.

Participant inclusion criteria included: 1) male or female aged ≥60 years, 2) English speaking, and 3) willing to participate and complete all study activities following randomization into the IG or CG. Those eligible, interested, and able to participate were recruited into the study.

Both IG and CG met at three specified time points, i.e., at baseline, week 8, and month 6, for the completion of study questionnaires and to provide anthropometric measures. In addition, the IG met weekly to receive the intervention outlined below.

The protocol was approved by the New England Institutional Review Board, and written informed consent was obtained from all participants. This study was registered at clinicaltrial.gov with identifier: NCT04991844. https://clinicaltrials.gov/ct2/show/NCT04991844.

Healthy Eating for Successful Living in Older Adults™

The HESL intervention was developed with strategies aimed to promote changes in lifestyle factors in adults aged ≥60 years.

The IG met once a week, for 2.5 h, with 10–16 participants/group, over a period of 6 weeks for the HES workshops. The HES workshops, offered in English, followed a scripted curriculum that incorporated information from the USDA's MyPlate™, and the USDA 2015–2020 dietary guidelines (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015). The HESL was designed to increase knowledge of healthy eating habits and identify food choices for healthy bones and heart, and for overall health. Participants were taught how to select healthier foods using the MyPlate™ app and to read labels, plan menus, and prepare meals in a lively interactive session of learning as a group. Among others, MyPlate™ emphasizes the importance of eating a variety of foods, eating foods from five major food groups (fruits, vegetables, grains, protein, and dairy), covering half their plates with fruits and vegetables, making half of the grain intake whole grains, choosing low or no fat dairy products, choosing foods with less added sugar and salt, avoiding

TABLE 1 Summary of healthy eating for successful living workshops' weekly curricula.

Week	HESL workshops' weekly curricula
Week 1	Use of MyPlate™, and the 2015–2020 dietary guidelines as a foundation for healthy eating, label reading, identifying and overcoming barriers to healthy eating, and importance of maintaining a healthy weight and physical activity
Week 2	How to make healthy choices from the Grains, Vegetables, and Fruits Groups, understand the importance of Water and Nutrition, Facts on food labels, and how to incorporate endurance exercises
Week 3	How to make healthy choices from the Protein and Dairy Groups, benefits of plant-based proteins in preventing certain chronic diseases, such as coronary heart disease, diabetes, and colon cancer, how to limit sodium intake, fats that are good and bad for health, healthy food preparation and storage methods, importance of calcium and vitamin D for bone health, and sources of these vitamins (from both foods and supplements), and importance of balance exercises
Week 4	How to create a well-balanced diet that includes Fats, and Sweets, and understanding the roles of Fats and Sweets in disease prevention, including heart disease prevention, how to modify recipes for health, importance of strength exercise, and preparation for Week 5 and Week 6 activities
Week 5	Applying Grocery Shopping Skills, practice label reading skills, identifying and modifying shopping list to include more heart healthy and bone healthy food choices
Week 6	Putting it all together—planning menu and preparing heart and bone healthy foods using techniques that are low in fat and added calories following MyPlate™ and flexibility exercises

transfat and saturated fat, reducing intake of cholesterol, eating more fiber, and being physically active. MyPlate™ is also individualized for each person based on his/her age, gender, and level of physical activity. Participants were thus guided on how to determine their caloric requirements and what to eat.

Participants were also taught goal setting, problem solving through brainstorming, group support, self-assessment, and management of dietary and physical activity patterns. At the end of every session, each participant was asked to set a goal for the new week related to healthy eating. The goals were focused on a nutrition challenge the participant would like to address and felt confident was achievable before the next week's session. Participants were taught to formulate their own SMART goals, i.e., goals that are specific, measurable, action-oriented, realistic, and time-sensitive, which incorporate accountability and monitoring (Frates et al., 2019). Participants kept a food and physical activity journal to monitor changes and identify problem areas in their eating habits and physical activity. By monitoring food choices, and physical activity type and intensity through the completion of a food and physical activity journal, participants were in a position to identify healthy changes made in their dietary and physical activity patterns as well as identify barriers to reaching goals. This self-assessment component is an important factor of the intervention.

These workshops were led by program leaders who were trained and certified to present materials and support brainstorming to solve a problem or overcome barriers to success, identified by participants, using culturally relevant solutions, as well as the promotion of socialization and group interaction. These are also important components of the intervention. In addition, participants were given supporting materials such as a "Participant Manual" with clear dietary intake and physical activity guidelines. Participants were also provided with information on the availability of community nutrition and health education resources. The intervention also included various "hands-on" activities such as visiting a grocery store, learning to read food labels, and making healthy choices in preparing meals and at a restaurant. A Registered Dietitian/Nutritionist was available to help answer questions that remained unanswered by the workshop leaders, and responses were brought back to participants the following week. Table 1 provides a summary of the workshops' weekly curricula.

Data collection

Demographic variables

Participant's age, gender (male = 1, female = 0), race (seven categories; recoded as 1 for White; 0, otherwise), education (eight categories, recoded as 1 for those with a college degree and above; 0, otherwise), and marital status (eight categories; recoded as 1 for married or having domestic partnership; 0, otherwise). Information on household income was excluded as almost 80% of participants did not respond to this question.

Healthy behavior questions

These self-reported healthy behavior questions were formulated and used in-house to specifically evaluate the impact of the HESL intervention in the past. These questions and their transformations into binary variables are listed in Table 2. In addition, we generated a composite healthy behavior index (HBI) by summing the dichotomous items. HBI scores ranged between 0 and 10; a higher score indicates more healthy behaviors.

Social connectedness

This was measured using the Berkman–Syme Social Connectedness Survey (Berkman and Syme, 1979), which contained 11 items, with questions such as "How many close

TABLE 2 Healthy lifestyle behavior questions (transformed to binary variables).

Healthy Lifestyle Behavior Questions	Original Responses	Transformed Binary Responses
Do you make food choices that are healthy for your bones?	Always; Most of the time; Sometimes; Rarely; Never	1 = always, most of the time, or sometimes; and 0 = rarely and never
Do you make food choices that are healthy for your heart?	Always; Most of the time; Sometimes; Rarely; Never	1 = always, most of the time, or sometimes; and 0 = rarely and never
Do you read nutrition labels when shopping or when planning meals?	Always; Most of the time; Sometimes; Rarely; Never	1 = always, most of the time, or sometimes; and 0 = rarely and never
Do you use MyPlate™ tools to help make food choices?	Always; Most of the time; Sometimes; Rarely; Never	1 = always, most of the time, or sometimes; and 0 = rarely and never
Are you currently a smoker?	Yes; No	1 = Yes; 0 = No
During the past month, how many hours of actual sleep did you get most nights?	<4 h; 4 h; 5 h; 6 h; 7 h; 8 h; 9 h; 10 h	1 = 6–8 h of sleep; 0 = ≤ 5 h and ≥ 9 h
During the past month, how would you rate your sleep quality overall?	Very good; Fairly good; Fairly bad; Very bad	1 = Very good or Fairly good; 0 = otherwise
How confident are you that you can manage most of your health problems	0–10; with 10 being highest confidence	1 = ≥ 6; 0 if ≤ 5
How understandable and useful is the information that your doctor or nurses have given you about your health problems or concerns	0–10, with 10 being most understandable and useful	1 = ≥ 6; 0 if ≤ 5
I play an active role in my health care and well-being	0–10, with 10 being most active	1 = ≥ 6; 0 if ≤ 5

friends do you have?" We generated a continuous score using the same approach as done by Salinas et al. (2017); a high score implies greater social connectedness. Evidence for the predictive validity of this self-reported survey is available in Berkman and Breslow (1983).

Physical activity

Using a physical activity questionnaire, participants reported their average amount of time spent per week (ranging from 0 min to ≥11 h) during the previous month doing various activities. Using the classification by Ainsworth et al. (1993), metabolic equivalent task (MET)-hours/week were estimated by multiplying the MET score for each activity by the reported hours per week and summing across all activities (Colditz et al., 2003; Eliassen et al., 2010). This self-reported physical activity measure is used extensively in research studies and has been validated in both men and women (Al-Shaar et al., 2022; Pernar et al., 2022).

Anthropometrics

Participants provided data on their height at baseline, weight, and waist and hip circumferences (WC and HC) measures at all time points. Body mass index (BMI, kg/m^2) and waist-to-hip circumference ratio (WHR) were computed. Self-reported anthropometric measures such as weight, height, and WC and HC have been observed to be valid measures in men and women (Rimm et al., 1990; Hodge et al., 2020). In this study, the workshop leaders trained the study participants on how to take their anthropometric measures and were there to supervise and assist participants with taking their measurements. A weighing scale was made available to participants at the workshop to take their weights, and a tape measure was given to each participant to take both their WC and HC.

Quality of life

Quality of life was measured using the Euro-QoL-5D-5L questionnaire (Janssen et al., 2013). The questionnaire solicits responses on mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Participants provided a rating of their own health on a scale of 0–100, where 0 means "death" and 100 means "the best health." This self-reported quality of life questionnaire is available in more than 150 languages and is used as a quantitative measure of health outcomes that reflects the patient's own judgment (Group E, 2022).

Statistical analyses

Descriptive analyses for all measures between the IG and CG at baseline, week 8, and month 6 were conducted using the student *t*-test for continuous variables or the chi-square test for categorical variables. The impact of the intervention at week 8 and month 6 without adjusting for covariates were estimated using a difference in differences (DID) approach (unadjusted DID) using the following linear regression model A:

$$y_{cit} = \beta_0 + \beta_1 \text{group} + \beta_2 \text{week 8} + \beta_3 \text{ month 6} + \beta_4 \text{group} * \text{week 8} + \beta_5 \text{group} * \text{month 6} + \varepsilon_{cit}$$

where y_{cit} represents outcome measure of interest (continuous or binary variables) specified in the data collection section above, for individual i, at time t, in cluster (or site) c; t implies the three study time points; group is the variable indicating whether the individual belongs to the IG (group = 1) or CG (group = 0); week 8 and month 6 indicate whether the measure is at the respective time points; group*week 8 and group*month 6 are the interaction terms

TABLE 3 Characteristics of participants at baseline.

Characteristics	Control (N = 142)	Intervention (N = 150)	T Value or Chi-Square
Age (Mean ± SD)	72.7 ± 8.0	72.8 ± 10.2	−0.03
Female (%)	82.3	82.2	0.002
White (%)	59.3	77.2	11.11***
Some college or above (%)	58.5	72.0	6.04*
Married (%)	38.2	28.8	1.48
Location (% in MA)	83.8	76.1	2.70

*$p < 0.05$; ***$p < 0.001$. p-values obtained using student t-test for continuous variables, or chi-square test for categorical variables.

between group and these time points, respectively; and ε_{cit} is random noise. The coefficients for group*week 8 and group*month 6 (β_4 and β_5 respectively) represent the net impact of the intervention on the outcome measures of interest at the respective time points. For simplicity, we did not add the site effect and individual effect to the model.

The impact of the intervention at week 8 and month 6 adjusting for covariates and addressing repeated measures (adjusted DID) were estimated using linear mixed-effects regression models by adding covariates of x_{it} (including age, gender, education, marital status, state, and race), individual random effects of α_i, and the site random effect of γ_c to the above model A. Model B below is used for our adjusted analyses.

$$y_{cit} = \beta_0 + \beta_1 \text{group} + \beta_2 \text{week 8} + \beta_3 \text{ month 6} + \beta_4 \text{group} * \text{week 8} + \beta_5 \text{group} * \text{month 6} + \beta_6 x_{it} + \alpha_i + \gamma_c + \varepsilon_{cit}$$

By including γ_c in the model, we addressed the effect of the clustering of individuals within a site in the analysis.

Although our outcome measures contain binary variables, we opted to use linear models for both DID models to ease the interpretation of our findings. However, we also presented the results from logit mixed-effects models (Rich-Edwards et al., 2019) for binary variables in Supplementary Table S1, which shows that there were no major differences in findings between the linear mixed-effects and logit mixed-effects models in terms of the significance of coefficients and the direction of the intervention impact. In addition, we presented relevant results between groups for participants who provided data at all three time points in Supplementary Tables S2,S3. All the analyses were conducted with Stata 16.0 software (STATA Corp, College Station, TX; Stata, RRID:SCR_012,763).

Results

Table 3 shows the baseline characteristics of the participants (N = 292) in the CG (N = 142) and IG (N = 150) (Figure 1). Most participants lived in Massachusetts. There were no statistical differences in age, marital status, and gender between groups. However, the IG had significantly more White participants compared with the CG ($p <$ 0.001). The IG also had more participants with some college education or above than the CG ($p < 0.05$).

Findings from the unadjusted linear regression model A are presented in Table 4. Compared to findings at week 8, most improvements in the unadjusted values were sustained at month 6, except for making food choices for healthy bones. Significantly more participants in the IG compared with the CG indicated that they read nutrition labels at 6 months ($p < 0.001$).

The impact of the intervention controlling for age, gender, race, educational and marital status, as well as baseline differences, on health behaviors are also presented in Table 4. (adjusted DID using model B). In general, findings remain consistent compared with the results from the unadjusted analyses for both week 8 and month 6. However, in contrast to the unadjusted findings, reading nutrition labels when shopping/planning meals and having fairly good or very good sleep quality were shown to be significantly improved in the adjusted results at week 8 ($p < 0.05$ in both cases). Adjusted findings also showed that at week 8, the IG showed significantly improved responses to these questions: making food choices that are healthy for bones ($p <$ 0.001) and heart ($p < 0.001$), using MyPlate™ ($p < 0.001$), confidence in managing own health ($p < 0.001$), and playing an active role in managing their own health care and well-being ($p <$ 0.05). A similar pattern was observed at month 6, except for responses to making healthy food choices for bones and sleep quality, where significant differences were no longer detected between groups in the adjusted results. The degree to which participants understood and found useful the information provided by their doctors and nurses about their health problems and concerns, at week 8 and month 6, was also not found to be statistically significant between groups in the adjusted findings. The continuous composite HBI scores showed a significant improvement at week 8 ($p < 0.001$) and month 6 ($p < 0.001$).

We used a logit mixed-effects model to examine the impact of the intervention on the binary variables as a sensitivity analysis. In general, we found similar results in the logit mixed-effects model (Supplementary Table S1). Adjusted findings on participants who provided data at all three time points are presented in Supplementary Table S2.

Table 5 presents unadjusted and adjusted results of our analyses showing the effects of the intervention on BMI, WC, HC, WHR, physical activity level, social connectedness, and quality of life at week 8 and month 6. Similar to the results from the unadjusted

TABLE 4 Intervention impact on lifestyle behaviors (all participants).

Evaluation questions (outcomes measures)	Control			Intervention			Unadjusted DID [α]		Adjusted DID [β]	
	Baseline	Week 8	Month 6	Baseline	Week 8	Month 6	Week 8	Month 6	Week 8	Month 6
	N = 134	*N* = 119	*N* = 100	*N* = 139	*N* = 136	*N* = 82				
Made food choices for healthier bones (%)	49.3	50.4	58.0	55.4	77.9	68.3	21.4*	4.14	24.0***	7.8
Made food choices for healthier heart (%)	54.7	52.5	55.9	56.9	86.2	81.7	31.6***	23.7**	32.6***	29.4***
Read nutrition labels when shopping/planning meals (%)	39.3	48.0	52.3	53.0	73.0	84.5	11.3	18.5*	12.0*	21.7***
Used MyPlate™ tools for food choices (%)	11.5	13.5	15.1	23.6	53.7	53.0	28.1***	25.9**	33.9***	29.0***
Current smoker (%)	2.7	4.0	2.8	5.2	3.7	2.4	−2.7	−2.79	−3.4	−1.4
Sleep hours in past month										
6–8 h (%)	77.8	78.4	77.6	76.0	75.2	80.7	−1.4	4.93	−3.5	5.2
Overall sleep quality in the past month										
Fairly good or very good (%)	75.9	72.6	75.7	79.5	85.5	86.4	9.3	7.11	9.9*	8.9
Confidence in managing own health										
Score 6 to 10 (%)	90.9	91.9	88.7	76.5	91.2	92.8	13.7*	18.5***	16.5***	21.1***
Found information provided by health care providers useful/understandable										
Score 6 to 10 (%)	84.3	85.8	86.8	81.9	86.7	89.9	3.2	5.5	6.2	5.0
Played active role in own health care and well-being										
Score 6 to 10 (%)	88.8	91.9	93.5	84.1	97.8	97.6	10.6*	8.80	9.7*	7.2
Healthy Behavior Index (HBI) (Mean ± SD)	5.7 ± 2.0	6.0 ± 1.9	6.0 ± 2.0	8.2 ± 2.4	7.3 ± 1.5	7.5 ± 1.4	0.8*	0.9*	1.2***	1.3***

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

[α]: Unadjusted DID denotes the difference in differences, accounting for the baseline difference between the control and intervention groups. For example, the unadjusted DID result was calculated as $(y^{intervention}_{week\ 8} - y^{control}_{week\ 8}) - (y^{intervention}_{baseline} - y^{control}_{baseline})$ for week 8, and positive and negative numbers under this column indicate the net increase or decrease in response (in percentage points) due to the intervention, respectively, at week 8; same at month 6 for all binary variables. For HBI, values are actual net increase or decrease due to the intervention. *p* -values were derived using a linear regression model.

[β]: Adjusted DID (i.e., adjusted intervention effect) and *p*-values derived from a random effects regression model that accounts for site clustering and repeated measures, with binary outcomes measures (%) (1 = healthy behavior, 0 = otherwise; note exception: current smoker = 1, 0 = otherwise), adjusting for group (intervention = 1, control = 0); week 8 (week 8 = 1, 0 = otherwise); month 6 (month 6 = 1, 0 = otherwise); age (continuous variable); gender (male = 1, female = 0); education (college and above = 1, 0 = otherwise), marital status (married = 1 and 0 = otherwise), state (Massachusetts = 1 and 0 = otherwise), and race (White = 1 and 0 = otherwise). Positive and negative numbers under this column indicate the net increase or decrease in response (in percentage points) due to the intervention, respectively, at week 8, same at month 6 for all binary variables. For HBI, values are actual net increase or decrease due to the intervention.

analyses, the adjusted analyses results did not show a significant impact of intervention at week 8 nor at month 6, except for a significant decrease in WHR at month 6 ($p < 0.05$) in the IG, in comparison with the CG. Adjusted findings on participants who provided data at all three time points are presented in Supplementary Table S3.

Discussion

The IG showed significantly improved responses to most healthy lifestyle behavior questions at week 8 compared with the CG. However, not all improved responses were sustained at month 6. Significant improvements detected at month 6 included responses to the question on making food choices that are healthy for the heart, using MyPlate™ tools for food choices, reading nutrition labels when shopping/planning meals, and confidence in managing own health. The HESL intervention was also associated with a statistically significant increase in the composite HBI at week 8 and month 6 and a significant decrease in WHR at month 6.

Various elements of the HESL intervention that contribute to its improvement in changing a healthy lifestyle include promoting changes in habits through small increments by setting goals for the week at the end of each session. As described, participants were taught to formulate their own SMART goals, i.e., goals that are specific, measurable, action-oriented, realistic, and time-sensitive,

TABLE 5 Intervention impact on body mass index, waist-to-hip circumference ratio, physical activity, social connectedness, and quality of life (all participants).

Outcome measures	Control (Mean ± SD)			Intervention (Mean ± SD)			Unadjusted DID [α]		Adjusted DID [β]	
	Baseline	Week 8	Month 6	Baseline	Week 8	Month 6	Week 8	Month 6	Week 8	Month 6
	N = 142	*N* = 121	*N* = 84	*N* = 137	*N* = 96	*N* = 59				
BMI kg/m^2	28.0 ± 5.8	27.9 ± 5.7	28.2 ± 6.0	29.6 ± 6.0	29.2 ± 5.9	29.4 ± 6.4	−0.30	−0.44	−0.55	0.18
Waist Circumference (WC) (inches)	37.7 ± 5.7	37.9 ± 5.9	37.2 ± 5.7	39.7 ± 5.5	40.6 ± 5.0	39.6 ± 5.9	0.70	0.34	−0.15	−0.15
Hip Circumference (HC) (inches)	42.2 ± 5.5	41.7 ± 5.6	41.5 ± 5.2	43.7 ± 5.0	44.1 ± 5.0	44.8 ± 6.9	0.84	1.73	−0.19	1.36
Waist to Hip Circumference Ratio (WHR)	0.89 ± 0.08	0.91 ± 0.08	0.90 ± 0.07	0.91 ± 0.09	0.92 ± 0.08	0.89 ± 0.09	−0.003	−0.02	0.001	−0.03*
Physical Activity (MET-hrs/week)	50.5 ± 55.2	52.5 ± 50.3	56.2 ± 48.4	52.8 ± 95.2	51.8 ± 86.3	40.9 ± 57.0	1.88	−16.6	1.91	−0.37
Social Connectedness Score	4.6 ± 3.1	4.7 ± 3.0	4.7 ± 3.2	5.2 ± 3.2	4.8 ± 3.2	4.8 ± 3.2	−0.37	−0.50	−0.58	−0.53
Quality of Life	80.3 ± 13.7	81.7 ± 11.9	80.8 ± 12.1	78.6 ± 16.5	80.8 ± 13.5	79.3 ± 17.7	0.81	0.17	0.91	0.14

*$p < 0.05$.

[α]: Unadjusted DID denotes the difference in differences, accounting for the baseline difference between the control and intervention groups. For example, the unadjusted DID result was calculated as $(y^{intervention}_{week\ 8} - y^{control}_{week\ 8}) - (y^{intervention}_{baseline} - y^{control}_{baseline})$ for week 8, and positive and negative numbers under this column indicate the net increase or decrease in value due to the intervention, respectively at week 8; sme at month 6. p -values were derived using a linear regression model.

[β]: Adjusted DID (i.e., adjusted intervention effect) and p-values derived from a random effects regression model that accounts for site clustering and repeated measures, with outcome measures as continuous variables, adjusting for group (intervention = 1, control = 0); week 8 (week 8 = 1, 0 = otherwise); month 6 (month 6 = 1, 0 = otherwise); age (continuous variable); gender (male = 1, female = 0); education (college and above = 1, 0 = otherwise), race (White = 1 and 0 = otherwise), state (Massachusetts = 1 and 0 = otherwise), and marital status (married = 1, 0 = otherwise). Positive and negative numbers under this column indicate the net increase or decrease in value due to the intervention, respectively at week 8; same at month 6.

which incorporate accountability and monitoring (Frates et al., 2019). By monitoring food choices, as well as physical activity type and intensity through the completion of a food and physical activity journal, participants were in a position to identify changes made in their dietary and physical activity patterns as well as identify barriers to reaching goals. Brainstorming to solve problems or overcome barriers to success using culturally relevant solutions and the promotion of socialization and group interaction were also likely to be contributing factors. In addition, participants were given supporting materials such as a "Participant Manual" with clear dietary intake and physical activity guidelines. Participants were also provided with information on the availability of community nutrition and health education resources. Further, workshop leaders had access to a registered dietitian/nutritionist who helped answer questions raised by participants that remain unanswered during the workshops.

Although the IG showed statistically significant improvement in making food choices that are healthy for bones at week 8, this improvement was not sustained at 6 months, and it is unclear why. The 2015–2020 USDA dietary guidelines for healthy bones include promoting the consumption of dairy products, including whole and skim milk, at least 2 to 3 times/day (U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015). However, it is questionable if such guidelines are protective of bone health, and there are concerns that such recommendations may actually increase adverse health outcomes (Ganmaa et al., 2002; Ganmaa and Sato, 2005; Qin et al., 2009; Aune et al., 2015; Willett and Ludwig, 2020). It may be too much for some participants to consume large amounts of dairy products daily long term, especially for those who are lactose intolerant or who may not like the taste of milk. However, HESL does promote the consumption of other sources of calcium, including plant-based calcium sources such as tofu, nuts beans, broccoli, and kale (Messina and Mangels, 2001; American Dietetic and Dietitians of, 2003), as well as calcium supplements, to promote healthy bones.

Although participants were instructed on the health benefits of physical activity of various types including endurance, strength, flexibility, and balance exercises, during the intervention, we did not find a significant increase in the level of physical activity. We were informed by AgeSpan that the intervention did not focus on physical activity as much as it did on making dietary changes and that some participants opted out of the physical activity component.

WHR showed a significant decrease of 0.03 at month 6 in the IG compared to the CG (Table 5). WHR is a measure of central obesity or visceral fat, whereas BMI is a measure of overall obesity (Paniagua et al., 2008). Central obesity as measured using WHR has been shown to be a stronger predictor of chronic diseases, including heart disease, diabetes, and cancer, as well as mortality, than measures of total body fat such as BMI (Lapidus et al., 1984; Fujimoto et al., 1999; Janssen et al., 2002; Vazquez et al., 2007; Mathieu et al., 2009; Czernichow et al., 2011). The significant

decrease in WHR at month 6 suggests that the healthy behavioral changes due to the HESL intervention may have a protective impact on an important risk factor of chronic diseases. In a study by de Koning et al. (2007), a 0.01 increase in WHR has been shown to be associated with a 5% increase in the risk of incident CVD events. It is not clear if the decrease in WHR in this study was due to changes in the WC and/or HC. However, a non-significant increase in HC was observed (Table 5).

Given that there was no follow-up of participants between week 8 and month 6, it is noteworthy that several significant improvements to healthy lifestyle behaviors observed at week 8, remained significant at month 6 (Table 4). It is difficult to predict if participants will continue to maintain the positive changes made to their lifestyle behaviors due to the intervention beyond the 6-month period. Future evaluations to determine factors associated with longer-term (i.e., beyond 6 months) effectiveness of HESL on healthy lifestyle behaviors, disease outcomes, and quality of life, among others, as well as to identify barriers to continuing adoption of these behaviors, are needed. We were not able to evaluate the impact of the intervention beyond 6 months due to a lack of resources. However, healthy lifestyle and behavioral changes can be maintained long term (Ornish et al., 1998; Ornish et al., 2005). It has been shown that those who made the greatest changes showed the biggest improvement (Ornish et al., 1998). In the BROAD study, overweight participants in the IG were empowered with knowledge of the benefits of a plant-based diet and encouraged to incorporate it into their lifestyles (Wright et al., 2017). The CG, also overweight participants, received standard medical care. Although the study lasted only 3 months, the plant-based group not only lost significant weight but also achieved greater weight loss at 6 and 12 months, derived physical and mental benefits, and stopped taking many of their medications, even though no further instructions were given beyond the 3 months intervention (Wright et al., 2017; Greger, 2020). Sustainable changes in dietary habits and behavioral lifestyles beyond the intervention period are possible when changes result in increased benefits and pleasure and/or are associated with positive emotions that naturally motivate the rewiring of the brain toward the adoption of such behaviors (Frates, 2016; Esselstyn, 2017; Fogg, 2020; Greger, 2020). Healthful habits repeated over time become a way of life (Fogg, 2020; Greger, 2020) and should be the ultimate goal of all interventions aimed at improving dietary and health behaviors. Although there was considerable variation across participants and behaviors (Gardner et al., 2012), it has been shown that automaticity plateaued on average around 66 days after the first daily performance (Phillippa et al., 2010). Working effortfully on a new behavior for 2 to 3 months may be appealing to participants interested in turning that behavior into a habit (Gardner et al., 2012).

Overall, findings in this work suggest that the HESL intervention positively impacts the healthy behaviors of its targeted population. However, several limitations should be acknowledged. These include a sample size of predominantly female and White, making the findings of this study likely not generalizable to males and persons of other ethnic groups. The present study used largely self-reported measures that were previously validated. Available resources did not allow for use of objective health measures (e.g., blood biomarkers), which would help further strengthen the study findings. Another limitation is missing pre-existing conditions and other data which may bias findings. For example, income was not included in the regression model. Although requested, only 21% of participants provided income data. The analyses, however, controlled for educational status, which serves as a surrogate variable for household income. As such, this bias is likely mitigated. Biases of randomization not being blinded were also limitations. The large loss to follow-up was another limitation of the study. While not receiving the intervention may be a reason for leaving the CG by approximately 23% of the CG participants, it is not clear why the intervention lost approximately 43% of its IG participants. Characteristics of participants in both groups who were lost to follow-up were not found to be significantly different when compared with those in these groups who remained in the study at week 8 and month 6 (data not shown). Future evaluations need to reach out to participants leaving the intervention to identify reasons for leaving, so future programs can remedy the situation.

The present study was able to explore the impact of the intervention based on self-reported measures. Although limited, findings suggest that the intervention has a positive impact on study participants' health behaviors as well as a risk factor of chronic diseases (WHR), as reported by the participants themselves. Objective outcome measures that directly point to bone, heart, and overall health, are needed to support and strengthen current study findings. As such incorporation of such measures is needed in future evaluations of this intervention.

A huge challenge in public health remains the need to minimize the large gap between existing evidenced-based knowledge on healthy diets and lifestyle behaviors and their actual adoption as a way of life by individuals in all age groups (Willett, 2019). Closing this gap requires multiple strategies. The HESL attempts to address this challenge in older adults through community education. The HESL program implementers will continue to adjust the program to optimize its effectiveness and its ability to change behaviors in the long term, beyond the intervention period, and to expand its reach to other states.

Data availability statement

The raw data supporting the conclusion 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 New England Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

Author contributions

JBB and WZ were responsible for conceptualizing and designing the evaluation component of the study as well as providing guidance on data collection. WZ was responsible for data analyses and wrote the first draft of the results section. JBB wrote the first draft of the article other than the results section. Both JBB and WZ were responsible for the interpretation of data, review of the overall article, and approval of the final paper for 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/fragi.2022.960986/full#supplementary-material

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Physical ability, cervical function, and walking plantar pressure in frail and pre-frail older adults: An attentional focus approach

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Aging and increased vulnerability define the clinical condition of frailty. However, while the cervical function is recognized as a determinant of balance and walking performance, no study simultaneously physical ability, cervical function, balance, and plantar pressure distribution in walking in nursing house population. Thus, the present study aimed to compare these parameters between Frail and Pre-Frail aged people. Thirty-one (12 men and 19 women) institutionalized participants (age: 89.45 ± 5.27 years, weight: 61.54 ± 9.99 kg, height: 160.34 ± 7.93 cm) were recruited and divided into Pre-Frail and Frail groups according to SPPB (Short Physical Performance Battery) score (Frail <6, Pre–Frail ≥6). Participants performed the Timed Up and Go Test (TUGT) and a static balance evaluation. The cervical range of motion (COM), knee extensor strength, and walking plantar pressure distribution have been measured. The Pre-Frail group showed a higher gait speed (ES = 0.78, $p \leq 0.001$) and a better TUGT, as well as higher knee extensor strength (ES = 0.4, $p = 0.04$). Furthermore, the Pre-Frail group presented a center of pressure (COP) displacement velocity on the sagittal axis (ES = 0.43, $p = 0.02$) and a more COP projection on this axis (ES = 0.43, $p = 0.02$). No significant difference has been observed between the two groups concerning the total contact time and most of the plantar pressure parameters except for the rear foot relative contact time which was lower in the Pre-Frail group. The Pre-Frail group also showed better cervical tilt mobility (ES = 0.35, $p = 0.04$). This study highlights the influence of some new parameters on frailty in older people, such as cervical mobility and plantar pressure distribution in walking.

KEYWORDS

functional capacity, aging, cervical, plantar pressure, TUG

Introduction

Aging is a global phenomenon often occurring in altered living conditions, with loss of mobility leading to incapacity and primary care input. Increased vulnerability defines the clinical condition of frailty and concerns all psychological, physical, and social capacities (Gobbens et al., 2010). Deconditioning is intrinsically linked to balance disorders and loss of mobility threatening autonomy in daily life. Nursing home populations are particularly concerned by frailty, with people affected heterogeneously (Sverdrup et al., 2018). However, due to a high percentage of institutionalized elderly suffering from cognitive impairment, psychological and social determinants of frailty could be challenging to investigate.

Consequently, the physical aspects appear to be the most accessible parameters to evaluate if tests are adapted. A relationship has already been established between frailty and Short Physical Performance Battery (SPPB), even for complex elders living in nursing homes (Tabue-Teguo et al., 2018). There is the same kind of relationship between frailty and the well-known SPPB test as between SPPB and aging, with a decrease in walking pace, an increase in static imbalance (Xie et al., 2016), and a loss of muscular strength in the lower limbs (Barbat-Artigas et al., 2016). An age-related decrease in walking speed is associated with a high risk of falls (Barack et al., 2006). Elderly fallers also exhibit postural instability on both sagittal and transversal axes and modifications in the static center of pressure, displacement, and velocity (Muir et al., 2013).

Aging is also at the origin of modifications in a global posture with accentuation of forwarding inclination of the trunk, deeper kyphosis, general asymmetry, flexed knees, and ankles (Drzał-Grabiec et al., 2013). This phenomenon makes older adults cautious when walking, reducing speed, step length, and symmetry with increased step variability, frequency, and bilateral contact phase time Field (Iosa et al., 2014), which negatively correlated with gait performance. Few studies have explored plantar pressure distribution in older adults with or without imbalance issues but have identified higher peak pressures with age localized on the forefoot (McKay et al., 2017). More precisely, it seems that peak pressure at the heel pose and metatarsophalangeal joint at the toe-off decreases while the contact time of the same parts increases. These results are observed when comparing young and old participants (Scott et al., 2007) and fallers and non-fallers (Nakajima et al., 2014). However, no studies for now compared gait patterns according to the stage of frailty.

Since age impacts spine statics and postural control, modifications could lead to balance strategy maladjustment with weakened postural control, altered visual feedback, proprioceptive and vestibular system impairment, and neuromuscular trouble (Woollacott, 2000). Previous studies show that the neck area is an anatomical and physiological crossroads for the balance (Armstrong et al., 2008). The inclination capacity of the cervical spine appears decisive for adaptation to everyday movement, particularly in case of loss of equilibrium, and could become a marker of frailty. Moreover, the cervical function is the last possibility for the spine to adjust the balance with plantar proprioception decrease and to compensate for vestibular alteration. Spine mobility is commonly affected by aging, and it has been demonstrated that decreased cervical mobility and asymmetry in rotation can impact anteroposterior swing in standing position in older adults (Quek et al., 2013). Even in young participants, cervical muscle tiredness could alter static balance parameters by modifying the speed displacement of the center of pressure (COP) (Liang et al., 2014). In addition, over-activation of superficial neck muscles appears with aging and a global decrease of muscle tone to the detriment of deep muscles. This particular pattern leads to a forward position of the head (Gogola et al., 2014). It is well known that part of the trunk and neck role stabilizes the head and cushions the acceleration during walking (Kavanagh et al., 2006). The cervical area is affected by aging, structurally and functionally, therefore influencing static and dynamic balance. Nevertheless, to our knowledge, no balance rehabilitation program includes prevention, enhancement, or rehabilitation of this body part. Consequently, it appears very important to analyze the modifications in neck muscle strength and mobility regarding physical capabilities and balance in frail elderly participants.

While many studies have focused on different components of frailty in older adults, none have simultaneously analyzed, in nursing home populations, gait speed, validated mobility tests, lower limb strength, COP variations in static standing posture, dynamic distribution of plantar pressure, and cervical strength and mobility. Thus, the principal aim of the present study was to compare mobility and balance parameters between Frail and Pre-Frail (Frail vs Pre-Frail) groups in nursing homes to highlight specific differentiation criteria useful for individualized injury prevention or rehabilitation. It was hypothesized that older adults at different frailty levels would present different walking plantar pressure patterns and spine mobility associated with strength losses that could impair their physical ability.

Materials and methods

Experimental approach

This cross-sectional study was designed to compare the different physical abilities between Pre-Frail and Frail groups. Short Physical Performance Battery (SPPB) test, TUGT, knee extensor strength, cervical strength and range of motion, static balance, and walking plantar pressure were measured in the two groups. Each measurement was realized three times, and the best score was considered for analysis. Afterward, the participants

were divided into Pre-Frail and Frail groups according to the SPPB threshold score of 6; thus, the Pre-Frail group (SPPB scores from 0 to 5) and the Frail group (SPPB scores from 6 to 12) (Pritchard et al., 2017).

Participants

Thirty-one participants were recruited in three nursing homes, including 12 men and 19 women (age: 89.45 ± 5.27 years, weight: 61.54 ± 9.99 kg, height: 160.34 ± 7.93 cm). The inclusion criteria for the participant recruitment were over 65, able to walk 10 m, and understanding simple orders. They completed an information and consent form before participation in the study, approved by the Ethics Committee of Université Claude Bernard Lyon 1, and complied with the Declaration of Helsinki.

Experimental sessions

Short Physical Performance Battery (SPPB) test

The Short Physical Performance Battery (SPPB) is an objective assessment tool for evaluating lower extremity functioning in older persons. The SPPB consists of three tests, including the ability to stand for 10 s with feet in 3 different positions (together side-by-side, semi-tandem, and tandem), the fastest gait speed, and the time to rise from a chair five times (de Fátima Ribeiro Silva et al., 2021).

Time up and go test (TUGT)

Subjects were required to rise from a chair, walk 3 m, turn around 180°, walk back to the chair, and sit down while rotating 180° (Barry et al., 2014). The time to perform the total test was measured and considered to assess the person's mobility. During the trial, the person was expected to use any mobility aids they would typically require.

Strength of the knee extensor and cervical muscles

A handheld dynamometer (HHD) (MicroFET2, Hoggan, Salt Lake City, United States) was used to measure the maximal isometric force of the quadriceps muscle of the dominant limb and the maximal cervical force in the three axes. A « make test » was performed to obtain the maximal isometric force. For the knee extensor force measurement, the force was normalized by each subject's body mass. The subject was also asked to perform cervical movements until maximal strength was reached (or 5 s) with resistance applied successively under the chin, under the occiput, at the right/left side of the mandible, and on the right/left side of the temporal. Four indicators were obtained: Flexion Strength, Extension Strength, Rotation Strength, and Tilt Strength.

Cervical mobility

Cervical mobility was obtained from a standard measuring tape (material information). The subject was placed in a neutral sitting position, looking straight ahead, back in contact with the chair back. Anatomic benchmarks were identified (tragus of ear, chin symphysis, anterior part of acromion, superior part of the sternum), and the subject was asked to perform cervical movements: flexion, extension, right/left rotation, and right/left tilt. Active range of motion (ROM) measurements were identified as the neutral distance difference between neutral and maximal movement expressed in centimeters (Chibnall et al., 1994) for 4 ROM measurements including cervical flexion, extension, rotation, and tilt.

Static balance measurement

The statics balance was measured using a posturographic platform (Fusyo, Medicapteur, Balma, France, 40 Hz) in the eyes open condition. The participants stood barefoot with two legs on the platform and were asked to look steadily at the fixed points on the wall in front of the participant. The center of pressure (COP) displacement was measured during 25.6 s (Bernard et al., 2010) and processed with the software W-IN POSTURO (Medicapteur, Balma, France). From COP displacement, several indicators were calculated, including the surface of displacement (SURF), the total length of displacement (LXY), length of displacement on the sagittal axis (LY), mean position on the transversal axis (X_{mean}), mean position on sagittal axis (Y_{mean}), length of displacement as a function of surface (LFS), the COP speed of displacement on the sagittal axis (VFY).

Foot pressure parameter measurement

Foot pressure was measured by W-INSHOE plantar sensors (Medicapteur, Balma, France, 100 Hz) during the walking phase of the Time Up and Go Test (TUGT) under standard conditions (Podsiadlo and Richardson, 1991). Nine pressure sensors were placed on the 3-foot locations, including hallux, forefoot, and rearfoot (Figure 1A). Data delivered by sensors concerned with pressure and duration. First, sensors were grouped according to their localization to compose the forefoot and the rearfoot to obtain a biodynamic pattern. Then, all parameters were normalized (%) according to total plantar pressure and total foot contact duration during the stance phase of the walking task. Six parameters were extracted: hallux pressure, forefoot pressure, rearfoot pressure, total pressure, rearfoot, and forefoot relative contact time. The average of the peak foot pressure of all the steps was calculated from the steps after stand-up for each location which was used for future analysis.

Statistical analysis

Prior to performing the statistical analysis, the Shapiro–Wilk and Levene's tests were used to assess the data's normality and variance equality for each variable. Non-parameter Wilcoxon test was used to determine the difference between the two groups

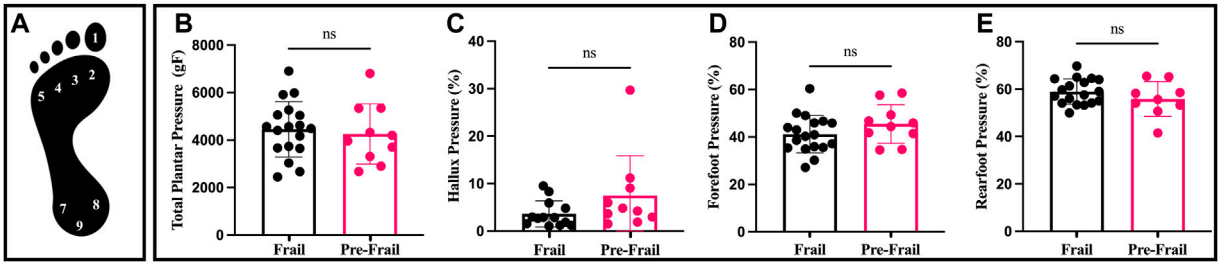

FIGURE 1
Plantar pressure and distribution; **(A)** sensors location on foot; **(B)** total plantar pressure; **(C)** percentage (%) of hallux pressure by total plantar pressure; **(D)** percentage (%) of forefoot pressure by total plantar pressure; **(E)** percentage (%) of rearfoot pressure by total plantar pressure. ns: non-significant.

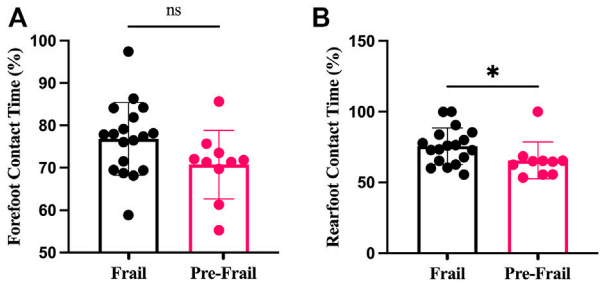

FIGURE 2
Plantar contact time; **(A)** forefoot contact time; **(B)** rearfoot contact time; ns: non-significant; *: $p < 0.05$.

(Pre-Frail vs Frail). The correlation coefficient r was calculated to estimate the effect size. The magnitude of the correlation coefficient was interpreted using criteria: very weak (0.11–0.19), weak (0.20–0.39), moderate (0.40–0.59), strong (0.60–0.79), and very strong (0.80–1.00). The critical p-value was set at 0.05. Descriptive statistics are presented as mean ± SD with 95% CI. All statistical procedures were performed with R software (R 3.5.0, R Core Team, Vienna, Austria).

Results

The Pre-Frail group indicated significantly higher SPPB score, gait speed, and TUGT (all $p < 0.002$, ES > 0.74). In addition, the frail group revealed lower knee extensor strength compared to the Pre-Frail group ($p = 0.05$, ES = 0.4) (Table 1). Moreover, the Pre-Frail group showed a higher cervical tilt ROM ($p = 0.04$, ES = 0.4), whereas no significant differences were found in cervical flexion, extension, and rotation (all $p > 0.05$) (Table 1). In contrast, no significant difference was found for any cervical strength (all $p > 0.05$). The static balance results revealed that only VFY and Y_{mean} significantly differed between the two groups ($p = 0.02$, ES = 0.43). In contrast, no significant difference was found in other statics parameters (all $p > 0.5$). Lastly, Pre-frail group presented significant shorter forefoot contact time ($p = 0.04$, ES = 0.39, Figure 2), but no other significant difference was found for other parameters (all $p > 0.05$, Figure 1).

Discussion

The present study aimed to determine whether there are differences in the measured parameters (mobility test, gait speed, knee extensor strength, static balance, plantar pressure, and cervical pressure) between Frail and Pre-Frail older adults. The main finding demonstrated that frail participants presented a significant difference in lower knee extensor strength and pressure distributions during walking, associated with an altered cervical function, especially in tilt mobility.

The results obtained in our nursing home population were in accordance with recent studies concerning TUGT or walking speed. Binotto et al. (2018) reviewed studies using gait speed as a marker of physical frailty in community elderly aged between 68 and 86 years. This review reported a systematic decrease in gait speed in frail people with a wide variability from 2.7% to 83.9%. In the same way, an association between functional test performance and knee extensor strength is well known, and a recent study by (Jacob et al., 2019) demonstrated the same repartition pattern according to SPPB score. Knee extensor force in the present study could not be compared to the literature due to our population specificity: older and more dependent than groups usually studied. However, the difference observed in maximal strength between Pre-Frail and Frail people associated with a lack of difference in BMI (body mass index) tends to indicate that dynapenia was more marked than sarcopenia in this population. Unfortunately, the present study did not enable the identification of the physiological determinants of this difference.

TABLE 1 Physical ability, cervical mobility, strength, static assessment, plantar pressure in Pre-Frail and Frail subjects. All data are presented as mean ± standard deviation with a 95% confidence interval (CI).

	Variable	Frail		Pre-frail		Difference [95% CI]	*p*-value	Effect size (r)	Magnitude
		Mean ± SD	[95% CI]	Mean ± SD	[95% CI]				
Physical ability	SPPB	3.2 ± 1.51	[2.49; 3.91]	7.18 ± 1.25	[6.34; 8.02]	3.98 [2.94; 5.03]	<0.001	0.82	Large
	Gait speed ($m.s^{-1}$)	0.32 ± 0.08	[0.28; 0.36]	0.58 ± 0.13	[0.49; 0.67]	0.26 [0.17; 0.36]	<0.001	0.78	Large
	TUGT(s)	29.33 ± 16.8	[21.47; 37.19]	13.06 ± 3.74	[10.54; 15.57]	−16.27 [−24.4; −8.14]	<0.001	0.74	Large
	Knee extensor strength (N/kg)	1.66 ± 0.66	[1.31; 2.01]	2.33 ± 0.78	[1.68; 2.98]	0.67 [−0.02; 1.37]	0.05	0.4	Moderate
Cervical mobility	Flexion (cm)	7.55 ± 2.63	[6.32; 8.78]	8.27 ± 1.68	[7.14; 9.4]	0.72 [−0.86; 2.31]	0.43	0.15	Small
	Extension (cm)	4.75 ± 2.02	[3.8; 5.7]	4.91 ± 1.58	[3.85; 5.97]	0.16 [−1.19; 1.51]	0.69	0.08	Small
	Rotation (cm)	8 ± 2.15	[7; 9]	8.23 ± 1.94	[6.92; 9.53]	0.23 [−1.34; 1.79]	0.85	0.04	Small
	Tilt (cm)	3.38 ± 2.45	[2.23; 4.52]	4.91 ± 1.02	[4.22; 5.59]	1.53 [0.24; 2.82]	0.04	0.35	Moderate
Cervical Strength	Flexion Strength (N/kg)	1.26 ± 0.32	[1.04; 1.48]	1.26 ± 0.34	[1.01; 1.5]	0 [−0.31; 0.3]	1	0	Small
	Extension Strength (N/kg)	1.48 ± 0.27	[1.29; 1.66]	1.48 ± 0.5	[1.13; 1.84]	0.01 [−0.37; 0.39]	0.86	0.05	Small
	Rotation Strength (N/kg)	0.88 ± 0.33	[0.66; 1.1]	1 ± 0.36	[0.74; 1.26]	0.12 [−0.2; 0.44]	0.5	0.15	Small
	Tilt Strength (N/kg)	0.92 ± 0.27	[0.74; 1.1]	1 ± 0.32	[0.77; 1.23]	0.09 [−0.19; 0.36]	0.6	0.12	Small
Static assessment	SURF(mm^2)	116.83 ± 91.15	[71.5; 162.15]	91.87 ± 58.61	[52.5; 131.25]	−24.96 [−82.05; 32.14]	0.74	0.07	Small
	LXY (mm)	534.03 ± 408.54	[330.86; 737.19]	319.66 ± 157.63	[213.76; 425.56]	−214.36 [−436.05; 7.33]	0.17	0.26	Small
	LY (mm)	264.11 ± 317.42	[106.26; 421.96]	185.45 ± 102.48	[116.6; 254.29]	−78.67 [−246.45; 89.12]	0.98	0.01	Small
	X_{mean} (mm)	58.17 ± 45.38	[35.6; 80.73]	35.54 ± 24.61	[19.01; 52.07]	−22.63 [−49.35; 4.09]	0.29	0.2	Small
	Y_{mean} (mm)	91.22 ± 43.56	[69.56; 112.89]	50.35 ± 44.81	[20.24; 80.46]	−40.87 [−76.19; −5.56]	0.02	0.43	Moderate
	LFS (mm)	1.19 ± 0.85	[0.77; 1.61]	0.74 ± 0.35	[0.51; 0.98]	−0.45 [−0.91; 0.02]	0.18	0.25	Small
	VFY(mm/s)	113.88 ± 64.2	[81.95; 145.8]	64.96 ± 45.72	[34.24; 95.67]	−48.92 [−90.98; −6.86]	0.02	0.43	Moderate
Plantar pressure	Hallux pressure (KgF)	3.6 ± 2.76	[1.93; 5.27]	7.5 ± 8.38	[1.51; 13.49]	3.9 [−2.21; 10]	0.08	0.38	Moderate
	Forefoot pressure (KgF)	2420.12 ± 666.41	[2088.72; 2751.52]	3183.56 ± 1673.62	[1897.1; 4470.01]	763.44 [−541.39; 2068.26]	0.14	0.29	Small
	Rearfoot pressure (KgF)	58.95 ± 5.33	[56.3; 61.61]	55.86 ± 7.37	[50.19; 61.53]	−3.1 [−9.09; 2.9]	0.4	0.17	Small
	Total pressure (KgF)	4453.62 ± 1161.2	[3876.17; 5031.07]	4255.41 ± 1266.68	[3349.28; 5161.53]	−198.22 [−1220.14; 823.71]	0.61	0.1	Small
	Forefoot contact time (%)	76.84 ± 8.61	[72.56; 81.12]	70.77 ± 8.07	[65; 76.55]	−6.07 [−12.88; 0.74]	0.11	0.31	Moderate
	Rearfoot contact time (%)	75.63 ± 12.9	[69.21; 82.04]	65.61 ± 13.11	[56.23; 74.99]	−10.01 [−20.79; 0.77]	0.04	0.39	Moderate

Static balance evaluation regarding frailty gave more heterogenous results. The Pre-Frail group had better static balance than the frail group: COP displacement velocity was a lower variable, and its projection on the anteroposterior axis was less retro-pulsed. This should enable lesser muscular stiffness and energy expenditure (Houdijk et al., 2009). Some previous results reported a similar finding, including the study by Wiśniowska-Szurlej *et al.* (Wiśniowska-Szurlej et al., 2019), which observed a negative correlation between frailty and LXY or VFY in 209 older adults. However, other studies like Marques *et al.* (Marques et al.,

2019) reported no difference in sway or mean value of COP displacement between frail and pre-frail groups. These differences may be partly explained by the specific high-frailty status of nursing populations. . Consequently, frail people may be influenced by postural control alteration and daily activities, showing difficulties and increased risk of falls, especially when handling objects at a height or getting up from a chair (Barry et al., 2014).

To our best knowledge, while the gait pattern is well documented, to our best knowledge, a few study has compared plantar pressure distribution between frail and pre-frail individuals. For example, Scott *et al.* (Scott et al., 2007) compared the foot pressure and contact time during gait between 50 young and 50 older participants. They reported that older participants presented significantly decreased peak pressure on the heel, forefoot, and hallux and increased contact time on the heel and forefoot compared to younger people. Our results are in accordance with theirs concerning the tendency for a higher Hallux pressure in pre-frail participants and significantly higher rear foot contact time in frail subjects ($p = 0.04$). Even if pressure distribution and frailty have not been studied together, a comparison between elderly fallers and non-fallers can be made. Indeed, Nakajima *et al.* (Nakajima et al., 2014) also reported a reduction of plantar peak pressure in fallers and an extension of the double support phase. In the present study, the higher rear foot contact time measured in frail people suggested that this population presented similar patterns to elderly fallers with a shortening swing phase up to its elimination, leading to a shuffling gait. This is confirmed by the trend observed in hallux pressure, which tends to be higher in pre-frail people. More recently, Anzai et al., 2022 found that the classification of participants relative to their frailty state primarily relied on features obtained from the different plantar pressure during the walking in line with the present study (Anzai et al., 2022). Considering that it is commonly accepted that the forefoot and hallux are the propulsive part of the foot, it could be hypothesized that frail people no longer use them. Even if this result could be a consequence of plantar deformation, it was mostly due to a particular gait pattern. Various explanations could be given, such as plantar tissue stiffness, decreased strength, sensitivity or mobility of the foot, or alteration of the somatosensory system. Future studies are needed to explore these parameters. In consequence, it suggests that the measurement of the plantar pressures may be used as the new approach to evaluating aspects of the degree of frailty related to physical ability such as SPPB.

Whereas cervical function is the last possibility for the spine to adjust the changes induced by the foot postural entry, among others, to the best of our knowledge, it has never been reported in the literature in frail and pre-frail older people. Although Pan *et al.* (Pan et al., 2018) described a global decrease in all cervical mobility, discontinuous across age, they could not conclude reference values due to the large variability of results. Swinkels *et al.* (Swinkels and Swinkels-Meewisse, 2014) made the same conclusions and found that tilt and extension were not changed before 60 years. In the present study, only one parameter was significantly discriminant between frail and pre-frail: tilt mobility. Rotation or flexion mobility and strength would also be expected to be discriminant regarding previous findings (Quek et al., 2013; Liang et al., 2014), but this was not the case. Different hypotheses could be made: first, some authors explored the position of the head related to the trunk with passive stiffness. At the same time, we measured active mobility considering that it was more representative of daily life requirements. Moreover, as participants included in the present study were older and frailer than in the literature, more considerable variability in measurement could be hypothesized and make comparison difficult. Concerning cervical strength measurement, although HHD was painless and not intrusive, it did not allow differentiation of deep and superficial muscles, unlike an intra-muscular sensor, and this could explain the lack of differences observed in strength measurement.

Finally, tilt mobility seemed a relevant parameter because of its strong direct impact on the vestibule and inner ear orientation. Some neurophysiological hypotheses could be mentioned to explain the present results according to previous studies, which explored the influence of age on postural reflex. It is well known that aging provokes an alteration of vestibular structure, which could lead to so-called “vestibular omission” with a distortion of vestibulospinal and oculo-vestibular reflexes. In healthy participants, the cervical-ocular reflex increases to compensate for this loss (Kelders et al., 2003), and this reflex is mainly driven by rotation. The physiological compensation could be modified by diminished neck movement like hypokinesia, causing an increase in cervical-ocular and vestibular-ocular reflex (Ischebeck et al., 2018). Specifically, this capacity is less significant than the other movements (Watier, 2006), so it did not permit intra-movement compensation as rotation or extension did. Thus, the inclination capacity of the cervical spine is decisive for adaptation to everyday movement, particularly in case of loss of balance, and seems to be a marker of frailty.

Limitation

The present study suffers some limitations, such as a relatively low number of participants, due to the inclusion and exclusion criteria, which excluded participants with cognitive impairment. However, this dimension is often altered in the elderly and drives to institutionalization, making recruiting a significant number of participants difficult. The further study requires recruiting more participants, especially younger participants, which permits confirmation of the current finding and investigates the impact of the age range on the current parameters. Finally, more features should be extracted

from pressure data for a combined spatio-temporal analysis and have a deeper insight in gait quality alteration.

Conclusion

In conclusion, as expected from the literature, some parameters like gait speed and muscular strength appear to be determinants for the level of frailty. Still, some new parameters, such as cervical tilt and plantar pressure distribution in walking, have also been observed. Considering that cervical mobility can be easily measured, it could become part of a clinical routine. Although plantar pressure measurements require specific equipment and competence, some professionals, such as podiatrists, could be involved in detecting frailty. Moreover, the combined use of technology and conventional support shows encouraging results in the prevention of falls (Giovannini et al., 2022). Further studies could enable exploration of the influence of cervical tilt and pressure plantar in walking on physical performance in older people. Evaluation or changes in one of these parameters should raise the attention of health practitioners and improve the individualization of prevention and rehabilitation programs.

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 studies involving human participants were reviewed and approved by Université Claude Bernard Lyon 1. The patients/participants provided their written informed consent to participate in this study.

Author contributions

LP, KM, and CH conceived and designed the experiments and wrote the manuscript. QZ and CH analyzed the data and contributed materials and analysis tools. LP, SB, KM, and QZ provided critical feedback and contributed to the final version. 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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Towards AI-driven longevity research: An overview

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While in the past technology has mostly been utilized to store information about the structural configuration of proteins and molecules for research and medical purposes, Artificial Intelligence is nowadays able to learn from the existing data how to predict and model properties and interactions, revealing important knowledge about complex biological processes, such as aging. Modern technologies, moreover, can rely on a broader set of information, including those derived from the next-generation sequencing (e.g., proteomics, lipidomics, and other omics), to understand the interactions between human body and the external environment. This is especially relevant as external factors have been shown to have a key role in aging. As the field of computational systems biology keeps improving and new biomarkers of aging are being developed, artificial intelligence promises to become a major ally of aging research.

KEYWORDS

artificial intelligence, machine learning, biomarkers, feature selection, deep aging clock, longevity medicine

1 Introduction

The process of aging is known to be dependent upon the interaction of different factors, such as the genome content of an individual, lifestyle factors, environmental interaction, and health facilities available to the individual (Newman and Murabito, 2013; Partridge et al., 2018; Singh et al., 2019). Increased lifespan and age represent the exceptional survival, maintenance of good health as compared to peers, delayed onsets of age-dependent diseases, and extreme phenotype of individuals (Kaeberlein, 2018; Pignolo, 2019).

Previous works have emphasized how modern Artificial Intelligence (AI) is already playing an important role in speeding up decision-making in medical sciences by means of advanced machine learning (ML) algorithms. For example, it is revolutionizing the drug discovery process, saving money and time (Kelemen et al., 2008), as it is already being used to create the structure of new drugs depending on the specific structure of the target disease-causing compound [see (Santus et al., 2021) for an overview]. In life sciences, the generation of high-throughput data such as proteomics, genomics, chemoproteomics and phenomics

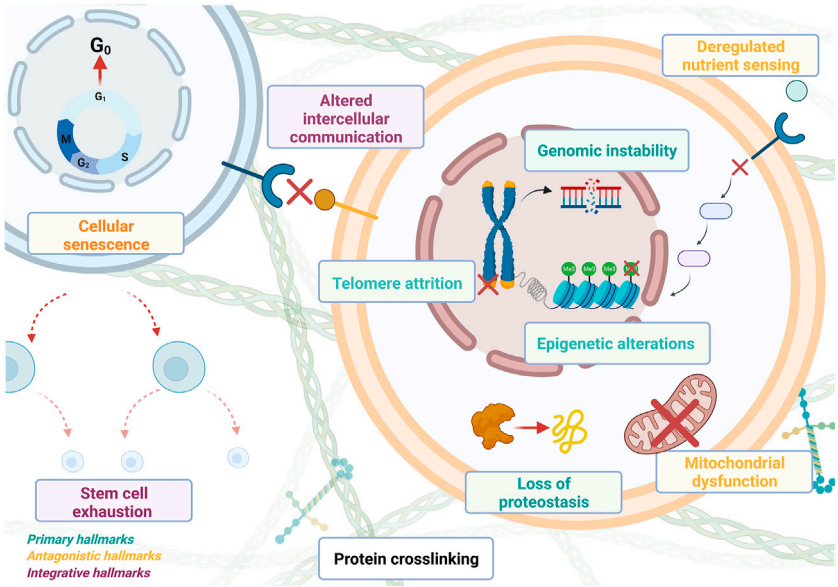

FIGURE 1
Summary of the hallmarks of aging. Primary hallmarks of aging, the primary causes of cellular damage, include genomic stability, epigenetic alterations, loss of proteostasis, and telomere attrition. Antagonistic hallmarks (this refers to factors that originated from body responses to the damage itself but end up exacerbating it) include mitochondrial dysfunction, deregulated sensing, and cellular senescence. Integrative hallmarks of aging (that result from the cumulative action of the previous two groups and are the main determiners of the functional decline) include stem cell exhaustion and altered intercellular communication. Each of these hallmarks has been the focus of intensive research to understand their involvement in the decline of biological functions. ML/AI technologies are used to deepen our understanding of the many components that are involved. This knowledge can help to improve not only our understanding of these mechanisms taken separately but also how the interplay between them unfolds.

combined with the recent development of AI technologies (Alberghina and Westerhoff, 2007; Santus et al., 2021) and the availability of increasingly powerful computational resources allow the deployment of complex ML methods to preprocess massive amounts of data, integrate different input modalities and identify insightful correlations (Kulaga et al., 2021).

The increasing availability of biological data of all types has contributed to greatly improve our understanding of the human body and the systemic nature of biological systems in general. This was accompanied by a conceptual change within biology with the transition from a qualitative, reductionist, structural and most of the time static description to a more systemic description in terms of functional and dynamical properties (Barabási and Oltvai, 2004; Bruggeman and Westerhoff, 2007; Liang et al., 2011). Now, biological entities are more and more often described as dynamical systems made of a multilayered hierarchy of sub-systems containing large numbers of highly connected components. ML techniques are well adapted to discover not only correlations but also causal relations between data to identify key interactions and key regulators that must be integrated into a model to uncover mechanisms that can explain the emergence of specific biological functions. In the context of aging research, there are several mechanisms; called hallmarks of aging (*cf.* Figure 1), that have been identified as playing a central role in the onset and propagation of aging. Understanding the causal relationships taking place within biological systems is a prerequisite to build dynamical, i.e., kinetic, models that can be used to simulate the integral response of a biological system during its development, the progression of a disease, or during pharmaceutical interventions. Dynamic models have been designed, for example, to describe the effects of inflammation, senescence, apoptosis, oxidative stress, accumulation of mutations and DNA damages, cell cycle deregulation, mitochondrial dysfunction, and telomere shortening (Auley et al., 2017). Interestingly, many of these mechanisms have been associated over time with specific pathologies, called aging-related-diseases (ARDs), which commonly appear when individuals get older. For example, various types of cancers are identified as ARDs and their origin is assumed to be connected to genomic instability and decreased capacity for DNA repair, two characteristics of both cancer and aging (Maslov and Vijg, 2009). Many of the mechanisms triggering ARDs have been used to elaborate specific theories of aging, which propose to explain the onset and propagation of aging from a set of molecular mechanisms and leading functions associated with ARDs.

The surge in aging research and associated R&D investments witnessed during the recent years should be put in perspective with the continuous increase in human life expectancy observed over the last decades which has significant long term social impacts and economic consequences. One estimated that over 700 million people were over 65 years old in 2019, a number that might double by 2050 (Prince et al., 2016). Hopefully, thanks to intense scientific research, we are continuously improving our understanding of the intertwined biological processes behind aging. This valuable knowledge combined with the possibilities created by technological developments can be used to develop novel treatments which are much needed in societies where the rise in human longevity is often accompanied with an increased burden of chronic diseases and ARDs including cardiovascular diseases, cancer, and neurodegenerative diseases such as Parkinson disease (PD) and Alzheimer's disease (AD) (Partridge et al., 2018). Beside

the fact that these ARDs result in reduced quality of life of the elderly population, they also present a healthcare and socioeconomic challenge. Many countries facing a continuously aging society have already embraced this challenge by initiating ambitious healthcare development programs and adaptation plans to be able to cope with these unavoidable societal trends. In this context, ML and AI combined with big data and other novel technologies can be deployed to monitor disease patterns within a population, develop adapted geriatric care systems, prioritize, and optimize drug development and design appropriate public health policies to foster healthy aging habits and improved lifestyle among all segments of the populations (Fang et al., 2020).

Aging research and its offspring, longevity research are two very active and rapidly evolving fields. In the present contribution, we propose to discuss a subset of studies which, in our opinion, should provide interested readers and researchers with a broad overview of how aging, when considered from a mechanistic perspective, can be investigated from different viewpoints using classical computational methods combined with ML/AI approaches, leveraging the opportunities offered by the continuously growing sets of health and biological data. There is a tremendous variety of questions and topics of interest to be covered and a large diversity of research methods deployed to investigate them. Scientific studies can investigate biological mechanisms at a fundamental level to clarify their links with the onset of ARDs. In several cases, the authors use the newly acquired knowledge to develop aging clocks. Other research works utilize the already established knowledge of aging mechanisms and associated signaling pathways to identify through computational means potential novel therapeutic targets. In some cases, such analysis proposes a complete workflow with either *in vitro* and/or *in vivo* validation to support the computational findings. These studies are also sometimes complemented with virtual screening experiments to identify compounds with suitable drug-like properties which could constitute the basis of novel therapies. Thus, to structure the discussion, we decided to follow the taxonomy introduced in the classical work by (López-Otín et al., 2013) and organized this article according to the three main areas of intervention of AI technologies. An outline of the article is as follows. Section 2 covers the primary hallmarks of aging, that is, the primary causes of cellular damage, Section 3 focuses on antagonistic hallmarks, namely, those factors that originated from body responses to the damage itself but end up exacerbating it. Section 4 discusses studies interested in the integrative hallmarks which result from the cumulative action of the previous two groups and are the main determiners of the functional decline. We discuss several possible clinical applications in Section 5 and end up with a conclusion in Section 6.

2 Primary hallmarks

2.1 Genomic instability

Genomic instability is the growing tendency of cells to accumulate mutations both in nuclear and mitochondrial DNA, and it is considered as one of the primary hallmarks of the aging process (López-Otín et al., 2013; Laffon et al., 2021). The micronucleus (MN) test and its evolution, the cytokinesis-block micronucleus (CBMN) test, are the most used methodologies to evaluate genomic instability and quantify DNA damage in different tissues. Therefore, their results can be used as biomarkers of genomic instability in aging (Laffon et al., 2021).

Such tests allow measuring the number of micronuclei in several types of cells (Laffon et al., 2021; Wills et al., 2021). Micronuclei are chromosome-derived structures, surrounded by a membrane, that arise from fragments of acentric chromosomes or from entire chromosomes that fail to bind to the mitotic spindle and to segregate properly in the daughter cells (Fenech et al., 2016). The frequency of micronuclei in peripheral lymphocytes has been shown to increase by age groups, with a frequency ratio of 1 in children (<10 years old) and a frequency over 2 in elder people (>70 years old) (Bonassi et al., 2001).

However, a limitation of the CBMN test is that it requires users' manual scoring which makes it time-consuming and subjective, with very low specificity and a high number of false positives (Wills et al., 2021). For these reasons in the last few years the CBMN test was associated with imaging flow cytometry and deep learning (DL) algorithms to automatize and accelerate the procedure (see Figure 2). In (Wills et al., 2021), the authors developed an automated image classification for the CBMN assay by training and cross-validating the "DeepFlow" neural network with data obtained in several laboratories that used different techniques for sample preparation, cytometer calibration, and image acquisition. They trained the system with tens of thousands of images from two laboratories and then tested it with the images of a third one. The "DeepFlow" neural network took less than 2 min to score thousands of images with an accuracy of 98% in mononucleate cells, 82% in mononucleate cells plus MN, 94% in binucleate cells, and 85% in binucleate cells plus MN. Interestingly when evaluating the cases in which the result of the software was different from the human-scored one it was found out that the software was sometimes more valid because it can classify the MN according to its area and to the size of the nucleus of the relative cell. Thus, in this case, integrating AI technology contributed to improve not only the average speed but also the accuracy of the procedure.

A similar automated method was developed by combining imaging flow cytometry with custom-designed software and AI to score the micronuclei in a 3D skin-based model (Allemang et al., 2021). The evaluation of reconstructed skin allows using cells that are naturally more exposed to genotoxic substances, and AI makes it a completely automatic method devoid of subjective scoring. By further training these systems or similar ones, even without user configuration, it will be possible to eliminate the variability of the CBMN test and significantly reduce the associated costs. In this way, the results of the CBMN test will be more accurate biomarkers of genomic instability. Although, until now, this type of test has been primarily used to evaluate genotoxicity, it would be possible to assess aging in the same way and to estimate the biological age of a patient.

2.2 Epigenetic alterations

The term epigenomics refers to mechanisms regulating genome activity independently of changes to DNA sequence (Felsenfeld, 2014). These mechanisms that induce reversible changes can be classified into four categories: remodeling of chromatin

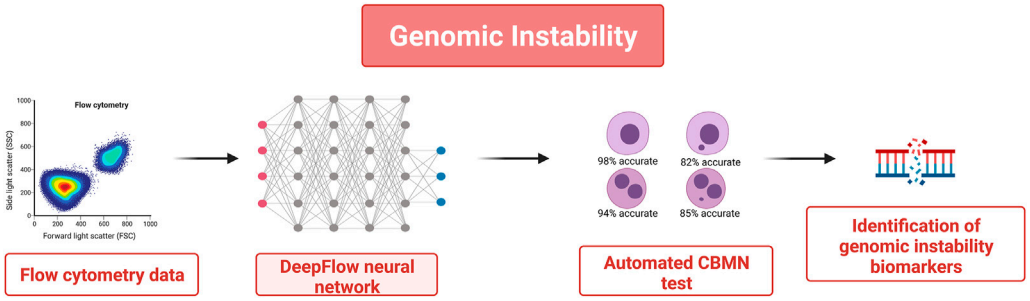

FIGURE 2
AI-based automated CBMN test to quantify genomic damages in tissues. Thousands of images from flow cytometry data were used to train a DL-based image classifier. This allows an automated scoring of the evaluation of the number of micronuclei while reducing time overload and false positives.

conformation, DNA modifications such as DNA methylation, histone post-translational modifications, and RNA-centered mechanisms (including non-coding RNAs and microRNAs) (Pagiatakis et al., 2021). Epigenetics is the main mechanism by which environmental factors such as stress, physical activity, diet but also alcohol consumption influence gene expression. Epigenetic mechanisms can also be modulated by physiological and pathological stimuli (Pagiatakis et al., 2021). Although epigenetic modifications attracted interest for their involvement in aging and in the onset of ARDs, they are also essential for development processes such as tissue and organ formation.

The investigation of epigenetic mechanisms, epigenetic changes and their relationships with aging must begin with the prediction of epigenetic-relevant features such as epigenetic sites and genetic alterations. Despite some successful applications of ML/AI for epigenome mapping (Angermueller et al., 2017) and for the identification of susceptible epimutation sites in the genome (Haque et al., 2015), it should be emphasized that these essential steps already present specific challenges. Indeed, experimental protocols to study epigenetic mechanisms are typically expensive to implement and to some extent ML/AI methods such as active learning must be deployed to reduce the expense of generating epigenetic data. Furthermore, analyzing epigenetic data can be cumbersome because like other biological datasets, raw epigenetic data are typically high dimensional, but the occurrences of interest, i.e., epigenetic marks and epimutation sites, are difficult to find. For instance, DNA methylation data usually contain a few differentially methylated DNA regions (DMR) and many non-DMR sites, although both are described with many DNA sequence and genomic features (Holder et al., 2017). In fact, the number of genomic features within epigenetic data is huge, and the selection of the most relevant ones that could be used to locate epigenetic sites requires specific approaches (see below for an example). In this context, ML/AI techniques are necessary not only to identify the regions of the genome where the epigenetic changes of interest could occur and that are susceptible to epimutations, but also to preprocess and carefully annotate epigenetic data prior to any analysis. Workflows used to that end combine techniques for feature generation and selection, and techniques to deal with the specific characteristics of epigenetic data. Concretely, ML/AI is commonly used to help define the most relevant genomic features. Moreover, imbalanced class learning has proven to be useful to compensate for the relatively low occurrence of relevant epigenetic events.

An example of how epigenetic marks can be selected and used to characterize a disease state is shown in (Bahado-Singh et al., 2022) where the authors determined whether epigenetic changes occur in patients with Coarctation of the aorta (CoA) (see Figure 3). CoA is a congenital heart defect that might have epigenetic origins because prior studies showed that significant methylation changes were found in the DNA of newborns with CoA. Thus, the authors decided to use Cytosine nucleotide (CpG) methylation changes in samples from patients with CoA and healthy patients (obtained from Genome-wide DNA methylation analysis of 24 newborn blood DNA with CoA cases and 16 unaffected controls) to build several classifiers to distinguish CoA samples from controls and identify epigenetic patterns specific to CoA. Feature selection was carried out using the probes with statistically significant methylation differences combined with a sufficiently high methylation fold change in CoA compared to controls. Multivariate approaches such as principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) confirmed the possibility to accurately segregate or differentiate the CoA group from controls based on CpG methylation levels. Different methods were used to build the classifiers: random forest (RF), support vector machine (SVM), linear discriminant analysis (LDA), prediction analysis for microarrays (PAM), and generalized linear model (GLM). Features with the highest predictive powers for the various classifiers were characterized by highly statistically significant methylation changes in several CpG loci in CoA relative to controls. To interpret these results from a biological viewpoint, further analysis was performed using Ingenuity pathway analysis to link key features to disease pathways associated with CoA. Furthermore, PCA and PLS-DA also showed that using a limited number of "principal components" (CpG markers) was enough to differentiate the CoA from the unaffected control groups. Those findings pave the way to the identification of novel epigenetic biomarkers for CoA.

The integration of systems biology, big data science and AI/ML can be a successful strategy to elucidate still largely unknown epigenetic mechanisms involved in aging and in ARDs. Recently,

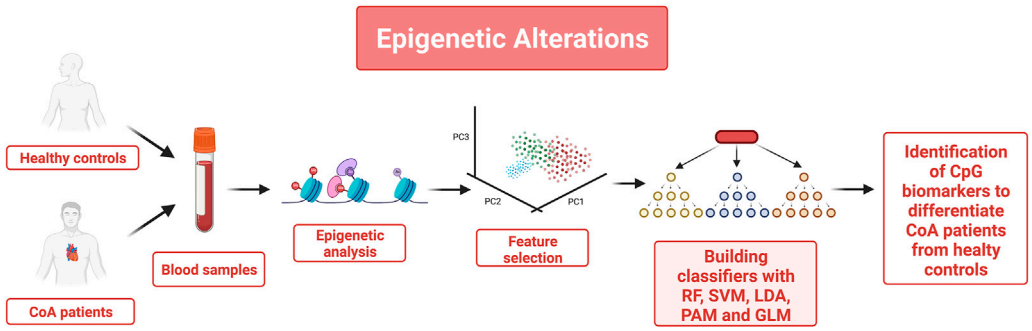

FIGURE 3
Blood samples were utilized to identify epigenetic changes that allow distinguishing healthy controls from patients with coarctation of the aorta (CoA). Cytosine nucleotide (CpG) methylation changes obtained from Genome-wide DNA methylation analysis were selected as mean features. Only the probes with statistically significant methylation differences combined with a sufficiently high methylation fold change were kept as features and Multivariate approaches such as principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) were used to confirm that these features could accurately distinguish the CoA samples from the controls. 5 classifiers were built and trained using these features. Features with the highest predictive capabilities are potential novel biomarkers candidates for CoA.

ML/AI has been successfully applied to analyze omics and clinical data gathered in epigenetic studies (Oh et al., 2015; Ladd-Acosta et al., 2016; Holder et al., 2017). These applications also included the detection of DNA methylation characterizing specific diseases and aging related pathologies (Crowgey et al., 2018). Another study focused on the identification of correlations among methylation marks and found that different methylation profiles exist for different diseases as well as for tissues of different types (Luedi et al., 2007). The findings supporting that aging and the onset of ARDs are often associated with specific and reproducible changes across DNA methylation sites were exploited to develop a class of biomarkers of aging usually known as epigenetic clocks. These clocks function by integrating information about DNA methylation sites known to be correlated with chronological age across multiple tissue types and different populations. These epigenetic clocks have been shown to be able to predict the possible onset of ARDs such cardiovascular diseases and different types of cancers (Bocklandt et al., 2011; Hannum et al., 2013; Horvath, 2013; Weidner et al., 2014). Other recent examples of epigenetic studies using ML/AI include the classification of prostate cancer (Aref-Eshghi et al., 2018) and heart disease (Dogan et al., 2018).

A point worth mentioning is that genetic and epigenetic studies are still generally accomplished independently and as a result, physiological relationships between genetics and epigenetics in diseases remain poorly understood (Hamamoto et al., 2019). Studies revealed that there is a degradation of functional transcriptional networks correlated with an increase in heterogeneity between the epigenome and the transcriptome during aging (Hernando-Herraez et al., 2019). Epigenetic alterations have also been shown to impact genome stability and promote genetic sequence mutations (Skinner et al., 2015; McCarrey et al., 2016). Thus, to improve our understanding of genetic variations and epigenetic deregulations, multimodal analyses of big omics data should be deployed using AI platforms that allow integration between genetics and epigenetics.

2.3 Telomere attrition

Telomeres are sequences of tens of kilobases formed by the repetition of six nucleotides associated with protective proteins located in the terminal regions of chromosomes. Telomeres have two main functions: to prevent DNA repair and recombination complexes from recognizing the linear ends of chromosomes as broken ends, and to protect the gene content of chromosomes from degradation due to the progressive shortening of DNA following replication (Blackburn et al., 2015). In fact, the DNA replication machinery fails to copy the final nucleotides of the chromosome causing a shortening of the telomeres. In specific types of cells, the telomerase, a reverse transcriptase, is active to prevent telomere shortening and maintain their length (Hou et al., 2017). When the telomere length reaches a certain threshold, known as Hayflick limit, the cell cycle stops, and the cell becomes senescent (Liu et al., 2019). Therefore, telomere shortening is associated with senescence and aging (López-Otín et al., 2013). Numerous meta-analyses have been conducted in recent years, especially to decipher the relationship between telomeres and environmental factors: those are very useful tools that allow increasing the samples used in statistical surveys but suffer from deep methodological differences (Wang et al., 2018).

The results of the meta-analyses suggest that telomere shortening is associated with increased mortality risk in the general population. They also provide insights on the impact of the environment on telomere shortening. For instance, several studies show that the Mediterranean diet and the absence of cigarette smoking are correlated with longer telomere length, while the relationship with physical activity is not clearly established (Arsenis et al., 2017; Astuti et al., 2017; Wang et al., 2018; Canudas et al., 2020). In all the above-mentioned studies, the authors suggested that larger-scale analysis and clinical trials would be necessary to confirm these conclusions with greater certainty.

Currently, the leukocyte telomere length is used as a biomarker for healthy aging, despite the fact that there are many inconsistent and contradictory data relating to its effectiveness (Hartmann et al., 2021; Vaiserman and Krasnienkov, 2021). In fact, as previously mentioned, there are environmental factors that contribute to the

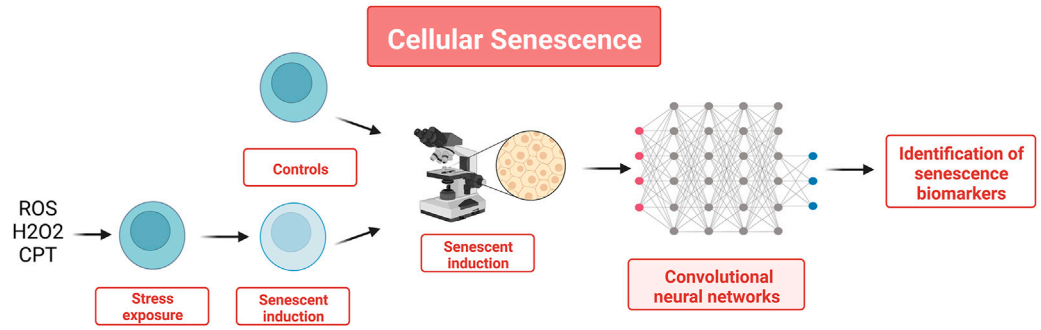

FIGURE 4
To identify novel aging-related proteins, the full set of human proteins was extracted from the Swiss-Prot database. Already known aging related proteins listed on the GenAge database were used as instances of the aging-related class. UniProt, Gene Ontology and GeneFriends databases were used to extract 21,000 protein characteristics that were subsequently used as features to train 3 classifiers to identify proteins likely to be associated with aging.

telomere shortening and it has been shown that the length of the telomeres fluctuates by 2%–4% monthly (Galkin et al., 2020). Therefore, without adequately accounting for most of the variables involved in the shortening of telomeres and in the oscillation of their length, it remains difficult to use this measure as a reliable biomarker.

Bioinformatics and deep mining approaches can be useful for better understanding the biological interactions of telomeres and telomerase. For example, (Hou et al., 2017), analyzed numerous databases with multiple bioinformatics approaches to map the interactions of telomerase reverse transcriptase (TERT, one of the two main components of telomerase) with other proteins and its function in different biological pathways. With similar bioinformatics approaches it will be possible, thanks to data already available, to obtain new information on the enzymes involved in the shortening of telomeres. A better understanding of the phenomenon of telomere attrition will make possible using this parameter as a reliable aging biomarker although recent articles and reviews have suggested that telomere attrition could just be used inside a panel together with other aging factors to improve the efficacy in the assessment of biological age (Galkin et al., 2020; Vaiserman and Krasnienkov, 2021).

2.4 Loss of proteostasis

Protein homeostasis, or proteostasis, refers to the balance that must be maintained between the newly folded proteins and the degradation of the superfluous, older proteins with the aim to prevent protein misfolding and accumulation of protein aggregates. Proteostasis is regulated through a complex network comprising molecular chaperones, proteolytic machinery, and their regulators (Hipp et al., 2019). The EU-funded project PROTEOSTASIS (Cell-type-specific modulation of protein homeostasis in health and disease) has investigated the proteostasis mechanisms and found that the identity and concentration of chaperones and proteostatic machinery are highly cell-type specific. Further investigation of the capacity of proteostasis in different tissues under various physiological and stress conditions shows that there is a strong decline in folding capacity in the transition to adulthood, followed by the loss of protein solubility and the accumulation of aggregates (when they exceed the physiological concentration). These results led to the conclusion that proteostasis dysregulation is the main cause of age-related protein misfolding diseases. From a broader perspective, it is important to develop methodologies to consider all the proteins, their different interactions, and the ways they change their expression through aging.

To obtain a better picture of which proteins intervene in the repair and maintenance mechanisms such as autophagy for instance which is well-known for being affected as individuals age, the authors of a recent study (Kerepesi et al., 2018) used ML methods to build classification models using multiple protein features to identify new aging-related proteins that have a particularly prominent role in repair and maintenance mechanisms (see Figure 4). Data used for this study included the set of aging-related genes available in GenAge, a manually curated database made of 305 aging-related genes. The corresponding proteins were used as instances of the aging-related class. All other human proteins in Swiss-Prot, were used as the instances of the non-aging-related class. Multiple features were extracted from the data. In total 21,000 protein features were extracted from different databases. This included co-expression and protein-protein interaction features such as the number of aging-related neighbors and several other topological properties of the protein-protein interaction networks associated with the selected proteins. Three ML methods (XGBoost, logistic regression (LR), and SVM) were deployed to build classifiers that could distinguish aging-related proteins from the non-aging-related ones. In the second part of the study, the trained models were utilized to predict new aging-related proteins. The score associated with the proteins by the classifiers gives an insight on the role of a protein in the aging process. To identify the most important aging-related features of human aging-related proteins, XGBoost feature selection capabilities were used to identify the top 36 protein features. A reduced and more easily interpretable model based on these 36 features was designed and demonstrated high prediction performance. It was used to select a list of the twenty new most relevant aging-related proteins. This study showed how the most

relevant protein features can provide an insight into the regulation of the aging process.

Proteins found within human plasma are another unvaluable source of information to be used to characterize and quantify the onset and propagation of aging. Intensive research has been undertaken to systematically identify proteins whose expression shows strong patterns of changes with age and that could constitute a reliable proteomic signature of the phenomenon. Proteins found in plasma are attractive for this endeavor because they affect phenotypes and are directly involved in the dynamics of signaling pathways regulating many of the physiological manifestations of aging. From this viewpoint, proteomic signatures are even more advantageous than epigenetic signatures for instance because the effects of molecular changes such a DNA methylations and other epigenetic changes are not always very well understood. In Tanaka et al. (2018), the authors measured a total of 1301 proteins in plasma in a cohort of 240 healthy individuals aged from 22 to 93 years old with the goal to identify proteins associated with chronological age while avoiding as much as possible the effect of clinically detectable diseases. After multiple adjustments for body mass index and serum creatinine for instance, they assembled a list of 210 age associated proteins. The association of these age-related proteins with several clinical characteristics was analyzed and the authors analyzed how proteome can predict chronological age by designing a proteomic signature of age using an elastic net regression model. Several predictors were built using different subsets of age associated proteins ranging from 76 predictor proteins to only one protein. This first study was later expended by considering a second population sample of almost one thousand participants in the Italy-based InCHIANTI study (https://www.nia.nih.gov/inchianti-study) (Tanaka et al., 2020). The purpose of this second analysis made on a much larger and diverse cohort was to confirm the age-associated proteins reported in the first study, and to uncover their relationship with ARDs. The authors used a two-sample Mendelian Randomization method to study the causal relationship between age-related proteins with ARDs. Interestingly, for some age-related proteins, DNA methylation was shown to partially explain the observed age associations. Another study that showed how proteins that significantly change their expression level with age are also often proteins that directly impact longevity and the onset of ARDs was presented in (Lehallier et al., 2020) where the authors measured the q-value and age coefficient of 529 previously identified aging plasma proteins (103 of which have a HAGR listing). The proteins were analyzed in a plasma proteomic dataset derived from 4263 individuals, using an online software tool. The authors found that approximately 95% of them significantly ($q < 0.05$) changed their expression level with age. They performed ML modeling and fitted a LASSO linear regression on a plasma proteomic dataset derived from 3301 individuals, finding an ultra-predictive aging clock composed of 491 protein entries. The latter was used to demonstrate, for example, that aerobic exercise trained individuals are predicted to be younger than their actual biological age, compared with physically sedentary subjects. Moreover, they unveiled a multitude of novel aging clocks that are made up of a smaller set of proteins (Lehallier et al., 2020). This has the obvious effect of reducing the costs, making the prediction of patient age logistically simpler and therefore easier to implement on a larger scale.

3 Antagonistic hallmarks

3.1 Mitochondrial dysfunctions

Mitochondria are dynamic structures well-known for primarily acting as cellular energy generators by producing adenosine triphosphate (ATP) either through mitochondrial oxidative phosphorylation or through anaerobic glycolysis, a second ATP production route in which nicotinamide adenine dinucleotide (NAD+), a small molecule that regulates many biological processes, plays an important role (Aman et al., 2019). Mitochondrial homeostasis and maintenance are unsurprisingly considered as key to health and paramount for healthy aging. Mitochondrial homeostasis and quality are strongly regulated by mitochondrial-autophagy, termed mitophagy, the biological process responsible for the elimination of defective mitochondria. Mitophagy was shown to be important in neurons for the maintenance of neuronal function and to prevent neuronal cell death and pathogenic brain ageing, which are partially caused by an impairment of mitophagy and subsequent accumulating dysfunctional mitochondria. Mitochondrial dysfunction has been recognized for a long time as an antagonistic hallmark of ageing and is an important component of the age-related cellular processes that contribute to the onset of ARDs. For instance, mutations of nuclear- or mitochondria-encoded mitochondrial proteins are known to trigger mitochondrial disorders (Scheibye-Knudsen et al., 2015), while mitochondria-mediated ATP deprivation and oxidative stress are associated to the pathogenesis of cancer and neurodegenerative diseases such as AD and PD. As impairment of mitophagy is common to many age-related neurodegenerative pathologies such as Alzheimer's disease, ML and AI approaches have been deployed to identify mitophagy modulators that could be used to design novel strategies to improve removal of dysfunctional mitochondria (Xie et al., 2022). In Xie et al. (2022), the authors carried out a computational screening of a large library of natural compounds using both supervised and unsupervised ML approaches to identify new mitophagy-inducing compounds. The workflow utilized vector representations of molecular structures, pharmacophore fingerprinting and conformer fingerprinting to identify two potent mitophagy inducers (Kaempferol and Rhapontigenin) whose activity was tested *in vivo* in nematode and rodent models of AD. Other strategies proposed to focus on the NAD + mitophagy axis. Indeed, the molecular mechanisms of the NAD + mitophagy axis are globally well understood and there is an accumulation of evidence that suggest a correlation between the onset of AD with the depletion of NAD + levels that impacts mitochondrial biogenesis and the clearance of damaged mitochondria. Therapeutic strategies to boost NAD + levels are thus considered as promising treatments against AD and neurodegenerative diseases in general. AI/ML technologies are going to play an important role in this endeavor in areas such as compound screening, lead compound discovery, drug target identification and biomarker development (Aman et al., 2019; Ruixue, et al., 2021).

3.2 Deregulated Nutrient Sensing

The highly conserved mechanistic target of rapamycin signaling pathway (mTOR) is the main nutrient sensor and works as a key controller of cellular metabolism and cell organization. mTOR

complexes (TORC1 and TORC2) have multiple interactions with various intracellular molecules, mainly the Insulin Growth Factor (IGF) and AMP-activated protein kinase (AMPK) contributing to coordinate a plethora of cellular processes including gene transcription, translation, autophagy, as well as cell metabolism (Bjedov and Rallis, 2020). mTOR activation is critically involved in the autophagy process, a degradation system that mediates the breakdown of major macromolecules (lipids, polysaccharides, and proteins). This process is constantly active and permits to maintain the physiological activity of cells and their survival also in states of metabolic imbalance. Its deregulation strongly contributes to biological effects occurring in aging and chronic diseases of elderly (Lamming and Bar-Peled, 2019; Bjedov and Rallis, 2020). Interventional strategies (genetic, pharmacological, and behavioral) are known to act *via* decreasing mTOR activity, suggesting that its hyperactivation supports the age-related functional failure (Lamming and Bar-Peled, 2019). As a definite dietary intervention that effectively increases healthy lifespan has not been delineated so far, caloric restriction (CR) regimen and alternative nutritional strategies have been reported as valuable strategies to promote healthy aging in animal models, also suggesting a similar effect in humans (Flanagan et al., 2020). Several drugs and other compounds have been shown to act as CR mimetics with various mechanisms, being directly or indirectly associated with mTOR-mediated autophagy regulation (Chung and Chung, 2019; Stead et al., 2019).

Bioinformatics combined with ML algorithms is commonly used to investigate the relationships between autophagy/apoptosis and aging. The consensus supports the role of numerous proteins and genes as predictors of aging-relatedness (Kurz et al., 2008; Kerber et al., 2009). For instance, using supervised ML systems, the gene AKT1 (associated with apoptosis) was revealed as being age-related with high probability (Xu et al., 2002). In addition, experiments on mice revealed that if a cellular protein has an influence on CDK1 (involved in apoptosis), then it is probably linked to aging-process (Xu et al., 2012; Fabris and Freitas, 2016). An autophagy flux sensor, named red-green-blue-LC3 (RGB-LC3) was developed to detect the different footsteps of autophagy progression and the deregulation of this process at different levels (Kim et al., 2020). In addition, different computational methods have been applied for the mathematical modeling of the core regulatory machine of autophagy (Sarmah et al., 2021). The integration of different data types can widen our knowledge of the molecular mechanisms governing autophagy. This could be helpful in the context of the development of targeted therapies (Sarmah et al., 2021).

3.3 Cellular senescence

Cellular senescence is defined as a condition in which a cell can no longer proliferate. The accumulation of senescent cells is one of the most important processes in aging (Silva-Álvarez et al., 2019). Senescent cells are in the G1 phase of the cell cycle, and even if they are not responsive to external stimuli, they are metabolically active and can modify gene expression. Senescent cells can be found in various tissues affected by various diseases (osteoarthritis, pulmonary fibrosis, atherosclerosis, Alzheimer's disease, liver fibrosis and cancer) and play an important role in tumor genesis, as demonstrated in mice that undergo senescence (Krizhanovsky et al., 2008; Naylor et al., 2013). Senescent cells are known to have a unique morphology that can be easily identified, and cell morphology images obtained by phase-contrast microscopy contain numerous biological data such as cellular identity and status that can be used as input for a morphology-based identification system that could be utilized to distinguish senescent cells from others. In this context, endothelial cells have attracted interest because they serve many functions in homoeostasis and are involved in the pathology of age-related diseases through cellular senescence. Recent studies (Kusumoto et al., 2018) have proposed to use convolutional neural networks (CNN) to identify endothelial cells derived from pluripotent stem cells, using phase-contrast microscopy images. This CNN system was later adapted by the same research team to identify senescent cells (Kusumoto et al., 2021). In this study, the images to be used as input data were obtained by inducing cellular senescence in human umbilical vein endothelial cells with hydrogen peroxide (H2O2) and camptothecin (CPT). 50 × 50 pixels of input datasets were prepared at the single-cell resolution level from phase-contrast images acquired under each condition. The final number of pictures was 92,242 for H2O2-induced senescence, 41,207 for H2O2 control, 134,097 for CPT-induced senescence, and 64,535 for CPT control. The images were then analyzed in a network to predict them as senescence or control. The predictions were compared with predetermined answers, and weights were optimized to train the CNN. The non-linear prediction of the CNN provides a binary output, meaning that the CNN classified cells as senescent or control (*cf.* Figure 5). These results were shown to be superior to the ones obtained with three other ML methods (SVM, RF, and LR). In that case, the features needed to generate the inputs were extracted using Histograms of Oriented Gradients, a commonly used feature descriptor (Dalal and Triggs, 2005).

The possibility to properly characterize and identify senescent cells is an important step toward the development of therapeutics that can be used to remove them from host tissues. The most well-known of these compounds are called "senolytics", a new family of natural compounds such as Dasatinib and Quercetin, that could be useful to neutralize senescent cells (Song et al., 2020). Several companies have started the development of novel therapeutic drugs in this area with AI, using different algorithms for the so-called Life Extending Medicine (Dolgin, 2020). The company Dorian Therapeutics developed a new class of therapeutics that can rejuvenate cells and tissues: the "senoblockers". They focused on the function of Usp16 that contrasts the self-renewal and senescence pathways, switching the entire genetic program of the cells into a more useful expression profile. In human tissues, overexpression of Usp16 reduces the expansion of normal fibroblasts and post-natal neural progenitors, while downregulation of Usp16 partially rescues the proliferation defects of fibroblasts and expands the stem cell compartment in blood, mammary epithelial tissue, and brain. Usp16 is known to have an important role in the accelerated aging observed in people with Down's Syndrome (Adorno et al., 2013). Another company, Rubedo Life Sciences, is developing a novel method using small molecules to selectively target and clear senescent cells from aged or pathological tissues using the platform ALEMBIC. Their new class of senolytic prodrugs promises

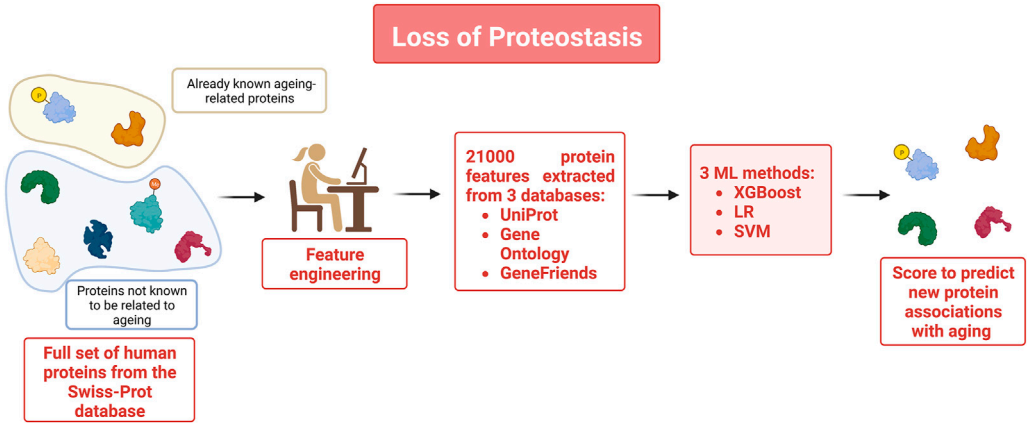

FIGURE 5
To identify senescent cells, this study relied on the fact that senescent cells elicit a very specific morphology to generate a set of features using cell morphology images obtained by phase-contrast microscopy. The set of 50 × 50 pixels images were used to train a CNN as a classifier that could distinguish senescent cells from healthy cells with a greater accuracy than classical ML methods (SVM, RF, and LR).

capabilities such as targeting selectively and safely specific senescent cell types in multiple tissues to treat age-related diseases in geriatric people (Doan et al., 2020).

It will interesting to see how these different techniques will be combined into an end-to-end biomarker development, target identification, drug discovery and real-world evidence pipeline that may help accelerate and improve pharmaceutical research (Zhavoronkov et al., 2019a).

4 Integrative hallmarks

4.1 Stem cell exhaustion

The regenerative capabilities of the tissues depend on the pool of stem cells, known to be able to differentiate into different predefined cell types. The newly differentiated cells play an important role for tissue and organ maintenance. It is known that these pools of stem cells tend to decrease over time and finding ways to replenish exhausted stem cell pools within tissues is a major axis of stem cell research and regenerative medicine. Stem cell exhaustion is thus intrinsically associated with aging. The decline of the regenerative potential of the tissues is a multifactorial process impacted by the accumulation of DNA damages (Rossi et al., 2007), telomere shortening (Flores and Blasco, 2010) and excessive proliferation of progenitor cells (Rera et al., 2011). There are three ways, ML/AI technologies may play a major role in this context. Firstly, AI/ML can be deployed to help to elucidate unknowns surrounding the mechanisms behind stem cell fate decision and cellular specialization. Secondly, the capabilities of ML/AI to handle classification tasks where multiple features and non-linear relationships between them must be considered can be highly beneficial for stem cell classification. Thirdly, ML/AI technologies can be used for the design of new systems for cellular engineering in the context of the development of novel stem cell therapies.

4.1.1 Stem cell fate decision and cellular specialization

Depending on their self-renewal and differentiation (specialization) capabilities, stem cells are classified into different categories. The most famous are probably pluripotent stem cells, such as embryonic stem cells (ESCs), which can give rise to every cell type in the formed body, but not the placenta and umbilical cord (Labusca and Mashayekhi, 2019). Studies demonstrated that adult skin tissues contain cell populations with pluripotent characteristics (Chunmeng and Tianmin, 2004). On the other hand, multipotent stem cells, for example, can develop into more than one cell type but are more limited than pluripotent stem cells. Multipotent stem cells from hair follicle and non-follicular skin for instance are found to have the differentiation capacity to generate multiple cell lineages. Other examples of multipotent stem cells are adult stem cells and cord blood stem cells. Sobhani et al. (2017). Observations show that pluripotency (or multipotency) state maintenance which is critical for tissue regenerative abilities is a function of the external environment of the stem cells. A dynamical balance between environmental factors and cellular signals help to preserve the tissue regenerative capacity of stem cells. Stem cell differentiation into a specific cell lineage is often induced by an alteration of this dynamical balance by external perturbations which activate or inhibit biological pathways. This impacts the transduction signals received by transcription factors (TFs) which form highly connected gene regulatory networks (GRNs) within the nucleus (Thomson et al., 2011; Tantin, 2013) and ultimately act as master regulators of the stem cell fate decision (Iglesias-Bartolome and Gutkind, 2011; Tsai and Hung, 2012). Thus, a realistic description of how stem cell fate decision operates should consider not only the GRNs localized inside the nucleus but also the network of signaling pathways located and operating inside the cytoplasmic compartment which transmit signals received from the stem cell environment.

The fact that stem cell fate decision can effectively be controlled through activation or inhibition of TFs was demonstrated in (Takahashi and Yamanaka, 2006) where the authors used AI

algorithms to identify key regulators of stem cell fate decision. Thanks to the discovery of key regulatory TFs, they were the first to generate Induced Pluripotent Stem Cells (iPSCs). Another study by Dunn and others used computational modeling to elucidate the dynamics of the GRNs controlling the fate of self-renewing mouse Embryonic Stem Cells (ESCs) (Dunn et al., 2019). They were able to show that a common deterministic gene regulation program might be sufficient to govern the maintenance and induction of naïve pluripotency.

Since then, the field of cellular specialization, which studies stem cell differentiation continued to study the molecular basis of stem cell fate decision as much behind the mechanisms of differentiation remain to be clarified. Regarding the role played by GRNs in stem cell fate decision, a recent study (Gheorghe et al., 2019) showed how to develop the ChIP-eat model, combining computational TF binding models and chromatin immunoprecipitation followed by sequencing (ChIP-seq) to automatically predict direct TF-DNA interactions. Other studies focused on developing a comprehensive evaluation of state-of-the-art algorithms for inferring GRNs from single-cell gene expression data. In (Pratapa et al., 2020), the authors developed BEELINE to use synthetic networks with predictable cellular trajectories and curated Boolean models to evaluate GRN inference algorithms' accuracy.

From a therapeutic perspective, understanding stem cell decision and specialization would open the doors to tremendous long-term opportunities. An important area of research aims at developing methods to predict the behavior or function of cells produced using methods from synthetic biology. Those cells do not mimic *in vivo* identity but are able to perform specific functions, as cell fate reprogramming is often performed by constant overexpression of specific TFs. However, this process can be unreliable and inefficient. Therefore, approaches based on mathematical analysis and computational methods are expected to be the way to go for the future developments of the discipline (Del Vecchio et al., 2017). For example, in Stumpf et al. (2017) the authors propose to study cell fate using a framework where stem cell differentiation is modeled as a non-Markov stochastic process. Another example is presented in Jones et al. (2020), where the authors generated an experimental lineage tracing dataset with 34,557 human cells continuously traced over 15 generations. In this case, the CRISPR/Cas9-based gene editing approach Jiang and Doudna, (2017) combined with AI can be highly effective.

4.1.2 Stem cell classification

AI systems are used to identify and analyze genes involved in stem cell differentiation and specialization (Haque et al., 2017). An example of such system is the GCTx-TFome, which was used to discover 240 previously unreported TFs involved in ESC differentiation. This system operates by performing large computational screening of the human transcriptome (Ng et al., 2021). CNN is another example of deep learning architecture which was originally a very popular tool in computer vision, given its efficiency in modeling two-dimensional data (LeCun et al., 1999). The development of CNN enables the automation of the cell type identification from phase-contrast microscope images without molecular labeling. The objective is to develop a program that can judge medical conditions as accurately as a physician (Kusumoto and Yuasa, 2019). As discussed above, CNN has been used to create an automated method to identify endothelial cells derived from iPSCs without the need for immunostaining or lineage tracing (Kusumoto et al., 2018).

Another approach to identify stem cells is based on scRNA-seq data. AI can be used to identify stem cells from scRNA-seq data to search for peculiarities of stem cells in the query data. RNA sequencing (RNA-seq) is a genomic approach for the detection and quantitative analysis of messenger RNA molecules in a biological sample and is useful for studying cellular responses (Haque et al., 2017). More recently, (Gulati et al., 2020), developed CytoTRACE, a computational framework based on the simple observation of transcriptional diversity, the number of genes expressed in a cell, and obtained promising results for the prediction of cellular differentiation states from scRNA-seq data.

4.1.3 Cellular engineering

The goal of cellular engineering is to design new therapeutics for patients leveraging the knowledge of the mechanisms behind stem cell fate decision and cellular specialization. In this context, AI is used to evaluate the quality of the engineered cells and to suggest improvements. One example is CellNet (Cahan et al., 2021), a network biology platform that assesses the fidelity of cellular engineering more accurately than any other existing methodology and generates hypotheses for improving cell derivations.

AI can be used to understand the molecular state of a cell in a tissue or within a population, which usually varies stochastically in response to its environment. In many situations, it may be difficult to quantify or even identify the different costs and benefits of a particular response for a cell, particularly for cells in multicellular organisms (Perkins and Swain, 2009). Trajectory inference (TI) is the computational task of determining the position of single cells on temporally regulated biological processes. This method can allow, for example, to study linear tracing with higher accuracy and fidelity. This method is relatively new, and much work will be done to develop it and make it more effective. When designing a system for TI, one needs to consider different indicators such as accuracy, usability, and stability (Saelens et al., 2019). All these features can offer the possibility to define new therapeutics to decrease stem cell exhaustion and allow to tailor the therapy to the specific needs of the patients.

4.2 Altered intercellular communication

The coordination of biological processes and activities between the different cells of an organism occur through the extracellular environment and is a necessary condition for the maintenance of homeostasis within any pluricellular organisms. In this context, cell-cell interactions (CCIs) across the various cell types and tissues are regulated through different types of molecules, including ions, metabolites, integrins, receptors, junction and structural proteins as well as ligands and other secreted proteins located in the extracellular matrix (Armingol et al., 2021). These molecules intervene to regulate CCIs in different ways with some like cell adhesion proteins supporting structural CCIs while other factors such as hormones, growth factors, chemokines, cytokines and neurotransmitters act as ligands to mediate cell–cell communication (Armingol et al., 2021).

Aging is associated with alterations of CCI characterized by a deregulation of endocrine, neuroendocrine, or neuronal signaling, a decrease of immunosurveillance as well as an alteration of the composition of peri- and -extracellular environment. Alterations of the CCI also appear with the emergence of senescent cells which utilize three means of intercellular communication known as classical, emerging, and non-classical (Fafián-Labora and O'Loghlen, 2020). The "senescence-associated secretory phenotype" (SASP) is considered as the classical mean of CCI of senescent cells and can result in both beneficial and detrimental effects according to the trigger factors and the context present when senescence is induced. In general, senescence reactivates the expression of multiple pro-inflammatory genes in many different cell types with a profound alteration of SASP composition enriched in pro-inflammatory cytokines and soluble factors such as IL-6, IL-8, membrane cofactor proteins and macrophage inflammatory. Such molecules tend to promote proliferation, angiogenesis, and inflammation, both in autocrine and paracrine manners (Lopes-Paciencia et al., 2019).

Recent results were obtained on the role of extracellular vesicles as novel SASP components as well as non-cellular metabolites and ions. The new emerging picture supports the hypothesis that a simultaneous combination of all these components may contribute to the deleterious effects associated with senescence (Lopes-Paciencia et al., 2019; Fafián-Labora and O'Loghlen, 2020). One of the major changes which takes place with aging is a chronic and systemic, low-grade dysfunctional inflammation, known as inflammaging, where most of the inflammatory factors involved are also part of the SASP. Thus, the SASP is a primary mediator of the detrimental effects of senescent cells, contributing to the development of a state of chronic, low-grade inflammation which is characterized by high levels of circulating cytokines and increased immune infiltration associated with an increased risk of diseases with age (Lopes-Paciencia et al., 2019). Strategies to eliminate senescent cells and/or to modulate SASP have been investigated with the hope that such approaches could bring therapeutic benefits (see also Section 3.3). One of the main focuses of this research is the development of a new class of drugs referred to as senotherapeutics. This class of drugs consists of two members: senolytics and senomorphics. While senolytics are small molecules that can selectively kill senescent cells through apoptosis, senomorphics drugs have the capacity to at block SASP thus reducing the senescent burden of the cells.

The promising capabilities of senotherapeutics to improve health and contribute to the substantial extension of healthy lifespan have already been reported, even if the results currently available showing the effects of these drugs were obtained from preclinical animal studies, which raise legitimate concerns about a potential underestimation of the side effects resulting from a long-term use and chronic administrations. Regarding senomorphics agents, the molecular identification of SASP factors with a detailed characterization of the different pathways responsible for the expected outcomes may enable future interventions in different tissues (Lagoumtzi and Niki, 2021). In a recent study, a drug-screening system for cellular senescence using a pre-trained CNN identified four compounds (terreic acid, PD-98059, daidzein, and Y-27632 2HCl) with the potential capability to repress senescence *in vitro* (Kumari and Jat, 2021). Through the analysis of transcriptome data, these compounds showed a) anti-inflammatory effects *via* the suppression of NF-KB signaling, a pathway that plays a central role in inflammation and b) the appearance of SASP, indicating that these compounds could be strong candidates for the design of new treatments against ARDs (Kusumoto et al., 2021).

Finally, many efforts have been undertaken to develop guidelines on stem cell applications because the field is still in its infancy. For instance, in (Cahan et al., 2021) the authors described four major applications of stem cell biology (cell typing, lineage tracing, trajectory inference, and regulatory networks) with a detailed overview of the future challenges to be addressed in the near future.

5 Longevity medicine: Translating and applying the hallmarks of aging into the practice

Despite the currently well-established scientific and technological foundations for clinically guided longevity medicine, there is still a large gap between the geroscience and AI-based tools. This translational bridge is challenging, since this new burgeoning medical discipline is of a distinct character, shaped by multi- (virtually all disciplines of the clinical medicine, including genetics, radiology and pathology, etc.) and interdisciplinarity (AI, computational science, gerontology, gerosciences, engineering, etc.), with strong roots in internal medicine dealing with the complexity of co and multimorbidity (Bischof et al., 2022). Longevity medicine has not yet been officially defined by a central medical body, but expert recommendations suggested that longevity medicine is an AI-driven precision medicine, guided by biological age determination with deep aging clocks (Zhavoronkov et al., 2019a). The formal definition might be further enriched by the core goal of longevity medicine, which is to establish and restore the biological age of an individual at each specific point of time to the biological age of the optimal individual performance (Bischof et al., 2022). Longitudinally and cumulatively, this leads to mitigation and ideally also elimination of risks of age-related and overall morbidity. Therefore, the main focus of longevity medicine is to prolong the life lived in good health, both physically and mentally, ergo: extension of the healthy lifespan and not solely the health span (simple prevention) or solely the lifespan (reactive medicine/sickcare) (Bischof et al., 2022).

The release, in 2018, of the first deep aging clocks was a crucial milestone for longevity medicine because it provided clinicians with a wide range of new possibilities to vigilantly track the progress of a patient's/individual's biological age, based on various modalities, e.g., hematological tests, methylation, metabolomic, microbiome, etc. (Zhavoronkov et al., 2019b). Since the progress has been tremendously rapid, most medical professionals are not exposed to the foundations of longevity medicine, nor to its latest fueling scientific and AI sources. Educational resources adapted to clinicians are scarce, with solely one Center for Medical Education offering an accredited course of Longevity Medicine for Physicians (Bischof et al., 2021). However, the autodidactic efforts are increasing, therefore academic programs started to implement healthy aging in the educational curriculum to equip healthcare professionals with the necessary understanding of aging research. Soon, the Healthy

Longevity Medicine Society (https://hlms.co/) will coordinate the development of recommendations and guidelines that will allow to credibly validate and further establish biomarkers of aging, while also educating medical clinicians on how to implement the knowledge in their work with the patients.

6 Conclusion

In the last few years, we have witnessed many AI-enabled technological and biological companies initiating collaborations to use a variety of algorithms to identify lifestyle characteristics that influence how individual age as well as to develop drugs and therapeutic medicine to counteract the deleterious effects of aging and ARDs (Dolgin, 2020). Moreover, AI technologies also hold great promises to study the molecular state of tissues, organs, or cells in response to physical or chemical change in the environment.

When applying ML/AI in the field of aging research, one should keep in mind one major characteristics of this phenomenon which is that, rather than being a localized event, aging is an intrinsically systematic process. The systemic characteristic of aging is well illustrated by the hallmarks discussed herein which despite being of different nature are also highly mechanistically intertwined. The systemic nature of aging can be seen as the result of the hierarchical organization of living systems (Han et al., 2004; Buescher et al., 2012; Nicolas et al., 2012). The human body in particular is a multi-level complex system consisting of billions of independent cells which form different types of tissues, organs and regulatory systems. Dysfunctions affecting even a restricted number of biological processes within some of the cells of one or several organs will often propagate to all parts of the body (Vanhaelen, 2015; Vanhaelen, 2018). The network theory of aging was specifically designed to overcome the reductive nature of the first individual dynamical models usually focusing on a restricted set of hallmark-related phenomena. The main purpose of the network theory of aging was to integrate different mechanisms of aging into a common framework to better understand the systemic consequences of their continuous mutual interactions (Kowald and Kirkwood, 1994; Kowald and Kirkwood, 1996; Franceschi et al., 2000; Slijepcevic, 2008). In this context, modern ML/AI can provide valuable modeling tools to investigate how all aging mechanisms work together, and to shape the global aging pattern.

In this overview, we have shown a variety of approaches from the technology and biology field, which can help in the development of biological markers, in the identification of new targets in the cells, in making the drug discovery process more efficient, and in therapeutics aimed at increasing the age expectation. The field of investigation is relatively young, and the early results have already shown the enormous application potential of the new technologies. Therefore, it is not difficult to foresee AI as an essential component for future life and health science research and for the pharmaceutical industry towards new discoveries that could guarantee a healthier and longer life for everyone (Zhavoronkov et al., 2019a; Zhavoronkov et al., 2021).

Author contributions

NM: Conceptualization, Investigation, Resources, Writing—Original Draft, Project administration; GP: Investigation, Resources, Writing—Original Draft; SC: Investigation, Resources, Writing–Original Draft; EC: Formal analysis, Resources, Writing—Review and Editing; AS: Writing—Review and Editing, Supervision; EB: Resources, Writing—Review and Editing; QV: Formal analysis, Resources, Writing—Review and Editing; AZ: Formal analysis, Supervision; GC: Investigation, Resources, Writing—Original Draft; BS: Resources, Writing—Original Draft; AM: Resources, Writing—Original Draft; ES: Supervision, Project administration.

Conflict of interest

Authors NM, GP, and AS, were employed by the company Women's Brain Project (WBP). Authors EB, QV, and AZ were employed by the company Insilico Medicine Hong Kong Ltd. Author ES was employed by company Bayer Corporation.

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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Prioritizing research on over-the-counter (OTC) hearing aids for age-related hearing loss

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Hearing aids are the most commonly used treatment for people with age-related hearing loss, however, hearing aid uptake is low, primarily due to high cost of the device, stigma, and a lack of perceived need. To address accessibility and affordability issues, the U.S. Food and Drug Administration created a new over-the-counter (OTC) hearing aid category. Various types of hearing devices are available for both individuals with hearing loss and for those with normal hearing, as hearing enhancement devices. Hearing aids (i.e., prescription hearing aids, self-fitting OTC hearing aids, and pre-set OTC hearing aids) are regulated by the FDA. The purpose of this article is to (a) provide a summary of existing research on direct-to-consumer (DTC) hearing devices such as Personal Sound Amplification Products (PSAPs) that informs OTC service delivery models; (b) provide an update on existing and ongoing randomized controlled trials on currently marketed OTC hearing aids; and (c) highlight the need for immediate research on OTC hearing aids and service delivery models to inform policy and clinical care. It remains to be seen what effect OTC hearing aids have on improving the uptake of hearing aids by individuals with mild-to-moderate hearing loss. However, there is scant research on all aspects of OTC hearing aids that are currently on the market. We conclude that high quality independent research must be prioritized to supplement evidence provided by the OTC hearing aid manufacturers for regulatory approval purposes.

KEYWORDS

hearing aids, over-the-counter hearing aids, direct-to-consumer hearing devices, mild-to-moderate hearing loss, healthcare research, age-related hearing loss

1 Introduction

Aging is the leading cause of hearing loss which affects an estimated 1.5 billion persons globally and age-related hearing loss is one of the most common chronic health conditions affecting nearly one third of the world's population over 60 years (World Health Organization, 2021). Age-related hearing loss has various physical, cognitive and emotional consequences including structural and functional changes to the brain (Glick and Sharma, 2020; Slade et al., 2020). Hearing aids are the most commonly used treatment

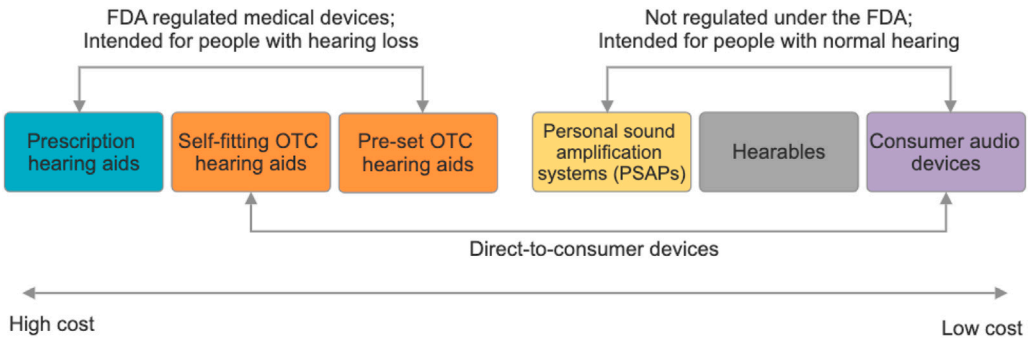

FIGURE 1
Hearing device categories.

for people with hearing loss and the 2020 Lancet Commission on Dementia Prevention, Intervention and Care, identified hearing loss as the leading modifiable, (e.g., through management options such as hearing aids), mid-life risk factor for later development of dementia (Livingston et al., 2020). However, hearing aid uptake is low with only one in four people with hearing loss in high-income countries using hearing aids (Reed et al., 2021). This low uptake has been attributed to several reasons including awareness, high cost of the device, stigma, and a lack of perceived need. To address accessibility and affordability issues with HAs, the Over-the-Counter (OTC) Hearing Aid act passed by the U.S. Congress, 2017 mandated the U.S. Food and Drug Administration (FDA) to release a new category of devices, OTC hearing aids, which consumers can purchase without consulting a licensed hearing healthcare provider. The FDA finalized this decision on 16 August 2022 calling it historic and OTC hearing aids began being sold in the U.S. from 17 October 2022.

Hearing devices have seen tremendous evolution in the last decade including rapid development in features, functionalities as well as look and feel of the device. Modern hearing aids have many new features such as Bluetooth connectivity, rechargeability and fitness tracking. Interestingly, several devices look more like an earbud rather than a traditional hearing aid. This has been possible due to the convergence of traditional hearing aids, which are medical devices, with consumer audio devices, creating a whole array of hybrid devices such as Personal Sound Amplification Products (PSAPs) and hearables. Currently, there are several hearing devices on the market, of which some are medical devices intended for individuals with hearing loss and regulated by the FDA (i.e., prescription hearing aids, self-fitting OTC hearing aids, pre-set OTC hearing aids), and other devices that serve as hearing enhancement devices for individuals with normal hearing who have an average hearing thresholds of 25 dB or better in frequencies 500 Hz, 1,000 Hz, 2000 and 4,000 Hz (i.e., PSAPs, hearables) or those used mainly for entertainment purpose (i.e., consumer audio devices) (Manchaiah et al., 2023) (Figure 1). Some manufacturers are blurring the lines between these categories and offering sound enhancement and personalization of acoustic output for persons with hearing loss using smartphone-based earphones with an accompanying smartphone app (Lin et al., 2022). Moreover, studies have documented that people with hearing loss tend to use devices such as PSAPs and hearables which are meant to be for people with normal hearing (Kochkin, 2010; Manchaiah et al., 2019).

Some of these devices have been available to consumers through direct-to-consumer (DTC) channels for several years (i.e., PSAPs, hearables) including a category of DTC online hearing aids. The OTC hearing aid category has now superseded the DTC online hearing aid category with devices becoming available in-store and online to consumers in the United States starting 17 October 2022 following the historic ruling of the U.S. Food and Drug Administration (Food and Drug Administration, 2022). The OTC hearing aids are for adults with perceived mild-to-moderate hearing loss which generally tend to be individuals with age-related hearing loss. Although consumers who purchase these devices without consultation with hearing healthcare professionals (i.e., audiologists, otolaryngologists) may have some risk of not having the opportunity to identify possible medical conditions (e.g., middle ear disorders) resulting in hearing loss (Hoff et al., 2020), consensus expert opinion is that the benefits of OTC hearing aids will outweigh the limitations (Warren, & Grassley, 2017).

There are some important pre-requisites for successful use of OTC hearing aids to achieve optimal benefit and satisfaction as illustrated in Figure 2. First, consumers must self-identify their hearing loss and also ensure that they have no ear disorders. This is important as OTC hearing aids are intended for individuals with self-perceived mild to-moderate-hearing loss. Hence, over- or under-estimation of hearing loss or not recognizing ear disorders may pose a potential barrier to optimal benefits. Second, users have to consider several device options on the market, choose and purchase an appropriate device *via* channels such as supermarkets, pharmacies, consumer electronic stores or online. Third, users may have to select one of the pre-set programs or self-fit the hearing aid *via* an accompanying smartphone app. Fourth, users have to self-learn handling skills such as putting on the hearing aid appropriately, charging, cleaning, etc. Finally, users must monitor on-going issues with the device (e.g., connectivity with the smartphone app, no sound due to earwax blocking the speaker of the device) and troubleshoot them as necessary. OTC hearing aids do come with instruction manuals and/or step-by-step help in the smartphone app. Moreover, users may also have remote customer support by a technician and/or remote clinical support by hearing healthcare professionals.

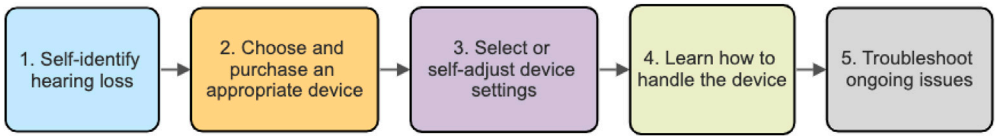

FIGURE 2
Pre-requisites for successful use of OTC hearing aids along the consumer journey.

Nevertheless, these are still pre-requisites that users need to be able to comply with to obtain optimal benefits. For these reasons, both consumers as well as the licensed hearing care professionals/providers (HCPs) who are assisting the consumers must consider aspects related to the (a) device, (b) service delivery model and (c) user when deliberating on the appropriateness of OTC hearing aids for specific persons.

In this article, we aim to 1) provide a summary of existing research on DTC hearing devices that informs OTC service delivery models; 2) provide an update on existing and ongoing randomized controlled trials (RCTs) on currently marketed OTC hearing aids based on the clinical trials registration; and 3) highlight the need for immediate research on OTC hearing aids and service delivery models to inform policy and clinical care.

2 Discussion

2.1 Previous research on DTC hearing devices and service delivery models

Much of the existing research on this area was conducted pre-2017 when the OTC hearing aid category did not exist in the United States. As the hearing devices (i.e., PSAPs, hearables, direct-mail hearing aids) used in the research discussed below were available to consumers *via* DTC channel, we can call them as DTC hearing devices. Nevertheless, a few systematic reviews on consumer hearing devices suggested that the available literature can be grouped into three key themes focusing on 1) acoustic quality of hearing devices, 2) consumer surveys, and 3) clinical trials as discussed below (Manchaiah et al., 2017; Maidment et al., 2018; Tran & Manchaiah, 2018; Chen et al., 2022). First, a series of studies examined electroacoustic characteristics in the test box (e.g., frequency response, distortion, equivalent input noise) of DTC hearing devices such as PSAPs and hearables which showed mixed results. Some studies showed that these devices were of very poor acoustic quality (Callaway & Punch, 2008; Chan and McPherson, 2015), whereas other studies concluded that some of these devices have appropriate acoustic characteristics for people with hearing loss (Reed et al., 2017). These results highlight the importance of quality of acoustic output in device selection. It is noteworthy that some of these studies point to the fact that higher priced devices generally have better acoustic quality (Almufarrij et al., 2019). It is also important to note that most of the evaluated devices are not currently offered as FDA-regulated OTC devices. Second, a few large-scale consumer surveys on DTC hearing aid users in the United States (Kochkin, 2010) and Japan (EHIMA, 2022) show that the benefit and satisfaction reported by users of devices such as PSAPs and direct-mail hearing aids is generally much lower when compared to users of prescription hearing aids fitted by HCPs. The reason for this can be attributed to the poor quality of DTC devices available a decade ago, as well as users may not have met one of the five pre-requisites discussed above. Finally, the third group of studies included clinical trials focused on the outcomes of DTC hearing devices (Maidment et al., 2018; Tran & Manchaiah, 2018; Chen et al., 2022). These studies generally showed positive outcomes in self-reported hearing aid benefit and satisfaction measures [e.g., Abbreviated Profile of Hearing Aid Benefit (APHAB)] as well as in behavioral measures (e.g., speech in quiet, speech in noise). However, the main criticism of these studies is that they generally used single-group pretest-posttest study designs without a control group (e.g., Sacco et al., 2016; Mamo et al., 2017).

In another study, Humes et al. (2017) performed a three-arm double-blind placebo-controlled trial comparing a gold standard audiologist fitted group with a consumer decides self-fit group and a placebo group (n = 154 across all groups). Participants from all three groups used prescription hearing aids, although the self-fit group used a device with pre-set programs and the placebo group used a device with no functional gain. The study results showed that the self-fit group presented with only slightly poorer outcomes in self-reported and behavioral measures when compared to an audiologist-fitted group demonstrating the efficacy of the OTC service delivery model. In a follow-up study, Humes et al. (2019) further examined the consumer decides self-fit model with less front-end screening in a double-blind clinical trial (n = 40). Participants were asked to choose one of the pre-programed hearing aids (like a pre-set OTC hearing aid) one of which included a placebo device with functional gain. The outcomes of the two groups with pre-programmed hearing aids with gain were comparable and were superior to the placebo group. The study also highlighted that the presence of red-flag conditions (e.g., cerumen) did not impact the purchase decision of the users raising some concerns about consumers ability to self-identify their candidacy for OTC hearing aids.

All the studies discussed above used either early generation DTC hearing devices (Manchaiah et al., 2017; Tran & Manchaiah, 2018) or prescription hearing aids with limited features to simulate an OTC hearing aid (Humes et al., 2017; Humes et al., 2019). Moreover, they focused on either the device or the service delivery model which limits the ecological validity and generalizability. Overall, the key takeaway from these studies is that if users choose an appropriate device, then they are likely to have some measurable benefit from using them.

TABLE 1 Controlled trials (completed and on-going) on OTC hearing aids and service delivery models.

Study sponsor or principal investigator	Status	Funding source	Arms and design (n)	Hearing device categories	Hearing aid brand/ model	Outcome domains studied
Sabin et al. (2020); Ear Machine LLC transferred to Bose Corporation	Complete	Federal (NIDCD)	2 arms parallel assignment: AF vs. SF (n = 75)	SF OTC hearing aid and AF version of the same device	Bose sound control	Self-reported, Behavioral
GN Hearing A/S	Complete*	Industry	2 arms cross-over design: AF vs. SF (n = 40)	SF OTC hearing aid and AF version of the same device	Jabra Enhance Plus	Self-reported, Behavioral
Sousa et al. (2023); hearX group	Complete	Industry	2 arms parallel assignment: AF vs. SF (n = 68)	SF OTC hearing aid and AF version of the same device	Lexie Lumen	Self-reported, Behavioral
Yu-Hsiang Wu	Ongoing	Federal (NIDCD)	3 arms parallel assignment: AF vs. SF vs. Hybrid (n = 240)	Prescription HAs in AF; Pre-set OTC in other two groups	Unknown	Self-reported, Behavioral
Northwestern University	Ongoing	Independent non-profit (PCORI)	3 arms parallel assignment: AF vs. SF-1 (consumer decides) vs. SF-2 (efficient fitting) (n = 591)	Prescription HAs in all three groups	Unknown	Self-reported, Behavioral
University of Minnesota	Ongoing	University of Minnesota	2 arms cross-over design: AF vs. SF (n = 40)	SF OTC hearing aid and AF version of the same device	Eargo	Self-reported, Behavioral
Whisper AI	Ongoing	Industry	2 arms cross-over design: AF followed by SF vs. placebo (n = 80)	SF OTC hearing aid and AF version of the same device	Whisper AI	Self-reported, Behavioral
Starkey Laboratories Inc.	Complete*	Industry	2 arms cross-over design: AF vs. SF (n = 40)	SF OTC hearing aid and AF version of the same device	Start Hearing One	Self-reported, Behavioral

; *Note: Unpublished study details were retrieved from the clinicaltrials.gov registry, Marked as complete in the Clinical trials registry but the results are not published yet; SF, self-fitting; AF, audiologist-fitted; HAs, hearing aids; NIDCD, national institute on deafness and other communication disorders; PCORI, patient-centered outcomes research institute.

2.2 Existing and ongoing research on OTC hearing aids and service delivery models

Table 1 presents a summary of completed as well as on-going RCTs studies examining OTC hearing aids and/or the service delivery models. Of these, only two studies have been published in a peer reviewed journal (Sabin et al., 2020; De Sousa et al., 2023) and two other studies are marked as complete in the clinical trials registry. It appears that most of these RCTs (4 of the 8 listed in Table 1) are industry sponsored studies examining self-fitting algorithms or process when compared to audiologist-fitted devices for regulatory approval from the FDA. Two of the ongoing studies that are funded by a federal agency (National Institute on Deafness and Other Communication Disorders; NIDCD) and a non-profit organization (i.e., Patient-Centered Outcomes Research Institute; PCORI) seem to have a large sample size (n = 240–591) and aim to investigate service delivery models. In most of the studies, OTC hearing aids that are currently on the market or likely to come to the market in the near future are being investigated which increases their ecological validity. All of the studies include self-reported and/or behavioral measures as primary and secondary outcome measures. Unlike blinded RCTs (e.g., Humes et al., 2017, 2019), these studies use devices that are currently on the market with the existing branding information. There may be some placebo effects that could potentially impact outcomes of both self-reported and behavioral outcomes (Dawes et al., 2013). This highlights a need for studies that also include more objective outcomes like electrophysiological markers to examine non-subjective benefits of these devices and associated service delivery models (Glick & Sharma, 2020).

2.3 Outstanding research questions about OTC hearing aids and service delivery models

There is an immediate need for research on all aspects of OTC hearing aids including hearing device characteristics, service delivery models, the user and the complex interaction between these three domains. The following are some aspects that we think are highly relevant and timely to inform hearing healthcare policy, clinical care, industry decisions and increase much-needed hearing aid uptake. The key question during the last decade was whether OTC hearing aids provide measurable benefit to users. This question continues to be important but since devices are already available on the marketplace through a regulatory framework (requiring FDA non-inferiority trials for self-fitting OTC hearing aids), other pressing questions should be prioritized. Important questions should consider for whom OTC devices and respective service delivery models work and what the predictors of success are. We outline some specific questions below:

Hearing devices related:

- What is the range in electroacoustic characteristics of OTC hearing aids that are currently on the market and how many of them are appropriate for individuals with mild-to-moderate hearing loss?
- How effective are self-fitting algorithms in personalizing hearing aid gain for individual users when compared to the gold standard prescription targets (NAL-NL2) based on pure tone audiometry thresholds? Also, is there a difference in different self-fitting methods (e.g., *in-situ* audiometry-based fitting vs. direct methods)?
- Is there a difference in outcomes between pre-set vs. self-fitting OTC hearing aids?
- What are the outcomes of OTC hearing aids that are currently available when compared to prescription hearing aids (e.g., Swanepoel et al., 2023) as well as other type of DTC hearing devices such as PSAPs or hearables?
- Is there incremental benefit and satisfaction from OTC hearing aid users from incremental technology?

Service delivery model related:

- What are the contextual facilitators and barriers to implementation of OTC service delivery models in different settings according to stakeholders such as users, HCPs, patient organizations, managers of the health systems, companies manufacturing and distributing OTC hearing aids as well as potential payers such as insurance companies?
- What role do HCPs play in facilitating the journey of users of OTC hearing aids? What guidance, additional training and support would HCPs need if they include OTCs in their practice.
- How cost-effective are OTC hearing aids from the payer as well as provider perspective?
- What effect do OTC hearing aids have on hearing aid market in terms of improving uptake rates, reducing hearing aid costs, improving access to people with low-incomes and ethnic minorities, and in improving the features and functionalities of all categories of devices?
- Are OTC hearing aids, including the use of consumer brands, improving the traditional stigma surrounding hearing loss and hearing aids.
- What outcome measures are best suited to evaluated OTC hearing aids (e.g., behavioral, subjective, cognitive, objective brain-based)?
- Are there measures [e.g., Digits-In-Noise (DIN) test] that can be administered over the internet (web or mobile phone) that can predict who will benefit from OTC hearing aids as well can be used as an outcome measure?

User related:

- Are there specific users based on their biographical, demographic, and audiological variables who are more likely to seek OTC hearing aids and successfully navigate the OTC service delivery model in terms of key prerequisites?
- How and where will consumers find OTC hearing aid models? How will they make decisions on which device to purchase?
- How will users determine whether they are benefitting from OTC hearing aids or whether they should return or exchange the hearing aids during the federally mandated trial period? It is possible to design a self-testing method that consumers can use for this purpose?
- Will there be a difference with respect to when and where users are more likely to wear OTC vs. prescription hearing aid? For example, are OTC hearing aids more likely to be used situationally while prescription hearing aids used daily? How does the duration of hearing aid use impact core outcomes?
- What is the cost-benefit ratio and for which users when it comes to lower versus higher cost OTC hearing aids and which features, and functionalities are most relevant?
- Can customized or personalized educational programs increase user uptake and motivation, supplement self-fitting and management of OTC hearing aids and enhance outcomes (e.g., Ferguson et al., 2021)?

3 Conclusion

OTC hearing aids have opened a new service-delivery avenue for hearing care with many potential consumer benefits especially for those with age-related hearing loss. The limited available research on OTC hearing aids currently on the market emphasizes the need for a stronger evidence-base to support the efficacy of these devices and their service-delivery models. In this article, we propose several questions regarding OTC hearing aids as well as service delivery models that need to be answered rigorously and urgently to inform hearing healthcare policy and clinical care. High quality independent research is important to supplement the evidence that is currently being provided by the OTC hearing aid manufacturers for regulatory approval. Moreover, Patient and Public Involvement (PPI) or Consumer and Community Involvement (CCI) should be considered in shaping future research priorities (Dawes et al., 2022).

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.

Author contributions

VM: conceptualization (Lead), writing the original draft (Lead), reviewing and editing (Lead). DS and AS: conceptualization (supporting), writing (Supporting), reviewing and editing (Supporting).

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Conflict of interest

DS is the Co-founder and scientific advisor at the hearX Group.

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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Recent clinical trials with stem cells to slow or reverse normal aging processes

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Aging is associated with a decline in the regenerative potential of stem cells. In recent years, several clinical trials have been launched in order to evaluate the efficacy of mesenchymal stem cell interventions to slow or reverse normal aging processes (aging conditions). Information concerning those clinical trials was extracted from national and international databases (United States, EU, China, Japan, and World Health Organization). Mesenchymal stem cell preparations were in development for two main aging conditions: physical frailty and facial skin aging. With regard to physical frailty, positive results have been obtained in phase II studies with intravenous Lomecel-B (an allogeneic bone marrow stem cell preparation), and a phase I/II study with an allogeneic preparation of umbilical cord-derived stem cells was recently completed. With regard to facial skin aging, positive results have been obtained with an autologous preparation of adipose-derived stem cells. A further sixteen clinical trials for physical frailty and facial skin aging are currently underway. Reducing physical frailty with intravenous mesenchymal stem cell administration can increase healthy life expectancy and decrease costs to the public health system. However, intravenous administration runs the risk of entrapment of the stem cells in the lungs (and could raise safety concerns). In addition to aesthetic purposes, clinical research on facial skin aging allows direct evaluation of tissue regeneration using sophisticated and precise methods. Therefore, research on both conditions is complementary, which facilitates a global vision.

KEYWORDS

aging, clinical trial, frailty, lomecel-B, mesenchymal stem cells, rejuvenation, skin aging, stem cells

1 Introduction

Stem cells (SCs) are undifferentiated cells which can proliferate indefinitely or differentiate into progenitor cells and end-phase differentiated cells (becoming pluripotent) (Mayo, 2021; Slack, 2022). Human embryonic SCs (*h*E-SCs) are found in the inner cell mass of the blastocyst; *h*E-SC research raises ethical concerns (Lo and Parham, 2009), and *h*E-SC transplantation *in vivo* can lead to the formation of large tumors called teratomas (Blum and Benvenisty, 2008).

Small numbers of adult SCs are found in some organ "niches", including the bone marrow, where hematopoietic progenitor cells (HPC) replenish blood and immune cells. In 1958, Mathe et al. (1959) successfully performed the first adult SC therapy on five workers who had received high-dose accidental irradiation at the Vinca Nuclear Institute in Yugoslavia. After transfusions and grafts of homologous adult bone marrow, all workers survived (Mathe et al., 1959).

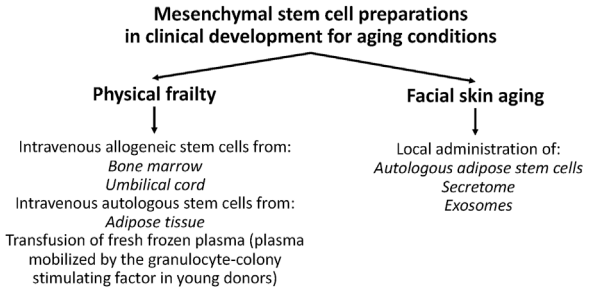

FIGURE 1
Mesenchymal stem cell (MSC) preparations in clinical development for aging conditions. MSC preparations are in development for physical frailty in older persons and facial skin aging. Reducing aging frailty can increase healthy life expectancy and decrease costs to the public health system. Clinical trials for facial skin aging are important because tissue regeneration is *directly assessed* by sophisticated and precise methods, and because cell entrapment in the lungs and safety issues of intravenous stem cells are avoided.

For years, the human umbilical cord was a waste material and, unlike *h*E-SCs, its use does not raise ethical concerns. In 1988, Gluckman et al. (1989) successfully performed the first human cord blood transplant in a child with Fanconi's anemia. Since then, numerous public and private cord blood banks have been established worldwide for the cryopreservation of cord blood in view of its transplantation (Gluckman, 2011).

In the United States, the only SC products that are approved by the FDA consist of allogeneic HPC from human cord blood, for use in patients with disorders affecting the hematopoietic system (FDA, 2020) (Figure 1). Such HPC cord blood products include: Allocord (SSM Cardinal Glennon Children's Medical Center), Clevecord (Cleveland Cord Blood Center), Ducord (Duke University School of Medicine), Hemacord (BHI Therapeutic Sciences), and some other HPC cord blood preparations (FDA, 2022).

In the EU, the EMA has approved two SC products for disorders that do not affect the hematopoietic system: 1) darvadstrocel (Alofisel®, Takeda Ireland), an allogeneic adipose-derived SC preparation to treat perianal fistulas in adults with Crohn's disease, and 2) holoclar (Holoclar®, Holostem Terapie Avanzate, Italy), an autologous corneal SC preparation for severe corneal SC deficiency caused by burns (Cynober, 2020). Several other SC products have been approved in South Korea, Japan, India, Canada and New Zealand (Levy et al., 2020).

Adult mesenchymal stem cells (MSCs) have been extensively investigated in clinical trials (Squillaro et al., 2016). In particular, human bone marrow MSCs (*h*BM-MSCs) have been widely used for clinical research, although they are obtained with low yields, through an invasive procedure (BM aspiration) (Varghese and Mosahebi, 2017) and their ability to proliferate and differentiate declines with age (Rao and Mattson, 2001).

In 2006, Lu et al., (2006) published a protocol to isolate abundant MSCs by enzymatic digestion of the human umbilical cord (hUC) and cell culture expansion. The UC is an easily accessible fetal tissue, and the *h*UC, which was previously discarded as waste material, quickly became an alternative source of MSCs to be investigated in clinical trials (Figure 1).

Another source of human SCs is adipose tissue (*h*AD-MSCs) (Coleman, 1994; Varghese and Mosahebi, 2017; Alexander, 2019; Khazaei et al., 2021; Surowiecka and Struzyna, 2022). Subcutaneous fat tissue contains many more SCs than bone marrow, large amounts of autologous *h*AD-MSCs are easily obtained by liposuction, and autologous *h*AD-MSCs do not require cell expansion.

Aging is associated with a decline in the regenerative potential of adult SCs, and this may play a crucial role in the pathogenesis of age-associated conditions (Rao and Mattson, 2001; Choudhery et al., 2014; Verdijk et al., 2014; Picerno et al., 2021; Zhu et al., 2021). Indeed, the use of SC preparations for aging conditions has a strong rationale:

1. Animal and human studies have shown that as they age, SCs decrease in number and tend to lose their potential for self-renewal and tissue-regeneration [for recent reviews, see (Picerno et al., 2021; Zhu et al., 2021)]. In human skeletal muscle biopsies, Verdijk et al. (2014) found that atrophy of type II ("fast-twitch") muscle fibers with aging is accompanied by a specific decrease in SC ("satellite cell") content.Choudhery et al. (2014) showed that *h*AD-MSCs from individuals older than 60 years displayed senescent characteristics compared to cells isolated from young donors, concomitant with reduced viability, proliferation, and differentiation potential.
2. Animal studies showed increased life expectancy with MSC transplantation. In mice, Shen et al. (2011) reported that transplantation of young MSCs prolongs the life span of old mice. Mansilla et al. (2016) found that intravenous administration of *h*BM-MSC to a 6-month-old rat increased its lifespan to 44 months, compared to an average of 36 months in control animals. Lavasani et al. (2012) showed that intraperitoneal administration of muscle-derived stem/progenitor cells from young wild-type mice significantly increased the lifespan and healthspan of progeroid mice (a rodent model of accelerated aging).
3. Within the animal kingdom, the healthy life expectancy of different animal species depends to a large extent on the regenerative capacity of their SC, notably in invertebrates such as planarians and hydra (Handberg-Thorsager et al., 2008).

In recent years, several clinical trials have been launched to evaluate the efficacy and safety of SCs on aging conditions (Figure 1). Here, those SC preparations were identified, and trials were analyzed from information extracted from national (United States, EU, China, and Japan) and international (World Health Organization, WHO) clinical trial databases. Stem cell-based therapies for age-related diseases are described elsewhere (Levy et al., 2020; Rezaie et al., 2022).

2 Methods

2.1 Identification of recent clinical trials with stem cell preparations for aging

2.1.1 Clinical trials databases

Clinical trials databases from the US (ClinicalTrials.gov), EU (clinicaltrialsregister.eu), China (chictr.org.cn/searchprojen.aspx),

Japan (https://rctportal.niph.go.jp/en/) and the World Health Organization (WHO; trialsearch.who.int) were accessed to identify recent clinical trials with SC preparations for aging conditions, using a previously developed approach (Garay et al., 2016; Garay, 2021) slightly modified. The WHO Clinical Trials Search Portal provided access to trials registered in 14 primary registries (Australia, Brazil, Cuba, Germany, India, Iran, ISRCTN, Korea, Lebanon, Netherlands, Pan African, Peru, Sri Lanka, and Thailand). For each website, the list of clinical trials was obtained by filling out the "Advanced Search" form (or "More Information" form).

2.1.2 Selection criteria

In order to be retained for this review, compounds needed to be in clinical trials with SC interventions for aging conditions and satisfy the following criteria:

1. Trial declared with "Aging" as "Condition", "stem cell" as "Other terms", and "Interventional Studies (Clinical trials)" as "Study type"
2. Trial updated on 1 January 2019 or later,
3. Trial not terminated,
4. Trial including healthy participants.

2.2 Data extraction and organization

For each selected clinical trial, the following relevant data were extracted: identifier number (and/or designated name, and/or bibliographic reference), aging condition, SC preparation, trial sponsor(s), main outcomes, duration of the study, number of patients, and trial status (results, if available, or expected completion date). Clinical trials were listed according to the aging condition and SC preparation investigated.

2.3 Additional sources of information

Relevant articles related to the selected SC interventions were searched in the following biomedical literature databases: PubMed (https://pubmed.ncbi.nlm.nih.gov), Science Direct (www.sciencedirect.com/search), Cochrane Library (www.cochranelibrary.com), and Google Scholar (https://scholar.google.com). For each website, relevant articles were found by using the name of the SC preparation, OR the clinical trial identifier number AND "aging condition". Clinical trial information was also obtained by consulting the websites of pharmaceutical and biotechnology companies working in the field of stem cells and aging.

2.4 Analysis

The current treatment and therapeutic needs of each aging condition were identified in the corresponding clinical practice guidelines (CPG). The therapeutic impact of the selected clinical trials was evaluated in the context of such competitive environment. Research analysis included four therapeutic aspects: 1) Key findings from SC interventions for aging, 2) research with ongoing clinical trials, 3) clinical trial limitations, and 4) future perspectives.

3 Clinical trials with SC preparations for aging

Clinical trial registries were accessed from 1 August 2022 to 16 January 2023, to identify trials with SC preparations for aging. The US database (ClinicalTrials.gov), included twenty-three clinical trials updated on 1 January 2019 or later. Of these, NCT03457870, NCT02642094, NCT04712955, NCT02456870, NCT01169831, NCT02790541, NCT03140319, NCT03535844, NCT04450602, and NCT04450589 were excluded from the present analysis because the interventions did not meet the inclusion criteria. Thirteen clinical trials met the inclusion criteria and were included in the analysis (Tables 1–3).

No other clinical trials were found in the EU database (clinicaltrialsregister.eu). The Chinese database (chictr.org.cn/searchprojen.aspx), included one clinical trial (ChiCTR2200061216) (Table 2). The Japanese database (https://rctportal.niph.go.jp/en/) included one clinical trial (jRCT2043200038) (Table 1). The WHO database (trialsearch.who.int) included two clinical trials (IRCT20141007019432N2, and RPCEC00000362) (Table 2).

3.1 SC preparations for the frailty of aging

Eleven clinical trials selected for analysis were investigating SC preparations for aging frailty (Table 1). These included six trials with allogeneic human bone marrow MSCs (*h*BM-MSCs), three trials with allogeneic human umbilical cord MSCs (*h*UC-MSCs), one trial with autologous adipose-derived MSCs (*h*AD-MSCs), and one trial with plasma mobilized by the granulocyte-colony stimulating factor (GMFFP).

The clinical development of MSCs preparations for physical frailty in older persons has a strong rationale (Figure 2). A large amount of evidence suggests that SC exhaustion is associated with the progression of aging frailty (Verdijk et al., 2014; Sousa-Victor and Munoz-Canoves, 2016; Zhu et al., 2021). In addition, human studies showed that MSCs possess therapeutic potential for musculoskeletal regeneration [for a review, see (Steinert et al., 2012)]. Finally, allogeneic *h*MSCs are rarely rejected, making them suitable for MSC therapy without the need for immunosuppression (Hare et al., 2009; Florea et al., 2019). These observations suggested that an intravenous infusion of allogeneic *h*MSCs may be a potentially effective therapy for physical frailty in older persons (Figure 2).

3.1.1 hBM-MSCs

Lomecel-B (or "allo-*h*MSCs", Longeveron, United States) is a formulation of allogeneic *h*BM-MSCs sourced from the posterior iliac crest of healthy young adult donors (aged 18–45 years) and expanded in culture (Golpanian et al., 2016; Yousefi et al., 2022). After a specific number of expansion cycles, the cells are harvested, separated into specific doses, and frozen until future use. Unlike an autologous bone marrow transplant (that is used for a single

TABLE 1 Recent clinical trials with allogeneic stem cell preparations for physical frailty in older persons (2019 and later).[a]

SC preparation	Sponsor	Identifier	Main outcomes	Time frame	N[c]	Results or status
*h*BM-MSCs[d]	Longeveron (United States)	NCT02065245	Safety[e]	1 month	15	Safe
*h*BM-MSCs[d]	Longeveron (United States)	NCT02065245	Efficacy[f]	6 months	30	Positive
*h*BM-MSCs[d]	Longeveron (United States)	NCT03169231	6 MWD	180 days	150	Completed
*h*BM-MSCs[d]	Longeveron (United States)	NCT02982915	Vaccine adjuvant[h]	12 months	62	September 2021[g]
*h*BM-MSCs[d]	Longeveron (Japan)	jRCT2043200038	6 MWD	180 days	45	NC
*h*vBM-MSCs[g]	VA's ORD (United States)[i]	NCT05284604	Adherence[j]	6 months	36	June 2025[g]
*h*UC-MSCs	Shanghai East Hosp (CHN)	NCT04314011	Safety and Efficacy	1 and 6 months	30	Completed
*h*UC-MSCs	Vinmec Research (VNM)[k]	NCT04919135	Safety and Efficacy	12 months	44	Not yet recruiting
*h*UC-MSCs	FOREM (United States)	NCT05018767	Safety	4 years	20	November 2025[g]
*h*AD-MSCs[l]	Healeon Medical (United States)	NCT03514537	Safety (Frailty)	6 months	200	March 2023[g]
GMFFP[m]	Maharaj Institute (United States)	NCT03458429	Safety (Efficacy[n])	24 M	30	February 2023[g]

[a]Most of the studies were randomized controlled trials (see text for details).
[b]Abbreviations: 6 MWD, 6-min walk distance; allo-*h*MSCs, allogeneic mesenchymal stem cells; FOREM, Foundation for Orthopaedics and Regenerative Medicine; *h*BM-MSCs, human bone-marrow mesenchymal SCs. Hosp, hospital. *h*UCM-SCs, human umbilical cord mesenchymal stem cells; NC, not communicated.
[c]Number of participants.
[d]Lomecel-B (also called "allo-*h*MSCs").
[e]Phase I safety trial, including frailty outcomes.
[f]Phase II RCT, investigating 1-month safety and 6-month efficacy on aging frailty.
[g]Primary completion date (past or estimated).
[h]Phase II RCT, to test the efficacy of Lomecel-B to improve influenza vaccine responses (12 months), including an initial phase I safety trial (30 days). Vertebral *h*BM-MSCs.
[i]Veterans Health Administration-Office of Research and Development.
[j]Percentage of study visits attended.
[k]Vinmec Research Institute of Stem Cell and Gene Technology (Vietnam).
[l]Cellular Stromal Vascular Fraction, an autologous *h*AD-MSCs, preparation.
[m]GCSG-Mobilized Fresh Frozen Plasma.
[n]Frailty Index and other secondary outcomes.

TABLE 2 Recent clinical trials with stem cell preparations for facial skin aging and photoaging (2019 and later).[a]

SC preparation	Sponsor	Identifier	Outcomes[b]	Time frame	N[c]	Results or status[b]
SVF[d]	Xuzhou Medical Univ (CHN)	NCT02923219	Volume; skin quality	6 months	50	Positive[e]
SVF[d]	Alexandria Univ (Egypt)	NCT03928444	Facial rejuvenation	6 months	15	Completed
SVF[d]	HA Hospital (Cuba)[f]	RPCEC00000362	Wrinkles and furrows	1 year	N.C.	December 2022[g]
SVF[d]	Tehran Univ (Iran)[h]	IRCT20141007019432N2	Wrinkles	6 months	46	Started
SC secretome[i]	SN Yusharyahya[j]	NCT05508191	Facial rejuvenation[k]	6 weeks	30	October 2022[g]
*h*BM-MSCs	Stemedica (United States)[l]	NCT01771679	Safety (Photoaging)	1 year	29	Suspended[m]
SC Exosomes	Sun Yat-sen Univ (China)[n]	ChiCTR2200061216	Photoaging[o]	N.A.	10	December 2024[g]

[a]Most of the studies were randomized controlled trials (see text for details). N.A., not applicable; N.C, not communicated; SC, stem cell; Univ, University.
[b]See text for details.
[c]Number of participants.
[d]Autologous stromal vascular fraction (SVF).
[e]The results of the study have been reported by Yin et al. (Yin et al., 2020).
[f]Hermanos Ameijeiras Surgical Clinical Hospital (Havana).
[g]Primary completion date (past or estimated).
[h]Tehran University Medical Sciences and Sinacell Corporation (Tehran).
[i]*h*AD-MSC, secretome developed by PT, Kimia Farma Tbk (Jakarta, Indonesia).
[j]Shannaz Nadia Yusharyahya (Indonesia University).
[k]Skin aging changes evaluated by several methods (see text).
[l]Stemedica Cell Technologies.
[m]The study has stopped early but it can start again.
[n]The seventh Affiliated Hospital of Sun Yat-sen University (Shenzhen).
[o]*h*AD-MSC, derived exosomes loaded with circcol elns (a circular RNA, circRNA) are tested for their ability to promote collagen and elastin synthesis in skin samples from 6 to 10 photoaged patients (55–75 years).

TABLE 3 Other clinical trials with stem cell preaparations for aging (2019 and later).

Intervention	Sponsor	Identifier	Outcomes[a]	Time frame	N[b]	Results or status
NT-020	North Texas Univ (United States)[c]	NCT01847027	Blood SC levels[d]	4 weeks	23	Negative
*h*UC-MSCs and *h*AD-MSCs	Landmark (Malaysia)[e]	NCT04174898	Safety[f]	1 year	100	April 2021[g]

SC, stem cell. Univ, University.
[a]See text for details.
[b]Number of participants.
[c]University of North Texas Health Science Center.
[d]CD34+ and CD133+ blood cell levels.
[e]Landmark Medical Centre Sdn Bhd.
[f]Safety, quality of life and inflammatory markers.
[g]Primary completion date (past or estimated).

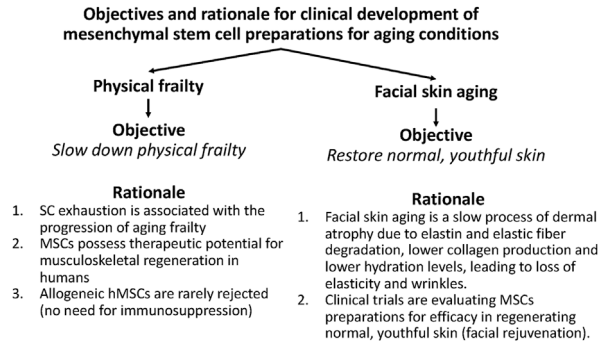

FIGURE 2
Objectives and rationale for clinical development of mesenchymal stem cell preparations for physical frailty and facial skin aging.

patient), tissue from a single donor is used to obtain many doses of Lomecel-B for use in multiple patients.

Longeveron has launched a clinical development program with intravenous Lomecel-B for aging frailty. The program includes five clinical trials designed to determine if Lomecel-B can improve physical function, reduce inflammation and improve quality of life in frail older adults (Table 1).

3.1.1.1 NCT02065245

CRATUS (NCT02065245) consisted of a phase I open label, escalated dose pilot trial (Golpanian et al., 2017), and a phase II randomized controlled trial (RCT) versus placebo (Tompkins et al., 2017) (Table 1). The phase I open label trial (Golpanian et al., 2017) included 15 elderly subjects (mean age: 78.4 years) with early signs and symptoms of frailty, and a frailty score between 4 and 7 on the Clinical Frailty Scale (CFS) (Rockwood et al., 2005). Participants were divided in three groups (n = 5/group) scheduled to receive 20-, 100- or 200-million *h*BM-MSCs, delivered *via* peripheral intravenous infusion (Golpanian et al., 2016). No therapy-related TE-SAE (treatment emergent-serious adverse event) occurred during the trial (Golpanian et al., 2017). There were no signs of T-cell activation (a marker of graft rejection) at 6-month. Only one subject (20-million group) developed mild to moderate donor-specific antibodies.

Significant increases in the 6-min walk distance (6 MWD) test were obtained: 1) in the group of 20-million *h*BM-MSCs at 6 months (mean value of the increase = 37.2 m), and 2) in the group of 100-million *h*BM-MSCs at 3 months (36.6 m) and at 6 months (76.6 m) (Golpanian et al., 2017). No significant increases were seen in the group of 200-million *h*BM-MSCs (Golpanian et al., 2017).

TNF-alpha levels (an inflammatory marker) significantly decreased in the groups of 100-and 200-million *h*BM-MSCs at 6-month. No significant changes were seen in Interleukin-6 (IL-6) or C-reactive protein (CRP) (Golpanian et al., 2017).

The phase II RCT (Tompkins et al., 2017) included 30 elderly subjects (mean age = 75.5 years) with frailty scores between 4 and 7 on the CFS (Rockwood et al., 2005). Subjects receiving 100 million cells (n = 10) or 200 million cells (n = 10) were compared with those receiving placebo (n = 10). The results confirmed those obtained in the phase I open label trial (Golpanian et al., 2017). In particular, the 6 MWD significantly increased in the 100 M-group from baseline (mean value = 345.9 m) to 6-month (410.5 m). Immuno-tolerability was acceptable (only three participants showed a mild to moderate increase in donor specific antibodies).

3.1.1.2 NCT03169231

The CRATUS trial was limited by its small sample size (Golpanian et al., 2017; Tompkins et al., 2017). NCT03169231 is a phase IIb multicenter RCT evaluating Lomecel-B *versus* placebo (Yousefi et al., 2022) (Table 1). A total of 150 older adults with CFS scores of 5"mildly frail" or 6"moderately frail" (Rockwood et al., 2005), and 6 MWD of >200 m and <400 m was included in the study. Primary outcome is the change from baseline in 6 MWD compared to placebo. Secondary outcomes are changes in overall physical function and TNF-alpha. This trial was recently completed.

In September 2021, Longeveron announced preliminary biomarker results from the NCT03169231 trial (Longeveron, 2021b). Administration of Lomecel-B was accompanied by a statistically significant reduction in serum soluble TIE-2 (sTIE-2) levels, in a dose-dependent manner, compared to placebo. TIE-2 is a cell surface receptor tyrosine kinase that plays a pivotal role in vascular barrier maintenance, and increased levels of sTIE-2 in the blood stream may indicate endothelial dysfunction (Idowu et al., 2020).

3.1.1.3 NCT02982915

HERA (NCT02982915) is a phase I/II RCT to test the safety and efficacy of intravenous Lomecel-B to improve influenza vaccine

(fluzone) responses in subjects with aging frailty (Table 1). Following an initial phase I safety trial (30 days), a phase II RCT will assess whether Lomecel-B may be an effective vaccine adjuvant to enhance influenza virus inactivation (assessed by hemagglutination inhibition assays) (time frame: 12 months). Primary completion date was expected for September 2021.

3.1.1.4 jRCT2043200038

jRCT2043200038 is a phase II RCT evaluating intravenous Lomecel-B in Japan (Table 1). The study includes people 70–85 years of age, who present the CHS (Cardiovascular Health Study) frailty phenotype (Fried et al., 2001) and serum levels of TNF-alpha <2.5 pg/mL. Participants are divided into three groups that receive a single intravenous infusion of 50 million *h*BM-MSCs or 100 million *h*BM-MSCs or placebo. The primary outcome is the change in the 6 MWD from baseline to 180 days post-infusion in the high-dose group compared to placebo. Secondary outcomes are: 1) the change in 6 MWD in the low dose group compared to placebo, and 2) the change in TNF-alpha levels in the high dose group. Recruitment status is pending and completion date was not communicated to https://rctportal.niph.go.jp/en/.

3.1.2 hvBM-MSCs

3.1.2.1 NCT05284604

NCT05284604 is a phase I/II RCT investigating *h*BM-MSCs derived from vertebrae (*h*vBM-MSCs, obtained from the vertebral bodies of deceased organ donors) versus placebo (Table 1). *h*vBM-MSCs are administered intravenously to older adults (65–85 years of age) who meet the following conditions: 1) Modified Physical Performance Test (mPPT) score of 18–31, 2) Clinical Frailty Scale (CFS) score of 5 or 6 and, 3) 6 MWD of >200 m and <400 m. The primary outcome is adherence (percentage of study visits attended). Secondary outcomes include: number of participants recruited, mPPT score, CFS score, and 6-min walk test (6 MWT). Other secondary outcomes include: adverse events, inflammatory markers and quality of life. The trial is not recruiting yet. Primary completion date is expected for June 2025.

3.1.3 hUC-MSCs

3.1.3.1 NCT04314011

NCT04314011 is a phase I/II RCT evaluating the safety and efficacy of intravenous *h*UC-MSCs in older adults (60–80 years of age) with a frailty score of 1–4 on the Fried Phenotype Scale (Op het Veld et al., 2015) (Table 1). Participants receive two intravenous infusions of *h*UC-MSCs (10^6 cells/kg) or saline separated by an interval of 1 month, and are followed for 6 months (after the first intervention). The primary outcome is the occurrence of serious adverse events (SAEs) during the month following the infusion. Secondary out outcomes are changes in: 1) Fried phenotype scale scores (Op het Veld et al., 2015), 2) blood proinflammatory cytokines and 3) quality of life, assessed at baseline, 1 month, 3 months and 6 months. The trial was recently completed, but the results have not yet been published on ClinTrials.gov.

3.1.3.2 NCT04919135

NCT04919135 is a phase I/II RCT investigating the safety and efficacy of adjunctive intravenous administration of allogeneic *h*UC-MSCs in patients receiving standard treatment for frailty in Vietnam [Hightamine (Hankook Korus Pharm, Korea), Total calcium (Nugale Pharmaceutical, Canada), and Bioflex (Ausbiomed, Australia)] (Table 1) (Hoang et al., 2022). The intervention group will receive two doses of *h*UC-MSCs (1.5×10^6 cells/kg) separated by a time interval of 3 months. The primary outcome is the occurrence of treatment-dependent SAEs. Secondary outcome measures include the 6 MWD test and $CD3^+$ cells. The trial is not yet recruiting.

3.1.3.3 NCT05018767

NCT05018767 is a single-arm, phase I trial designed to assess the long-term safety of a single intravenous infusion of cultured allogeneic *h*UC-MSCs (100 million cells) in subjects with aging frailty (Table 1). Patients will be evaluated at baseline and at 1, 6, 12, 24, 36, and 48 months. NCT05018767 is currently recruiting participants and primary completion is expected in November 2025.

3.1.4 hAD-MSCs

3.1.4.1 NCT03514537

NCT03514537 is an open trial to investigate the safety (and efficacy) of an autologous preparation of *h*AD-MSCs (cellular SVF, cSVF) for aging frailty (Table 1). The study includes adult and older adults (40–90 years) who have noted compromise to activities or work requirements due to increasing age and loss of energy. Participants receive intravenous infusions of cSVF isolated from subdermal adipose tissue removed from the trunk or upper thigh area. The primary outcome is the occurrence of Treatment-Emergent Adverse Events (TEAEs) during 6 months following the infusion. Secondary outcomes are changes in weight, activity level, mobility, and fatigue (at 6 months). NCT03514537 is currently recruiting participants and primary completion is expected in March 2023.

3.1.5 GMFFP

Granulocyte-colony stimulating factor (G-CSF) stimulates the BM to produce granulocytes and SCs, and release them into the bloodstream (Patterson and Pelus, 2017). GMFFP (GCSF-Mobilized Fresh Frozen Plasma) is a fresh frozen plasma preparation harvested from young, healthy donors (Maharaj, 2020) (Table 1).

3.1.5.1 NCT03458429

NCT03458429 is a single-arm, phase I/II trial of GMFFP in elderly (55–95 years) and frail people (score of 4–7 on the Clinical Frailty Scale and/or abnormal Immune Risk Profile) (Table 1). Participants receive 12 once monthly transfusions of GMFFP (initial treatment period of 12 months) and are followed for a total of 24 months. The primary outcome is the number of participants with treatment-related adverse events. Secondary efficacy outcomes include frailty index (mobility, energy, strength, physical activity, nutritional status, mood, cognition, and social support), immune risk profile, and cognitive function. Primary completion date was expected for February 2023.

3.2 SC preparations for facial skin aging and photoaging

Skin aging is due to natural causes, as well as extrinsic factors (especially Sun exposure: Photoaging) (Zhang and Duan, 2018;

Wong and Chew, 2021). Several SC preparations are investigated for facial skin aging and photoaging (Table 2).

Long-term natural aging is a slow process of dermal atrophy due to elastin and elastic fiber degradation, lower collagen production and lower hydration levels, leading to loss of elasticity and wrinkles (Zhang and Duan, 2018; Wong and Chew, 2021). Clinical trials with MSCs preparations for facial skin aging are evaluating their efficacy in regenerating normal, youthful skin (facial rejuvenation) (Figure 2).

Exposure to ultraviolet (UV) radiation facilitates skin aging (photoaging), characterized by the degradation of collagen and elastin, with deposition of collagen breakdown products and abnormal elastin fibers in the dermis (solar elastosis) (Huang and Chien, 2020). Clinical trials with MSCs preparations are evaluating their efficacy in restoring a normal skin.

3.2.1 Stromal vascular fraction (SVF) for facial skin aging

The "stromal vascular fraction" (SVF) is a preparation of autologous *h*AD-MSCs obtained by liposuction, followed by collagenase digestion, filtration, centrifugation and separation of the SVF (Coleman, 1994; Varghese and Mosahebi, 2017; Alexander, 2019; Khazaei et al., 2021; Surowiecka and Struzyna, 2022). The SVF represents about 10% of the adipose tissue volume, and is composed of *h*AD-MSCs, adipocyte progenitors, fibroblasts, endothelial cells, vascular smooth muscle cells, lymphocytes, and a variety of immune cells (T-cells and M2 macrophages). The efficacy and tolerability of SVF-enriched autologous fat grafting is currently being investigated in facial skin aging.

3.2.2.1 NCT02923219

NCT02923219 was an RCT comparing the efficacy of SVF-assisted autologous fat grafting (intervention) versus fat transfer alone (control) for facial volume restoration and improvement of skin quality (Table 2) (Yin et al., 2020). Fifty women (mean age: 35.4 years) participated in the study. At 6 months: 1) Whole face volumes (assessed by 3D scanner and Geomagic software) were significantly higher in the intervention group (77.6%) compared to the control group (56.2%, $p < 0.001$), 2) wrinkles and texture (assessed by VISIA detector) improved significantly more in the intervention group than in the control group, and 3) graft survival rate was significantly higher in the intervention group than in the control group.

3.2.2.2 NCT03928444

NCT03928444 is an RCT comparing intradermal autologous SVF injection on one side of the face versus saline injection on the other side (Table 2). Fifteen female participants with facial aging (35 years or older) are included in the study and will be followed for 6 months. The primary outcome is the degree of aesthetic improvement using the global aesthetic improvement GAIS 5-point scale (Savoia et al., 2014). The trial was completed, but the results were not posted to ClinicalTrials.gov.

3.2.2.3 RPCEC00000362

RPCEC00000362 is an RCT (single-blind) comparing the efficacy of SVF-enriched fat transfer versus conventional fat transfer (Table 2). Participants with facial aging (30–59 years of age) are included in the study and will be followed for 12 months. Outcomes include clinical evaluation and evolution of furrows and wrinkles. Trial completion date was expected for December 2022.

3.2.2.4 IRCT20141007019432N2

IRCT20141007019432N2 is a single-arm clinical trial, designed to investigate the efficacy of autologous SVF transplantation in reducing facial wrinkles (Table 2). Forty-six (46) participants with facial aging (35–65 years of age and with grade 2 to 4 wrinkle type) were included in the study and will be followed for 6 months. The primary outcome is biometric evaluation (with visioface and skin ultrasound) of the amount and extent of facial wrinkles. The trial completion date was not reported.

3.2.2 Soluble paracrine SC factors (secretome) for facial skin aging

3.2.2.1 NCT05508191

NCT05508191 is a single-blind RCT comparing two methods of AD-MSCs "secretome" administration for facial aging (fractional CO_2 laser treatment on one side of the face and microneedle treatment on the other half) (Table 2; Figure 1). The term "secretome" designates the soluble paracrine factors produced by SCs (Xia et al., 2019). Thirty female participants with facial aging (35–59 years) are included in the study and will be followed for 6 weeks. Primary outcomes are: 1) Skin aging changes evaluated by dermoscopy photoaging scale and by Janus-3® skin analyzer, 2) skin capacitance evaluated by the Corneometer® and 3) total water content in the stratum corneum of the skin. Primary completion date was expected for October 2022.

3.2.3 Facial photoaging

3.2.3.1 hBM-MSCs

NCT01771679 is a phase I/II safety trial to evaluate the safety and efficacy of a single intravenous injection of allogeneic (non-hematopoietic) *h*BM-MSCs for the treatment of facial photoaging in men and women 40–70 years of age (Table 2). Recruitment was suspended.

3.2.3.2 Exosomes

SCs secrete exosomes (40–120 nm extracellular vesicles), which contain cytokines, growth factors, messenger RNAs, and different non-coding RNAs, especially micro-RNAs (mi-RNAs) (Hamdan et al., 2021) (Figure 1).

3.2.3.2.1 ChiCTR2200061216. ChiCTR2200061216 investigates if *h*AD-MSC derived exosomes loaded with circcol elns (a circular RNA, circRNA) can promote collagen and elastin synthesis in skin samples from 6 to 10 photoaged patients (55–75 years). The study compares samples of facial skin tissue (part exposed to light), with skin tissue of the hip or upper arm (part protected from light) (Table 2). The study is open for recruitment. Completion is expected for December 2024.

3.3 Other SC therapies for aging

3.3.1 NT-020

NT-020 (NutraStem®) is a patented nutraceutical formulation containing green tea extract, blueberry extract, carnosine, and

vitamin D3 (EurekAlert, 2008). In 2006, Bickford et al. (2006) reported that these agents (as well as catechin) synergistically stimulated the *in vitro* proliferation of human bone marrow and human CD34$^+$ and CD133+ cells. CD34$^+$ are often used clinically to quantify H-SC numbers in H-SC transplantation (Remberger et al., 2020). CD133 is a well-characterized biomarker of normal and cancer SCs (Barzegar Behrooz et al., 2019).

3.3.1.1 NCT01847027

NCT01847027 is a phase II RCT that investigated whether NT-020 in combination with an exercise stimulus was able to increase blood levels of CD34$^+$ and CD133+ SCs in persons aged 50–70 years (Table 3). No significant increases in CD34$^+$ and CD133+ SCs (primary outcome) were found at 2 or 4 weeks after starting the intervention.

3.3.2 hUC-MSCs and hAD-MSCs for quality of life and morbimortality risk

3.3.2.1 NCT04174898

NCT04174898 is a single-arm, phase I trial investigating safety, quality of life and morbimortality risk of *h*UC-MSC and *h*AD-MSC infusion in adults and older adults (Table 3). Morbimortality risk is assessed by measuring inflammatory markers of aging (IL-6, CRP and TNF-alpha) (Giovannini et al., 2011; Puzianowska-Kuznicka et al., 2016; St Sauver et al., 2022). Primary completion was expected in April 2021. The study is not yet recruiting.

4 Discussion

Clinical research with SC interventions for aging has only recently begun. SC interventions are in development for the treatment of two important aging conditions: physical frailty and facial skin aging.

4.1 Physical frailty in older persons

Physical frailty in older adults is characterized by reduced locomotor activity and decreased immunological functioning (Fedarko, 2011; Dent et al., 2019). Aging frailty was recognized as a disease in the WHO ICD-11 (International Classification of Diseases, 11th Revision: MG2A ageing associated decline in intrinsic capacity; https://icd.who.int/browse11/l-m/en#/http://id.who.int/icd/entity/835503193). In addition, the International Conference of Frailty and Sarcopenia Research (ICFSR) edited Clinical Practice Guidelines (CPGs) for the identification and management of physical frailty (Dent et al., 2019) [other CPGs for frailty can be found in (Zheng et al., 2022)].

In Western countries, the prevalence of physical frailty is around 15% in adults ≥65 years and increases to more than 25% in adults >85 years (Dent et al., 2019). Locomotion frailty increases the risk of falls, disability and hospitalization (Xue, 2011). The SHARE study (24,634 European people over 50 years of age, followed for 11 years) showed that frailty status was associated with increased all-cause mortality (Grabovac et al., 2019).

Therapeutic interventions for physical frailty have focused on exercise and nutritional supplementation (Dent et al., 2019; Mohd Suffian et al., 2020; Zheng et al., 2022). The ICFSR-CPGs (Dent et al., 2019) recommend a multi-component physical activity programme as first-line therapy for physical frailty in older adults, and protein/caloric supplementation when there is weight loss or malnutrition. There are no specific medical or biological treatments to prevent, delay, or reverse aging frailty (Tompkins et al., 2017; Cesari et al., 2022), and the ICFSR-CPGs (Dent et al., 2019) do not recommend any currently available non-specific pharmacological treatment.

4.1.1 Key findings of SC interventions for physical frailty in older adults

The allogeneic *h*BM-MSC preparation Lomecel-B (Longeveron, United States) is the leading SC preparation in the area of physical frailty. The CRATUS trial (Golpanian et al., 2017; Tompkins et al., 2017) showed that intravenous Lomecel-B was well tolerated and modestly but significantly increased the 6 MWD in frail elderly participants. The maximum increase in 6 MWD was obtained with the 100-million *h*BM-MSCs dose [+64 m in the phase II study of Tompkins et al. 2017)]. This 6 MWD value is higher than the "substantial meaningful change" (47–49 m) estimated by Perera et al. (2006).

The results of the CRATUS trial can also be compared with those obtained in a meta-analysis of 13 studies (Bohannon, 2007). In healthy subjects aged 70–79 years, Bohannon (2007) reported mean 6 MWD values of 510 m (490 m and 530 m for men and women, respectively). In the CRATUS trial (phase II sub-trial), the 100 million-*h*BM-MSCs-dose significantly increased mean 6 MWD from 345.9 m (baseline value) to 410.5 m (6-month value) (Tompkins et al., 2017). This means a recovery of about 39% of normal values for the elderly.

Another interesting observation from the CRATUS trial was a significant reduction in TNF-alpha levels in the groups treated with Lomecel-B (Golpanian et al., 2017; Tompkins et al., 2017). Acute inflammation is a natural defense process that eliminates infectious agents and toxins and promotes tissue repair (Rea et al., 2018; Furman et al., 2019). Aging and frailty are associated with chronic inflammation, driven by abnormal secretion of proinflammatory cytokines (at least in part by senescent cells). A review by Heinze-Milne et al. (2022) identified: 1) 31 of 37 studies reporting that circulating IL-6 levels increase with increasing degree of frailty, and 2) 9 of 17 studies reporting a positive association between TNF-alpha and frailty. In the CRATUS trial, serum TNF-alpha levels were significantly decreased in the Lomecel-B groups, but no significant changes in IL-6 were observed (Golpanian et al., 2017; Tompkins et al., 2017).

4.1.2 Ongoing SC trials for physical frailty in older adults

4.1.2.1 Ongoing trails with intravenous Lomecel-B

The positive results obtained in the CRATUS trial (Golpanian et al., 2017; Tompkins et al., 2017) were very encouraging to continue clinical development. Therefore, two additional RCTs (phase 2b NCT03169231 and phase 2 jRCT2043200038) were launched to further assess the efficacy and safety of Lomecel-B for aging frailty. The CRATUS trial also suggested that Lomecel-B might reduce age-related chronic inflammation ("inflammaging"). Therefore, a RCT (HERA, NCT02982915)

was launched to evaluate the ability of Lomecel-B to improve influenza vaccine responses in frail subjects (Table 1).

Lomecel-B is also being evaluated in multiple clinical trials for aging-related conditions and chronic diseases (https://www.longeveron.com/clinical-pipeline). In 2021, the FDA granted Rare Pediatric Disease (RPD) designation for Lomecel-B (direct injection into the heart tissue) for the treatment of Hypoplastic Left Heart Syndrome, a rare and life-threatening congenital heart defect in infants (Longeveron, 2022b). Lomecel-B is undergoing clinical trial development for Alzheimer's disease and Acute Respiratory Distress Syndrome (ARDS) due to COVID-19 (Longeveron, 2022a).

4.1.2.2 hBM-MSCs derived from vertebrae from deceased donors (hvBM-MSCs)

An important limitation of BM-MSC therapy is the low number of cells obtained. This requires extensive cell expansion *ex vivo*, with the risk of cell senescence and reduced regenerative potency (Ganguly et al., 2017). Barbanti Brodano et al. (2013) compared the biological properties of MSCs derived from different sites in the human body, and found that *h*vBM-MSCs: 1) Can be maintained in culture for a greater number of passages, and 2) more efficiently generate mature cells of all mesenchymal lineages (osteogenic, adipogenic and chondrogenic differentiation). The number of *h*vBM-MSCs obtained from deceased donors is much higher than that obtained from traditional types of *h*BM-MSCs aspirated from living donors. NCT05284604 will assess the feasibility of *h*vBM-MSC therapy for aging frailty.

4.1.2.3 hUC-MSCs

An *h*UC-MSC preparation investigated by the Shanghai East Hospital (China) recently completed a phase I/II study (Table 1). Moreover, a phase I/II RCTs (NCT04919135) and a phase I safety trial (NCT05018767) have been recently launched to evaluate the efficacy and safety of *h*UC-MSCs in aging frailty (Table 1).

*h*UC-MSC possess several advantages compared with *h*BM-MSCs: 1) The UC is a waste material, while the collection of *h*BM-MSC involves painful invasive procedures, and 2) the UC is a source of young SCs, while adult *h*BM-MSCs exhibit reduced cellular regenerative potency with increasing age (Ganguly et al., 2017). *h*UC-MSC have been extensively investigated to treat hematological disorders [reviewed in (Shang et al., 2021)], as well as to treat age-related or immune disorders [including metabolic and cardiovascular diseases and systemic lupus erythematosus; reviewed in (Xie et al., 2020)].

4.1.2.4 hAD-MSCs

NCT03514537 is a safety trial of intravenous *h*AD-MSCs in frail adult and older adults (40–90 years) (Table 1). Comparisons with other studies are difficult to make because: 1) The doses of *h*AD-MSC used have not been found in the ClinicalTrials.gov database, 2) to my knowledge, no safety trial of intravenous *h*AD-MSC in frail subjects has been published previously, and 3) frail older people are susceptible to adverse drug reactions (Hilmer and Kirkpatrick, 2021).

4.1.2.5 GMFFP

NCT03458429 investigates whether transfusion of GMFFP (GCSF-Mobilized Fresh Frozen Plasma) from young persons may be a safe and effective treatment for frailty and immune dysfunction in older people (Maharaj, 2020). A great advantage of GMFFP is that it is easy to collect and prepare in large quantities. However, there are some limitations regarding NCT03458429 (see Section 4.1.3).

4.1.3 Limitations of current clinical trials

Current clinical trials with SCs for aging frailty use have three main limitations: 1) There is no standard protocol, 2) few pharmacokinetic and dosing data are available, and 3) SC interventions investigate older people, a "special population" who are poly-medicated and have comorbidities (Grimsrud et al., 2015).

4.1.3.1 Lack of consensus on efficacy outcomes

The 6 MWD was one of the efficacy outcomes of the CRATUS trials with Lomecel-B (Golpanian et al., 2016; Golpanian et al., 2017; Tompkins et al., 2017), and 6 MWD is the primary outcome measure in the current multicenter trial of Lomecel-B (NCT03169231). Some but not all ongoing trials include 6 MWD as an outcome measure (see, for example, NCT04314011 in Section 3.1). These differences in evaluation tools do not allow for a precise comparison of efficacy results between clinical trials.

4.1.3.2 Lack of consensus on diagnostic tools

A 6 MWD of >200 m and <400 m is an inclusion criterion of the current multicenter trial with Lomecel-B (NCT03169231), but this 6 MWD criterion was not included in the previous CRATUS trials (which included patients with CSF fragility scores of 4–7) (Golpanian et al., 2016; Golpanian et al., 2017; Tompkins et al., 2017). Differences in diagnostic tools are also common in ongoing trials (see Section 3.1) and make clinical efficacy comparisons even more difficult

4.1.3.3 Very few pharmacokinetic studies of intravenous MSCs in humans are available

MSC distribution studies in rodents showed that 1) MSCs are transplantable by the intravenous route of administration, 2) more than 90% of intravenous MSCs are trapped in the lung and then cleared (by monocyte phagocytosis) with a half-life of 24 h and 3) local MSC administration is more appropriate for a regenerative effect *in situ* [for review, see (Elman et al., 2014; Salvadori et al., 2019)]. These results suggest that intravenous MSCs act, at least in part, through secreted factors.

Few studies have been dedicated to investigating the distribution of intravascular MSC in humans (Levy et al., 2020). In patients with myocardial infarction, Kang et al. (2006) showed that 2 h after intracoronary administration of radiolabeled H-MSCs, only 1.5% of the injected H-MSCs accumulated in the infarcted myocardium. Similarly, Hofmann et al. (2005) showed that after intravenous administration of radiolabeled BM cells, only background activity was detected in the infarcted myocardium. In men with localized prostate cancer, Schweizer et al. (2019) failed to detect intravenously infused allogeneic MSCs targeting the tumor.

4.1.3.4 Further dosing studies are needed

Commenting on the CRATUS trial, Larrick and Mendelsohn (Larrick and Mendelsohn, 2017) noted that "*. . .modest improvement outcomes were limited to the lower dose, a finding that remains difficult to explain*", and suggested that "*Future studies are definitely*

warranted given the magnitude of this increasingly important medical syndrome". Indeed, further studies in frail elderly are needed to optimize the dosage of intravenous MSCs, that is, to find the intravenous dose and frequency of administration that guarantee an optimal efficacy/safety ratio

4.1.3.5 Influence of polypharmacy and comorbidities in frail elderly

Elderly subjects frequently have comorbidities, are poly-medicated, have reductions in hepatic and/or renal function, and have changes in the bioavailability of concomitant drugs (Grimsrud et al., 2015). In addition, such harm is amplified in frail people (Ibrahim et al., 2021).

In multiple myeloma patients older than 65 years and treated with autologous SC transplantation, Marini et al. (Marini et al., 2019) found that a reduction in the conditioning dose of melphalan was needed to maintain a safety profile similar to that in young subjects. Therefore, MSC therapy in a poly-medicated frail elderly patient may interact with concomitant medications, increasing or decreasing their bioavailability, with the risk of revealing adverse events, or reducing their therapeutic efficacy.

Older adults are considered by regulatory authorities to be a "special population" that has a therapeutic profile that cannot be directly extrapolated from what is known in adults (Grimsrud et al., 2015). Regarding safety issues, MSC therapies have a good safety profile, both in adults and in the elderly (Marini et al., 2019; Wang et al., 2021). However, severe comorbid disease is often considered an exclusion criterion in clinical trials, which carries the risk that the results obtained will be different from those obtained in "real life" conditions.

4.1.3.6 Limitations concerning NCT03458429 with GMFFP

There are some limitations regarding NCT03458429 (GMFFP). First, file NCT03458429 (ClinicalTrials.gov database) assumes that GMFFP contains factors secreted by mobilized SCs, but does not specify which are present in the transfused preparations (Maharaj, 2020). Second, nothing was found in the medical literature to clarify this issue (a PubMed search using GMFFP as a keyword returned no results). Finally, the FDA issued a statement in 2019 (FDA, 2019) warning consumers not to receive plasma infusions from young donors that are promoted as an unproven treatment for various conditions, and NCT03458429 does not have a control (placebo) arm to assess the intervention efficacy.

4.1.3.7 Negative results with NT-020

The nutrient combinations of NT-020 (NutraStem®) stimulate *h*SC proliferation *in vitro*, equal to or better than human granulocyte-macrophage colony-stimulating factor (*h*GM-CSF). However, NT-020 and exercise were unable to significantly increase blood levels of SCs in men and women aged 50–70 years, at two or 4 weeks from the start of the trial. Unlike the *in vitro* studies, *h*GM-CSF was not used as an active comparator

4.1.3.8 Other SC preparations

No clinical trials for aging using E-SCs or induced pluripotent stem cells (iP-SCs) were found. The main reasons for this are: 1) their teratoma-forming tumorigenicity (Miyawaki et al., 2017), and 2) that the use of E-SCs raises ethical concerns (Lo and Parham, 2009).

4.1.4 Future perspectives

The present investigation has not identified any SC preparation in late clinical development (Phase III RCTs) for physical frailty in older adults. Lomecel-B showed modest, but significant results in recent phase II RCTs (Golpanian et al., 2017; Tompkins et al., 2017), and if it successfully completes the current phase II RCTs, it would have the potential to initiate phase III trials and later become the first effective targeted intervention for aging frailty.

4.1.4.1 What study protocol for phase III RCTs with SCs preparations?

It would be preferable to perform phase III RCTs using a standard protocol, but this is not currently available. A previously proposed frailty protocol is the one used in the phase III RCT NCT02582138 (SPRINTT: Multicomponent Intervention for Physical Frailty and Sarcopenia) (Marzetti et al., 2018; Bernabei et al., 2022) (Figure 3). SPRINTT was designed to test interventions in older people with physical frailty and sarcopenia. The primary outcome is the ability to independently walk 400 m in <15 min. Inclusion criteria are: 1) short physical performance battery score between 3 and 9, 2) low lean appendicular mass, and 3) ability to independently walk 400 m (Marzetti et al., 2018; Bernabei et al., 2022). SPRINTT has the advantage of including the assessment of sarcopenia as an inclusion criterion.

In currently ongoing trials, 6 MWD is increasingly accepted as a primary outcome measure (and is also often an inclusion criterion, i.e., 6 MWD of >200 m and <400 m) (see Section 3.1). The 6MWT (6 Minute Walk Test) is simple, easy to perform, and better reflects activities of daily living than other walking tests (Chetta et al., 2006). Adopting it as the primary outcome to assess physical frailty (as well as inclusion criteria) may increase the relevance of comparisons with current clinical trials (Figure 3).

4.1.4.2 Inflammatory biomarkers

In the CRATUS trial, serum TNF-alpha levels were significantly decreased in the Lomecel-B groups, but no significant changes in IL-6 were observed (Golpanian et al., 2017; Tompkins et al., 2017). Most of the clinical trials in Table 1 are investigating the effect of SC preparations on cytokine levels. Their results may help confirm the reduction in TNF-alpha levels as a valid criterion of efficacy (and/or better understand the role of IL-6 in therapeutic response).

4.1.4.3 The efficacy of MSCs in reversing aging frailty can be improved

Most of the trials with MSC for aging frailty (Table 1) have not yet been completed (with results). If the results are not better than those obtained with Lomecel-B (Golpanian et al., 2017; Tompkins et al., 2017), other alternative types of SC preparations could enter preclinical and clinical research development.

4.1.4.3.1 Preconditioning and/or genetic modification of naive MSCs. A large number of preclinical studies have shown that preconditioning naïve MSCs (with growth factors, drugs, and/or other factors), as well as genetic modification, can improve their therapeutic efficacy in many animal models of disease [for a review, see (Ocansey et al., 2020)]. The modified MSCs could enter cell therapy development, first in animal models of frailty [for animal

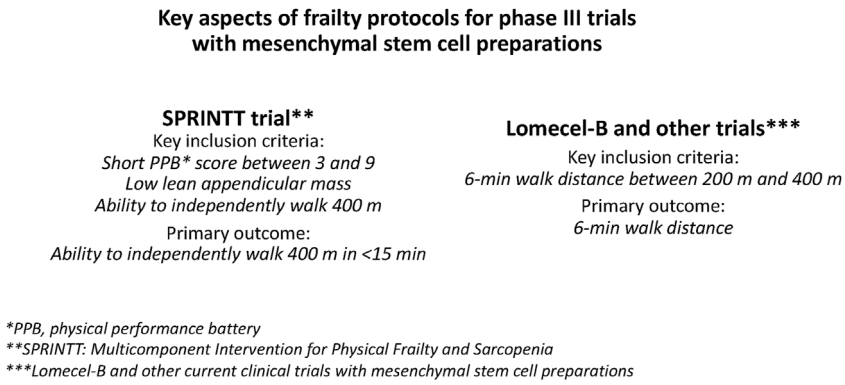

FIGURE 3
Key aspects of physical frailty protocols for phase III trials with mesenchymal stem cell (MSC) preparations. Two study protocols appear suitable for conducting phase III RCTs for aging frailty: (i) SPRINTT (Multicomponent Intervention for Physical Frailty and Sarcopenia) (Marzetti et al., 2018; Bernabei et al., 2022) and (ii) that used by ongoing clinical trials for aging frailty.

models of frailty, see (Heinze-Milne et al., 2019; Heinze-Milne et al., 2022)] and then in clinical trials.

4.1.4.3.2 Exosomes as another option to reverse the fragility of aging. Exosome therapies are intensively investigated in various clinical development programs. ClinicalTrials.gov lists 166 exosome intervention trials, but none for aging. The Chinese clinical trial database (chictr.org.cn/searchprojen.aspx) included a clinical trial with MSC-derived exosomes for photoaging (ChiCTR2200061216; see Section 3.2.3.2; Table 2). Interestingly, the exosomes used were loaded with circular RNA, a genetic modification procedure that can improve therapeutic efficacy [for information on genetic modification, preconditioning and engineering of MSC-derived exosomes, see (Ahmed and Al-Massri, 2022; Chen et al., 2022)].

Yoshida et al. (2019) reported that administration of nicotinamide phosphoribosyltransferase (NAMPT)-containing exosomes significantly enhanced wheel-running activity and prolonged lifespan in aged mice [for review of studies of MSC-derived exosomes in preclinical models of age-related diseases, see (Ahmadi and Rezaie, 2021); for perspectives and challenges of clinical trials with exosomes, see (Rezaie et al., 2022)]. Then, MSC-derived exosomes constitute an additional option to enter in preclinical and clinical development for the fragility of aging.

4.1.4.4 Repurposing of approved SC products and related agents for aging frailty

Repurposing medicinal agents means finding new therapeutic indications for existing ones (Ayyar and Subramanian, 2022). Repurposing is a cost- and time-effective mechanism that can be applied to develop new SC therapies for aging frailty (Begley et al., 2021).

Hemacord (BHI Therapeutic Sciences, United States) is an FDA-approved HPC cord blood product for disorders affecting the hematopoietic system (FDA, 2022). Hemacord is now in clinical development to be repurposed for acute ischemia stroke (NCT03735277), and could also be repurposed for aging frailty (Figure 4). It is important to mention that a large number of clinical studies are underway with allogeneic umbilical cord blood infusion

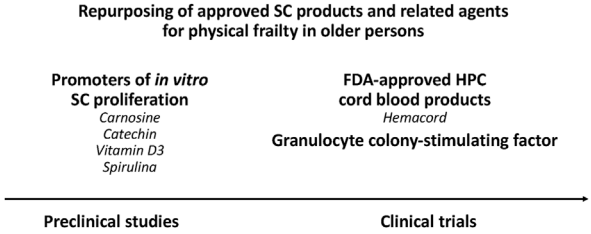

FIGURE 4
Repurposing of approved SC products and related agents for physical frailty in older persons. Several biological candidates can be repurposed for aging frailty: (i) Hemacord and other FDA-approved HPC cord blood products, (ii) the granulocyte colony-stimulating factor (G-CSF), and (iii) some nutraceuticals (carnosine, catechin, vitamin D3, spirulina).

for stroke and several other (non-hematopoietic) therapeutic indications (Ray and Mukherjee, 2021; Paton et al., 2022), but not for aging frailty. The current search identified only one study with allogeneic cord blood infusion for aging [case report of efficacy for a rare premature aging disorder, the Hutchinson-Gilford progeria syndrome (Suh et al., 2021)].

Darvadstrocel (Alofisel®, Takeda Ireland) and holoclar (Holoclar®, Holostem Terapie Avanzate, Italy) are EMA-approved SC products, but neither manufacturer has announced the launch of clinical trials for aging conditions (https://www.takeda.com/worldwide/) (https://www.holostem.com/news/?lang=en).

An important candidate to be repurposed for aging frailty is G-CSF (Figure 4). The G-CSF receptor (G-CSFR) is expressed in mouse and human skeletal muscle (Hara et al., 2011; Wright et al., 2014). Rodent studies have shown that: 1) Following skeletal muscle injury, G-CSF administration enhances satellite cell proliferation and muscle strength (Stratos et al., 2007), and 2) G-CSF enhances load-induced muscle hypertrophy (Ohashi et al., 2018). More importantly, muscle secreted G-CSF ameliorated satellite cell loss in the muscle of aged mice (Li et al., 2019).

Some nutraceuticals (carnosine, catechin, vitamin D3, spirulina) promote SC proliferation *in vitro* (Bickford et al., 2006; Bachstetter et al., 2010), and are candidates to be repurposed for aging frailty (Figure 4). Before entering clinical trials for aging frailty, these compounds should show their ability to increase SC blood levels in animal models (using GM-CSF as active comparator).

4.1.4.5 MSCs as "longevity candidates"

Aging frailty is associated with increased all-cause mortality (Grabovac et al., 2019) suggesting that Lomecel-B and other MSC therapies may increase life expectancy in frail older persons [for the relationship between longevity and all cause-mortality, see (Garay, 2021)]. In addition, animal studies consistently showed increased life expectancy with MSC transplantation (Shen et al., 2011; Lavasani et al., 2012; Mansilla et al., 2016). Therefore, MSCs can be considered as "longevity candidates".

The National Institute on Aging (NIA, United States) has launched an Intervention Testing Program (ITP) dedicated to identifying longevity drug candidates in mice (Nadon et al., 2017), for further testing in human clinical trials (Garay, 2021) (https://www.nia.nih.gov/research/dab/interventions-testing-program-itp). Therefore, MSCs deserve to be investigated for their ability to increase healthy lifespan in the ITP.

4.2 Facial skin aging

Various non-invasive methods have been used to prevent or treat facial aging, such as creams and lotions, without really satisfying results. In contrast, autologous fat grafting is efficacious for facial plastic and reconstructive purposes, and is widely used to restore volume and improve skin quality (facial skin rejuvenation) (Vasavada and Raggio, 2022).

4.2.1 Key findings of SC interventions for facial skin aging

Facial skin rejuvenation is another area of clinical research with SC preparations [for a review, see (Surowiecka and Struzyna, 2022)]. Recently, an RCT by Yin et al. (2020) clearly showed that SVF-assisted autologous fat grafting increases graft survival, facial volume, and skin quality.

4.2.2 Ongoing clinical trials for facial skin aging

4.2.2.1 hSVF preparations for facial skin aging

Two RCTs (NCT03928444 and RPCEC00000362) and one single-arm clinical trial (IRCT20141007019432N2) are investigating *h*SVF preparations for facial rejuvenation (Table 2). The positive results obtained by Yin et al. (2020) with autologous *h*SVF transplantation suggest that similar protocols could be used in other clinical settings for regulatory purposes (see Section 4.2.3)

4.2.2.2 AD-MSCs "secretome" preparation

The SC "secretome" comprises diverse soluble factors (chemokines, cytokines, growth factors, angiogenic factors, and exosomes) produced in the endosomal compartment, and released for SC migration, apoptosis, proliferation, and angiogenesis [for review, see (Xia et al., 2019)]. Recent work suggests that the regenerative mechanism of SC transplantation could involve a modulatory paracrine effect of the SC secretome (Xia et al., 2019).

Compared to SC preparations, SC secretome has several advantages, including ease of manufacture, freeze-drying, packaging, and easier transportation (Xia et al., 2019). In addition, the SC secretome has shown potential to counteract facial aging (Kerscher et al., 2022). PT Kimia Farma Tbk (Jakarta, Indonesia; https://www.kimiafarma.co.id/) develops an *h*D-MSC secretome preparation for facial aging. This preparation is being investigated in NCT05508191, which compares two methods of secretome administration (microneedle and fractional CO_2 laser).

4.2.3 Weakness of clinical research with hAD-MSCs for facial skin aging

4.2.3.1 SVFs are regulated as biologicals in the US

A large number of studies with SVFc for facial rejuvenation have been conducted in the US (Surowiecka and Struzyna, 2022), but very few are registered on ClinicalTrials.gov (Table 2). One important reason is that the FDA regulates SVF preparations as biologics, because mechanical processing is required (ASCS, 2019). This means that any surgeon wishing to use SVF preparations must submit an Investigational New Drug Application (INDA) to the FDA and be approved by an ethics committee. Consequently, the FDA initiated legal action against SC clinics using unauthorized SC products (El-Kadiry et al., 2021).

4.2.3.2 RCT in surgery are difficult to conduct

The efficacy results of the available SC studies are difficult to compare due to the very different techniques used for the extraction of fatty tissue, and for preparation and injection of SVFs (Surowiecka and Struzyna, 2022). In addition, outcome results are not similar for all surgeons (Demange and Fregni, 2011). Robinson et al. (2021) analyzed 388 RCT in surgery and identified several limitations: 1) trial registration was suboptimal, 2) sample sizes were small, 3) only a few trials were focused on major clinical events, and 4) few trials controlled the quality of the intervention or the experience of the surgeon.

4.2.4 Future perspectives

4.2.4.1 Research advantages of clinical trials on facial aging

Regardless of aesthetic considerations, clinical trials with SC preparations for facial rejuvenation are important because: 1) Tissue regeneration is directly assessed by sophisticated and precise methods and 2) SC trapping in the lung (Elman et al., 2014; Salvadori et al., 2019) and safety problems of intravenous MSC administration are avoided

4.2.4.2 Regulatory aspects of clinical trials with autologous SVFs preparations

The FDA regulates allogeneic SVF products as biologics (ASCS, 2019), but the risk of skin rejection prevents clinical development of allogeneic SVF preparations for facial rejuvenation. The final consequence is that the very numerous US clinical trials with autologous SVF preparations for facial rejuvenation (Surowiecka and Struzyna, 2022) are not registered on ClinicalTrials.gov.

Some RCT with autologous SVF preparations have recently been registered on ClinicalTrials.gov (see Table 2). The application of

similar RCT protocols could pave the way for autologous SVF preparations to be registered on ClinicalTrials.gov and comply with FDA regulations (Investigational New Drug applications and FDA-approvals).

4.2.5 Cutaneous photoaging

Photoaging of the skin ("EJ20 Photoaging of the skin") was recognized as a disease in the WHO ICD-11 (https://icd.who.int/browse11/l-m/en#/http://id.who.int/icd/entity/673647195). Topical retinoids such as tretinoin are effective in improving the clinical appearance of sun-damaged skin (Serri and Iorizzo, 2008). Topical treatment is often supplemented with orally administered vitamins, polyphenols, and carotenoids (Parrado et al., 2018). Among surgical approaches, fractionated laser is widely used for treating cutaneous signs of photoaging (Wu et al., 2016).

Preclinical studies suggested that AD-MSC preparations possess anti-wrinkling properties (Chen et al., 2020), and Charles-de-Sa et al. (Charles-de-Sa et al., 2020) recently reported full regeneration of solar elastosis by subdermal AD-MSC injection.

There are two ongoing clinical trials for cutaneous photoaging: 1) NCT01771679 which was evaluating intravenous allogeneic *h*BM-MSCs for the treatment of facial photoaging (recruitment was suspended), and 2) ChiCTR2200061216 which is currently exploring whether *h*ADSC-derived exosomes (loaded with circcol elns) can promote collagen and elastin synthesis in photoaged skin (Table 2).

Circular RNAs (circRNAs) include a large family of non-coding RNAs, which can regulate gene expression (acting on transcription, mRNA turnover and translation, by sponging microRNAs and RNA-binding proteins) (Panda, 2018). Intensive research of non-coding RNA therapy for photoaging is currently being carried out at Sun Yat-Sen University Hospital (China) (ChiCTR2200061216, Table 2) (Peng et al., 2017; Hou et al., 2021). If ChiCTR2200061216 yields positive results, treatment with circcol elns-loaded exosomes would hold great promise for cutaneous photoaging.

5 Concluding remarks

Clinical research with SC therapies for aging focuses on two main objectives: Physical frailty and facial skin aging. The advantages and disadvantages of these two objectives are complementary (which facilitates a global vision). Physical fragility affects organs that are usually accessed parenterally, where the pulmonary filter makes it even more difficult for SCs to access the target organ. Rejuvenating the skin is above all an aesthetic objective, but the effectiveness is evaluated directly on a visible and easily accessible organ.

With regard to aging frailty, the allogeneic *h*BM-MSC preparation Lomecel-B (Longeveron, United States) is the leading SC preparation in the area (Golpanian et al., 2016; Golpanian et al., 2017; Tompkins et al., 2017; Longeveron, 2021a; Longeveron, 2021b; Yousefi et al., 2022). Positive results have been obtained in preliminary phase II studies. An *h*UC-MSC preparation investigated by the Shanghai East Hospital (China) recently completed a phase I/II study. Several other clinical trials are currently underway for aging frailty (Table 1).

Facial skin aging is another area of clinical research with SC preparations. An RCT conducted by Yin et al. (2020) has shown positive results with an autologous *h*SVF preparation. Several other clinical trials are currently underway for facial skin aging (Table 2).

Clinical research with SC interventions for aging has only recently begun. This area of research has received a great initial impetus, as demonstrated by the twenty clinical trials launched worldwide and reviewed here. Let's hope that all these efforts will be rewarded with the arrival of the first SC anti-aging product in the near future.

Author contributions

The author confirms being the sole contributor of this work and has approved it for publication.

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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Metformin mitigates SASP secretion and LPS-triggered hyper-inflammation in Doxorubicin-induced senescent endothelial cells

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Introduction: Doxorubicin (DOX), a chemotherapeutic drug, induces senescence and increases the secretion of senescence-associated secretory phenotype (SASP) in endothelial cells (ECs), which contributes to DOX-induced inflammaging. Metformin, an anti-diabetic drug, demonstrates senomorphic effects on different models of senescence. However, the effects of metformin on DOX-induced endothelial senescence have not been reported before. Senescent ECs exhibit a hyper-inflammatory response to lipopolysachharide (LPS). Therefore, in our current work, we identified the effects of metformin on DOX-induced endothelial senescence and LPS-induced hyper-inflammation in senescent ECs.

Methods: ECs were treated with DOX ± metformin for 24 h followed by 72 h incubation without DOX to establish senescence. Effects of metformin on senescence markers expression, SA-β-gal activity, and SASP secretion were assessed. To delineate the molecular mechanisms, the effects of metformin on major signaling pathways were determined. The effect of LPS ± metformin was determined by stimulating both senescent and non-senescent ECs with LPS for an additional 24 h.

Results: Metformin corrected DOX-induced upregulation of senescence markers and decreased the secretion of SASP factors and adhesion molecules. These effects were associated with a significant inhibition of the JNK and NF-κB pathway. A significant hyper-inflammatory response to LPS was observed in DOX-induced senescent ECs compared to non-senescent ECs. Metformin blunted LPS-induced upregulation of pro-inflammatory SASP factors.

Conclusion: Our study demonstrates that metformin mitigates DOX-induced endothelial senescence phenotype and ameliorates the hyper-inflammatory response to LPS. These findings suggest that metformin may protect against DOX-induced vascular aging and endothelial dysfunction and ameliorate infection-induced hyper-inflammation in DOX-treated cancer survivors.

KEYWORDS

endothelial senescence, doxorubicin, metformin, SASP, LPS, senomorphics

1 Introduction

Doxorubicin (DOX) is a chemotherapeutic drug used for the treatment of solid and blood cancers (Chatterjee et al., 2010). Although DOX has contributed to improved survival rates in cancer patients, these survivors experience premature aging and frailty (Armenian et al., 2019). DOX induces premature aging primarily by accumulated DNA damage due to inhibition of topoisomerase II (Zhang et al., 2012) and increased reactive oxygen species (ROS) resulting from mitochondrial dysfunction (Asensio-Lopez et al., 2017). Both mechanisms can initiate a signaling cascade that prevents cells from undergoing replication, termed senescence.

DOX induces senescence in different cardiovascular cells, including cardiomyocytes, endothelial cells (ECs), cardiac fibroblasts, and cardiac progenitor cells (Abdelgawad et al., 2020). However, a landmark study demonstrated that, following DOX administration in p16-3MR male mice, the majority of senescent cardiac cells were ECs (Demaria et al., 2017). Moreover, a recent study by Yousefzadeh et al. (2020) used an accelerated aging mouse model and screened the expression of the senescence markers p21 and p16 in different tissues. Interestingly, the aorta demonstrated the highest expression of p16 and the second highest expression of p21 compared to other organs, with no significant increase in the expression of these markers in the heart (Yousefzadeh et al., 2020). Collectively, these studies suggest that ECs are a salient target for the induction of senescence, and that endothelial senescence plays a major role in DOX-induced cardiovascular complications.

Endothelial senescence is associated with multiple cellular and functional alterations that contribute to endothelial dysfunction, including impairment of vascular permeability, altered angiogenic response, and decreased endothelium-dependent dilation (Lesniewski et al., 2017). Importantly, senescent ECs secrete pro-inflammatory cytokines and metalloproteases known as senescence-associated secretory phenotype (SASP) (Abdelgawad et al., 2020). Accumulation of SASP promotes chronic low-grade inflammation, known as "inflammaging" (Franceschi et al., 2000), which can affect endothelial function (Soysal et al., 2020). Indeed, vascular senescence has been identified as a significant contributor to multiple cardiovascular diseases [reviewed in (Katsuumi et al., 2018; Jia et al., 2019)]. Furthermore, cancer survivors treated with anthracyclines exhibit endothelial dysfunction and vascular damage (Terwoord et al., 2022), which can be partially attributed to endothelial senescence as we recently reviewed (Abdelgawad et al., 2022b). Therefore, there is a compelling need for pharmacological strategies that target endothelial senescence to preserve endothelial function and potentially mitigate the related adverse effects in cancer survivors.

The hypothesis that senescent cells contribute to the pathogenesis of age-related diseases has led to the development of a new class of drugs called senotherapeutics (Baker et al., 2011). Senotherapeutics can be divided into two categories: senolytics and senomorphics, both of which mitigate senescence. Senolytics induce apoptosis and selectively eliminate senescent cells (Tse et al., 2008), whereas senomorphics modulate the secretion of SASP from senescent cells, thereby improving cellular functions (Moiseeva et al., 2013; Laberge et al., 2015).

Metformin, a widely used drug for the treatment of type 2 diabetes, was recently demonstrated to exert senomorphic and anti-aging effects (Chen et al., 2022b; Khodadadi et al., 2022). These effects are mediated by the ability of metformin to reduce ROS levels (Acar et al., 2021) and prevent DNA double-strand breaks (Park et al., 2022). Metformin has also been shown to have anti-inflammatory effects as evidenced by its ability to suppress SASP secretion in IMR90 fibroblasts (Moiseeva et al., 2013), bronchial-alveolar epithelial cells (Hansel et al., 2021; Wang et al., 2021), and lens epithelial cells (Chen et al., 2022a). Additionally, metformin has been shown to protect against endothelial senescence in different models of senescence including radiation- (Park et al., 2022), lipopolysaccharide (LPS)- (Raj et al., 2021), and high glucose-induced senescence (Arunachalam et al., 2014; Zhang et al., 2015). However, the effects of metformin on DOX-induced endothelial senescence have not been reported. Therefore, the current study aims to identify the senomorphic effects of metformin against DOX-induced endothelial senescence.

In recent years, there has been growing interest in the role of senescent ECs in LPS-induced inflammation, as ECs are of the major cellular targets of LPS-induced inflammation. Newly arising evidence show that radiation-induced and replicative senescent ECs are more vulnerable to LPS-induced inflammation than non-senescent ECs (Suzuki et al., 2019; Camell et al., 2021). Given that a significant percentage of cancer survivors received DOX during their treatment, we determined the effect of LPS stimulation on DOX-induced senescent ECs and identified the effects of metformin on LPS-induced hyper-inflammation.

2 Materials and methods

2.1 Cell culture

Human umbilical vein endothelial cells (HUVECs) and EA.hy926 human endothelial-derived cell lines were purchased from American Type Culture Collection (ATCC, Manassas, FL, United States). HUVECs were cultured in vascular cell basal medium (ATCC) supplemented with endothelial cell growth kit–VEGF, including 5 ng/mL rh VEGF, 5 ng/mL rh EGF, 5 ng/mL rh FGF basic, 15 ng/mL rh IGF, 10 mM L-glutamine, 0.75 U/mL heparin sulfate, hydrocortisone 1 μg/mL, ascorbic acid 50 μg/mL, fetal bovine serum 2%, 10 U/mL penicillin, and 10 μg/mL streptomycin. EA.hy926 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin (MilliporeSigma, St. Louis, MO, United States). Both cell lines were incubated at 37°C in 75 cm^2 tissue culture-treated flasks in a 5% CO_2 humidified incubator. Every other day, the media were replaced and the cells were subcultured at 80% confluence.

2.2 Cell treatments

Both EA.hy926 cells and HUVECs were pretreated for 24 h with increasing concentrations of metformin (0.5 mM, 1 mM, 2 mM, and 5 mM for EA.hy926 cells or 2 mM and 5 mM for HUVECs). Then,

cells were co-treated with DOX and metformin for an additional 24 h. Based on our previous study (Abdelgawad et al., 2022a), the clinically-relevant concentration 0.5 μM of DOX was selected to induce senescence in ECs since this concentration was associated with highest induction of senescence markers. Thereafter, cells were washed with PBS to remove DOX, metformin was added back to the medium, and cells were incubated for a further 72 h for protein extraction or 120 h for SA-β-gal staining.

For LPS experiments, HUVECs were either treated with DOX for 24 h, followed by 72 h incubation without DOX to establish senescence, or left untreated as non-senescent cells. The effect of LPS was then determined by stimulating both senescent and non-senescent HUVECs with LPS (30 ng/mL) for an additional 24 h. Notably, the media were changed before adding LPS so that the assessed SASP factors in the media reflect mainly the response of control and senescent ECs to LPS. Metformin was added as described above to determine its effect on LPS stimulation.

DOX, metformin, and LPS were purchased from Sigma (St. Louis, MO, United States) and stock solutions were prepared by dissolving them in the corresponding media of each cell line. All the cell treatments were performed between passages 5 and 10 in EA.hy926 cells and between passages 3 and 6 in HUVECs.

2.3 Protein extraction and western blotting

Following the treatments described above, EA.hy926 cells and HUVECs were washed twice with PBS and harvested in lysis buffer containing 20 mM Tris, 10 mM sodium pyrophosphate, 100 mM sodium fluoride, 5 mM EDTA, and 1% NP-40 supplemented with protease and phosphatase inhibitors. Cells were passed through a 28 gauge needle 10 times to further lyse the cells. Thereafter, the cell lysate was centrifuged at 2,000 x g for 10 min at 4°C and the supernatant was collected for western blotting. Protein concentration was measured using Pierce™ bicinchoninic acid (BCA) protein assay kit according to manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA, United States). Cell homogenates were denatured by boiling at 100°C for 5 min in sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) loading buffer (G Biosciences, St. Louis, MO, United States) containing 20 mM dithiothreitol. Thereafter, 20 μg homogenates were separated on 8%, 12%, or 15% SDS-PAGE gels and electrophoretically transferred to nitrocellulose membranes. The blots were then blocked at room temperature for 1 h using a blocking buffer consisting of 5% skim milk powder in Tris-buffered saline (20 mM Tris, 150 mM NaCl, pH 7.4) with 0.05% (v/v) Tween-20 (TBST). Following blocking, blots were incubated overnight at 4°C with primary antibodies diluted in 1% milk solution in TBST. Blots were then washed in TBST and incubated for 1 h at room temperature with horseradish peroxidase (HRP)-conjugated secondary antibodies diluted in blocking buffer, then washed with TBST. Blots were visualized using Pierce™ ECL substrate (Thermo Fisher Scientific) according to the manufacturer's instructions. Primary mouse antibodies against p53 (catalog 2,524, 1:1000 dilution) and primary rabbit antibodies against phospho-p53 (Ser15) (catalog 9284, 1:1000 dilution), p21 (catalog 2,947, 1:1000 dilution), MMP-3 (catalog 14,351, 1:1000 dilution), ICAM-1 (catalog 4915, 1:1000 dilution), phospho-SAPK/JNK (Thr183/Tyr185) (catalog 4668, 1:1000 dilution), SAPK/JNK (catalog 9252, 1:1000 dilution), phospho-p38 (Thr180/Tyr182) (catalog 4511, 1:1000 dilution), p38 (catalog 8690, 1:1000 dilution), AMPK alpha (catalog 2,532, 1:1000 dilution), phospho-NF-κB p65 (catalog 3033, 1:1000 dilution), and alpha-tubulin (catalog 2144, 1:1000 dilution) were purchased from Cell Signaling Technology (Danvers, MA, United States). Primary rabbit antibodies against phospho-AMPK alpha (Thr172) (catalog 07–681, 1:1000 dilution) were purchased from MilliporeSigma (Burlington, MA, United States). HRP-conjugated horse anti-mouse secondary antibodies were purchased from Cell Signaling (catalog 7076; 1:1000 dilution) and HRP-conjugated goat anti-rabbit secondary antibodies were purchased from Jackson ImmunoResearch (catalog 111–035–144, West Grove, PA, United States; 1:10,000 dilution). ImageJ software (National Institutes of Health, Bethesda, MD, United States) was used to quantify band intensities using alpha-tubulin protein levels as normalizing loading controls. Phospho-protein band intensities were measured relative to the respective total protein level. In some experiments, the blots were cut at separate molecular weight marks, thereby allowing the same blot to be incubated with more than one primary antibody at the same time.

2.4 Senescence associated-B-galactosidase (SA-β-gal) assay

For the detection of senescence, the SA-β-gal staining kit (Cell Signaling Technology) was used to stain senescent cells according to the manufacturer's protocol. Incubation time and pH were optimized as previously described (Abdelgawad et al., 2022a). To calculate the percentage of cells that were positive for SA-β-gal, the number of stained cells was counted relative to the total number of cells (at least 100 cells) using a bright-field microscope with a ×4 objective lens.

2.5 Assessment of senescence-associated secretory phenotype (SASP) factors in cell culture media

After the specified treatments, the media from HUVECs was collected and stored at −80°C until use. The supernatants were analyzed by the Cytokine Reference Laboratory at the University of Minnesota for the detection of human-specific interleukin 6 (IL-6), tumor necrosis factor-alpha (TNF-α), macrophage inflammatory protein-1 alpha (MIP-1α), monocyte chemoattractant protein-3 (MCP-3), monocyte chemoattractant protein-1 (MCP-1), C-X-C motif chemokine ligand 1 (CXCL1), C-X-C motif chemokine ligand 2 (CXCL2), interleukin-1 beta (IL-1β), interleukin 8 (IL-8), matrix metalloproteinase-3 (MMP-3), intracellular adhesion molecule-1 (ICAM-1), and E-selectin using the Luminex multiplex platform. The cytokines were analyzed according to the manufacturer's

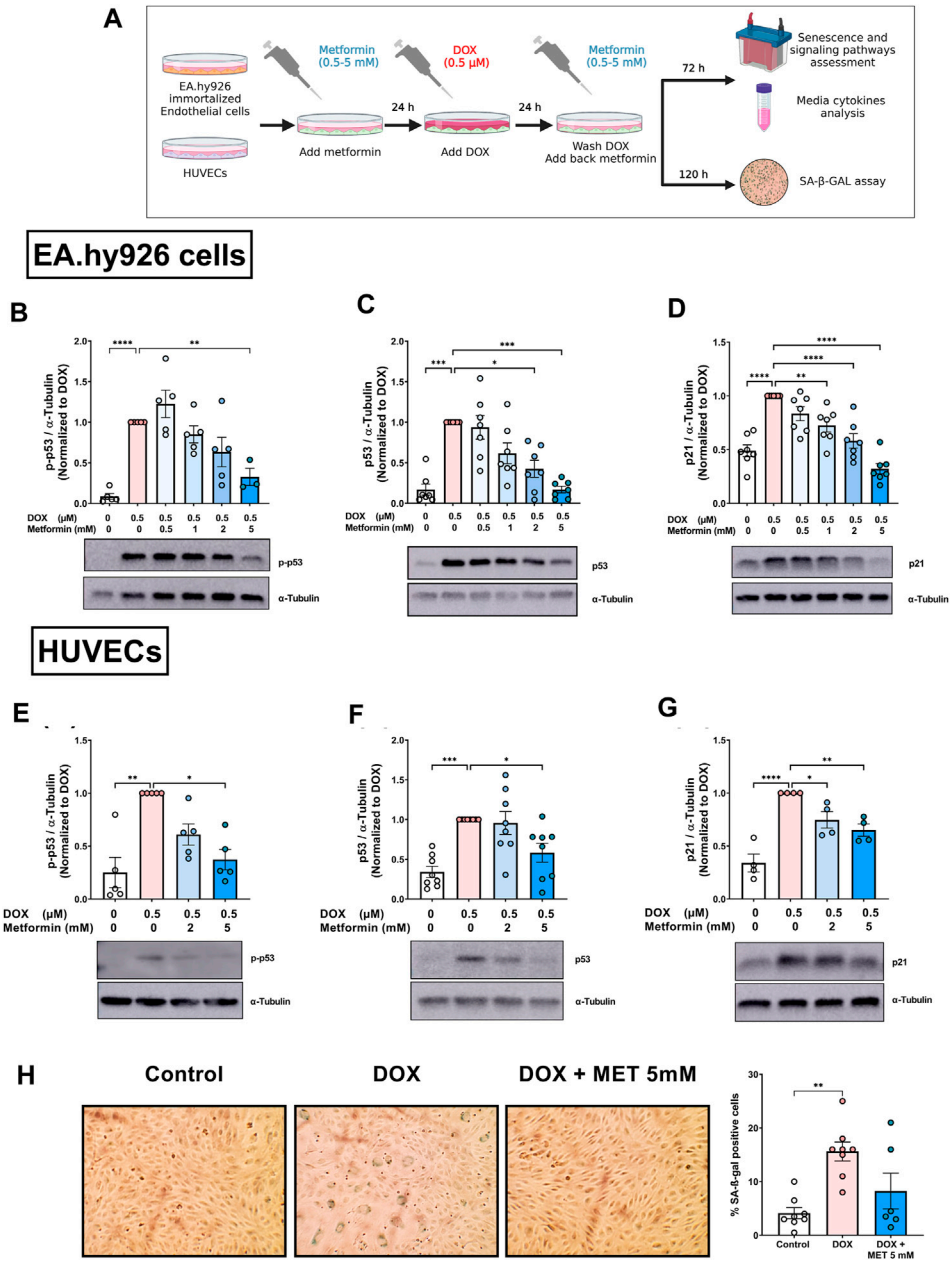

FIGURE 1
Metformin inhibited DOX-induced upregulation of senescence markers and SA-β-gal activity in endothelial cells. **(A)** Schematic diagram of the experimental design. Both EA.hy926 endothelial derived cells and HUVECs were treated for 24 h with 0.5 μM DOX ± metformin (0.5–5 mM, added 24 h before DOX). Thereafter, DOX was removed and the cells were incubated in DOX-free media with or without metformin for an additional 72 h for protein expression experiments or 120 h for measurement of SA-β-gal staining. Expression levels of senescence markers including p-p53, p53, and p21 in EA.hy926 cells **(B–D),** and HUVECs [**(E–G)**, respectively] were measured using western blot (n = 4–8). Representative images of western blots are shown. Values were normalized to α-tubulin and expressed relative to cells treated with DOX alone. **(H)** Images of SA-β-gal staining in control, DOX-treated, and DOX + metformin co-treated cells are shown in HUVECs. Images were analyzed and the percentage of SA-β-gal positive cells were calculated (n = 6–8). Values are presented as means ± SEM. Data were analyzed by one-way ANOVA followed by a Dunnet's multiple comparisons test (Figures 1B,D,F–H) or non-parametric Kruskal–Wallis tests followed by Dunn's *post hoc* test (Figures 1C, E); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Schematic diagram created with BioRender.com.

guidelines by lab personnel who were unaware of the experimental design. Samples were run in duplicate and the values were interpolated from 5-parameter-equipped standard curves. Cytokine concentrations were reported after being normalized to the protein content of the cells, which was determined by BCA.

2.6 Statistical analysis

Data analysis was performed using GraphPad Prism software (version 8.3.0, La Jolla, CA, www.graphpad.com) and the data are presented as mean ± standard error of the mean (SEM). Normality

was checked using the Shapiro-Wilk test. Comparisons between control, DOX, and metformin treatments were performed using a one-way analysis of variance (ANOVA) followed by pair-wise comparisons relative to DOX treatment using Dunnet's multiple comparison test (if the normality test was passed) or non-parametric Kruskal–Wallis tests followed by Dunn's *post hoc* test (if the normality test was failed). For LPS experiments (Figures 5, 6), comparisons were performed by ordinary two-way analysis of variance (ANOVA), followed by Tukey's multiple comparison *post hoc* analysis. A p-value of <0.05 was chosen to indicate statistical significance.

3 Results

3.1 Metformin inhibits DOX-upregulated expression of senescence markers in ECs

Previously, we characterized DOX-induced senescence phenotype in two types of endothelial cells: immortalized EA.hy926 endothelial-derived cells and primary human umbilical vein endothelial cells (HUVECs) (Abdelgawad et al., 2022a). However, the effect of metformin on DOX-induced endothelial senescence phenotype has not been reported yet. In this study, we first evaluated the effects of metformin in EA.hy926 endothelial-derived cells. Cells were treated with 0.5 μM DOX with or without metformin in a concentration range of 0.5–5 mM as illustrated in Figure 1A. The senescence phenotype was evaluated by measuring the protein expression of senescence markers p-p53, p53, and p21. DOX alone upregulated all the assessed senescence markers including p-p53 (Figure 1B), p53 (Figure 1C), and p21 (Figure 1D) by 11.5-, 5.9-, and 2-fold, respectively. These markers were expected to be upregulated by DOX since the p53/p21 pathway is activated in response to DNA damage (Abdelgawad et al., 2022a). Importantly, pretreatment with the highest concentration of metformin (5 mM) downregulated the phosphorylation of p53 compared to cells treated with DOX alone (Figure 1B). Additionally, pretreatment with 2 and 5 mM metformin resulted in significant concentration-dependent inhibition of the expression of p53 compared to cells treated with DOX alone (Figure 1C). The same concentrations of metformin, in addition to the 1 mM concentration, significantly inhibited the expression of downstream target p21 compared to cells treated with DOX alone (Figure 1D).

We have recently demonstrated that immortalized EA.hy926 cells and primary HUVECs have a differential response to the senolytic ABT-263 (Abdelgawad et al., 2022a). Therefore, we repeated similar experiments in HUVECs to determine whether metformin has similar effects in both endothelial cell lines. Only higher concentrations of metformin (2 and 5 mM) were used because they were associated with the largest reduction in senescence markers in EA.hy926 cells. Pretreatment of HUVECs with 5 mM metformin significantly inhibited DOX-induced upregulation of p-p53 compared to cells treated with DOX alone (Figure 1E). Similarly, only 5 mM metformin significantly downregulated p53 compared to cells treated with DOX alone (Figure 1F). Both 2 mM and 5 mM metformin significantly decreased the expression of p21 in a concentration-dependent manner compared to cells treated with DOX alone (Figure 1G). Since there is no single specific marker for senescence, the activity of senescence-associated beta-galactosidase (SA-β-gal), which is upregulated in senescent cells, was also evaluated. As shown in Figure 1H, the percentage of SA-β-gal-positive cells significantly increased in HUVECs treated with DOX alone compared to control cells (15.6% vs. 4.1%, respectively). Additionally, DOX-treated cells demonstrated enlarged morphology which is another marker of senescence. Importantly, pretreatment with 5 mM metformin ameliorated the increase in SA-β-gal activity (Figure 1H). However, no statistical difference was observed between DOX-treated HUVECs with and without metformin. Considering that metformin had similar effects in HUVECs and EA.hy926 cells, we chose to use HUVECs as our main endothelial model for subsequent experiments, as they are a more clinically relevant model.

3.2 Metformin inhibits DOX-induced secretion of SASP factors and endothelial adhesion molecules in senescent ECs

Endothelial senescence is also characterized by overexpression of SASP factors which contribute to inflammaging and endothelial dysfunction. We measured the levels of secreted SASP factors in the media of HUVECs following treatment with DOX in the absence or presence of metformin. Treatment of HUVECs with DOX alone induced a significant increase in the concentrations of IL-6 (Figure 2A), TNF-α (Figure 2B), MIP-1α (Figure 2C), and MCP-3 (Figure 2D). The same trend was observed in the expression of other SASP factors including MCP-1, CXCL1, CXCL2, IL-1 β, and IL-8, although the observed increases were not statistically significant (Figures 2E–I). Importantly, pretreatment with 2 mM and 5 mM metformin normalized the levels of cytokines including IL-6 (Figure 2A), TNF-α (Figure 2B), MIP-1α (Figure 2C), MCP-3 (Figure 2D), and CXCL2 (Figure 2G) in a concentration-dependent manner. Treatment with 5 mM metformin, but not 2 mM, significantly reduced the levels of MCP-1 (Figure 2E) and CXCL1 (Figure 2F) in DOX-treated cells. Both IL-1β (Figure 2H) and IL-8 (Figure 2I) were reduced by metformin, although the observed reductions did not reach statistical significance.

In addition to determining changes in the expression of SASP pro-inflammatory cytokines and chemokines, we assessed the effect of metformin on the SASP protease MMP-3. MMP-3 plays an important role in promoting inflammation, not only in the cardiovascular system, but also in different organs (recently reviewed in (Wan et al., 2021)). DOX alone significantly increased MMP-3 concentration in the culture media (Figure 3A) and its protein expression in cell lysate (Figure 3B). In agreement with the previous SASP results, metformin normalized DOX-induced increase of MMP-3 (Figures 3A,B). Moreover, the expression of endothelial adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1) and E-selectin, was determined since these endothelial markers were previously shown to be highly expressed in senescent HUVECs in models of radiation-induced senescence (Sermsathanasawadi et al., 2009) and replicative senescence (Korybalska et al., 2012). The expression of ICAM-1, in both the culture media (Figure 3C) and cell lysate

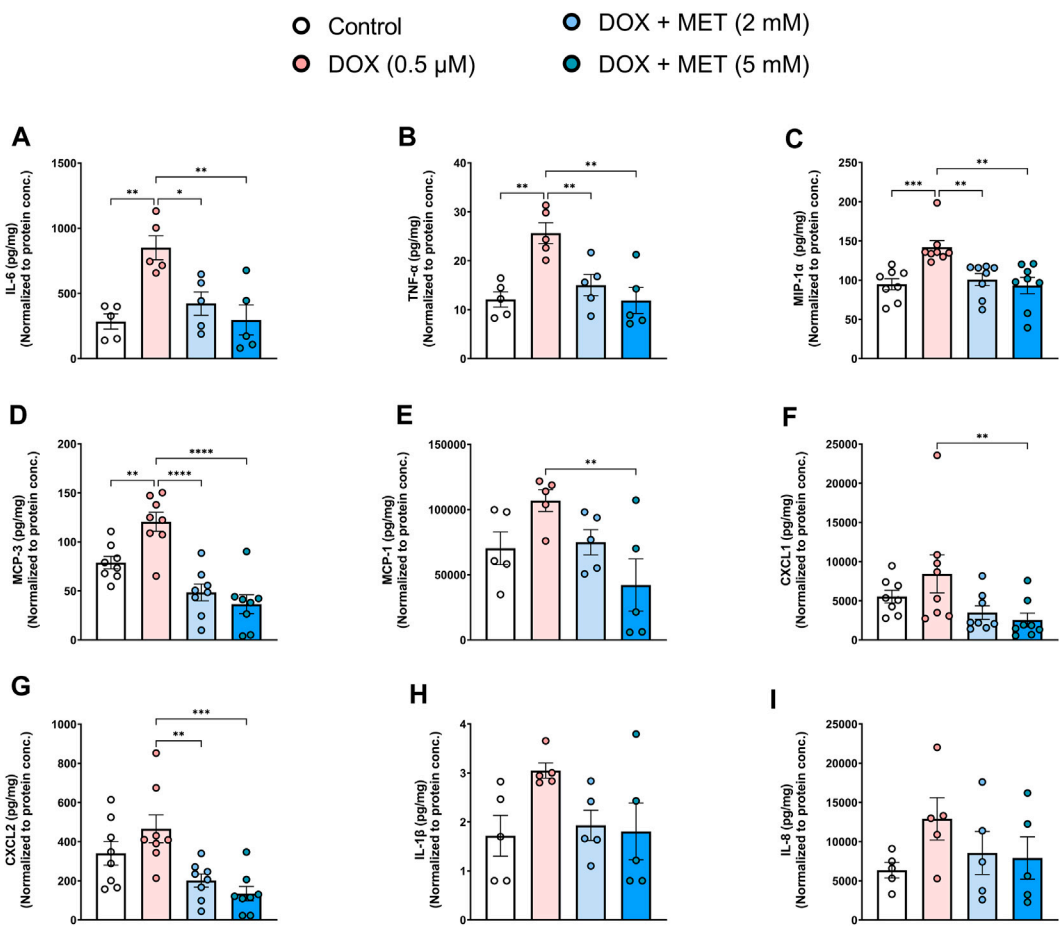

FIGURE 2
Metformin decreased DOX-induced SASP factors in conditioned media of HUVECs. HUVECs were treated for 24 h with 0.5 μM DOX ± metformin (2 and 5 mM, added 24 h before DOX). Thereafter, DOX was removed and the cells were incubated in DOX-free media with or without metformin for an additional 72 h. Conditioned media were collected and the protein expression of SASP factors including IL-6, TNF-α, MIP-1α, MCP-3, MCP-1, CXCL1, CXCL2, IL-1 β, and IL-8 [**(A–I)**, respectively (n = 5–8)] was determined by Luminex multiplex platform. Values were normalized to the protein concentration of the cells determined by BCA. Values are shown as means ± SEM. Data were analyzed by one-way ANOVA followed by a Dunnet's multiple comparisons test (Figures 2A,B, D–E, G, I) or non-parametric Kruskal–Wallis tests followed by Dunn's *post hoc* test (Figures 2C,F,H); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

(Figure 3D), and E-selectin in the culture media (Figure 3E) were increased in DOX-induced senescent cells, although the observed increases were not statistically significant. Metformin significantly decreased the expression of both ICAM-1 and E-selectin compared to cells treated with DOX alone (Figures 3C–E).

3.3 Signaling changes associated with the protective effect of metformin against endothelial senescence

Metformin has pleiotropic properties and can modulate multiple pathways. As a result, many aspects of the mechanisms by which metformin exerts its senomorphic effects are still not fully understood. To gain insights into the mechanistic pathways associated with the modulatory effect of metformin on endothelial senescence, we sought to measure the expression of mitogen-activated protein kinase (MAPK) signaling pathways including c-Jun N-terminal kinase (JNK) and p38 MAPK (p38). MAPKs are activated by stress stimuli, such as the DNA damage response induced by DOX and have been shown to be involved in senescence (Spallarossa et al., 2010; Altieri et al., 2017). JNK was previously demonstrated to be activated in senescent irradiated fibroblasts (Vizioli et al., 2020). Importantly, inhibition of JNK ameliorated the induction of SASP genes without affecting the expression of senescence markers (Vizioli et al., 2020). Our results show that DOX alone resulted in a robust 12-fold increase in JNK phosphorylation (Figure 4A), which is in agreement with previous findings reporting the activation of JNK by DOX in neonatal rat cardiomyocytes (Spallarossa et al., 2009) and endothelial progenitor cells (Spallarossa et al., 2010). Importantly, pretreatment with metformin significantly abrogated DOX-induced JNK activation (Figure 4A). This inhibitory effect of metformin on JNK activation was previously demonstrated in hypoxia/reoxygenation injury model in cardiomyocytes (Hu et al., 2016).

On the other hand, inhibition of p38 was previously reported to suppress both senescence markers and SASP in different models of

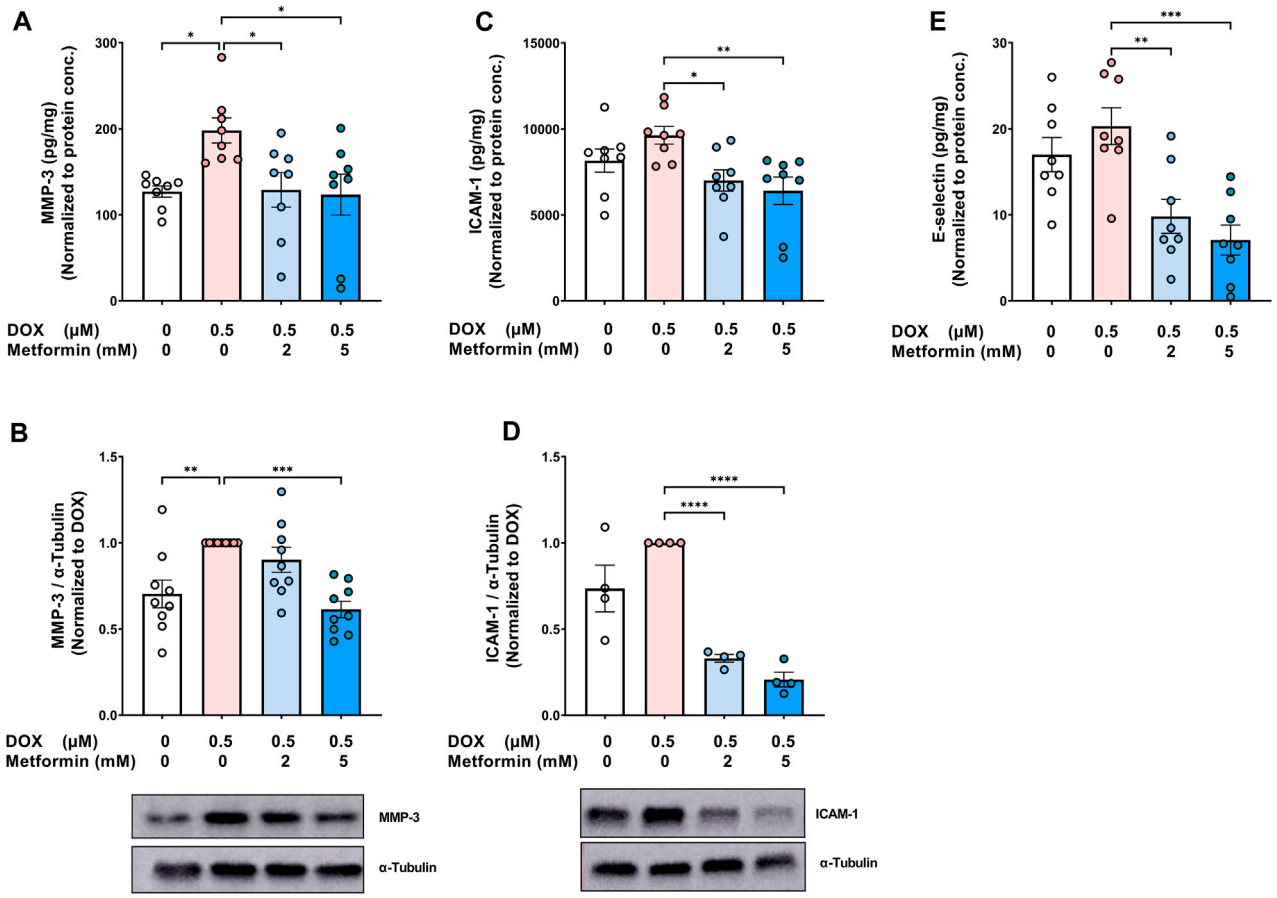

FIGURE 3
Metformin decreased the protein expression of SASP protease, MMP-3, and endothelial adhesion molecules in HUVECs. HUVECs were treated for 24 h with 0.5 μM DOX ± metformin (2 and 5 mM, added 24 h before DOX). Thereafter, DOX was removed and the cells were incubated in DOX-free media with or without metformin for an additional 72 h. The protein expression of the SASP protease MMP-3 was determined in **(A)** culture media by Luminex and **(B)** cell lysate by western blotting (n = 8–9). Expression of the endothelial adhesion molecules ICAM-1 in **(C)** culture media (n = 8) and **(D)** cell lysate (n = 4), and **(E)** E-selectin (n = 8) in the culture media were determined. Representative images of western blots are shown. Expression values in the media were normalized to the protein concentration of the cells determined by BCA. Values are shown as means ± SEM. Data were analyzed by one-way ANOVA followed by a Dunnet's multiple comparisons test (Figures 3A,B,D–E) or non-parametric Kruskal–Wallis tests followed by Dunn's *post hoc* test (Figure 3C); * p <0.05, ** p <0.01, *** p <0.001, **** p < 0.0001.

senescence (Altieri et al., 2017; Huang et al., 2021). In contrast to JNK, no significant changes in the phosphorylation of p38 were observed following treatment with DOX alone (Figure 4B). Surprisingly, 5 mM metformin caused a modest 1.5-fold increase in p38 phosphorylation when compared to DOX alone (Figure 4B). In agreement with our results, no inhibitory effect of metformin was observed on p38 phosphorylation in RAS-induced senescent fibroblasts (Moiseeva et al., 2013), further suggesting that the protective effect of metformin is independent of p38.

The nuclear factor κB (NF-κB) signaling pathway can be activated in response to DNA damage and was shown to be the major inducer of SASP (Salminen et al., 2012). Therefore, it was important to determine the effect of metformin on NF-κB activity. As expected, DOX alone induced NF-κB activation by 1.3-fold compared to control cells, as shown by higher phosphorylation of NF-κB p65 (Figure 4C). Pretreatment with 5 mM metformin abrogated DOX-induced NF-κB activation (Figure 4C). In agreement with this finding, it has been reported that metformin inhibits NF-κB activation in senescent fibroblasts (Moiseeva et al., 2013).

Conflicting results have been reported regarding the involvement of AMPK in the senomorphic effects of metformin. While some studies have reported that metformin inhibits senescence phenotype independently of AMPK (Moiseeva et al., 2013; Park et al., 2022), other studies have reported that AMPK activation is necessary for metformin's senomorphic and anti-inflammatory effects (Hattori et al., 2006; Chen et al., 2022a). Therefore, we evaluated the effect of DOX ± metformin on AMPK activation. No significant changes were observed in AMPK phosphorylation following DOX treatment with or without metformin (Figure 4D). The lack of AMPK activation by metformin may be attributed to the culture conditions, higher activation of AMPK by metformin was previously shown to be achieved in normoglycemic media than hyperglycemic conditions (Zordoky et al., 2014). Another explanation is that AMPK activation is time-dependent (Zhao et al., 2020). Considering that the ECs were incubated with metformin for 5 days, we speculate that activation may

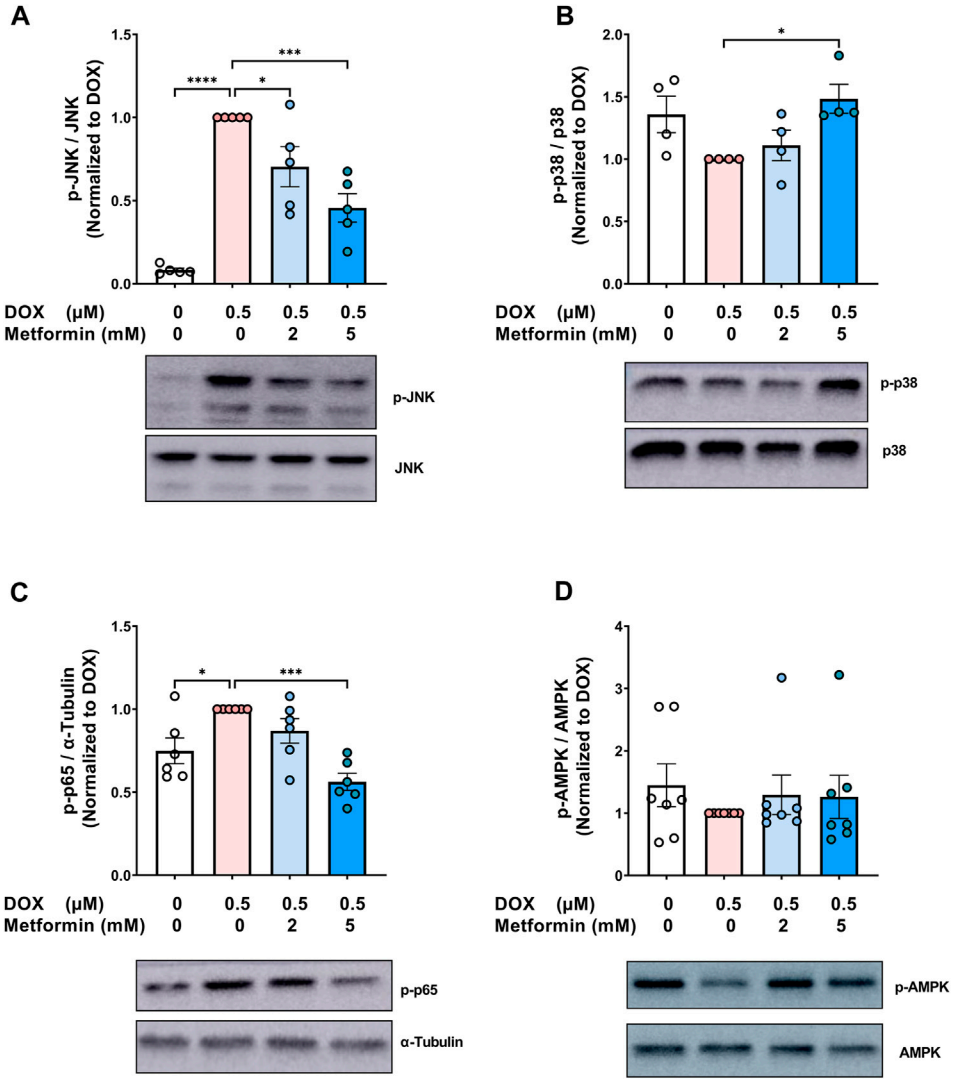

FIGURE 4
Signaling pathways associated with the protective effect of metformin on endothelial senescence in HUVECs. HUVECs were treated for 24 h with 0.5 μM DOX ± metformin (2 and 5 mM, added 24 h before DOX). Thereafter, DOX was removed and the cells were incubated in DOX-free media with or without metformin for an additional 72 h. Protein expressions of phospho- **(A)** JNK, **(B)** p38, **(C)** NF-κB p65, and **(D)** AMPK (n = 4–7) were quantified using western blot. Representative images of western blots are shown. Values were normalized to total protein or α-tubulin and expressed relative to cells treated with DOX alone. Expressed values are presented as mean ± SEM. Data were analyzed by one-way ANOVA followed by a Dunnet's multiple comparisons test (Figure 4A,C) or non-parametric Kruskal–Wallis tests followed by Dunn's *post hoc* test (Figure 4B,D); * p <0.05, *** p <0.001, **** p < 0.0001.

have occurred at an earlier time point. Collectively, our results demonstrated that the protective effects of metformin against DOX-induced endothelial senescence are associated with inhibition of DOX-induced JNK and NF-κB activation.

3.4 Metformin protects against LPS-induced hyper-inflammation and exacerbated SASP factors expression in DOX-induced senescent ECs

Lipopolysaccharide (LPS) is a bacterial toxin found on the outer membrane of gram-negative bacteria. When LPS enters the body, it can stimulate the immune system and cause a severe, systemic inflammatory response. Notably, ECs express specific receptors such as toll-like receptor 4 (TLR4) that interact with LPS, making them susceptible to LPS-induced inflammation. Recent studies demonstrate that senescent ECs are even more vulnerable to LPS-induced inflammation compared to non-senescent cells (Suzuki et al., 2019; Budamagunta et al., 2021; Camell et al., 2021). Importantly, these studies used other models of senescence than DOX-induced senescence. Therefore, we sought to characterize the effect of LPS-induced inflammation in DOX-induced senescent HUVECs. We exposed non-senescent and DOX-induced senescent HUVECs to LPS for 24 h following establishment of senescence and removing the culture medium that contain already-secreted SASP

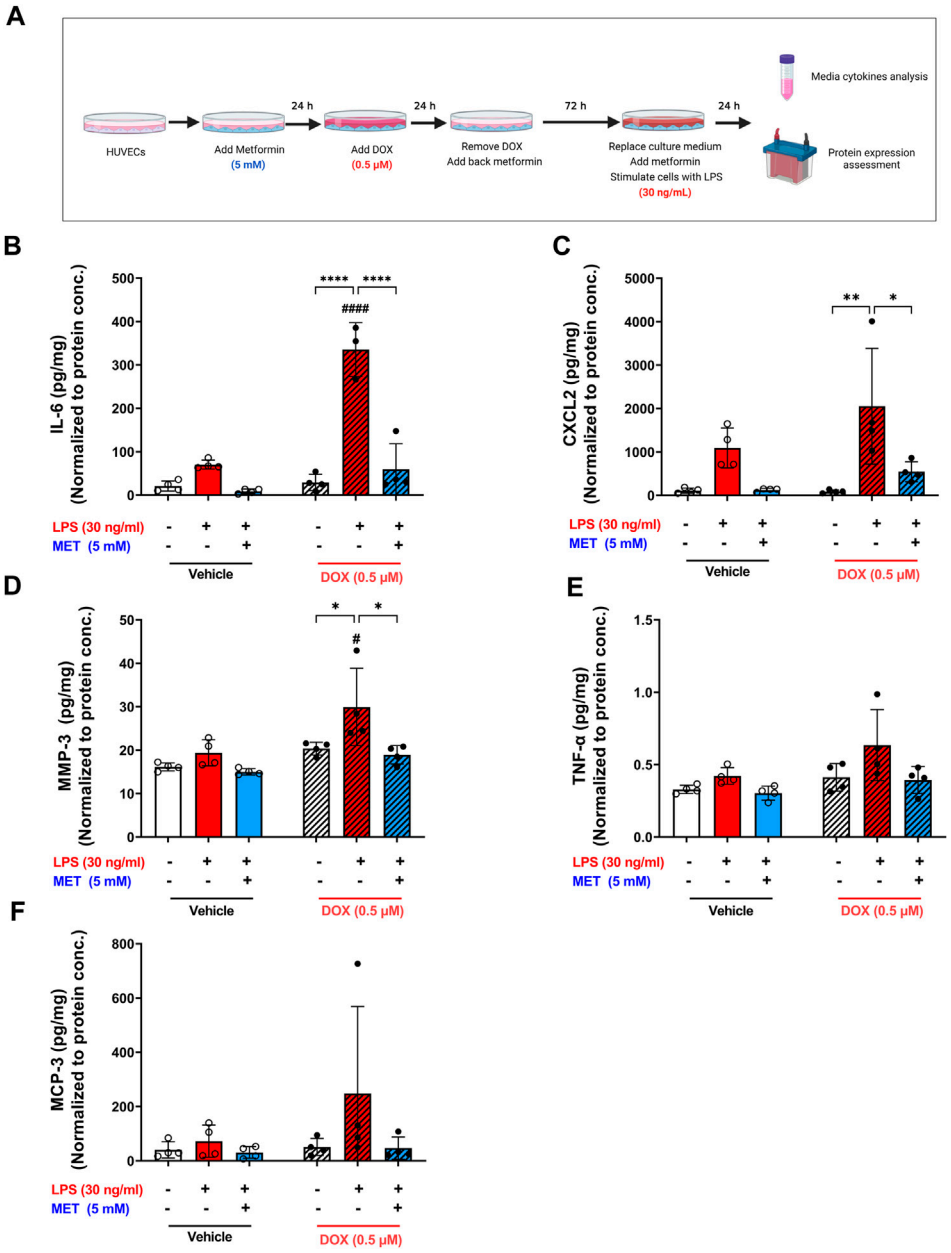

FIGURE 5
Metformin ameliorated LPS-triggered hyper-inflammation in DOX-induced senescent HUVECs. **(A)** Schematic diagram of the experimental design. HUVECs were treated for 24 h with 0.5 μM DOX ±5 mM metformin (added 24 h before DOX) or left untreated. Thereafter, DOX was removed and the cells were incubated in DOX-free media with or without metformin for an additional 72 h. The media were changed so that the assessed SASP factors in the media reflect only the effect of LPS. Then, cells were stimulated with LPS (30 ng/mL) for an additional 24 h. Thereafter, conditioned media were collected and the protein expression of SASP factors including **(B)** IL-6, **(C)** CXCL2, **(D)** MMP-3, **(E)** TNF-α, and **(F)** MCP-3 was determined by Luminex (n = 4). Values were normalized to the protein concentration of the cells determined by BCA. Expressed values are presented as mean ± SEM. Data were analyzed by two-way ANOVA followed by a Tukey multiple comparisons test. * compared to different treatment within the same group; * p <0.05, ** p <0.01, **** p < 0.0001. # compared to non-senescent cells with the same treatment; # p <0.05, #### p < 0.0001.

factors as illustrated in Figure 5A. A significant hyper-stimulatory response to LPS was observed in senescent HUVECs compared to non-senescent cells, demonstrated by remarkable increase of the secretion of SASP factors in the media including IL-6, CXCL2, and MMP3 (Figures 5B–D). Importantly, 5 mM metformin significantly inhibited LPS-induced hyper-inflammation and almost normalized the level of these cytokines in the media. Other SASP factors including TNF-α (Figure 5E) and MCP-3 (Figure 5F) demonstrated the same trend; however, they were not significant.

The same trend was observed in the protein expression of the adhesion molecule ICAM-1. LPS triggered a 9-fold upregulation in the expression of ICAM-1 in DOX-induced senescent HUVECs

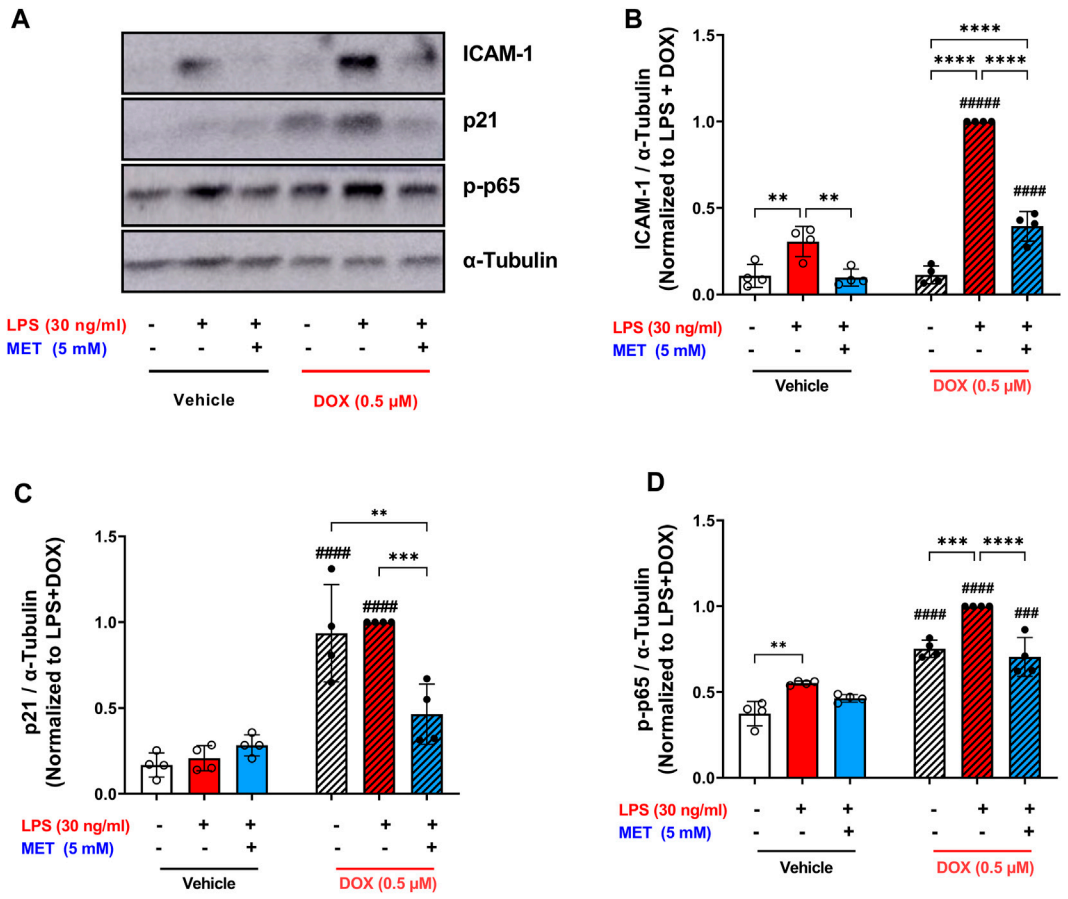

FIGURE 6
Metformin suppressed the expression of ICAM-1 and prevented NF-κB activation following LPS stimulation. HUVECs were treated for 24 h with 0.5 μM DOX ± 5 mM metformin (added 24 h before DOX) or left untreated. Thereafter DOX was removed and the cells were incubated in DOX-free media with or without metformin for an additional 72 h. Then, cells were stimulated with LPS (30 ng/mL) for an additional 24 h. **(A)** Representative images of western blot are shown and the expression levels of **(B)** ICAM-1, **(C)** p21, and **(D)** phospho-NF-κB p65 were measured (n = 4). Values were normalized to α-tubulin and expressed relative to DOX + LPS treated cells. Expressed values are presented as mean ± SEM. Data were analyzed by two-way ANOVA followed by a Tukey multiple comparisons test. * compared to different treatment within the same group; ** p <0.01, *** p <0.001, **** p < 0.0001. # compared to non-senescent cells with the same treatment; # p <0.05, ### p <0.001, #### p < 0.0001.

compared to 2.8-fold increase in non-senescent HUVECs (Figures 6A,B). Metformin significantly reversed this upregulation (Figure 6B).

To confirm the senescence phenotype, the expression of p21 was measured as a surrogate marker of senescence. Consistent with our previous results (Figure 1G), cells treated with DOX alone demonstrated higher expression of p21 which was maintained after LPS stimulation (Figure 6C). Pretreatment with metformin significantly downregulated the expression of p21 (Figure 6C). Mechanistically, exposure to LPS increased the phosphorylation of NF-κB p65 both in senescent and non-senescent HUVECs (Figure 6D). Metformin significantly inhibited NF-κB activation as shown by decreased phosphorylation of NF-κB p65 (Figure 6D). Together these findings suggest that metformin maintains its senomorphic properties even in the presence of hyper-inflammation in senescent ECs. Of note, since metformin was added before DOX and throughout all steps, these observed effects may either be due to its senomorphic effects against DOX-induced senescence, direct anti-inflammatory effects against LPS, or a combination of both.

4 Discussion

Doxorubicin (DOX) is a widely used chemotherapy drug that has been in use since the 1960s. Despite its effectiveness, DOX's clinical utility is limited due to its adverse cardiovascular effects. Others and we have previously demonstrated that DOX induces senescence in endothelial cells (ECs) (Yang et al., 2015; Chen et al., 2021a; Matacchione et al., 2021; Misuth et al., 2021; Abdelgawad et al., 2022a), which may contribute to the deleterious outcomes associated with DOX. ECs play several important roles such as maintaining vascular tone, initiating angiogenesis, and acting as a barrier to molecules circulating in the blood, all of which are impaired when ECs become senescent (Abdelgawad et al., 2022b). One characteristic feature of senescent cells is the secretion of SASP, which consists of multiple components including pro-inflammatory cytokines, chemokines, and proteases (Coppe et al., 2008). A recent study showed that senescent ECs have higher levels of SASP expression compared to other senescent cell types (Schafer et al., 2020). Importantly, the accumulation of SASP

can have deleterious effects on the cardiovascular system. Of note, these deleterious effects extend beyond the cardiovascular system, as overexpression of SASP has been shown to promote cancer progression (Hwang et al., 2020) and trigger a hyper-inflammatory response to inflammatory stimuli such as infections (Budamagunta et al., 2021; Camell et al., 2021).

Targeting senescent ECs could be a promising strategy to mitigate the complications of DOX-induced endothelial senescence. Recent findings have demonstrated that the removal of senescent cells using a genetic approach following DOX administration in mice restored endothelium-dependent dilation, suggesting the important role of senescence in mediating DOX-induced vascular dysfunction (Hutton et al., 2021). Others and we have shown that pharmacological approaches such as senolytics can selectively induce apoptosis in ECs (Zhu et al., 2017; Abdelgawad et al., 2022a). However, senolytics have limitations, including the potential to kill non-senescent cells and the risk of some senolytics to trigger thrombocytopenia (Leverson et al., 2015; Hwang et al., 2018). As a result, senomorphics may be another pharmacological alternative because they modulate the senescence phenotype by downregulating the detrimental effects of SASP without eliminating senescent cells.

Accumulating evidence has previously shown metformin to demonstrate senomorphic and anti-aging actions in different models of senescence including radiation (Hansel et al., 2021; Park et al., 2022), hyperglycemia (Arunachalam et al., 2014), and oxidative stress-induced senescence (Chen et al., 2021b). Two clinical trials, including MILES (Metformin In Longevity Study), and TAME (Targeting Aging with Metformin), have been designed to further identify the anti-aging effects of metformin (Barzilai et al., 2016; Kulkarni et al., 2018). However, the effect of metformin on DOX-induced endothelial senescence has not been established. Recent evidence suggests that the signature of senescence displayed by cells can vary greatly depending on the cell type and the inducer of senescence (Casella et al., 2019; Abdelgawad et al., 2022b). Moreover, we have recently shown that the effect of senolytics can also differ, as demonstrated by the differential response to ABT-263 in different senescent endothelial cell lines (Abdelgawad et al., 2022a). Therefore, we evaluated for the first time the effect of metformin on modulating DOX-induced senescence in ECs. Our results demonstrate that metformin abrogates DOX-induced endothelial senescence, as evidenced by the suppressed expression of senescence markers and decreased SA-β-gal activity. These findings were validated using two different endothelial cell lines: primary HUVECs and immortalized EA.hy926 endothelial-derived cells, and demonstrated that metformin suppressed DOX-induced senescence markers to the same extent in both cell lines.

The present study demonstrates for the first time that treatment of DOX-induced senescent ECs with metformin inhibits the secretion of SASP factors including pro-inflammatory cytokines (IL-6, TNF-α), chemokines (CXCL1, CXCL2, MCP 1 and 3, and MIP-1α), and proteases (MMP-3). Excessive secretion of SASP promotes vascular inflammation and can contribute to DOX-induced cardiovascular complications. Venturini et al. (2020) recently demonstrated through an *in vitro* model that SASP factors released due to DOX-induced endothelial senescence stimulate platelet activation and aggregation, which can contribute to atherothrombotic events. In agreement with the observed anti-inflammatory effect, metformin was previously shown to suppress the expression of SASP factors in RAS-induced senescent fibroblasts (Moiseeva et al., 2013) and angiotensin-II-induced senescent vascular smooth muscle cells (Tai et al., 2022). Moreover, a recent clinical study demonstrated that treatment with metformin was associated with a lower expression of SASP factors (IL-6 and TNF-α) in B cells isolated from elderly population (Frasca et al., 2021). Our findings also showed that metformin markedly decreased the expression of adhesion molecules ICAM-1 and E-selectin in DOX-induced senescent ECs. Higher expression of adhesion molecules can increase the risk of vascular complications by facilitating the recruitment and aggregation of leukocytes on the endothelial surface, which triggers vascular inflammation. Together, these findings suggest that metformin can be a promising senomorphic approach to protect against DOX-induced vascular aging and vascular inflammation.

Recent evidence suggests that the effects of SASP can go beyond the cardiovascular system. Indeed, SASP-induced inflammaging was recently shown to induce a hyper-inflammatory response upon exposure to further inflammatory insults, such as the cytokine storm induced by COVID-19 (Camell et al., 2021). The same study showed that treating aged mice with the pathogen-associated molecular pattern factor LPS increased the serum levels of inflammatory SASP factors compared to young mice (Camell et al., 2021). The same concept was demonstrated *in vitro*, where the stimulation of radiation-induced senescent ECs with LPS resulted in a higher induction of inflammation compared to non-senescent cells (Budamagunta et al., 2021; Camell et al., 2021). Together, these results suggest that the higher vulnerability of the aged population to COVID-19 related mortality may be attributed, at least in part, to the accumulation of senescent cells and the resulting hyper-inflammatory response. The same explanation may also be applicable to other conditions with a high burden of senescence, such as cancer survivors. In agreement with this hypothesis, a recent retrospective observational study demonstrated that childhood cancer survivors are at a higher risk of developing severe infections that require hospitalization (Chehab et al., 2022). Furthermore, a recent population-based study in Italy showed that cancer survivors are at the same risk of infection with COVID-19, but have an increased risk of mortality once infected (Mangone et al., 2021). The current study showed that LPS induced a hyper-inflammatory response in DOX-induced senescent ECs compared to non-senescent cells. To our knowledge, this is the first study to report the effect of LPS on DOX-induced senescent cells. These results can provide a mechanistic explanation for the clinical findings in cancer survivors.

Consequently, decreasing the burden of senescence can be a promising strategy for mitigating the severity of bacterial infections in these vulnerable populations. Recently, senolytics were demonstrated to decrease the mortality of aged mice exposed to a mouse β-coronavirus, further supporting the detrimental role of senescence in infections (Camell et al., 2021). In the current work, we showed that metformin significantly abolished LPS-induced hyper-inflammation and normalizes the level of SASP factors in DOX-induced senescent ECs. The demonstrated anti-inflammatory effects support, at least in part, recent data from the COVID-OUT trial demonstrating a 42% relative decrease in the incidence of Long

Covid in patients treated with metformin (Bramante et al., 2022). Moreover, a recent review highlighted the anti-inflammatory effect of metformin on the microvasculature as a major contributor to better outcomes in COVID-19 patients (Wiernsperger et al., 2022).

In conclusion, the current study showed that metformin protected against DOX-induced endothelial senescence as evidenced by the abrogation of senescence markers and downregulation of SASP factors and adhesion molecules. This protective effect was associated with inhibition of JNK and NF-κB pathways. Additionally, we showed that DOX-induced senescent ECs exhibited a hyper-inflammatory response to LPS compared to non-senescent cells. Importantly, metformin ameliorated this hyper-inflammation. Together, these findings suggest that metformin may serve as a promising drug for mitigating DOX-induced endothelial senescence and the resulting complications.

Data availability statement

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

Author contributions

Conceptualization, BZ, IA, and KA; methodology, IA, KA, BS, and MG; formal analysis, IA and KA; data curation, IA, MG, and KA; writing-original draft preparation, IA and KA; writing-review and editing, IA, KA, MG, and BZ; supervision, BZ; project administration, MG and BZ; funding acquisition, BZ All authors have read and agreed to the published version of the 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

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fragi.2023.1170434/full#supplementary-material

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Highlighting the value of Alzheimer's disease-focused registries: lessons learned from cancer surveillance

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Like cancer, Alzheimer's disease and related dementias (ADRD) comprise a global health burden that can benefit tremendously from the power of disease registry data. With an aging population, the incidence, treatment, and mortality from ADRD is increasing and changing rapidly. In the same way that current cancer registries work toward prevention and control, so do ADRD registries. ADRD registries maintain a comprehensive and accurate registry of ADRD within their state, provide disease prevalence estimates to enable better planning for social and medical services, identify differences in disease prevalence among demographic groups, help those who care for individuals with ADRD, and foster research into risk factors for ADRD. ADRD registries offer a unique opportunity to conduct high-impact, scientifically rigorous research efficiently. As research on and development of ADRD treatments continue to be a priority, such registries can be powerful tools for conducting observational studies of the disease. This perspectives piece examines how established cancer registries can inform ADRD registries' impact on public health surveillance, research, and intervention, and inform and engage policymakers.

KEYWORDS

public health, aging, cancer, disease surveillance, disease registries, Alzheimer's disease and related dementias, data management, data infrastructure

1 Introduction: Registries as a powerful source of data surveillance and for enhancing public health

Evidence-based decisions in public health are often guided by the interpretation of surveillance data (Bauer, 2014). Surveillance is the ongoing systematic collection of health-related data that public health researchers aggregate, analyze, and disseminate for making informed public health practice decisions (Langmuir, 1963). *Public health surveillance*—as first coined by Thacker and Berkelman in 1988 and most recently refined in 2012 by the Centers for Disease Control and Prevention's (CDC) surveillance working group, addresses a defined public health problem and is intended to reduce morbidity and mortality and improve population health (L. M. Lee and Thacker, 2011; Thacker et al., 2012; Thacker and Berkelman, 1988).

Registries are a powerful source of data surveillance (Declich and Carter, 1994) and may be classified as a product registry, health service registry, or disease (condition) registry (Gliklich et al., 2014). Disease registries are based on data from people who share a standard feature that defines the registry's purpose. The information in disease registries is updated, predefined, systematic, and periodic, usually based on a geographically defined population (Donaldson, 1992). Disease incidence is reported to one of the three levels of registries, including local hospitals, central registries (hospitals/regions), and population-based registries (Rankin and Best, 2014). The four aims of a disease registry include the intention to: 1) improve patient care, 2) enhance public health, 3) advance medical knowledge, and 4) disseminate information (Rankin and Best, 2014).

Beyond being a representative source of de-identified data for a defined population (Gliklich et al., 2014), registries provide rich resources for observational studies (Hlatky et al., 1984), improving study design, process, and hypothesis testing (Porten et al., 2011; Gliklich et al., 2014). Linking this aggregation of complete, high-quality, timely data to other data collections like biobanks and randomized control trials (RCTs) has expanded population-based studies (Maudsley and Williams, 1999; Li et al., 2016; Hoskin et al., 2019; Karanatsios et al., 2020; Hoopes et al., 2021).

Using standard nomenclature (for disease etiologies, stages, and treatments) has allowed registry-based trials to compare studies in the real world with existing clinical care practices to determine real-world outcomes (Karanatsios et al., 2020). Involving the diverse perspectives of users, creators, and sources of data—cancer registrars, patients, caregivers, and providers—in creating and evaluating the registries provides critical perspectives for relevant, high-functioning, sustainable registries (Maudsley and Williams, 1999; Parkin, 2006; Bray and Parkin, 2009; Bray et al., 2014; Gliklich et al., 2014; MacIntyre and MacKay, 2018). Cancer registries, in particular, are regulated, linked, and share nomenclature across disease types, stages of diagnosis, and treatments (NAACCR, 2020), expanding the possibilities of innovative cancer prevention and control approaches to address the most common and rarer cancers competently, powerfully, cost-effectively, and compassionately across the continuum of cancer care (B. Lee et al., 2022; Mariotto et al., 2011; Piñeros et al., 2017; Ribisl et al., 2017; Wingo et al., 2005). This paper examines how established cancer registries can inform Alzheimer's disease and related dementias (ADRD) registries' impact on public health surveillance, research, and intervention.

2 History of the development of cancer registries

Investigators interested in establishing disease registries have much to learn from the most extensively developed disease registries in the United States (US)—cancer registries (Cromley and McLafferty, 2012, p. 93). In the case of cancer, the second leading cause of death worldwide, tracking characteristics of cases and the morphology of such a heterogenous disease through registries is well-established and essential to surveillance and cancer control programs worldwide (Parkin, 2006; Ferlay et al., 2021). The core purpose of a cancer registry is to estimate the burden of cancer with a focus on risk (incidence) based on the defined regional or national population. In the US, all states mandate cancer reporting (Coates et al., 2015). This state-required reporting, combined with federal collaboration with both the CDC and the National Cancer Institute (NCI), creates a vast and complex network of data sources that ultimately support the infrastructure for two national cancer registries, NCI's Surveillance, Epidemiology, and End Results (SEER) and CDC's National Program for Cancer Registries (NPCR).

In 1973, the SEER program established a coordinated system of cancer registries from the research of two pre-existing cancer surveys, the Third National Cancer Survey (Cutler and Young, 1975) and the End Results Program (Ederer, 1961). At that time, SEER included five states and two large metropolitan cities. Since then, it has collected quality data on patient demographics, tumor locations, morphology, and stage of diagnosis, as well as treatment and follow-up information for approximately 30% of the US population, encompassed by ten states and seven regions (Bray and Parkin, 2009; White et al., 2017). A key strength of SEER is its expandability and linkages with administrative data, including that from the National Death Index, Social Security Administration, Medicare, Medicaid, and state vital records departments. Linking to such complementary databases provides opportunities for researchers to identify disparities (Francoeur et al., 2022; Lawson et al., 2022), costs (Islami et al., 2022; Shih et al., 2022), risk reduction interventions (Hurwitz et al., 2022), and emerging trends (Chang et al., 2022; Shen et al., 2022), among many other findings. In 1992, through Public Law (PL 102–515), the US Congress created the Cancer Registries Act charging the CDC to form and fund the National Program for Cancer Registries (NPCR), incentivizing and standardizing state registries across all 50 states. The NPCR encompasses the remaining states and territories not included in the SEER database and overlapping areas, ultimately covering 96% of the US population. SEER and NPCR work closely with NAACCR (North American Association of Central Cancer Registries). NAACCR is the collaborative umbrella organization for North American cancer registries. It notably develops and promotes uniform data standards for cancer registration; provides education and training; certifies population-based registries; aggregates and publishes data from central cancer registries; and promotes cancer surveillance data from such systems as SEER and NPCR (NAACCR, 2016b). It combines registry information from

Canada for even more power in cancer data aggregation (NAACCR, 2016a).

In South Carolina (SC), for example, the SC General Assembly passed the SC Central Cancer Registry Act in 1996, creating the South Carolina Central Cancer Registry (SCCCR), the state's population-based cancer surveillance system. The SCCCR has consistently achieved Registry of Distinction and Gold Certification status with the NAACCR. The SC Department of Health and Environmental Control (DHEC) collects, processes, analyzes and publishes SC cancer incidence data which annually feed into the networks of the CDC and the NPCR (SCDHEC, 2022). With SCCCR keeping comprehensive and accurate records for surveillance, cancer researchers have identified: risk and prevention factors (Wagner et al., 2011; Tantamango-Bartley et al., 2013; Orlich et al., 2015; Fraser et al., 2020b; Babatunde et al., 2021), the timing of diagnosis-to-treatment and access to care (Virgo et al., 2010; Babatunde et al., 2022), and disparities in prevalence among different racial groups (Adams et al., 2006; Meyer et al., 2007; Adams et al., 2009; Babatunde et al., 2021; Thomas et al., 2021; Adams et al., 2022), geographic groups (Adams et al., 2006; Meyer et al., 2007; Georgantopoulos, 2018; Nicoli et al., 2019; Babatunde et al., 2021; Adams et al., 2022; Babatunde et al., 2022) and religious groups (Fraser et al., 2020a). Using the SCCCR, researchers have found trends suggesting efficient and effective treatment (Yen et al., 2006; Overton et al., 2013; Noxon and Bennett, 2015; Xirasagar et al., 2015) and intervention elements needed to address the person and community coping with cancer (Coker et al., 2006). When the SCCCR data gets aggregated into the national and multinational registries, NPCR and NAACCR, respectively, researchers can define the impacts of the more robust surveillance in a richer context (Ferlay et al., 2021; Zahnd et al., 2021).

3 Learning from cancer registries in the surveillance of Alzheimer's disease and related dementias

Like cancer, Alzheimer's disease and related dementias (ADRD) represent an insidious global health burden that can significantly benefit from the power of accumulating disease registry data. With an aging population, the incidence, treatment, and mortality from ADRD are increasing and changing rapidly (Alzheimer's and Dementia, 2023). In the same way that current cancer registries work toward control and prevention, so do ADRD registries. ADRD registries maintain a comprehensive and accurate registry of ADRD within their state, provide disease prevalence estimates to enable better planning for social and medical services, identify differences in disease prevalence among demographic groups, help those who care for individuals with ADRD, and foster research into risk factors for ADRD. Additionally, ADRD registries offer a unique opportunity to efficiently conduct high-impact, scientifically rigorous research without the burden of primary data collection. With the explosion of electronic health record systems mandated by our federal government, the efficiency of ADRD registries as a research resource through key data linkages could be expanded exponentially and strengthened through the establishment of these registries on a national level.

Given the nearly 50-year history available and the expansive scope across all 50 states, lessons learned from developing, implementing, and maintaining cancer registries can help decrease the time investment needed to develop, implement, and maintain such a system in ADRD. Perhaps even more importantly, identifying the weaknesses of our current cancer registry system is critical to creating a surveillance system that can surpass cancer registries in their utility. These established systems also inform us about mistakes that can be avoided with the hindsight offered by the cancer registry process. These may include the lack of a unified data collection system for all states and the creation of multiple national registries as opposed to one comprehensive registry. Finally, the vision of the future for cancer registry operations enables ADRD systems to strategically plan and begin early implementation of systems and processes that exceed our cancer registry system.

4 Formation and description of existing ADRD registries

Currently, there are three statewide ADRD registries in the US, all of which are geographically located in the southeast. While gaining attention, the registries are currently underutilized. The SC Alzheimer's Disease Registry began in 1988. On 31 May 1990, Governor Carroll A. Campbell, Jr. signed a state law authorizing the Registry. This law (R653, H4924) amended Title 44, Code of Laws of South Carolina 1976, relating to health, by adding Chapter 36, establishing a voluntary Statewide Alzheimer's Disease and Related Dementias Registry located within the School of Public Health at the University of South Carolina. The law has strict confidentiality requirements for data collection using existing sources. Still, it does allow Registry staff to contact the families and physicians of persons diagnosed with ADRD to collect relevant data and provide information about public and private healthcare resources and services available to them. It is maintained by the University of South Carolina's Office for the Study of Aging with the support of the South Carolina Department of Health and Human Services and the Revenue and Fiscal Affairs Office. An annual report (65) with summary data is published in fulfillment of the requirement of the South Carolina Code of Law Section 44 36 10 and Section 44 36 50, which established the registry for the state and tasked the University of South Carolina's Arnold School of Public Health Office for the Study of Aging with managing the registry data. Since 1 January 1988, the Registry has identified 340,921 cases of ADRD in South Carolina. Data from the SC Alzheimer's Disease Registry is pulled from multiple sources, including in-patient hospitalizations, emergency room visits, long-term care evaluations, state health plans, Medicaid, Vital Records, Home Health, Community Mental Health Centers, Mental Health and Rehabilitation Clinics, and Program of All-inclusive Care for the Elderly (PACE).

Based on the model developed in SC, faculty of the West Virginia (WV) University School of Medicine, together with representatives of the WV Chapter of the Alzheimer's Association, the Department of Health and Human Resources, the Blanchette Rockefeller Neurosciences Institute, and the South Carolina Alzheimer's Disease and Related Dementias Registry formulated and proposed legislation that would establish a registry of people in West Virginia with AD and related dementias. This legislation was introduced to the West Virginia Legislature on 11 January 2006, as Senate Bill 112 by Senator Roman Prezioso, Chair of the Senate Health and Human Resources

Committee, and sponsored by all the committee members. SB 112 passed on 11 March 2006, and became law on 11 June 2006, (WV Code §16-5R-7). Following the legislative process, procedural rules governing the type and manner of data collection for the West Virginia Alzheimer's Disease Registry (WVADR) were written and went into effect on 27 December 2007 (CSR64-94). WVADR is an electronic population-based registry that collects demographic, diagnosis, and treatment information about ADRD and serves as an information repository for policy, planning, and research concerning ADRD. It is password-protected, encrypted, and located on servers in a secure facility.

During the 2013 Georgia legislative session, the Georgia General Assembly created the Georgia Alzheimer's and Related Dementias (GARD) State Plan Task Force. The GARD Healthcare Research and Data Collection subcommittee found a paucity of data about ADRD in Georgia, yet no central repository existed for these data. Thus, it created a barrier to estimating accurate ADRD prevalence rates in Georgia to inform planning, research, and reporting efforts. The task force created a State Alzheimer's Disease Plan, including recommendations to collect statewide data to inform the evaluation and care infrastructure. A key recommendation was establishing a statewide ADRD registry to provide accurate, current data to address these urgent needs. During the 2014 Georgia Legislative Session, legislation to establish an ADRD registry within the Georgia Department of Public Health (DPH) (HB 966) was introduced and subsequently passed (OCGA 31-2a-17). The Georgia Department of Public Health (DPH) was identified as a prime coordinator of stakeholders and partners in the registry planning and development effort.

In 2019 the OCGA 31-2a-17 further defined the purpose, procedures, rules and regulations, and data confidentiality of the ADRD registry. Based on Georgia Law, the purpose of the ADRD registry is to assist in the development of public policy and planning, provide a central database of individuals with ADRD, establish procedures and promulgate rules and regulations for establishing and operating the registry. According to the Georgia Law, such procedures, rules, and regulations were intended to provide for 1) collecting and evaluating data regarding the prevalence of ADRD in Georgia, including who reports the data to the registry; 2) determining what information shall be maintained in the registry and the length of time such data shall be available; 3) sharing of data for policy planning purposes; 4) disclosing non-identifying data to support ADRD research; and 5) information about public and private resources. The methodology by which families and physicians of persons who are reported to the registry are contacted to gather additional data is also provided. The law stated that the collected ADRD registry data should be confidential. All persons to whom the data are released should maintain patient confidentiality under the requirements of 42 USC Section 1301, et seq., and PL 104–191, the federal Health Insurance Portability and Accountability Act of 1996.

5 Current and potential impact of ADRD registries on research, workforce, education, and policy

In addition to research on the statewide prevalence of ADRD (Office for the Study of Aging, 2022) and prevalence in special populations (Miller et al., 2023), ADRD registries have been used to investigate potential risk factors for the disease (Miller et al., 2019) and impacts on those who provide care for individuals with ADRD (Porter et al., 2016; Carpenter et al., 2020; Alhasan et al., 2021). With continued advancement in research on ADRD treatment and prevention in the scientific community and mass media, having such ADRD registries for observational studies with such a large resource of patient records will also be extremely important for understanding the real-world impact of emerging interventions.

While the scientific community is becoming increasingly diverse, there is still an underrepresentation of individuals from minority groups pursuing research careers in aging and ADRD despite the increased risk of ADRD within those groups (Johnson et al., 2022). Research centers encourage using ADRD registry data to encourage population-level studies to decrease ADRD-related health disparities. Currently, the Carolina Center on Alzheimer's Disease and Minority Research (CCADMR; P30 AG059294), a National Institute on Aging (NIA)-funded center dedicated to increasing the capacity of underrepresented and minority (URM) scholars, is working to advance the science of ADRD research focused on population health and determinants of ADRD disparities through research education in population-based, secondary data analysis, interdisciplinary co-mentoring teams, well-established strategies for recruitment of AD-RCMAR Scientists, and education on Health Disparities and Minority Aging Research. The SC Alzheimer's Disease Registry is a key data source for CCADMR Scientists (Ingram et al., 2021; Johnson et al., 2022).

Additionally, a 5-year NIA-funded grant (R13 AG074603) offers an annual virtual conference on how to use data from all three statewide registries for studying ADRD disparities. Furthermore, it involves a follow-up High-Impact Alzheimer's Disease Registry Workshop for Scholars of Color—a 1-day workshop for mentored URM scientists interested in developing a research project using SC Alzheimer's Disease Registry data with the support of a mentor. The workshop aims to introduce scholars to available registry data opportunities to brainstorm project ideas, network with other scholars, and connect with a group of senior mentors whom they meet with over the next full year. No prior research experience with registries is required.

ADRD registry data can also inform education, including the nationally registered Dementia Dialogues program for caregivers of persons who exhibit signs and symptoms of ADRD (Byers et al., 2022). The six-module program, which presents data from the SC Alzheimer's Disease Registry, has at its mission to provide the most current and practical evidence-based information about how to care for people living with ADRD. The target audience includes formal and informal caregivers and other community members interested in learning more about ADRD caregiving. Communities across the state, especially those with the highest ADRD rates based on Registry data, are engaged with this evidence-informed education and programming.

Finally, ADRD registries have the potential to inform policy. For example, county-level fact sheets are developed annually for the Alzheimer's Association SC Chapter to present to legislators and community partners, and state agencies to promote awareness of ADRD across urban and rural counties of the state with the goals of improving ADRD education, access to care, and advocate for

TABLE 1 Comparing available data across registries.

	Cancer registry	Alzheimer's disease registry
Demographic Information	yes	yes
Family/Caregiver Demographic and Health information	no	no
Disease Type	yes	yes
Age at Diagnosis	yes	yes
Year of Diagnosis	yes	yes
Geographic Location at Diagnosis	yes	yes¥
Staging of the Disease at Diagnosis	yes	no
Plan of Treatment		
First course of treatment	yes	**
Outcome of treatment	no	no
Medication use	no	**
Imaging data	limited*	no
Biomarkers	some↑-limited*	no
Medical procedures	limited*	**
Genetic testing	some↑-limited*	no
Hospitalization information	no	**
Death Data		
Vital Status	yes	yes
Date of Death	yes	* (year only)
Underlying cause of death	yes¥	yes
Other information from death certificate	no	**
Quality of life measures	no	no
Long-term follow-up data	no	no

↑- Indicates that it is not a required data element, but some registries will independently capture this information.
*limited-indicates that registry will capture some data related to this outcome, but do not collect a comprehensive treatment record.
**limited data is available through linkages to other data sources after receiving proper permissions.
¥—is a required data element, but privacy restrictions can limit its availability for research.

additional funding for patients and caregivers. A similar county-level effort in WV by the WV Alzheimer's Disease Registry and Alzheimer's Association WV Chapter volunteers led to a series of datasets sorted by legislative district and used to raise awareness and generate support for Alzheimer's disease among WV legislators. One tangible result was the passage of legislation (Senate Bill 570) to provide training to police and fire personnel engaging with people with Alzheimer's disease. A second bill (Senate Bill 526) was passed in 2023 to increase ADRD training for healthcare providers.

6 Concluding remarks: Lessons learned for ADRD registries moving forward

ADRD registries are impactful in many areas, including surveillance, research, and policy. There is also enormous potential for enhancement and expansion. As highlighted in Table 1, ADRD registries currently lack consistent and standard reporting of disease staging (e.g., preclinical, early-late), outcomes of treatment, imaging (e.g., PET, fMRI), biomarker ascertainment (e.g., pathogenic proteins, markers of synaptic dysfunction, and markers of inflammation in the blood), genetic testing (e.g., APOE gene), long-term follow-up information (e.g., preclinical/early stage through late stage), quality of life measures, or caregiver/family demographic and health information. Some similar information is captured in cancer registries and the availability of these data has led to groundbreaking discoveries over the past few decades.

As the ADRD registry system is still in the development stage, there are several pitfalls to avoid and lessons learned from the development of cancer registries which, if considered early in the process, have the potential to benefit the ADRD system.

- One of the greatest barriers to cancer research is the lack of a single unified system. The infrastructure allows for research to be conducted on the entire SEER database, however, research using state data from NAACCR registries has to be conducted on a state-by-state basis. Thus, having a truly national cohort of cancer survivors for research is impossible.
- Furthermore, states that are members of the NAACCR system operate autonomously and can opt in or out of research studies that want to utilize their data, even though all cancer registries are funded at the federal level. As ADRD registries develop, it would be wise to keep them unified under a single system.
- Another key point is to establish a way to systematically link ADRD registries to multiple other data sources, including claims data. Similar to how the NAACCR brings interdisciplinary teams together to set cancer definitions, data dictionaries, and data performance measures, bringing experts who are knowledgeable in information technology and

data sharing, perhaps including, but not limited to public health, will be critical to have at the table during all development phases of ADRD registries to ensure that we build a system with the flexibility to evolve over time and stay relevant with health data sources. A key strength of SEER is its expandability and linkages with administrative data. Regarding ADRD registries, once expanded and developed, data linkages such as SEER-Medicare would also add treatment and cost for the Medicare population which would include most patients with ADRD. For non-Medicare patients, SEER specifically has linked cancer registry data with administrative data and pharmacy data to enhance and expand the use of cancer registry data. This type of expansion would enhance future risk reduction and treatment interventions for ADRD.

- Finally, utilizing the fullest extent of existing protocols and processes from the NAACCR data standard will be essential to allow ADRD registries to become operational efficiently while allowing experts and key stakeholders to focus on issues that may be unique to ADRD.

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.

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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Sex differences in pharmacological interventions and their effects on lifespan and healthspan outcomes: a systematic review

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With an increasing aging population, the burden of age-related diseases magnifies. To alleviate this burden, geroprotection has been an area of intense research focus with the development of pharmacological interventions that target lifespan and/or healthspan. However, there are often sex differences, with compounds mostly tested in male animals. Given the importance of considering both sexes in preclinical research, this neglects potential benefits for the female population, as interventions tested in both sexes often show clear sexual dimorphisms in their biological responses. To further understand the prevalence of sex differences in pharmacological geroprotective intervention studies, we performed a systematic review of the literature according to the PRISMA guidelines. Seventy-two studies met our inclusion criteria and were classified into one of five subclasses: FDA-repurposed drugs, novel small molecules, probiotics, traditional Chinese medicine, and antioxidants, vitamins, or other dietary supplements. Interventions were analyzed for their effects on median and maximal lifespan and healthspan markers, including frailty, muscle function and coordination, cognitive function and learning, metabolism, and cancer. With our systematic review, we found that twenty-two out of sixty-four compounds tested were able to prolong both lifespan and healthspan measures. Focusing on the use of female and male mice, and on comparing their outcomes, we found that 40% of studies only used male mice or did not clarify the sex. Notably, of the 36% of pharmacologic interventions that did use both male and female mice, 73% of these studies showed sex-specific outcomes on healthspan and/or lifespan. These data highlight the importance of studying both sexes in the search for geroprotectors, as the biology of aging is not the same in male and female mice.

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KEYWORDS

sex differences, lifespan, healthspan, systematic review, pharmacological interventions, aging, mice

Introduction

The world population is aging. Life expectancy has increased by 30 years over the last century (Olshansky, 2018) and in 2018, people over 65 years of age outnumbered children below 5 years for the first time (Shetty, 2012; United Nations, 2019). This demographic shift is predicted to continue, as the number of people over 65 years old is expected to triple by 2050 (Shetty, 2012). The extension of human lifespan does not always guarantee an extension of healthspan (defined as the period free from disease), as the two are not necessarily linked (Prince et al., 2015; Fischer et al., 2016; Hansen and Kennedy, 2016; Mitchell et al., 2016; Atella et al., 2019; GBD Ageing Collaborators, 2022; GBD Ageing Collborators, 2022; Statzer et al., 2022). This is demonstrated by a global increase in disease burden related to old age which goes hand in hand with the rise of the aging population (Prince et al., 2015; Atella et al., 2019; GBD Ageing Collborators, 2022; GBD Ageing Collborators, 2022). Research has focused on understanding the biological mechanisms of aging in hope of finding ways to extend lifespan and healthspan (Sinclair, 2005; Sierra, 2016; Weir et al., 2017; Olshansky, 2018). For many, extending the years lived in good health with a reduced burden of chronic diseases is a more actionable and perhaps more attractive goal than an extended lifespan (Sierra, 2016; Olshansky, 2018; Mitchell et al., 2019; Aon et al., 2020).

While lifespan was classically considered the gold standard for determining the success of geroprotectors, over the last 10 years, researchers have started to include differential measures of healthspan in their studies. The concept of frailty as a state of overall decline is increasingly utilized to assess the risk of disease and mortality in old age (Kane and Howlett, 2017; Rockwood et al., 2017; Palliyaguru et al., 2019). Tools for assessing frailty in mice (Liu et al., 2014; Whitehead et al., 2014; Hession et al., 2022), which have been reverse-translated from human scales, have become more widely utilized in recent years as markers of healthspan (Sukoff Rizzo et al., 2018; Bellantuono et al., 2020; Palliyaguru et al., 2021a) and have been shown to be modifiable by dietary and pharmacological interventions that increase lifespan (Kane et al., 2016; Palliyaguru et al., 2019). Beyond the mouse frailty index, other important assays to assess health broadly span the domains of muscle function and coordination, cognitive function and memory, metabolic function, and tumor incidence (Ackert-Bicknell et al., 2015; Bellantuono et al., 2020). While there is no established stringent set of measures agreed upon by the entire community to fully define healthspan in mice, a number of important publications have established at least a panel of markers with demonstrated utility in the assessment of healthspan (Richardson et al., 2016; Bellantuono et al., 2020).

Interventions to increase lifespan and healthspan comprise behavioral, dietary, and pharmacological approaches (Longo et al., 2015), and are commonly referred to as geroprotectors (Moskalev et al., 2016). Potential geroprotectors are defined as interventions which may extend lifespan and/or healthspan by targeting one or more of the hallmarks of aging (Moskalev et al., 2016; Janssens and Houtkooper, 2020; López-Otín et al., 2023). Examples of successful geroprotectors include rapamycin and metformin (Martin-Montalvo et al., 2013; Bitto et al., 2016; Glossmann and Lutz, 2019; Selvarani et al., 2020; Moskalev et al., 2022). The development of geroprotectors is based on the "Geroscience hypothesis" (Sierra and Kohanski, 2017), in which aging plays a central role in many, if not all, chronic diseases. Interventions that retard aging should simultaneously delay the onset of many diseases according to this hypothesis. This foundational framework has proposed a roadmap for how geroprotectors should impact aging. A number of recent reviews have eloquently described the role of dietary interventions as potential geroprotectors (Brandhorst and Longo, 2019; Green et al., 2022; Longo and Anderson, 2022; Mitchell and Mitchell, 2022), so they will not be included here.

TABLE 1 Search strategy and eligibility criteria.

Search strategy	Eligibility criteria	
	Exclusion criteria	Inclusion criteria
Healthspan OR (health AND span) OR health span	No measurements of lifespan and/or healthspan	Lifespan and/or healthspan measured
AND longevity OR longevities OR lifespan OR lifespans OR mortality OR survival OR survivability OR survivable OR survivals OR survive OR survived OR survives OR surviving	No pharmacological intervention included	Only pharmacological studies included
AND male OR males OR (male AND female) OR female OR females	Non-wildtype mice	Only wildtype animals
AND English	Different species	In mice and/or rats
NOT review OR review literature as topic	Non-original research	Original research
NOT human OR humans	*In-vitro* and *in silico* studies	No *in-vitro* or *in silico* analysis
AND mice OR rats	Toxicity studies	Male and/or female animals
NOT in vitro NOT cell NOT clinical	Cancer study	Text in English
	No full-text available	Full text available via PubMed
	Presence of concomitant interventions	
	Intervention not in aged mice (<18 months)	

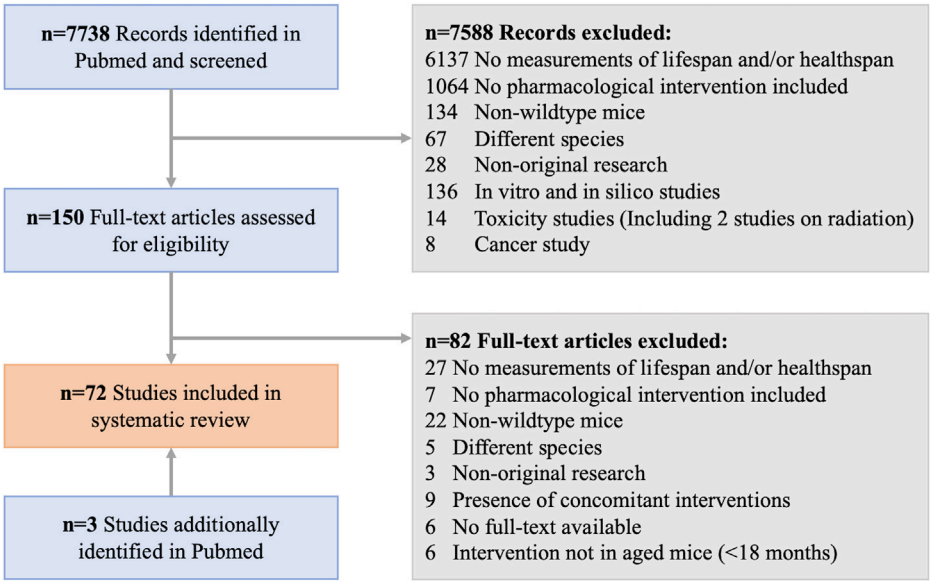

FIGURE 1
Workflow for the systematic review from identified records to all included studies in this manuscript.

It is well-known that there are sexual dimorphisms in the aging process, including in healthspan, muscle mass maintenance and physical performance, sex-hormones, age-related diseases and lifespan (Austad and Fischer, 2016; Le Couteur et al., 2018; Sampathkumar et al., 2019; Decaroli et al., 2021; Bronikowski et al., 2022; Viecelli and Ewald, 2022; Della Peruta et al., 2023). It is noteworthy that men and women have different susceptibility to various age-related diseases, such as women being more likely to develop osteoporosis, and men being more prone to cardiovascular diseases (Crimmins et al., 2019). This is partly influenced by sex-specific alterations in sex hormones with age, including a decrease in estrogen levels during menopause for women and a decline in testosterone with age for men (Guarner-Lans et al., 2011; Horstman et al., 2012; Decaroli et al., 2021). Further, women and females of other species tend to have significantly longer lifespans, but experience higher levels of frailty at a given age when assessed clinically (Le Couteur et al., 2018; Gordon and Hubbard, 2019; Kane and Howlett, 2021). In mice, sex-related differences can be seen in physical performance, which was shown to be lower in aging males (Tran et al., 2021), while anxiety-like behaviors were increased in aging males (Kobayashi et al., 2021). Even on the tissue and molecular level, there are vast sex-specific differences in mice's gene expression signatures associated with longevity (Vitiello et al., 2021). Further, sexual dimorphisms can be observed in geroprotective interventions aiming to increase lifespan or healthspan (Sampathkumar et al., 2019). Both dietary and pharmacological interventions, such as rapamycin and calorie restriction, have been shown to have sexually dimorphic effects when tested in mice (Anisimov et al., 2010; Harrison et al., 2014; Miller et al., 2014; Mitchell et al., 2016; Bielas et al., 2018; Cabo and Mattson, 2019; Sampathkumar et al., 2019; Berry et al., 2020; Henderson et al., 2021). These findings suggest underlying biological differences in the mechanisms of aging between the sexes and highlight the importance of considering sex as a biological variable. Despite a 2016 NIH mandate requiring both sexes to be used in preclinical research (NOT-OD-15-102, 2015: Consideration of Sex as a Biological Variable in NIH-funded Research," 2015), many fields, including the aging field, still face challenges to the inclusion of both sexes in their studies (Plevkova et al., 2020; Shansky and Murphy, 2021; Carmody et al., 2022; Merone et al., 2022). To comprehensively compile the current literature and provide a summary of findings, we performed a systematic review of original research publications from 1970 to 2022 and reviewed what is known about sexual dimorphisms in the lifespan and healthspan outcomes of mice undergoing some form of pharmacological intervention. Our findings are presented herein.

Methods

A systematic review of the literature was conducted according to the PRISMA guidelines (Page et al., 2021) to identify publications reporting on pharmacological interventions in mice and their effects on lifespan and/or healthspan, in a sexually dimorphic manner. PubMed (RRID:SCR_004846) was utilized as the search tool and database to screen the title, abstract, and keywords of all articles (excluding reviews) using the search terms with Boolean operators as outlined in Table 1. The search period was limited to all published within the period of 1970 to 1. January 2022. All identified records were exported to excel, where the authors (M.K. and S.J.M) screened them for the eligibility criteria and removed duplicate records, irrelevant titles/abstracts, as well as non-original research (re-analysis of previously published data, commentaries, etc.). To ensure all relevant research was included, an additional manual review of the literature was performed via PubMed, which produced four further studies. Of the remaining potential records, the full-text

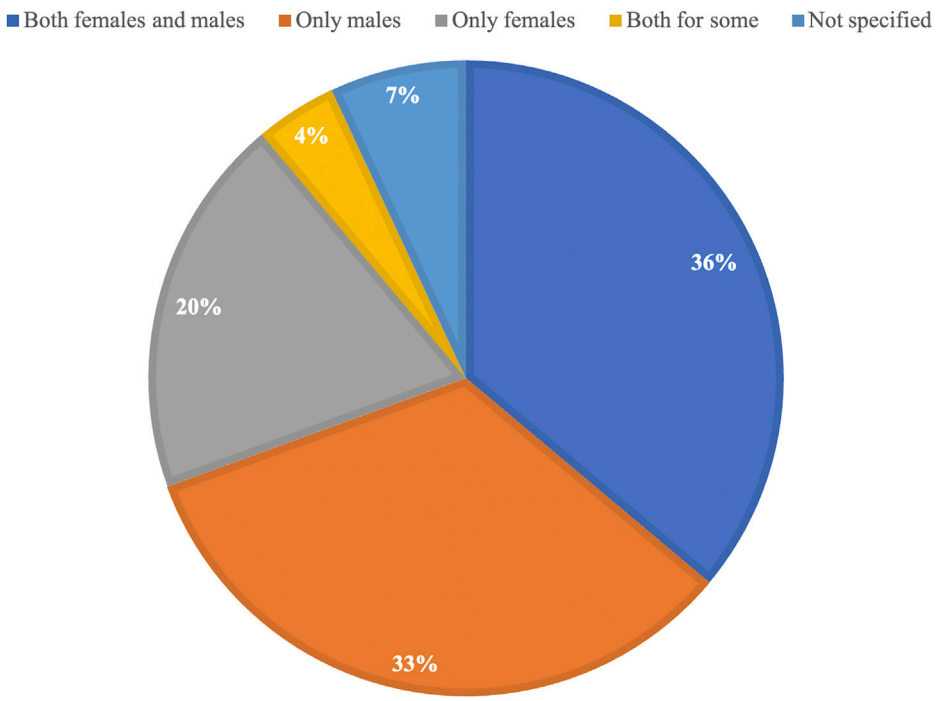

FIGURE 2
Proportional representation of the use of female and male mice in all studies included in this manuscript.

articles were screened against the exclusion criteria (Table 1) and the resulting 72 eligible articles were used as the basis for this systematic review. Figure 1 shows the workflow for the literature search and study selection, including the identification of the final 150 full-text articles, of which 72 were included in the final analysis. From the final articles, we extracted all relevant information for this review. This included details of the study design as well as the study outcomes for lifespan and healthspan parameters. For the study design, the compound used in the intervention, its dose and method of administration, the age at onset of the intervention and its duration, and the mouse strain used were recorded. The lifespan measures were separated into measures of median lifespan and maximal lifespan due to inherent differences in how authors report these findings. Due to the diversity of the healthspan measurements in mice, we defined healthspan parameters according to published recommendations (Richardson et al., 2016; Bellantuono et al., 2020) to include frailty, muscle function and coordination, cognitive function and memory, metabolic function, and cancer incidence. The limitation of these assays is the missing consensus of what measure(s) reliably demonstrate improvements in healthspan. For all outcomes it was reported whether there was a significant improvement (↑) or worsening (↓) of the parameter during the study in the intervention group relative to the control group stratified by sex. We also reported whether there was a sexual dimorphism (defined as opposing directionalities of the effect, i.e., improved in males, worsened in females) in the measured outcome. If the outcome was not reported in the respective study this was denoted with a "n.m." (not measured). For median and maximal lifespan, *p*-values were added if reported by the authors. All the information was then structured according to the drug class of the compound used, covering repurposed FDA drugs, novel small molecules, probiotics, traditional Chinese medicine, and supplements, including vitamins and antioxidants.

Results

The final 72 eligible studies (Figure 1) that were included in this review and published between 1970 and 2022 reveal that 36% (26/72) included both female and male mice in their research, while 33% (24/72) used only male mice and 20% (14/72) used only female mice (Figure 2). Out of the 26 studies including both sexes, a large part showed sex-specific results (19/26). Next to measurements of lifespan, a wide variety of healthspan metrics started to be included in studies from the year 2000 onwards, with a continuous increase in their implementation over time. All but one study conducted since 2020 included some sort of healthspan parameter. Of all compounds tested, 22 out of 64 were able to show positive effects on both lifespan and healthspan measures.

Repurposed FDA drugs

Repurposing FDA drugs for use in age-related diseases has been a popular strategy for identifying new geroprotectors (Table 2). One of the attractive benefits of this strategy is the wealth of pharmacology, safety, and efficacy data already available for these compounds. This means investigators can direct resources to validating the compound in the appropriate model, rather than

TABLE 2 Study details and results for interventions with repurposed FDA drugs.

Study	Compound and dose	Start-age and duration	Strain	Outcome lifespan		Outcome healthspan				
				Median lifespan	Max lifespan	Frailty	Muscle function and coordination	Cognitive function and learning	Metabolism	Cancer
Zhu et al. (2020)	Metformin, 100 mg/kg/day (water)	20 months, until end of life	No info	↓ f ($p < 0.05$)	n.m	n.m	n.m	n.m	n.m	n.m
Hiramoto et al. (2020)	Tranexamic acid, 12 mg/kg 3 times a week (water)	2 months, for 24 months	ICR-CD1	n.m	n.m	n.m	↑ m	↑ m	n.m	n.m
Sciorati et al. (2020)	Etanercept, 1 mg/kg/week (injection)	16 months, for 12 months	C57BL/6	n.m	↑ f ($p = 0.028$)	n.m	↑ f	n.m	n.m	n.m
Miller et al. (2020)	Canagliflozin, 30 mg/kg/day (food)	7 months, until end of life	UM-HET3	↑ 14% m ($p < 0.001$)	↑ 9% m ($p < 0.001$)	n.m	n.m	n.m	↑ f and m	= f and m
				= f	= f					
Strong et al. (2020)	Rapamycin, 42 mg/kg/day (food)	20 months, until end of life	UM-HET3	↑ 11% m ($p < 0.001$)	↑ 9% m ($p = 0.04$)	n.m	n.m	n.m	n.m	n.m
				↑ 15% f ($p < 0.0001$)	↑ 12% f ($p < 0.0001$)					
		20 months, 1-month cycles until end of life		↑ 9% m ($p = 0.002$)	↑ 9% m ($p = 0.001$)					
				↑ 8% f ($p < 0.0001$)	↑ 10% f ($p < 0.001$)					
		20 months, for 3 months		↑ 11% m ($p < 0.024$)	= m ($p = 0.08$)					
				= f ($p = 0.15$)	= f ($p = 0.12$)					
Palliyaguru et al. (2020)	Metformin, 500 mg/kg*bw/day (food) + HFD	14 months, until end of life	C57BL/6 J	= m	= m	n.m	↑ m	n.m	n.m	n.m
	Metformin 500 mg/kg*bw/day + SRT1720 100 mg/kg*bw/day (food) + HFD			↓ m ($p < 0.0001$)	↓ 35% m ($p < 0.0001$)		↑ m			
Harrison et al. (2021)	Candesartan cilexetil, 30 ppm	8 months, until end of life	UM-HET3	= f and m	= f and m	n.m	n.m	n.m	n.m	n.m
Hiramoto et al. (2019)	Tranexamic acid, 12 mg/kg 3 times a week (water)	2 months, until end of life	Hairles mouse (Hos:HR-1)	n.m	↑ m ($p < 0.01$)	n.m	n.m	n.m	n.m	↑ m
Smith et al. (2019)	Acarbose, 1,000 ppm (food)	8 months, until end of life	UM-HET3	↑ 5% f ($p = 0.003$)	n.m	n.m	n.m	n.m	n.m	n.m
				↑ 17% m ($p < 0.001$)						
Bielas et al. (2018)	Rapamycin, 14 ppm (food)	9 months, for 13 months (careful, control under 40% dietary restriction)	UM-HET3	n.m	n.m	n.m	= f and m	n.m	n.m	n.m
	Rapamycin, 42 ppm (food)						= f and m			
Thangthaeng et al. (2017)	Metformin, 219–297 mg/kg/day (water)	22 months, for 3 months	C57BL/6 J	n.m	n.m	n.m. (with met visual acuity decreased)	= m (with met took longer to initiate walking)	= m (with met spatial memory worse)	= m	n.m
Bitto et al. (2016)	Rapamycin, 8 mg/kg/day (intraperitoneal)	20–21 months, for 3 months	C57BL/6JNia	↑ 14% m ($p = ?$)	n.m	n.m	n.m	n.m	n.m	↓ f
				= f						
	Rapamycin, 126 ppm (food)			↑ 14% m ($p = ?$)	n.m	n.m	↑ f and m	n.m	n.m	n.m
				↑ 9% f ($p = ?$)						
Fischer et al. (2015)	Rapamycin, 14 ppm (food)	4 months, until end of life	C57BL/6 J	n.m	n.m	n.m	↑ f (grip strength, ↓ m (rotarod)	n.m	n.m	n.m

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TABLE 2 (*Continued*) Study details and results for interventions with repurposed FDA drugs.

Study	Compound and dose	Start-age and duration	Strain	Outcome lifespan		Outcome healthspan				
				Median lifespan	Max lifespan	Frailty	Muscle function and coordination	Cognitive function and learning	Metabolism	Cancer
							= m and f (activity, stride length)			
Miller et al. (2014)	Rapamycin, 4.7 ppm (food)	9 months, until end of life	UM-HET3	↑ 16% f ($p < 0.0001$) = m (3%, $p = 0.19$)	↑ 5% f ($p < 0.0001$) = m (6%, $p = 0.23$)	n.m	n.m	n.m	n.m	n.m
	Rapamycin, 14 ppm (food)			↑ 21% f ($p < 0.0001$) ↑ 13% m ($p = 0.0015$)	↑ 11% f ($p < 0.0001$) ↑ 8% m ($p = 0.003$)					
	Rapamycin, 42 ppm (food)			↑ 26% f ($p < 0.0001$) ↑ 23% m ($p < 0.0001$)	↑ 11% f ($p < 0.0001$) ↑ 8% m ($p = 0.004$)					
Harrison et al. (2014)	Acarbose, 1,000 ppm (food)	4 months, until end of life	UM-HET3	↑ 5% f ($p = 0.01$)	↑ 9% f ($p = 0.001$)	n.m	↑ f	n.m	= f	n.m
				↑ 22% m ($p < 0.0001$)	↑ 11% m ($p < 0.001$)		= m (activity)		↑ m (insulin reduced, but higher fasting glucose)	
Zhang et al. (2014)	Rapamycin, 14 ppm (food)	19 months, until end of life	C57BL/6Nia	n.m	↑ f ($p = 0.047$)	n.m	↑ f and m (f more active, ↑ gait and rotarod, not grip)	n.m	n.m	↑ f (fewer neoplastic lesions and adenomas, careful interpretation) = m
					= m ($p = 0.275$)					
Flynn et al. (2013)	Rapamycin, 14 ppm (food)	24 months, for 3 months	C57BL/6 J	n.m	n.m	n.m	↑ f (activity)	n.m	= f (after 3 months, initial increase in glucose)	n.m
Neff et al. (2013)	Rapamycin, 14 ppm (food)	4 months, for 12 months	C57BL/6 J Rj	n.m	n.m	n.m	↑ m (exploration OF, no effect grip strength)	↑ m (not in object rec., but in maze and far cond., for all)	n.m	↑ m (less cancer)
		13 months, for 12 months		n.m	n.m	n.m	= m	↑ m	n.m	= m (careful)
		20–22 months, for 12 months		n.m	n.m	n.m	= m	↑ m	n.m	= m (careful)
Martin-Montalvo et al. (2013)	Metformin, 100 ppm (food)	12 months, until end of life	C57BL/6	↑ 5.83% m ($p = 0.02$, mean ls)	n.m	↑ m (cataracts)	↑ m	n.m	↑ m (glucose)	= m
	Metformin, 1,000 ppm (food)			↓ 14.4% m ($p < 0.001$, mean ls)	n.m	n.m	n.m	n.m	n.m	n.m
	Metformin, 100 ppm (food)		B6C3F1	= 4.15% m ($p = 0.064$, mean ls)	n.m	n.m	↑ m	n.m	n.m	= m
Wilkinson et al. (2012)	Rapamycin, 4.7 ppm (food)	9 months, for 13 months	UM-HET3	n.m	n.m	↓ f and m (only cataracts)	= f and m (only spont. activity)	n.m	n.m	↑ f and m (only adrenal, not others)
	Rapamycin, 14 ppm (food)			n.m	n.m	↓ f and m (only cataracts)	= f	n.m	n.m	↑ f and m (only adrenal, not others)
							↑ m (only spont. activity)			
	Rapamycin, 42 ppm (food)			n.m	n.m	↓ f and m (only cataracts)	↑ f	n.m	n.m	↑ f and m (only adrenal, not others)
							= m (only spont. activity)			
Majumder et al. (2012)	Rapamycin, 14 mg/kg food	2 months, for 16 months	C57BL6/129svj	n.m	n.m	n.m	n.m	↑ (?)	n.m	n.m
		15 months, for 3 months		n.m	n.m	n.m	n.m	= (?)	n.m	n.m
Smith et al. (2011)	Sibutramine, 1.25 or 5 or 20 mg/kg/day (food)	1 month, until end of life	CD-1	= f and m	n.m	n.m	n.m	n.m	n.m	n.m

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TABLE 2 (*Continued*) Study details and results for interventions with repurposed FDA drugs.

Study	Compound and dose	Start-age and duration	Strain	Outcome lifespan		Outcome healthspan				
				Median lifespan	Max lifespan	Frailty	Muscle function and coordination	Cognitive function and learning	Metabolism	Cancer
Miller et al. (2011)	Rapamycin, 14 ppm (food)	9 months, until end of life	UM-HET3	↑ 18% f ($p < 0.0001$) ↑ 10% m ($p < 0.0001$)	↑ 13% f ($p < 0.01$)	n.m	↑ m (activity)	n.m	n.m	= f and m
					↑ 16% m ($p < 0.01$)		= f			
	Simvastatin, 12 or 120 ppm (food)	10 months, until end of life		= f andm	= f andm	n.m	= f andm	n.m	n.m	n.m
Anisimov et al. (2010)	Metformin, 100 ppm (water)	3 months, until end of life	129/sv	↑ 7.8% f ($p < 0.05$, median ls)	= f and m	n.m	n.m	n.m	= m (glucose, cholesterol, trigly, insulin)	↑ f (less malignant tumors)
				↑ 4.4% f ($p < 0.05$, mean ls)						= m
				↓ 13.4% m ($p < 0.05$, mean ls)						
Harrison et al. (2009)	Rapamycin, unknown dose	21 months, until end of life	UM-HET3	n.m	↑ 14% f ($p < 0.0001$) ↑ 9% m ($p < 0.0001$)	n.m	n.m	n.m	n.m	= f and m
Strong et al. (2008)	Aspirin, 21 ppm (food)	4 months, until end of life	UM-HET3	= f	= f and m	n.m	n.m	n.m	n.m	n.m
				↑ m ($p = 0.01$)						
Anisimov et al. (2008)	Metformin, 100 ppm (water)	3 months, until end of life	SHR	↑ 37.9% f ($p < 0.01$, mean ls)	↑ 10.3% f ($p = ?$)	n.m	n.m	n.m	= f	= f
				↑ 91.9% f ($p = ?$, median ls)						
Popovich et al. (2003)	Deltaran (Ibuprofen), 2.5 mg (5x per months, injection)	3 months, until end of life	SHR	= f	↑ f ($p < 0.01$, last 10%)	n.m	n.m	n.m	n.m	↑ f
Forbes (1975)	Prednisolone sodium phosphate 15–16 mg/day (water)	8 months, until end of life	DBA/2 J	= f (mean ls)	n.m	n.m	n.m	n.m	n.m	n.m
Cotzias et al. (1974)	L-Dopa	1 month, for 18 months	Swiss albino	↑ m ($p < 0.001$, only measured at 19 m)	n.m	↓ m (corneal opacity)	= m	n.m	n.m	n.m
	5,000 mg/kg*bw/day									

Notes: f, female; m, male; n.m., outcome was not measured (?), the sex was not specified; The arrows denote a significant improvement (↑) or worsening (↓) of the respective outcome in the intervention group relative to the control group, while a (=) denotes no difference to control.

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re-hashing safety data that already exists. Many proposed geroprotectors have been tested in the NIA Interventions Testing Program (ITP) in the United States (Miller et al., 2007). This program solicits investigator-proposed compounds and tests their lifespan potential in genetically heterogeneous UM-HET3 mice. Started in the early 2000s, this program to date has tested more than 60 different compounds.

One of the most promising compounds that came out of the ITP is rapamycin. Rapamycin has potent antitumor and immunosuppressive activity and was originally discovered in soil samples from the Easter Island. In mice, rapamycin has been tested at various doses, as well as at different ages of onset. Most data are consistent with the notion that rapamycin extends lifespan in both males and females, with stronger effects shown in females (Harrison et al., 2009; Miller et al., 2011; Miller et al., 2014; Zhang et al., 2014; Strong et al., 2020). Healthspan data supports the concept that age-related deficits are mitigated with rapamycin treatment (Flynn et al., 2013; Neff et al., 2013; Zhang et al., 2014), although specific tests may or may not show sexually dimorphic results (Table 2). Recent work has demonstrated that rapamycin treatment in the first 45 days of life is sufficient to improve healthspan, reduce frailty and extend median lifespan, at least in males (Shindyapina et al., 2022). Moreover, rapamycin treatment for 3 months during middle age (20–21 months) increased median lifespan by 14% for males and 9% for females (Bitto et al., 2016). This data highlights the importance of considering the age of onset of these therapeutics and that lifelong treatment may not be necessary. Other drugs tested in the ITP include aspirin, canagliflozin, candesartan, metformin, sibutramine, simvastatin, and acarbose (Strong et al., 2008; Miller et al., 2011; Smith et al., 2011; Harrison et al., 2014; Smith et al., 2019; Miller et al., 2020; Harrison et al., 2021). Canagliflozin, a diabetes drug, showed sexually dimorphic effects on lifespan, with an increase in both median and maximal lifespan of 14% and 9%, respectively, only in males (Miller et al., 2020). In a parallel study, canagliflozin was found to retard age-related lesions in males only, suggesting that the lifespan extension in the treated males is likely a reflection of delay in lethal neoplasms (Snyder et al., 2022). Interestingly, another diabetes drug, metformin, did not show lifespan extension in genetically heterogeneous males but did have small but significant effects on median lifespan in C57BL/6 J males, and a trend towards an effect in B6C3F1 male mice (Martin-Montalvo et al., 2013). Metformin at 0.1% improved markers of health in these mice, however, it must be noted that 1% metformin caused significant kidney damage and significantly reduced lifespan by 14% (Martin-Montalvo et al., 2013). Others have also tested metformin and shown that 1% metformin improved median and maximal lifespan in female SHR mice by 91.9% and 10.3%, respectively (Anisimov et al., 2008). When tested in 129/sv mice, the same concentration improved median lifespan in females by 7.8% but reduced it by 13.4% in males. In a recent study in female mice of an unknown strain, 1% metformin reduced their median lifespan (Zhu et al., 2020). Taken together, there are clear sexual dimorphic effects of metformin in different mouse strains on lifespan, with a lack of a clear directionality effect across strains. Several studies were able to show positive healthspan effects of metformin doses ranging from 1% to 5% in C57BL/6 J mice (Martin-Montalvo et al., 2013; Palliyaguru et al., 2020), illustrating the uncoupling of lifespan and healthspan outcomes. A third diabetes drug, acarbose, showed promising effects on lifespan in both female and male genetically heterogenous mice, with larger effects in males (Harrison et al., 2014; Smith et al., 2019). Healthspan was not tested in these studies. Aspirin, a classic anti-inflammatory drug, extended median lifespan in male, but not in female UM-HET3 mice (Strong et al., 2008). No effects on lifespan or healthspan were shown by the drugs Candesartan (Harrison et al., 2021), an antihypertensive drug, Sibutramine (Smith et al., 2011), an appetite suppressant, or Simvastatin (Miller et al., 2011), a statin reducing cholesterol. As these compounds were tested as part of the ITP, healthspan measures were not included in these studies.

Drugs that were tested outside of the ITP include tranexamic acid, Deltaran, Etanercept, L-Dopa, and Prednisolone. Deltaran, Etanercept, and Prednisolone are all anti-inflammatory drugs that were tested in female mice, from which the first two had positive effects on lifespan and healthspan (Popovich et al., 2003; Sciorati et al., 2020). Prednisolone showed no effects on lifespan (Forbes, 1975). L-Dopa, a precursor to the neurotransmitters dopamine, noradrenaline, and adrenaline, showed positive effects on male lifespan but had no impact on healthspan (Cotzias et al., 1974). Tranexamic acid, an antifibrinolytic, positively impacted male lifespan and healthspan parameters (Hiramoto et al., 2020; 2019).

Overall, using repurposed FDA drugs as geroprotectors is a promising strategy. Still, more research is needed to determine the optimal doses, ages of onset, and specific indications for these drugs, as well as the effectiveness in both sexes.

Novel small molecules

Beyond repurposing already approved drugs, a common approach in drug development is developing novel small molecules, which allows for a more target-specific approach. Examples of pathways that novel small molecule may target in the aging field, include oxidative stress, inflammation, AMPK, or senescence (Table 3). These are some of the processes implicated as hallmarks of aging (López-Otín et al., 2023).

Promising results have been shown with a carboxy-fullerene superoxide dismutase (SOD) mimetic and 17-α-estradiol. The SOD mimetic with its antioxidant properties was able to extend female and male lifespans by 11% and improved the mice's cognition and learning (Quick et al., 2008). In the ITP, 17-α-estradiol, a synthetic form of the hormone estradiol with proposed neuroprotective properties, has been found to extend lifespan in male mice in repeated studies but not in females (Harrison et al., 2014; Harrison et al., 2021). Lifespan effects have ranged from a median lifespan increase in male mice of 12% (Harrison et al., 2014) up to 19% (Harrison et al., 2021). While healthspan was not measured in these two studies, independent studies have shown that healthspan benefits are seen in both male rats and mice with 17-a-estradiol (Mann et al., 2020), highlighting the importance of cross-species validation of potential geroprotectors.

Two small molecules, SRT1720 and SRT2104, which were developed as specific sirtuin 1 (SIRT1) activators, have shown benefits in both healthspan and lifespan measures in both a high-fat diet (HFD) background, as well as a standard diet background. Mitchell et al. found that SRT1720 improved several measures of healthspan in male mice as well as mean lifespan, but only a trend

TABLE 3 Study details and results for interventions with novel small molecules.

Study	Compound and dose	Start-age and duration	Strain	Outcome lifespan		Outcome healthspan				
				Median lifespan	Max lifespan	Frailty	Muscle function and coordination	Cognitive function and learning	Metabolism	Cancer
Dorigatti et al. (2021)	Beta-guadinidinopropionic acid, 300 ppm ad libitum (food)	18–19 months, for 17–22 (m)/25–26 (f) weeks	UM-HET3	n.m	n.m	n.m	↑ f and m (only gait, not muscle strength)	n.m	= f and m	n.m
Palliyaguru et al. (2020)	SRT1720, 100 mg/kg*bw/day (food)	14 months, until end of life	C57BL/6 J	↑ m ($p < 0.0001$)	= m	n.m	= m	n.m	n.m	n.m
Harrison et al. (2021)	17-α-estradiol, 14ppm	16 months, until end of life	UM-HET3	↑ 19% m ($p < 0.0001$)	↑ 7% m ($p < 0.004$)	n.m	n.m	n.m	n.m	n.m
		20 months, until end of life		↑ 11% m ($p < 0.007$)	= m ($p = 0.17$)					
	Geranylgeranyl-acetone, 600 ppm	9 months, until end of life		= f and m	= f and m					
	MIF098, 240 ppm	8 months, until end of life		= f and m	= f and m					
Sun et al. (2019)	Dimethylamino-micheliolide, 10 mg/kg/EOD (orally)	12 months, for 15 months	C57BL/6	= m	n.m	n.m	↑ m (only treadmill, not rotarod)	= m	↑ m	n.m
	Dimethylamino-micheliolide, 25 mg/kg/EOD (orally)			= m	n.m	n.m	= m	↑ m	↑ m	n.m
	Dimethylamino-micheliolide, 50 mg/kg/EOD (orally)			= m	n.m	n.m	↑ m (only open field tot distance)	= m	= m	n.m
Krut'ko et al. (2016)	Alpha-fetoprotein, 10 mg/kg*bw/day (intraperitoneal)	18 months, for 2 weeks	BALB/c	n.m	n.m	↑ f (coat condition and hair loss)	↑ f (but statistics not very good)	n.m	n.m	n.m
Harrison et al. (2014)	17-α-estradiol, 4.8 ppm (food)	10 months, until end of life	UM-HET3	= f ($p = 0.8$)	= f ($p = 0.9$)	n.m	n.m	n.m	n.m	n.m
				↑ 12% m ($p = 0.0012$)	= m ($p = 0.13$)					
Mitchell et al. (2014)	SRT1720, 100 mg/kg*bw/day (food)	6 months, until end of life	C57BL/6 J	= m (trend $p = 0.096$)	= m	↑ m (less cataracts)	↑ m (improved rotarod 13 and 18 months)	n.m	↑ m (lower glucose)	= m
				↑ 8.8% m ($p = 0.04$, mean ls)						
Quick et al. (2008)	Carboxy-fullerene SOD mimetic, 10 mg/kg/day (water)	12 months, until end of life	C57BL/6	↑ 11% f and m ($p = 0.004$, mean ls, analyzed together)	↑ f and m	n.m	n.m	↑ f and m	n.m	n.m
Strong et al. (2008)	Nitroflurbiprofen, 200 ppm (food)	4 months, until end of life	UM-HET3	= f and m	= f and m	n.m	n.m	n.m	n.m	n.m

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TABLE 3 (*Continued*) **Study details and results for interventions with novel small molecules.**

Study	Compound and dose	Start-age and duration	Strain	Outcome lifespan		Outcome healthspan				
				Median lifespan	Max lifespan	Frailty	Muscle function and coordination	Cognitive function and learning	Metabolism	Cancer
	4-OH-PBN, 350 ppm (food)	4 months, until end of life		= f and m	= f and m	n.m	n.m	n.m	n.m	n.m

Notes: f, female; m, male; n.m., outcome was not measured (?), the sex was not specified; The arrows denote a significant improvement (↑) or worsening (↓) of the respective outcome in the intervention group relative to the control group, while a (=) denotes no difference to control. EOD, every other day; SOD, superoxide dismutase; 4-OH-PBN, 4-OH-a-phenyl-N-tert-butyl nitrone.

towards increasing median lifespan (Mitchell et al., 2014). More striking effects were seen when HFD-fed mice were treated with SRT1720 (Minor et al., 2011). Although in a second study of SRT1720 on an HFD, bodyweight and rotarod performance were not significantly different from the control group (Palliyaguru et al., 2020). A significant limitation of this work was that these compounds were not tested in female mice, thereby limiting the generalizability of these results.

Age-related changes in the immune system, often referred to as "inflamm-aging" (Franceschi et al., 2018), contribute to the pathogenesis of many age-related diseases. Alpha-fetoprotein, an immunoregulator, improved muscle function and coordination in female mice (Krut'ko et al., 2016). The anti-inflammatory molecules MIF098 (Harrison et al., 2021), dimethylaminomicheliolide (Sun et al., 2019), and nitroflurbiprofen (Strong et al., 2008), as well as the antioxidant 4-OH-PBN (Strong et al., 2008) and the insulin sensitivity promoting geranylgeranyl-acetone (Harrison et al., 2021) showed no effect on lifespan when tested in male and female UM-HET3 mice. Beta-guadinidinopropionic acid, an AMPK activator (Dorigatti et al., 2021), showed improvements in muscle function and coordination independent of sex when measured with gait and rotarod performance, but not with grip strength or exercise tolerance tests, while lifespan was not measured.

Probiotics

We only identified one probiotic, *Akkermansia muciniphila*, that has been tested as a potential geroprotector and fulfilled the criteria to be included in this review. Two studies, displayed in Table 4, found that *Akkermansia muciniphila* had no effect on lifespan, but did show minor improvements in healthspan measures such as frailty, muscle function, and cognitive function in female mice (Cerro et al., 2021; Shin et al., 2021). Further research is needed to fully understand the potential of *A. muciniphila* and other probiotics as potential geroprotectors including determining the optimal dosage and administration for use in humans.

Traditional Chinese medicine

Traditional Chinese medicine (TCM) is another field where researchers have tested compounds for their effects on longevity and healthspan (Zhao and Luo, 2017) (Table 5). TCM has a long history, and many herbs and their components are being studied now in a variety of diseases where they show beneficial effects, including aging (Chen et al., 2019; Bi et al., 2022; Xue et al., 2022). The flavanol Icariin, an ingredient of the herb *Epimedium*, improved the median lifespan in male mice by 8%, accompanied by improved muscle function and coordination. These beneficial effects could be attributed to its purported anti-inflammatory and anti-oxidant properties (Zhang et al., 2015; Bi et al., 2022). A late-onset treatment (22–23 months of age) with Liuwei Dihuang, an anti-oxidant TCM formula comprised of six different herbs, increased maximal lifespan significantly with a

TABLE 4 Study details and results for interventions with probiotics.

Study	Compound and dose	Start-age and duration	Strain	Outcome lifespan		Outcome healthspan				
				Median lifespan	Max lifespan	Frailty	Muscle function and coordination	Cognitive function and learning	Metabolism	Cancer
Shin et al. (2021)	*Akkermansia muciniphila*, 4.9 × 10^8 CFU/ 150 mL/day (orally)	24–25 months	C57BL/ 6 J	= (?)	= (?)	↑ (?)	↑ (?)	↑ (?)	n.m	n.m
Cerro et al. (2021)	*Akkermansia muciniphila*, 2 × 10^8 CFU/ 100 mL/day (orally)	18 months, for 1 month	ICR-CD1	n.m	= f	n.m	= f	↑ f	n.m	n.m

Notes: f, female; m, male; n.m., outcome was not measured (?), the sex was not specified; The arrows denote a significant improvement (↑) or worsening (↓) of the respective outcome in the intervention group relative to the control group, while a (=) denotes no difference to control. CFU, stands for "Colony Forming Unit".

dose of 4 ppm, but showed no significant effect at a higher dose of 7 ppm (Chen et al., 2019). Healthspan parameters, including frailty and cognitive function, were improved by extracts of the medicinal mushroom *Hericium erinaceus*, when given for 2 months to 21–23 months old male mice (Roda et al., 2021). Their healthspan measure was an alternative version of a frailty index, the locomotor frailty index which is determined via average speed and resting time. Their study found a 10% reduction in frailty in the treatment group and no sex-specific effects were observed. In a similar study design, the anti-oxidant acer truncatum seed oil was shown to improve cognitive function in male mice as measured with the Morris Water Maze test (Li et al., 2021). These results are promising and show the potential of medicinal herbs and their bioactive components as geroprotectors. However, further research is needed to understand what exactly is mediating the beneficial effects, especially in formulas such as Liuwei Dihuang, which mixes six different herbs.

Vitamins, supplements, antioxidants, and other compounds

Many vitamins, supplements, and antioxidants have been studied for their ability to improve healthspan and/or lifespan, as shown in Table 6. Interestingly, the strains of mice used in these studies vary beyond just the standard C57BL/6 mice commonly used in biomedical and aging studies (Palliyaguru et al., 2021b). In the case of SkQ1, for example, the authors used three different strains of mice (Anisimov et al., 2011) which ensures any effects observed are tested across different genetic backgrounds. A number of these compounds, which were tested in UM-HET3 mice as part of the Interventions Testing Program, did not have any significant effects on lifespan outcomes in either sex (Miller and Chrisp, 1999; Strong et al., 2013), with the exceptions of methylene blue and nordihydroguaiaretic acid (NHGA) showing sexually dimorphic lifespan effects (Strong et al., 2008; Harrison et al., 2014). Methylene blue improved lifespan only in female mice (Harrison et al., 2014), while NHGA improved only male lifespan (Strong et al., 2008; Harrison et al., 2014). Healthspan metrics were not measured in these animals. Additionally, glycine, which was also tested as part of the Interventions Testing Program, increased median lifespan by 4% in females and by 6% in males and significantly reduced the risk for lung adenocarcinomas (Miller et al., 2019).

Of the studies that used standard C57BL/6 mice, several found improvements in lifespan as well as healthspan metrics. Alpha-ketoglutarate was tested in both female and male mice and showed positive effects on lifespan and healthspan in a sex-independent manner, although stronger lifespan effects were observed in female mice (Shahmirzadi et al., 2020). Healthspan showed similar effects in both sexes, with particularly good improvements in female fur color. A sex-independent increase in median and maximal lifespan was also achieved with D-glucosamine, with a treatment onset at 25 months, and additional improvements in glucose metabolism were observed (Weimer et al., 2014). Treatment with procyanidin C1 (PCC1) from grape seeds (Xu et al., 2021), as well as treatment with sodium rutin, a flavonoid (Li et al., 2022), in male mice increased lifespan and several measures of healthspan, such as frailty, muscle function, and cognitive function. Multiple other studies measuring solely lifespan in C57BL/6 mice showed improvements with compounds including antioxidants and polyphenol mixtures, however, most were tested in male mice only (Bezlepkin et al., 1996; Saito et al., 1998; Kitani et al., 2007). Future studies with these compounds should involve healthspan outcomes as well as validating findings in female mice.

Declining NAD levels with age are thought to be one contributor to age-related degeneration (Gomes et al., 2013; McReynolds et al., 2020), with supplementation of NAD precursors evaluated as therapeutic avenues. In C57BL/6 mice, 400 ppm of nicotinamide riboside increased the maximal lifespan as well as muscle function and coordination in mice when given late in life (22–24 months) (Zhang et al., 2016), however, sex of the mice in this study was not specified. A higher dose of

TABLE 5 Study details and results for interventions with traditional Chinese medicine.

Study	Compound and dose	Start-age and duration	Strain	Outcome lifespan		Outcome healthspan				
				Median lifespan	Max lifespan	Frailty	Muscle function and coordination	Cognitive function and learning	Metabolism	Cancer
Roda et al. (2021)	*Hericium erinaceus*, 1 mg/day (orally)	21–23 months, for 2 months	C57BL/6 J	n.m	n.m	↑ m	n.m	↑ m	n.m	n.m
Li et al. (2021)	Acer truncatum seed oil, 0.01 mL/g/day (orally)	20 months, for 1 month	C57BL/6	n.m	n.m	n.m	n.m	↑ m	n.m	n.m
Chen et al. (2019)	Liuwei Dihuangh, 0.432 g/kg/day (water)	22–23 months, until end of life	C57BL/6 J	n.m	↑ (?) (p = 0.048)	n.m	n.m	n.m	n.m	n.m
	Liuwei Dihuangh, 0.72 g/kg/day (water)			n.m	= (?) (p = 0.078)					
S.-Q. Zhang et al., 2015)	Icariin, diet with 0.02%	12 months, until end of life	C57BL/6	↑ 8% m (p = 0.03)	= m	n.m	n.m	n.m	n.m	n.m
		12 months, for 12 months		n.m	n.m	n.m	↑ m	n.m	n.m	n.m

Notes: f, female; m, male; n.m., outcome was not measured (?), the sex was not specified; The arrows denote a significant improvesment (↑) or worsening (↓) of the respective outcome in the intervention group relative to the control group, while a (=) denotes no difference to control.

1,000 ppm of nicotinamide riboside started early in life (8 months) did not improve lifespan parameters in female and male UM-HET3 mice (Harrison et al., 2021). Supplementation with nicotinamide started at 12 months improved glucose metabolism but was also not able to improve lifespan parameters in male C57BL/6 mice (Mitchell et al., 2018).

The already mentioned SkQ1, which was tested in different genetic backgrounds, showed sexually dimorphic effects in C57BL/6 mice as well as BALB/c mice, where it was only able to extend male, but not female lifespan (Anisimov et al., 2011). Of the compounds in this category, magnesium thiazolidine carboxylate was the only compound that extended female median lifespan, even though p-values are missing in this study (Miquel and Economos, 1979). Studies testing spermidine, trehalose, polyphenol-rich grape skin extract, and resveratrol found improvements in several healthspan metrics in male C57BL/6 mice (Pearson et al., 2008; Asseburg et al., 2016; Berry et al., 2020; Wirth et al., 2021), however, they either observed no effects on lifespan (Pearson et al., 2008; Asseburg et al., 2016; Wirth et al., 2021), or lifespan was not measured (Berry et al., 2020). Of note, a number of these studies included males only. No effects on lifespan or healthspan could be shown with C_{60} in olive oil in either female or male C57BL/6 mice (Grohn et al., 2021). Interestingly, b-aminopropionitrile was shown to reduce female lifespan (Davies and Schofield, 1980), despite an earlier study reporting it increased lifespan in male LAF/J mice (LaBella and Vivian, 1978). This highlights the importance of using both sexes and a variety of mouse strains.

Studies on vitamin E discovered an interesting sexual dimorphism, as it only had an effect on male, but not female lifespan (Morley and Trainor, 2001; Navarro et al., 2005). Vilon and Epithalon, both synthetic peptides, were shown to significantly improve female lifespan as well as multiple metrics of healthspan (Khavinson and Anisimov, 2000; Khavinson et al., 2000). Anisimov et al. also observed an interesting effect of melatonin on uncoupling lifespan and healthspan. At a low dose (2 mg/L), tumor incidence was reduced, but lifespan was unaffected; meanwhile at a higher dose (20 mg/L), lifespan was increased, but tumor incidence was unaffected (Anisimov et al., 2003). Dose-dependent effects were also observed by Soda and colleagues (Soda et al., 2009) when testing a combination of polyamines (spermidine and spermine), with the highest dose improving lifespan when compared to lower doses, which were solely tested in male mice. Further, 2-mercapto-ethanol was shown to improve lifespan and healthspan, but it was only tested in male mice (Heidrick et al., 1984). Ethoxyquin, a quinoline-based antioxidant, was shown to improve lifespan and healthspan in both males and females (Comfort et al., 1971). However, interventions with ubiquinone (Lönnrot et al., 1998; Lee et al., 2004) and alpha-lipoic acid in male mice did not show any effects on lifespan (Lee et al., 2004). While some of the discussed compounds have shown promising results in extending lifespan and improving healthspan in mice, most studies have only been conducted on one sex, leaving questions about their potential benefit on the opposite sex.

TABLE 6 Study details and results for interventions with vitamins, supplements, antioxidants, and other compounds.

Study	Compound and dose	Start-age and duration	Strain	Outcome lifespan		Outcome healthspan				
				Median lifespan	Max lifespan	Frailty	Muscle function and coordination	Cognitive function and learning	Metabolism	Cancer
Xu et al. (2021)	PCC1, 20 mg/kg biweekly (orally)	20 months, for 4 months	C57BL/6 J	n.m	n.m	n.m	↑ m	n.m	n.m	n.m
		24–27 months, until end of life		↑ 64.2% m ($p < 0.0001$)	↑ 9.4% m ($p < 0.0001$)	n.m	= m	n.m	n.m	= m
Wirth et al. (2021)	Spermidine, 3 mM ad libitum (water)	17 months, for 6 months	C57BL/6 J	n.m	n.m	↑ m (only hair loss)	n.m	n.m	= m	n.m
			Rj							
Grohn et al. (2021)	C_{60} in olive oil, 1.7 mg/kg (injection), for 1 week daily, then for 1 month weekly, then for 7 months biweekly	25–27 months, for 7 months	CB6F1	= f	= f	n.m	n.m	n.m	n.m	n.m
	C_{60} in extra virgin olive oil, 4 mg/kg*bw/day (orally), for 1 week daily, then for 1 month weekly, then for 7 months biweekly	23 months, for 8 months	C57BL/6	= f and m	= f and m	n.m	= f and m	n.m	n.m	n.m
Li et al. (2022)	Sodium rutin, 0.2 mg/mL *ad libitum* (water)	8 months, until end of life	C57BL/6	↑ m ($p < 0.01$)	= m (trend, 3 months longer)	↑ m (kyphosis, cataract, hair loss)	↑ m	↑ m	n.m	n.m
Harrison et al. (2021)	Nicotinamide riboside, 1,000 ppm	8 months, until end of life	UM-HET3	= f and m	= f and m	n.m	n.m	n.m	n.m	n.m
Shahmirzadi et al. (2020)	Alpha-ketoglutarate, 2% w/w (food)	18 months, until end of life	C57BL/6 J	↑ 10.5%/16.6% f	↑ 19.7%/8% f	↑ f and m	↑ f and m (gait and activity, not treadmill)	n.m	n.m	n.m
				↑ 9.6%/12.8% m (cohort 1/2)	= m (cohort 1/2)					
Berry et al. (2020)	Trehalose, 0.1 mg/day (water)	25 months, for 1 month	C57BL/6N	n.m	n.m	n.m	= /↑ m (only coordination, not strength)	n.m	n.m	n.m
Miller et al. (2019)	Glycine, 8% in food	9 months, until end of life	UM-HET3	↑ 4% f ($p = 0.006$)	= f	n.m	n.m	n.m	n.m	↑ f and m
				↑ 6% m ($p = 0.002$)	↑ 6% m ($p = 0.0005$)					
Mitchell et al. (2018)	Nicotinamide, 37.5 mg/g*bw/day (food)	12 months, until end of life	C57BL/6 J	= m	= m	n.m	= m	= m	↑ m (only glucose)	n.m
				= m	= m	n.m	= m	= m	= m	n.m

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TABLE 6 (*Continued*) Study details and results for interventions with vitamins, supplements, antioxidants, and other compounds.

Study	Compound and dose	Start-age and duration	Strain	Outcome lifespan		Outcome healthspan				
				Median lifespan	Max lifespan	Frailty	Muscle function and coordination	Cognitive function and learning	Metabolism	Cancer
	Nicotinamide, 75 mg/g*bw/day (food)									
Zhang et al. (2016)	Nicotinamide riboside, 400 mg/kg/day (food)	24 months, until end of life	C57BL/6JRj	n.m	↑ (?) (p = 0.034)	n.m	n.m	n.m	n.m	n.m
		22–24 months, for 6 weeks	C57BL/6 J	n.m	n.m	n.m	↑ (?)	n.m	n.m	n.m
Asseburg et al. (2016)	Polyphenol-rich grape skin extract, 200 mg/kg*bw/day (ST: orally, LT: food, LS: water)	22–24 months, for 6 weeks	C57BL/6 J	n.m	n.m	n.m	↑ (?)	n.m	n.m	n.m
		13 months, for 6 months (LT)		n.m	n.m	n.m	= m	n.m	n.m	n.m
		6 months, until end of life (LS)		= m	= m	n.m	↑ m (only locomotor activity)	n.m	n.m	n.m
Weimer et al. (2014)	D-Glucosamine	25 months, until end of life	C57BL/6NRj	n.m	↑ m and f (p = 0.0143)	n.m	n.m	n.m	↑ m and f	n.m
Harrison et al. (2014)	Nordi-hydroguaiaretic acid, 800 ppm (food)	6 months, until end of life	UM-HET3	↑ m (p = 0.04)	n.m	n.m	n.m	n.m	n.m	n.m
	Nordi-hydroguaiaretic acid, 2500 ppm (food)			↑ m (p = 0.0053)	n.m	n.m	n.m	n.m	n.m	n.m
	Nordi-hydroguaiaretic acid, 5,000 ppm (food)			= f	n.m	n.m	n.m	n.m	n.m	n.m
				↑ m (p = 0.0048)						
	Methylene blue, 28 ppm (food)	4 months, until end of life		= f (p = 0.17)	↑ f (p = 0.004)	n.m	n.m	n.m	n.m	n.m
				= m (p = 0.27)	= m (p = 0.6)					
Strong et al. (2013)	Resveratrol, 300 ppm (food)/50 mg/kg*bw/day	4 months, until end of life	UM-HET3	= f and m	= f and m	n.m	n.m	n.m	n.m	n.m
	Green tea extract, 2000 ppm (food)/333 mg/kg*bw/day			= f and m	= f and m	= f and m	n.m	n.m	n.m	n.m
	Curcumin, 2000 ppm (food)/333 mg/kg*bw/day			= f and m	= f and m	n.m	n.m	n.m	n.m	n.m
	Oxaloacetic acid, 2200 ppm (food)/367 mg/kg*bw/day			= f and m	= f and m	n.m	n.m	n.m	n.m	n.m

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TABLE 6 (*Continued*) Study details and results for interventions with vitamins, supplements, antioxidants, and other compounds.

Study	Compound and dose	Start-age and duration	Strain	Outcome lifespan		Outcome healthspan				
				Median lifespan	Max lifespan	Frailty	Muscle function and coordination	Cognitive function and learning	Metabolism	Cancer
	Medium-chain triglyceride oil, 60′000 ppm (food)/ 10′000 mg/kg*bw/day			= f and m	= f and m	n.m	n.m	n.m	n.m	n.m
R. A. Miller et al., 2011)	Resveratrol, 300 or 1,200 ppm (food)	12 months, until end of life	UM-HET3	= f andm	= f andm	n.m	= f andm	n.m	n.m	n.m
Anisimov et al. (2011)	SkQ1, 5 or 250 nmol/kg/day water?	lifelong	129/sv	= f	n.m	n.m	n.m	n.m	n.m	n.m
	SkQ1, 1 or 30 nmol/kg/day water? (analyzed together)		BALB/c	= f	n.m	n.m	n.m	n.m	n.m	n.m
				↑ m ($p < 0.05$)						
	SkQ1, unknown dose		C57BL/6	= f	n.m	n.m	n.m	n.m	n.m	n.m
				↑ m ($p < 0.05$)						
Soda et al. (2009)	Polyamine high (Spermidine 1,540 nmol/g, Spermine 374 nmol/g)	3 months, for 19 months	Jc1:ICR	↑ m ($p = 0.011$, compared to normal and low)	n.m	n.m	n.m	n.m	n.m	n.m
	Polyamine normal (Spermidine 434 nmol/g, Spermine 160 nmol/g)			= m ($p = 0.432$, normal vs low)	n.m	n.m	n.m	n.m	n.m	n.m
	Polyamine low (Spermidine 224 nmol/g, Spermine 143 nmol/g)			= m ($p = 0.432$, normal vs low)	n.m	n.m	n.m	n.m	n.m	n.m
Strong et al. (2008)	Nordihydro-guaiaretic acid, 2500 ppm (food)	9 months, until end of life	UM-HET3	= f	= f and m	n.m	n.m	n.m	n.m	n.m
				↑ m ($p = 0.0006$)						
Pearson et al. (2008)	Resveratrol, 100 ppm (food)	12 months, until end of life	C57BL/6NIA	= m	= m	= m	= m	n.m	n.m	n.m
	Resveratrol, 400 ppm (food)			= m	= m	↑ m (less cataracts)	↑ m (improved rotarod)	n.m	n.m	n.m
	Resveratrol, 2400 ppm (food)			= m	= m	n.m	n.m	n.m	↑ m (lower cholesterol)	n.m
Kitani et al. (2007)	Tetrahydro-curcumin, 0.2% (food)	13 months, until end of life	C57BL/6JHsd	↑ m ($p < 0.01$)	↑ m ($p < 0.01$)	n.m	n.m	n.m	n.m	n.m
		19 months, until end of life		= m	= m					

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TABLE 6 (*Continued*) Study details and results for interventions with vitamins, supplements, antioxidants, and other compounds.

Study	Compound and dose	Start-age and duration	Strain	Outcome lifespan		Outcome healthspan				
				Median lifespan	Max lifespan	Frailty	Muscle function and coordination	Cognitive function and learning	Metabolism	Cancer
	Green tea polyphenols, 80 mg/L (water)	13 months, until end of life		↑ m ($p < 0.05$)	= m					
Navarro et al. (2005)	Vitamin E (dl-RRR-α-tocopherol, 5 g/kg (food)	7 months, until end of life	CD-1	= f	= f	n.m	↑ m	↑ m	n.m	n.m
				↑ m ($p < 0.0001$)	↑ m ($p < 0.0001$)					
Lee et al. (2004)	α-lipoic acid, 600 ppm (food)	14 months, until end of life	$B6C3F_1$	= m	= m	n.m	n.m	n.m	n.m	= m
	Coenzyme Q_{10}, 100 ppm (food)			= m	= m	n.m	n.m	n.m	n.m	= m
Anisimov et al. (2003)	Melatonin, 2 mg/L (5x per months, water)	3 months, until end of life	SHR	= f	= f	n.m	n.m	n.m	n.m	↑ f
	Melatonin, 20 mg/L			= f	↑ f ($p < 0.05$, last 10%)	n.m	n.m	n.m	n.m	= f
Morley and Trainor (2001)	Vitamin E, 20, 40 and 400 mg/kg (food)	Conception, until end of life	Balb/c	= f	= f	n.m	n.m	n.m	n.m	n.m
Khavinson et al. (2000)	Vilon (Lys-Glu), 0.1 mg (5x per months, injection)	6 months, until end of life	CBA	= f	↑ f ($p < 0.05$, last 10%)	n.m	↑ f	n.m	n.m	↑ f
Khavinson and Anisimov (2000)	Vilon (Lys-Glu), 0.1 mg (5x per months, injection)	6 months, until end of life	CBA	= f	↑ f ($p < 0.05$, last 10%)	n.m	↑ f	n.m	n.m	↑ f
	Epithalon (Ala-Glu-Asp-Gly), 0.1 mg (5x per months, injection)			↑ f ($p < 0.05$)	= f	n.m	↓ f	n.m	n.m	↑ f
Miller and Chrisp (1999)	DHEA sulfate, 100 mg/mL (water)	Birth, until end of life	UM-HET3	= f and m	= f and m	n.m	n.m	n.m	n.m	= f and m
Saito et al. (1998)	N-tert-butyl-a-phenylnitrone, 0.25 mg/mL (water)	24.5 months, until end of life	C57BL/6 J	↑ m ($p < 0.005$, mean ls)	n.m	n.m	n.m	n.m	n.m	n.m
Lönnrot et al. (1998)	Ubiquinone Q10, 10 mg/kg/day	2 months, until end of life	C57/B17	= m	= m	n.m	n.m	n.m	n.m	n.m
Bezlepkin et al. (1996)	Antioxidant mixture: 7.5 mg beta carotene, 15 mg α-tocopherol, 50 mg ascorbic acid, 25 mg rutin, 25 μg	2 months, until end of life	C57BL/6	↑ m ($p < 0.05$, mean ls)	↑ m ($p < 0.05$, last 10%)	n.m	n.m	n.m	n.m	n.m
		9 months, until end of life		↑ m ($p < 0.05$, mean ls)	↑ m ($p < 0.05$)					

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TABLE 6 (*Continued*) Study details and results for interventions with vitamins, supplements, antioxidants, and other compounds.

Study	Compound and dose	Start-age and duration	Strain	Outcome lifespan		Outcome healthspan				
				Median lifespan	Max lifespan	Frailty	Muscle function and coordination	Cognitive function and learning	Metabolism	Cancer
	selenium, 5 mg zinc per kg*bw	16 months, until end of life		= m	= m					
		23 months, until end of life		= m	= m					
Heidrick et al. (1984)	2-mercaptoethanol, 0.25% of food	4 months, until end of life	$BC3F_1$	↑ m 13.2% ($p < 0.005$, mean ls)	↑ m ($p < 0.001$, last 10%)	n.m	n.m	n.m	n.m	↑ m
Davies and Schofield (1980)	β-aminopropio-nitrile, 0.5–2 mg/mL water	3–4 months, until end of life	C57BL/Icrfa	↓ f	↓ f	n.m	n.m	n.m	n.m	n.m
	β-aminopropio-nitrile, 1 mg/mL water	9 months, until end of life		= f	= f					
Miquel and Economos (1979)	Magnesium thiazolidine carboxylate, 0.07% of food	23 months, until end of life	C57BL/6	↑ f 7% (no *p*-value)	n.m	n.m	n.m	n.m	n.m	n.m
LaBella and Vivian (1978)	β-aminopropio-nitrile, 1 or 3 mg/mL water	2 months, for 6/12/18 months	LAF/J	↑ m ($p < 0.05$, mean ls)	= m	n.m	n.m	n.m	n.m	n.m
	β-aminopropio-nitrile, 3 mg/mL water	2 months, for 6 months		= m	= m					
Comfort et al. (1971)	Ethoxyquin, 0.5% of food	3 months, until end of life	C3H	↑ f and m ($p < 0.005$, not specified)	n.m	n.m	↑ f and m	n.m	n.m	= f and m

Notes: f, female; m, male; n.m., outcome was not measured (?), the sex was not specified; The arrows denote a significant improvement (↑) or worsening (↓) of the respective outcome in the intervention group relative to the control group, while a (=) denotes no difference to control. PCC1, procyanidin C1; DHEA, dehydroepiandrosterone.

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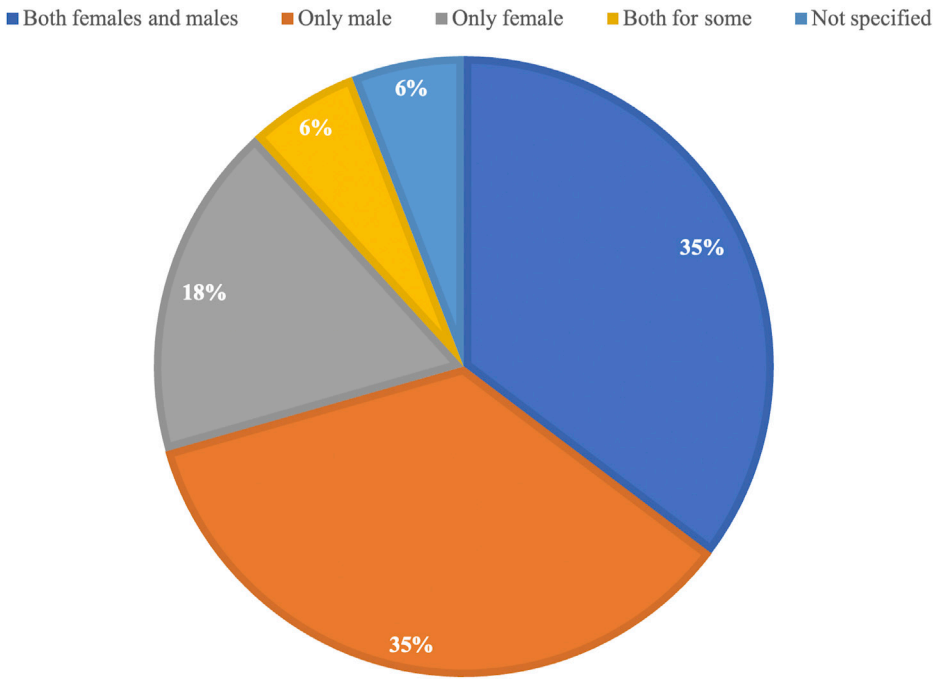

FIGURE 3
Proportional representation of the use of female and male mice in all studies included in this manuscript since 2020.

Adherence to the SABV mandate for preclinical studies

Since 2020, the NIH mandate has been in place, which requires authors to include both males and females in NIH-funded preclinical research studies. Of the 17 studies published since 2020 included in this systematic review, only six used both female and male mice and compared their outcomes (35%) (Figure 3). This is the same number of studies that used only male mice for their research (35%). When looking at the proportion of studies that used both sexes in all included studies, which is 36% (26/72) (Figure 2), nothing has changed despite the mandate being in place. This highlights the need for journals to further encourage or require compliance with the mandate and to promote the integration of sex as a biological variable in preclinical research studies. This could lead to a better understanding of the potential sex-specific differences in the outcomes of these studies and lead to improved treatments for all patients, regardless of their biological sex. It is also important for researchers to be aware of the potential impact of sex on the outcomes of their studies and to design studies that accurately represent the populations they aim to serve.

Discussion

The goal of this manuscript was to systematically review the available literature on sexual dimorphism in the use of pharmacological compounds as potential geroprotectors. We focused on lifespan and healthspan outcomes using mice as a model organism. Of the more than 7000 potentially eligible studies identified through our search, only 72 original research publications met the stringent inclusion and exclusion criteria.

Our results showed that of the 72 included studies, 40% (29/72) of studies only used male mice or did not clarify the sex, 20% (14/72) of studies used only female mice, and only 36% (26/72) of studies used both sexes for all their measurements (Figure 2). Additionally, of all studies using both sexes, 73% (19/26) showed sex-specific outcomes. These data highlight the importance of considering sex as a biological variable (SABV) when testing novel geroprotector interventions. The failure to do so prevents a clear understanding of the sex-specific effects of the tested compounds, particularly as our systematic review found that 73% of studies showed sex differences in the effects of the tested compound on the health or lifespan outcome. It is tempting to speculate how many geroprotectors that have "failed" preclinical testing may have been successful if they were tested in females.

In 2016, the National Institutes of Health mandate came into force, requiring the use of both males and females in NIH-funded research, unless there was a strong scientific justification. This mandate resulted from the workshop on sex as a biological variable. Since then, a number of authors (Garcia-Sifuentes and Maney, 2021; Shansky and Murphy, 2021; Carmody et al., 2022) have looked at adherence to these policies across different scientific disciplines, with a general consensus that adherence should be improved. In addition to inclusion, it is important that authors also provide statistical evidence supporting the difference. A recent report examining sex differences across nine biological disciplines (in 147 articles) found incorrect use

of statistics by authors to support their claims, which they suggest may lead to over-reporting or masking of sex-specific differences (Garcia-Sifuentes and Maney, 2021). These examples argue for continuing discussion on the importance of SABV and ongoing efforts to train biomedical researchers in how to test for and report sex differences correctly in their studies. It may be of importance for leading SABV journals to put together a white paper detailing the best practice for incorporating SABV in biomedical research, including how to appropriately use statistical tests to report effects, much like the PRISMA guidelines for systematic reviews. It is, however, encouraging to see journals such as the American Journal of Physiology-Heart and Circulatory Physiology requiring the inclusion of sex as a biological variable in the reporting of published articles (Denfeld et al., 2022) in their journal. Other journals, such as the Journals of Gerontology and Arteriosclerosis, Thrombosis, and Vascular Biology, have published statements recommending this to their authors (Le Couteur et al., 2018; Robinet et al., 2018). In the studies included here and published since 2020, there is no change observable regarding the use of both sexes when compared to all studies that were included.

Two more recent studies and therefore not yet included in this review have implemented the use of both sexes and found improvements in lifespan as well as healthspan. In the first study, the NADase CD38 inhibitor 78c increased median lifespan by 17% in males, but not in females, and improved exercise performance, endurance, and metabolic function in males (Peclat et al., 2022). In the second study, the PI3K p110α inhibitor, which targets the insulin receptor/insulin-like growth factor receptor pathway, extended median and maximal lifespan of both male and female mice and improved muscle function, with more significant effects in females (Hedges et al., 2023). These results further emphasize the importance of considering biological sex in preclinical research.

While including both sexes in preclinical research is critical, it is equally important to consider the genetic diversity of the mouse strains used in these studies. Testing interventions in heterogenous mouse strains provides a more accurate representation of how treatments may perform in a diverse human population, improving our ability to develop safe and effective treatments. Studies comparing genetically diverse inbred mouse strains have found significant differences in lifespan parameters (Yuan et al., 2009; Yuan et al., 2020), highlighting the importance of using multiple mouse strains when researching a potential geroprotector. While the studies included in this review exhibit some level of genetic diversity, there is room for improvement in terms of testing a specific compound on several genetic backgrounds to ensure greater generalizability.

In addition to the healthspan parameters focused on in this review, there are further health assessments that can be useful in intervention studies in aging mice. These include blood chemistry analysis, which provides information on glucose homeostasis, lipid metabolism, liver and kidney function, and inflammatory markers (O'Connell et al., 2015; Palliyaguru et al., 2021a; Zhang et al., 2022). Live animal imaging techniques, such as magnetic resonance imaging (MRI) (Chen et al., 2011) and positron emission tomography (PET) (Borrás et al., 2011; Hulsmans et al., 2018), can allow for the non-invasive visualization of organs and tissue and can therefore provide insights into structural and functional changes occurring with an intervention. Analysis of metabolomics (Adav and Wang, 2021; Tian et al., 2022), proteomics and transcriptomics (Takemon et al., 2021) can be used to identify changes in metabolic pathways, protein expression and gene expression in response to an intervention. Finally, tissue histology can assess changes on a tissue and cellular level (Pettan-Brewer and Treuting, 2011). Generally, it is important to use a wide variety of health assessment tools to get a more comprehensive understanding of the efficacy of geroprotective interventions.

Limitations of the systematic review

There are a number of limitations to consider when interpreting the findings of this systematic review. One limitation is that only one database (Pubmed) was used, which means that there may be a selection bias, as the studies included in the review may not be representative of the overall population of geroprotector studies. Additionally, the studies included in the review used a variety of outcomes and statistical methods, making it harder to compare the results across studies. Some studies also had missing information, which can impact the ability to accurately interpret the results. Furthermore, the quality of the studies included in the review may vary, with some studies having more robust designs, higher statistical power, and more reliable results compared to others. Overall, these limitations should be considered when interpreting the results of the review and planning future research on geroprotectors and their effects on healthspan and lifespan.

Conclusion

Pharmacological interventions represent an attractive therapeutic avenue for modulating age-related diseases and frailty, especially in those individuals for whom dietary interventions are not feasible. The results from our systematic review show that most studies have only been performed in males, meaning the generalizability of these findings to females is unknown. Given that females represent roughly 50% of the population, the knowledge gap surrounding the translational value of these interventions is large, as for half the population we do not know how these may impact healthspan or lifespan. Thus, we reiterate the point that only by studying both males and females can we leverage sex-specific differences to provide novel insights into the pathophysiology of aging and improve healthy aging for all.

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

MK and SM conceptualized and designed the study. MK collected articles. MK and SM reviewed all articles and formulated the results. MK wrote the first draft of the manuscript. SM, MM, and CE edited the

manuscript. All authors that contributed to the manuscript revision, read 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.

Publisher's note

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Impact of hearing aid technology level at first-fit on self-reported outcomes in patients with presbycusis: a randomized controlled trial

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To provide clinical guidance in hearing aid prescription for older adults with presbycusis, we investigated differences in self-reported hearing abilities and hearing aid effectiveness for premium or basic hearing aid users. Secondly, as an explorative analysis, we investigated if differences in gain prescription verified with real-ear measurements explain differences in self-reported outcomes. The study was designed as a randomized controlled trial in which the patients were blinded towards the purpose of the study. In total, 190 first-time hearing aid users (>60 years of age) with symmetric bilateral presbycusis were fitted with either a premium or basic hearing aid. The randomization was stratified on age, sex, and word recognition score. Two outcome questionnaires were distributed: the International Outcome Inventory for Hearing Aids (IOI-HA) and the short form of the Speech, Spatial, and Qualities of Hearing Scale (SSQ-12). In addition, insertion gains were calculated from real-ear measurements at first-fit for all fitted hearing aids. Premium hearing aid users reported 0.7 (95%CI: 0.2; 1.1) scale points higher total SSQ-12 score per item and 0.8 (95%CI: 0.2; 1.4) scale points higher speech score per item, as well as 0.6 (95%CI: 0.2; 1.1) scale points higher qualities score compared to basic-feature hearing aid users. No significant differences in reported hearing aid effectiveness were found using the IOI-HA. Differences in the prescribed gain at 1 and 2 kHz were observed between premium and basic hearing aids within each company. Premium-feature devices yielded slightly better self-reported hearing abilities than basic-feature devices, but a statistically significant difference was only found in three out of seven outcome variables, and the effect was small. The generalizability of the study is limited to community-dwelling older adults with presbycusis. Thus, further research is needed for understanding the potential effects of hearing aid technology for other populations. Hearing care providers should continue to insist on research to support the choice of more costly premium technologies when prescribing hearing aids for older adults with presbycusis.

Clinical Trial Registration: https://register.clinicaltrials.gov/, identifier NCT04539847.

KEYWORDS

presbycusis, hearing aids, technology level, self-reported outcomes, real-ear measurements

1 Introduction

Hearing loss is one of the most common chronic health conditions today (Stevens et al., 2013; Besser et al., 2018; World Health Organization, 2021). According to the Global Burden of Disease studies, hearing loss is the third leading cause of disability worldwide (GBD Hearing Loss Collaborators, 2021). Age-related hearing loss (presbycusis) is projected to be one of the top 10 leading causes of burden of disease by 2030, following a global demographic shift towards an aging population (Mathers and Loncar, 2006; Davis et al., 2016). It is estimated that approximately 50 percent of people older than 60 years and 80 percent of those older than 85 years have a hearing deficit (Cunningham and Tucci, 2017), and that 30% of men and 20% of women in Europe have a pure-tone average hearing loss across 0.5, 1, 2, and 4 kHz (PTA-4) of 30 dB hearing level (HL) or more in the better ear by the age 70 years (Roth, 2011).

Presbycusis is described as the cumulative effect of aging resulting from a degeneration of the cochlea and characterized by reduced hearing sensitivity and speech understanding in noisy environments (Working Group on Speech Understanding and Aging, 1988; Gates and Mills, 2005; Cunningham and Tucci, 2017). The audiometric profile is a bilateral symmetrical sensorineural hearing loss (ISO:7029, 2017) that progresses over the years, especially in the high-frequency region (Davis et al., 2016). Due to complex genetics and environmental factors that affect hearing throughout the entire lifespan, the underlying pathology is complex and contributes to an extensive variation in audiometric profiles (Dubno et al., 2013).

Hearing aids (HA) are the conventional choice of rehabilitation for older adults with presbycusis (Kochkin, 2009; Burton, Adams and Rosenfeld, 2014; Maidment et al., 2016), and the technology has improved rapidly over the last few decades (Edwards, 2007). The most substantial change was the transition from analog to digital sound processing, allowing for more advanced signal processing strategies (Levitt, 2007; Bertoli, 2010). Hearing aids can be more or less advanced in terms of feature settings and speech processing, but essentially, they all consist of four basic blocks: a microphone, a signal processor, a loudspeaker, and a power source (Levitt, 2007; Dillon, 2013). Manufacturers produce HA families that include different models at different levels of technology. The more advanced the technology level is, the higher the cost of the HAs will generally be (Chung, 2004). When fitting patients with HAs in clinics today, the choice of HA technology level is one of the challenges clinicians encounter (Walden et al., 2000; Cox, 2014; Johnson, 2016). The decision is often based on the clinician's individual preferences and the patient´s hearing needs. Cost is an important decision factor when total HA reimbursement is not provided. Studies have shown that patients perform a cost-benefit analysis to decide if the HAs provide sufficient value to justify their expenses (Newman, 1998; Cox, 2014). Devices that provide more benefit for a given cost are considered to provide greater value. Thus, cost has also been identified as a contributing factor for low HA uptake (Chien and Lin, 2012).

There is a lack of knowledge regarding what level of technology should be recommended for patients with a hearing loss, and further research is needed to clarify the relative benefits of premium-level *versus* basic-level HA technologies. From the HA users' point of view, it is important to know whether there is evidence suggesting greater benefit with premium compared to basic HA technology. In other words, in the decision process it is important to know, if a premium-level HA is worth the cost, which is a highly relevant topic from both a clinical and a commercial perspective.

Real-ear insertion gain can be used to verify the actual gain provided by the HA and is defined as the sound pressure level (SPL) near the eardrum when aided, minus the SPL at the eardrum when unaided (Ching and Dillon, 2003). Variations in the individual outer ear cause a mismatch between the predicted insertion gain and the measured real-ear insertion gain (Bell, 2009), and research has shown that different gain levels are prescribed for the same type of hearing loss depending on the device model and manufacturer (Keidser, 2003; Sanders et al., 2015; Sanchez-Lopez et al., 2021). Therefore, evidence suggests that it is important to use insertion gain when fitting HAs because the first-fit of HAs cannot be relied on to provide an accurate fit (Aazh and Moore, 2007; Aazh, 2012), and guidelines from professional organizations are available to guide matching the insertion gain to target (e.g., British Society of Audiology [BSA], 2007). The number of compression channels in the HA, the option for modifications of the HA and the acoustics of the unoccluded or occluded ear canal are determining the closeness between the target and insertion gain (Bell, 2009). Research has shown that fittings made according to a verified target prescription improve speech intelligibility in quiet and noise, and that real-ear measurement (REM)-based fittings improve the self-reported HA outcomes (Moore, 2001; Ching et al., 2010; Abrams et al., 2012; Almufarrij, 2021). In premium-level HAs, the more advanced signal-processing features and greater number of compression channels, noise reduction, feedback reduction, and microphone systems, are designed to improve speech-understanding compared to more basic levels of technology (Chung, 2004; Edwards, 2007). More complex technology such as environmental adaptation and binaural data streaming are often included in premium HA models (Levitt, 2007). Premium technologies have been suggested to yield improved access to speech cues, thus reducing the attentiveness required for speech understanding and thereby decreasing listening effort (Hornsby, 2013; Johnson, 2016). Some studies have shown performance advantages in laboratory tests with modern HA technology compared to basic technology (Walden et al., 2000; Wood and Lutman, 2004; Kießling and Kreikemeier, 2013; Wu et al., 2019).

Clinical assessments of HA performance are often not predictive of real-world outcomes which underlines the importance of assessing the perceived outcome of the patients. Real-world outcomes of a HA fitting can be assessed using different methods, and one reasonable way would be to ask for the HA user´s opinion. Thus, the patient's perspective has been argued to be the gold standard to assess HA effectiveness (Cox, 2014; Cox, 2016). Over the years, several instruments for measuring real-world outcomes have been developed, but only a limited number of these instruments are translated to Danish. The International Outcome Inventory for Hearing Aids (IOI-HA) (Cox et al., 2000; Jespersen, 2014) and the short version of the Speech, Spatial and Qualities of Hearing Scale (SSQ-12) (Gatehouse and Noble, 2004; Jensen, 2009) are among the few translated and validated Danish questionnaires. The IOI-HA measures the perceived HA effectiveness and comprises seven items, targeting different outcome domains (Cox and Alexander, 2002), whereas the SSQ addresses perceived hearing abilities in three domains (speech, spatial, and qualities of hearing) and originally entails 49 questions. An abbreviated version, SSQ-12, was developed to encourage implementation into routine clinical practice (Noble et al., 2013).

Previous research has demonstrated limited differences between premium-level and basic-level HA technologies using self-reported outcome measures. Walden et al. (2000) compared differences in perceived benefit between more advanced digital HAs and basic linear technology using the Profile of Hearing Aid Benefit outcome questionnaire and found that the participants did not perceive the performance advantages shown in laboratory testing. Cox and colleagues investigated differences in the effectiveness of premium *versus* basic HAs among 25 and 45 older individuals (mean age 70 years), respectively, with mild-to-moderate bilateral hearing loss and included both first-time and experienced HA users (Cox, 2014; Cox, 2016; Johnson, 2016; Johnson, 2017). They used both subjective and objective outcome measures to explore technology differences and included laboratory speech understanding and sound localization tests, along with four standardized questionnaires (the Abbreviated Profile of Hearing Aid Benefit (APHAB), the SSQ-B version, the Device-Oriented Subjective Outcome (DOSO), and Hearing-Related Quality of Life questionnaire). Listening effort outcomes, assessed using both laboratory tests and subjective ratings, showed no significant differences between the technology levels (Johnson, 2016), neither did the self-reported outcome measures (Cox, 2014; Cox, 2016). Wu et al. (2019) investigated technology differences related to directional microphones and noise reduction and found that premium HAs outperformed basic HAs in laboratory setting, but this was not apparent in real-world. The findings of a more recent study by Plyler et al. (2021) are consistent with Cox and colleagues, but they found that noise acceptance and satisfaction for speech in larger groups were significantly improved with premium devices and that those in more demanding listening environment received significant improvements with premium HAs.

The current study contributes to further research on the efficacy of premium-feature devices compared to basic-feature devices. The study was conducted as a randomized controlled trial to strengthen the results, which provided a homogenous patient group and minimized patient related variation. To provide clinical guidance in HA prescription, we aimed to investigate if arguments for prescribing a more costly premium HA for older adults with presbycusis could be found in a clinical set-up where HAs are costless for the patients. Thus, the main purpose of the study was to test the hypothesis that premium technologies provide better self-reported hearing abilities and greater perceived HA effectiveness compared to basic technologies among older adults with symmetric presbycusis. Secondly, as an explorative analysis, we investigated if differences in gain prescription between the six chosen HA models as verified with REM could explain differences in reported outcomes between premium-level and basic-level HA users.

2 Materials and methods

2.1 Study design and ethics

The study was designed as a two-arm parallel randomized controlled trial. Data were collected at the Odense site as part of the Danish national Better-hEAring-Rehabilitation (BEAR) project that aims to improve audiological rehabilitation in Denmark and worldwide through an evidence-based renewal of clinical practice. Data in the BEAR study were collected from the Department of Audiology at Odense University Hospital (OUH), Region of Southern Denmark and the Department of Audiology at Aalborg University Hospital (AAUH), North Denmark Region from January 2017 to May 2018. Adults (≥18 years of age) referred for public HA treatment were enrolled in the BEAR study, regardless of previous HA experience. The BEAR project was evaluated by The Regional Committees on Health Research Ethics for Southern Denmark (S-20162000-64), and the present study was registered (Clinical trial registration NCT04539847).

2.2 Population and procedure

Data were collected from 1,159 patients with hearing loss who were a subgroup of patients enrolled from Region Southern Denmark accepting to participate in the BEAR project (mean age 68 ± 12 years, 45% women). These patients were distributed across the whole BEAR study period. They were recruited by private ear-, nose-, and throat (ENT) physicians that were informed about the study, and project information letters were sent to the ENTs to distribute to the patients. All patients received a letter including information about study details, a consent form, and a note on the patient´s rights related to study participation. The consent to participate was forwarded in a referral letter to the Department of Audiology, OUH. The inclusion criteria for the current study were patients with bilateral presbycusis with no previous experience with HAs. Presbycusis was defined as a symmetrical hearing loss (less than 10 dB difference in PTA-4 between right and left ear) in patients older than 60 years where the high-frequency hearing loss were greater than the low-frequency hearing loss, and no disclosed history of hearing loss besides age. Patients were excluded if they were not native Danish speakers, not able to complete the consent form or the questionnaires, or if they were experienced HA users. Each patient's audiogram and general medical history was contained in the referral

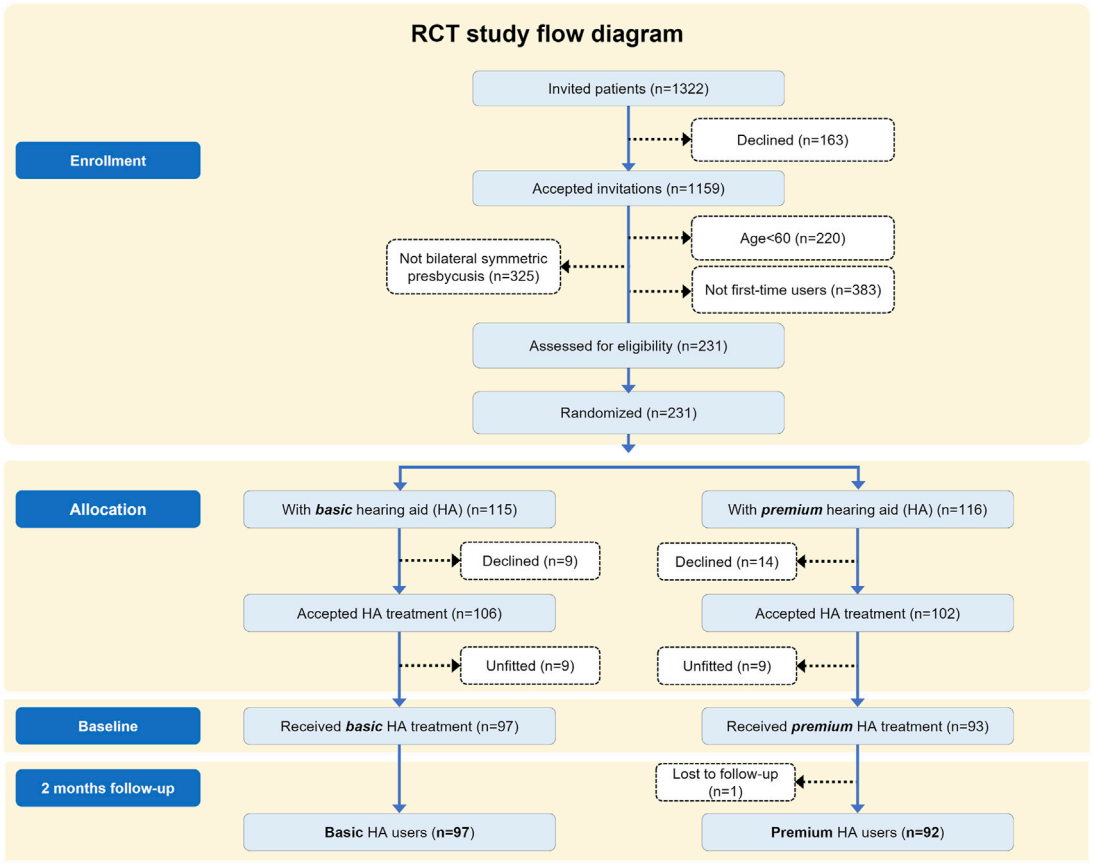

FIGURE 1
Patient flow diagram.

letter from the private ENT specialists and checked by the responsible researcher. If the inclusion criteria were met, patients were randomly assigned to either a premium-feature or basic-feature HA, stratified on age (60–69 years, 70–79 years, 80 years or above), sex, and word recognition scores (WRS) (WRS≥80% and WRS<80%). Block randomization with varying block sizes (12 and 18) was managed in the electronic REDCap database enabling equal distribution of the stratification variables in the two groups. Allocation was concealed.

In total, 231 patients were eligible for the study and randomized into two different groups (n = 115/116) (Figure 1). Twenty-three patients declined the HA treatment after given consent to participate, resulting in a study population of 208 patients. One hundred and six patients were allocated to basic-feature HAs, and 102 patients were allocated to premium-feature HAs. Patients were only presented to the randomly assigned HA model, but the audiologist could select another HA, or the patient could decline treatment with the selected HAs. Eighteen patients were fitted with HAs other than the allocated model and therefore excluded, leaving a study population of 190 patients (97 basic HA users and 93 premium HA users) (Figure 1). Patients were informed that the research was about improving hearing rehabilitation, but they were blinded towards the random selection of HAs and about taking part in the randomized study. No special attention was given towards this randomized subgroup within the BEAR project with regards to the HA fitting and counselling process, and the researchers were not actively involved in the intervention to promote the study. Patients were informed of the standard 2-months trial period with the option of choosing another HA model, or discontinue the HA treatment, if they were dissatisfied with the fitted HAs. The name of the individual HA manufacturer was visible to the patients, and patients therefore had the possibility to obtain information on the level of technology, but the potential cost or level of technology of the HAs were not discussed.

2.3 Hearing aids

Six different pairs of commercially available HAs (Resound Enya 4, Resound LiNX2 7, Oticon Nera2 Pro, Oticon Opn 1, Widex Dream 220 Fusion, and Widex Unique 440 Fusion) were evaluated as examples of basic-feature and premium-feature technologies from three manufacturers contributing to the BEAR project. One pair of basic-feature and premium-feature HA was included from each manufacturer. The HAs were released in different years and used different platforms, and the fitting strategy in one of the three companies differed between the two levels of HA technology, which

TABLE 1 Differences between premium- and basic-features in the six hearing aids as described by manufacturers (A, B, and C).

Features	Hearing aids					
	Premium A	Basic A	Premium B	Basic B	Premium C	Basic C
Number of compression channels	16	8	15	5	17	10
Adaptive microphone directionality	More advanced	Less advanced	More advanced	Less advanced	More advanced	Less advanced
Pinna simulation	N/A	N/A	Yes	No	Yes	No
Noise management	More advanced	Less advanced	Yes	No	More advanced	Less advanced
Wind Noise reduction	Yes	No	Yes	No	More advanced	Less advanced
Feedback management	Yes	Yes	Yes	Yes	Yes	Yes
Environmental adaptation	Yes	No	Yes	No	Yes	No
Binaural data streaming	Yes	Yes	Yes	No	Yes	Yes
Proprietary high-frequency boost	More advanced	Less advanced	Yes	No	Yes	Yes
Sound quality enhancer	Yes	No	Yes	No	Yes	No
Speech enhancer	More advanced	Less advanced	Yes	No	N/A	N/A
Number of programs	4	4	5	3	4	4
Tinnitus support	Yes	Yes	Yes	No	Yes	Yes
Connectivity (iPhone, Android)	Yes	No	Yes	No	Yes	No

Not applicable, N/A, was applied if the feature was not relevant for the given HA model, or if no information was available on the specific feature

TABLE 2 Distribution of the different types of acoustic fit (n = 190) by level of hearing aid technology.

Acoustic fit	Frequency	
	Premium	Basic
Open dome	58	57
Closed dome	14	7
Tulip dome (semi-open)	9	7
Custom made (silhouette)	5	9
Micro mold	4	7
Double Tulip (power)	1	2
Casted mould (shell)	0	1
N/A	2	7
Total	**93**	**97**

Total number of fitted hearing aids marked in bold.

is reported in Smeds et al. (2016). The HA models are anonymized in the following sections to comply with the collaboration agreement of the BEAR project. In Denmark, it is possible to receive free HAs as part of the public healthcare system, and therefore, patients can be treated with selected types of HAs that have been included as part of a regular ongoing public tender. All selected HAs in this study were available for the public tender in Denmark, and thus, representative of the HAs accessible for patients receiving their HAs free of charge from the Danish public healthcare system. A balanced design was applied with an intended representation of approximately one-third for each of the three manufacturers to avoid the dominance of a specific HA product and accompanying fitting rationale. To ensure equal distribution of the HAs from the three manufacturers, a randomization tool was used. The six different HA models were all behind-the-ear devices corresponding to the most popular style currently marketed. A list of the advertised features in each model of HA is presented in Table 1. The three premium HAs contained more compression channels and more advanced processing features (e.g. adaptive microphone directionality, noise management including wind noise reduction, environmental adaptation, and proprietary high-frequency boost). Besides, the three premium models included connectivity which enabled the use of HAs with smartphones.

2.4 Hearing aid fittings

All patients were bilaterally HA fitted by an experienced audiologist according to standard clinical practice, and the HAs were linked to the fitting software with wireless communication. The fitting and fine tuning of HAs were carried out in a single session following hearing evaluation. HAs were fitted using the proprietary fitting rationales by the specific HA manufacturers with NAL-NL2 target gains only being used for reference purposes in the analysis. Based on feedback from patients, If necessary, some gain adjustments in the high-frequencies were carried out to achieve a fit acceptable to the HA user. The decision about the acoustic coupling was based on individual characteristics of the ear canal and recommendations in the fitting software. Table 2 provides an overview of the final choice of acoustic earpiece per level of HA technology.

All feature settings were set according to the recommendations of the manufacturer and therefore left in default setting. As all patients were first-time HA users, no additional programs were added to the default listening program, and patients were given a short instruction on how to use the HAs, clean the earmolds, and changing of batteries. If connectivity was available for the given HA model, the HAs were connected via Bluetooth. Patients in need of assistive listening devices (e.g. remote controls) were referred to the responsible agencies or personnel. They were all recommended to use the devices during waking hours for as long as possible. Besides, they were informed of the opportunity to ask for additional counselling if needed.

2.5 Measures

2.5.1 Questionnaires

All patients completed a questionnaire survey 2 weeks before the first visit to the clinic and 2 months after HA fitting. The outcomes were designed to capture the perceived hearing abilities and the effectiveness of HAs and included the SSQ-12 (Gatehouse & Noble, 2004; Noble et al., 2013) and the IOI-HA (R. Cox et al., 2000). As all patients were first-time users, they only responded the IOI-HA 2 months following HA fitting. The SSQ was designed to assess people's perception of their listening capabilities in various situations and consists of the three domains: speech, space, and sound quality. In the SSQ-12 version, the three domains are represented by fewer questions (speech domain: 5 questions, space: 3 questions, sound quality: 4 questions) compared to the original version that entails 49 questions. The scale is ordinal and ranges from 0 to 10. A higher score reflects better hearing ability. The IOI-HA is a seven-item questionnaire intended to probe the experience with HAs during the recent past (2 weeks), reflecting the overall HA effectiveness. The scale is ordinal and ranges from 1 to 5. A higher score indicates a better outcome in the specific domains. Using principal component analysis or factor analysis, previous studies have identified two subscales within the IOI-HA that is described as factor 1 and factor 2 scores and reflect two different aspects: the HA benefit and the remaining difficulties with HAs (Cox and Alexander, 2002; Kramer et al., 2002; Brännström and Wennerström 2010; Jespersen et al., 2014). A non-standardized health-related questionnaire was also included in the baseline questionnaire survey and contained questions on demographical details such as sex, age, occupational status, HA experience, and motivation. The patients' motivation for HA treatment was assessed by two questions from an online evidence-based motivation tool developed by the Ida Institute (idainstitute.com): 'How important is it for you to improve your hearing?' and 'How much do you believe in your ability to use hearing aids?'. The scale is ordinal and ranges from 0 to 10. A higher score indicates higher motivation (Clark, 2010).

The questionnaire survey was managed by the research electronic data capture (REDCap) software that was developed by Vanderbilt University, Nashville, Tennessee, United States and hosted by Odense patient explorative network (OPEN) in the Region of Southern Denmark (Harris et al., 2009; 2019). All patients received and answered the questionnaire survey through an online link generated by REDCap. Due to the online versions of the questionnaires, some modifications were made to the SSQ-12. The response scale from 0–10 was marked by a cursor placed at the point of the scale corresponding to one's specified score. The response option, 'not applicable,' was substituted with an option to leave the cursor untouched, corresponding to the answer, 'not applicable,' or 'do not know.' A paper version was also available at the clinic for patients who were unable to fill out the form electronically. The responses were manually entered into the database by a research assistant.

2.5.2 Audiological assessment

As part of the first visit to the clinic, all patients underwent standard audiometry according to current clinical practice. The audiological assessment included a pure-tone audiometry measuring air-conduction thresholds for left and right ears at 250 Hz, 500 Hz, 1 kHz, 2 kHz (3 kHz), 4 kHz (6 kHz) and 8 kHz; bone-conduction thresholds at 250 Hz to 4 kHz when air-conduction thresholds showed low-frequency hearing thresholds >20 dB hearing lossor were asymmetric between the two ears; and a measure of WRS. Tympanometry was measured to rule out any middle-ear diseases. Air- and bone-conduction thresholds were measured according to ISO8253-1:2010 (International Organization for Standardization). TDH39 headphones, or ER-3A insert earphones, were used during the tests. The WRS was obtained by presenting 25 different monosyllabic words in quiet at the most comfortable listening level from the validated DANTALE-I wordlists (Elberling et al., 1989). The result is expressed as the percentage of correct responses to the words presented. All measurements were conducted in a soundproof booth in the Audiological Department at OUH and were carried out by experienced audiologists.

2.5.3 Real-ear measurements

A follow-up appointment was scheduled approximately 2 months after HA fitting. Real-ear measurements were performed both before and after any adjustments of the HAs and included measuring real-ear unaided gain at 65 dB SPL to record the natural gain provided by the outer ear followed by measuring real-ear aided gain at three different input levels (55, 65, and 80 dB SPL). Finally, the real-ear insertion gain was derived by subtracting the unaided gain from the aided gain. Only the REMs that were obtained before any adjustments of the HAs were used in the analysis. The International Speech Test Signal (ISTS) was used as the stimulus, and the HAs were set to default so that all features were activated during the measurement. The REM module (REM440) of Affinity 2.0 (Interacoustics) was used and followed the standards: ANSI/ASA S3.46 (2013); IEC 61669 (2015); ISO 12124 (2001). Calibration of the REM headset was repeated before each session. The REMs were only used for documentation and not as a basis for adjusting the HAs. Hence, the NAL-NL2 fitting prescription was only used as a hypothetical reference target. In addition, the HA usage time in hours per day was extracted from the fitting software. Some of the logged data showed usage time of >18 h per day. This could have been due to patients not turning off the HAs during the night, and these data were therefore excluded. The follow-up visit was carried out by the two researchers at the Department of Audiology at OUH responsible for collecting the outcome data.

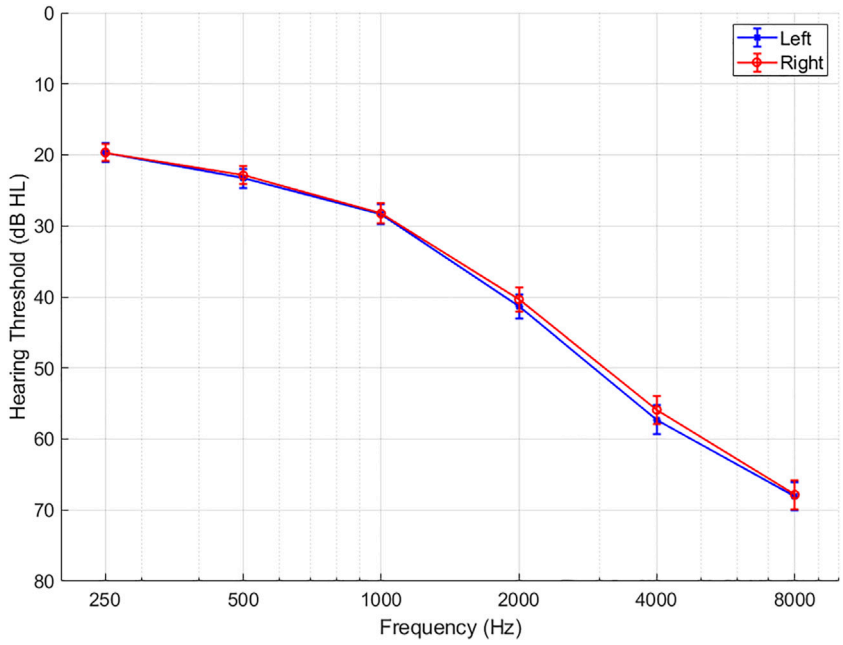

FIGURE 2
Average hearing thresholds in dB HL for left and right ear in the total study population (n = 190). Error bars show 1 standard error of the mean.

2.6 Statistical analysis

PTA-4 was calculated as the average of hearing thresholds at 0.5, 1, 2, and 4 kHz for each ear. Data normality was verified using Q-Q plots. Multiple linear regression (MLR) analyses were used to test the hypothesis that premium-feature HAs yielded better self-reported outcomes than basic-feature technologies in terms of overall SSQ score, SSQ domain scores, IOI-HA total score, and IOI-HA factor 1 and factor 2 scores. Baseline SSQ-12 scores were included in the models with SSQ-12 as an outcome to adjust for the unaided score, thereby minimizing the effect of individual differences that existed before HA treatment. Using linear regression as statistical model, the outcomes are treated as an interval scale despite ordinal scale properties. Since ordered logistic regression did not show any significant differences to the model estimates, linear regression was used in the analyses. Bootstrapping was applied to compensate for non-normally distributed residuals. Significance levels were set at $p < 0.05$ for the MLR analyses (command regress in STATA). Variance inflation factor (VIF) was used to test multi-collinearity between independent variables in all linear regression models and showed no indications for multi-collinearity in the models (VIF<2.5). The dependent variables used in the models were: IOI-HA total score, IOI-HA factor 1 and factor 2 score, SSQ-12 speech, SSQ-12 space, SSQ-12 sound quality score, and SSQ total score. The primary predictor used in the analyses was HA technology level, and secondary predictors were sex, age, WRS, motivation (Q1 and Q2), baseline SSQ-12 scores for the given domain, and mean gain deviation from NAL-NL2 targets at 1, 2, and 4 kHz. As an extended model, it was investigated whether the difference in manufacturer was a significant factor related to the outcome. The significant results from parametric analysis were checked and confirmed with non-parametric analysis using Mann-Whitney U tests as well.

Data management and analyses were performed using STATA SE version 16.0 (Stata Corp., College Station, TX).

2.6.1 Power calculation (SSQ-12 and IOI-HA)

Given an observed standard deviation (SD) of 1.54 and 1.61 scale points for the overall SSQ-12 score in the two study arms, a sample size of n = 190 and a power = 0.80, we can detect a difference in the overall SSQ-12 of 0.64. Also, based on the SD of 4.39 and 4.94 scale points for IOI-HA total score in the two groups and with the same sample size of $n = 190$ and power = 0.80, we can detect a difference in IOI-HA total score of 1.91. Alpha was set to 0.05.

3 Results

3.1 Demographics

Figure 2 depicts the mean audiograms of the right and left ear for the 190 included patients. Table 3 describes the characteristics of the patients allocated to either premium or basic level technologies (n = 97/93). The median age of the two groups of HA users was 72 (interquartile range (IQR) 10) years and 70 (10) years, respectively. Forty-one to 45% were female HA users. The median WRS for the left and right ears of the two groups of HA users were 92% and the IQRs were 8% and 12%, respectively. The average PTA-4 levels for the left and right ears were 37.5 (12.5) dB HL and 36.4 (11.0) dB HL, respectively. The two groups were highly similar in terms of sex (p = 0.8), age (p = 0.2), PTA-4 right and left ear (p = 0.5, p = 0.5), WRS right and left ear (p = 0.9, p = 0.7), level of motivation Q1 and Q2

TABLE 3 Characteristics of patients allocated to premium- or basic-feature hearing aids (HA).

Median (IQR)	Basic HA users (N = 97)	Premium HA users (N = 93)	*p*-value*
Sex, Women (%)	45	41	0.8
Age, years	72.0 (10.0)	70.0 (10.0)	0.2
Range	(61–90)	(60–93)	
PTA-4, dB HL			
Right ear	37.5 (12.5)	36.3 (10.0)	0.5
Left ear	37.5 (12.5)	36.6 (12.1)	0.5
Range	(15–60)	(18–64)	
WRS, %			
Right ear	92.0 (12.0)	92.0 (8.0)	0.9
Left ear	92.0 (8.0)	92.0 (8.0)	0.7
Severity of hearing loss based on better ear PTA-4, n (%)			0.7
Normal Hearing, ≤19 dB HL	3 (3)	1 (1)	
Mild, 20–34 dB HL	43 (44)	45 (48)	
Moderate, 35–49 dB HL	45 (46)	44 (47)	
Moderate-Severe, 50–64 dB HL	6 (6)	4 (4)	
Severe & Profound, >65 dB HL	0 (0)	0 (0)	
Motivation Q1 score	7.9 (3.1)	8.1 (3.2)	0.9
(Range 0–10)	(3–10)	(2–10)	
Motivation Q2 score	8.1 (3.2)	8.5 (2.6)	0.4
(Range 0–10)	(1–10)	(1–10)	
Occupational status, n (%)			
Active	9 (9)	12 (13)	0.5
Retired	81 (84)	75 (81)	
Missing	7 (7)	6 (6)	
Average HA usage time, hours per day	9.0 (7.0)	9.0 (7.5)	0.8
Range (3–18)	(3–16)	(3–18)	
IOI-HA total score (follow-up)	29 (6)	30 (7)	0.86
Range (7–35)	(15–35)	(14–35)	
Overall follow-up SSQ-12 score (item score)	6.3	7.3	0.01**
Range (0–10)	(3–10)	(3–10)	
Speech domain (5 items)	5.6	7.0	0.01**
Spatial domain (3 items)	7.4	7.8	0.12
Qualities domain (4 items)	6.9	7.7	0.03**
Overall baseline SSQ-12 score (item score)	5.1	5.1	0.69
Range (0–10)	(3–10)	(3–10)	
Speech domain (5 items)	4.2	3.9	0.88
Spatial domain (3 items)	6.3	6.7	0.48
Qualities domain (4 items)	5.6	5.9	0.61

Hearing aids, HA; Inter-Quartile Range, IQR; Hearing level, HL; Pure-Tone Average at 0.5, 1, 2, and 4 kHz, PTA-4; Word Recognition Score, WRS. *p-values using Fisher´s exact test, Mann-Whitney U test, t-test, and Chi-Square test; **significance at $p < 0.05$

($p = 0.9$, $p = 0.4$), and occupational status ($p = 0.5$). Also, there was no statistically significant difference in how much they used their HAs in hours per day in the two groups ($p = 0.9$).

3.2 Self-reported outcomes using the IOI-HA and SSQ-12

Table 4 shows the results from the regression analyses that investigated if premium-feature HA users reported significantly higher overall HA effectiveness using the IOI-HA and better hearing abilities using SSQ-12 than basic-feature HA users. The results show that premium-feature HA users did not report a significantly higher IOI-HA factor 1 score (diff = 0.03, 95%CI: 1.1; 1.2, $p = 0.96$), factor 2 score (diff = -0.11, 95%CI: 0.7; 0.5, $p = 0.73$), or IOI-HA total score (diff = -0.03, 95%CI: 1.6; 1.7, $p = 0.97$) compared to basic-feature HA users. Using the SSQ-12 speech, space, and qualities domain score as outcome variables showed that premium-feature HA users reported a 0.8 (95%CI: 0.2; 1.4, $p = 0.01$) scale points higher speech score per item in the

speech domain and a 0.6 (95%CI: 0.2; 1.1, $p = 0.01$) scale points higher qualities score per item in the qualities domain compared to basic-feature HA users. Using the SSQ-12 total score, results showed that premium-feature HA users reported a 0.7 (95%CI: 0.2; 1.1, $p < 0.001$) scale points higher total score than basic-feature HA users. SSQ-12 differences similarly ranged from 0.5-1 between two different noise management settings in Andersson et al. (2021) whereas the differences between the two device technology levels in Plyler et al. (2021) ranged from 0.2–0.5. Including manufacturer in the analysis did not change the significance of the results or the coefficients, hence it was eliminated from the regression model.

Patients with 10% higher WRS reported 0.4 (95%CI: 0.01; 0.1, $p = 0.02$) scale points higher SSQ-12 score per item in the space domain, and those with higher motivation for HA treatment reported a 0.2 (95%CI: 0.1; 0.4, $p = 0.03$) scale points higher SSQ-12 space score per one unit change in motivation score. WRS and motivation had similar significant effects on the total SSQ-12 score. Female HA users reported 0.7 (95%CI: 1.3; -0.2, $p = 0.01$) units lower SSQ-12 space score per item in the space domain than male HA users. These results indicate that WRS, motivation, and sex can also modestly affect SSQ-12 scores.

3.3 Real-ear measurements

3.3.1 Differences in insertion gain deviation from NAL-NL2 target at first-fit

Table 5 shows the mean difference between the real-ear insertion gain at 65 dB SPL input level and NAL-NL2 target at 0.5, 1, 2, and 4 kHz for the six HAs at first-fit. There was no statistically significant difference in how much the measured insertion gain deviated from NAL-NL2 target gains between premium and basic HAs across the three companies (A, B, and C). However, comparing the gain deviations between premium and basic HAs within company A, B, and C revealed that there was a statistically significant difference in gain deviation from NAL-NL2 within company A at 2 kHz (mean diff: 3.7 dB; $p < 0.001$) between the premium and basic HA and for company B at 1 and 2 kHz (mean diff: 2.3 dB and 2.7 dB; $p < 0.05$) The mean difference between the insertion gains and NAL-NL2 target gains at the measured frequencies varied from 2 to 3 dB between the two levels of technology.

3.3.2 Differences in real-ear insertion gain at first-fit

Figure 3 depicts the mean insertion gain from 125 to 8,000 Hz for the first-fit of premium and basic HAs at 65 dB SPL. Data are given for the average of the left and right ears and NAL-NL2 is included as a reference target. The figure demonstrates the average prescribed gain is highly similar for the three premium and basic HAs used in the current study. However, looking at the gain levels for premium and basic HAs within each company (A, B, and C) at the three input levels (55, 65, and 80 dB SPL) shown in Figure 4, a difference in the prescribed gain between premium and basic HAs can be observed. The largest difference between premium and basic HA within each company is observed at 1 kHz (company B) and 2 kHz (company A and C). The absolute value of the (mean) difference between premium and basic HA was calculated to be approximately 3 dB gain at 1 kHz in company B and 2 dB gain at 2 kHz in company A and C, which are rather small gain differences.

4 Discussion

One of the main findings from the current study was that presbycusis patients without previous HA experience using premium HAs reported better hearing abilities in terms of speech and qualities of hearing compared to those using basic HAs using the SSQ-12 as outcome measure. These results suggest that patients with symmetric presbycusis might benefit from premium technologies with regards to improved hearing abilities. Using the IOI-HA, showed no significant difference in reported HA effectiveness between the two levels of HA technology. In total, four out of seven outcome variables failed to show a significant difference between premium and basic HAs, and with larger samples sizes, even small effects can yield statistical significance. Hence, it is important to acknowledge the limited clinical relevance of the results. Also, given the rather small differences in SSQ-12 scores between the premium- and basic-feature HA users (mean differences ranged from 0.6 to 0.8 scale points), it is questionable if these observed differences would be relevant to daily life. Although minimal clinically important differences have not been established for the SSQ-12, previous research has used one scale point of change to demonstrate a clinical significant change between assessments in SSQ (Noble & Gatehouse, 2006; Lenarz et al., 2017)). However, Lenarz et al. (2017) looked at the change in SSQ at an individual level (within-effect) while our study investigated mean group differences (between-effect), and therefore the clinically relevant difference in SSQ might be less than one scale point. In Plyler et al. (2021), the observed differences in SSQ between the two device technology levels were smaller (0.2–0.5) compared to our study and was not found statistically significant.

In contrast, previous studies did not provide evidence to suggest that premium HAs yield better self-reported real-world outcomes or laboratory outcomes (Cox et al., 2014; 2016; Johnson et al., 2016; 2017; Plyler et al., 2021; Saleh et al., 2021). Although Wu et al. (2019) found that premium HAs provided better speech understanding and sound localization in the laboratory, this improvement was not transferred to the real-world setting. Cox et al. (2014) combined three sets of questionnaire data (SSQ-B, DOSO, and APHAB) to provide one single benefit score which may have affected the sensitivity of the original scales and concealed any observed differences in the reported benefit. One possible explanation for the different findings is that in the present study, patients used either a premium or a basic device, whereas the participants in previous studies tried both levels of technology. This could have biased their experience by comparing between different HA models, but it also could have helped them to decide which one they preferred, and the fact that they were all blinded to the model name and technology level limits this bias. Experienced users commonly exhibit a more severe hearing loss, which is why the inclusion of both first-time and experienced users in the previous studies might have contributed to a larger variation in hearing thresholds that could influence differences in reported hearing abilities related to the level of

TABLE 4 Pannel (A) Associations between self-reported hearing aid (HA) outcome using the international outcome inventory for hearing aids (IOI-HA) score and level of technology. Pannel (B) Associations between self-reported hearing aid (HA) outcome using the speech, spatial, and qualities of hearing (SSQ-12) score and level of technology.

Explanatory variables	IOI-HA factor 1 (n = 157)		IOI-HA factor 2 (n = 157)		IOI-HA total (n = 157)	
	Adj. R^2 = 0.08		Adj. R^2 = 0.05		Adj. R^2 = 0.08	
	Coef. (95% CI)	*p-value*	*Coef. (95% CI)*	*p-value*	*Coef. (95% CI)*	*p-value*
Primary explanatory variable						
HA technology level (Ref: Basic)	0.03 (-1.15; 1.22)	0.96	-0.11 (-0.74; 0.52)	0.73	-0.03 (-1.62; 1.68)	0.92
Secondary explanatory variables						
WRS (average left and right ear)	0.01 (-0.07; 0.09)	0.82	0.03 (-0.01; 0.08)	0.14	0.04 (-0.06; 0.14)	0.47
Motivation Q1	0.28 (-0.07; 0.64)	0.12	-0.02 (-0.22; 0.18)	0.84	0.23 (-0.24; 0.7)	0.43
Motivation Q2	0.28 (-0.06; 0.61)	0.09	0.05 (-0.14; 0.24)	0.54	0.32 (-0.11; 0.77)	0.13
Sex (ref: men)	0.17 (-0.96; 1.30)	0.77	-0.22 (-0.86; 0.42)	0.50	-0.18 (-1.55; 1.45)	0.84
Age	0.00 (-0.08; 0.09)	0.94	-0.01 (-0.06; 0.03)	0.59	-0.01 (-0.12; 0.10)	0.88
REIG deviation from target at						
1 kHz	-0.09 (-0.24; 0.07)	0.32	-0.03 (-0.11; 0.06)	0.57	-0.16 (-0.32; 0.10)	0.29
2 kHz	0.07 (-0.15; 0.29)	0.56	0.07 (-0.06; 0.19)	0.29	0.03 (-0.16; 0.43)	0.86
4 kHz	0.00 (-0.15: 0.15)	0.97	0.01 (-0.08; 0.09)	0.84	0.14 (-0.19; 0.21)	0.17
Constant	10.34 (-1.33; 22.02)	0.09	11.03 (4.45; 17.61)	0.00	21.37 (5.84; 36.91)	0.01

Explanatory variables	SSQ speech (n = 158) Adj. R^2 = 0.22		SSQ spatial (n = 158) Adj. R^2 = 0.38		SSQ qualities (*n* = 157) Adj. R^2 = 0.22		SSQ total (n = 157) Adj. R^2 = 0.33	
	Coef. (95% CI)	*p-value*	*Coef. (95% CI)*	*p-value*	*Coef. (95% CI)*	*p-value*	*Coef. (95% CI)*	*p-value*
Primary explanatory variable								
HA technology level (ref: Basic)	0.80 (0.2; 1.4)	**0.01**	0.41 (-0.1; 0.9)	0.11	0.64 (0.2; 1.1)	**0.01**	0.67 (0.2; 1.1)	**<0.001**
Secondary explanatory variables								
WRS (average of left and right ear)	0.04 (0.0; 0.1)	0.06	0.04 (0.01; 0.1)	**0.02**	0.02 (-0.01; 0.1)	0.24	0.03 (0.0; 0.1)	**0.05**
Motivation Q1	0.19 (-0.1; 0.4)	0.08	0.21 (0.1; 0.4)	**0.03**	0.12 (-0.03; 0.3)	0.09	0.19 (0.1; 0.3)	**0.01**
Motivation Q2	0.01 (-0.2; 0.9)	0.96	0.02 (-0.1; 0.2)	0.77	0.01 (-0.1; 0.1)	0.93	0.00 (-0.1; 0.1)	0.98
Sex (ref: men)	-0.36 (0.9; 0.2)	0.22	-0.74 (-1.3;-0.2)	**0.01**	-0.09 (-0.6; 0.4)	0.71	-0.35 (-0.8; 0.1)	0.12
Age	-0.04 (-0.1; 0.0)	0.06	-0.01 (-0.1; 0.03)	0.59	-0.02 (-0.1; 0.01)	0.22	-0.02 (-0.1; 0.01)	0.19
REIG deviation from target at								
1 kHz	-0.02 (-0.1; 0.1)	0.16	-0.05 (-0.1; 0.02)	0.17	0.02 (-0.04; 0.1)	0.50	-0.02 (-0.1; 0.04)	0.56
2 kHz	0.04 (-0.1; 0.2)	0.35	0.05 (-0.1; 0.02)	0.33	0.03 (-0.1; 0.1)	0.55	0.03 (-0.1; 0.1)	0.45
4 kHz	-0.02 (-0.1; 0.1)	0.64	-0.02 (-0.1; 0.1)	0.62	-0.01 (-0.1; 0.1)	0.66	0.00 (-0.1; 0.1)	0.92
SSQ-12 (baseline) for corresponding domain	0.32 (0.2; 0.5)	<0.001	0.48 (0.4; 0.6)	<0.001	0.41 (0.3; 0.6)	<0.001	0.49 (0.3; 0.6)	<0.001
Constant	2.72 (-3.3; 8.7)	0.37	-0.66 (-6.1; 4.8)	0.81	3.40 (-1.7; 8.5)	0.19	1.35 (-3.3; 6.0)	0.57

Statistical method: Multiple linear regression analysis with applied bootstrapping with 5,000 replications. Word Recognition Scores, WRS; Motivation Question One and Two from Ida Institute (Clark, 2010), Motivation Q1, Motivation Q2; Real-Ear Insertion Gain, REIG.

Statistical method: Multiple linear regression analysis with applied bootstrapping with 5,000 replications. Word Recognition Scores, WRS; Motivation Question One and Two from Ida Institute (Clark, 2010), Motivation Q1 and Motivation Q2; Real-Ear Insertion Gain, REIG, Baseline SSQ-12 score, B_SSQ-12.

Significant *p*-values marked in bold.

TABLE 5 Mean differences (in dB) between real-ear insertion gain at manufacturers' first-fit and NAL-NL2 prescription target for the six hearing aids.

Frequency, kHz	Hearing aids					
	Basic A	Premium A	Basic B	Premium B	Basic C	Premium C
0.5	0.6 (2.0)	0.3 (0.9)	0.8 (1.9)	0.1 (2.7)	0.1 (1.5)	0.3 (1.7)
1.0	−3.2 (2.7)	−3.3 (2.1)	2.1 (4.1)*	−0.2 (3.6)	−2.6 (3.8)	−3.1 (3.7)
2.0	−5.7 (3.3)**	−2.0 (3.6)	−0.9 (4.6)*	−3.6 (4.4)	−2.8 (3.6)	−1.3 (4.3)
4.0	−11.4 (4.0)	−9.3 (4.4)	−8.4 (5.1)	−9.9 (4.4)	−6.9 (4.5)	−6.1 (5.1)

Data for left and right ears were averaged for each patient (n = 162). Standard deviations are given in parentheses. *significance at $p < 0.05$, **significance at $p < 0.001$when comparing data for each pair of premium and basic hearing aid.

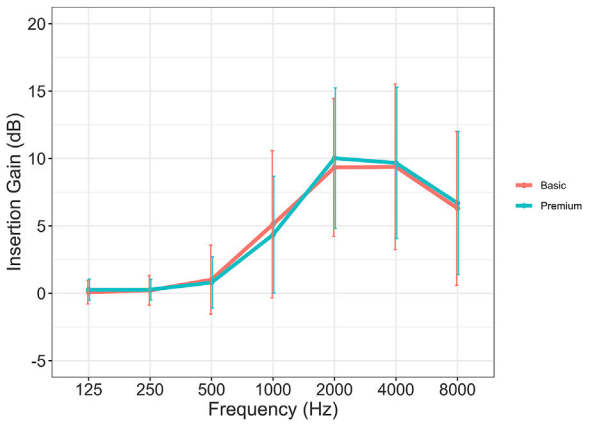

FIGURE 3
Average insertion gain at 65 dB SPL input level by level of device technology (n = 162). Error bars show 95%CI.

device technology. The mean audiograms in Cox et al. (2014; 2016) shows PTA-4 thresholds above 70 dB HL and SDs above 20 dB HL, whereas in our study that only included first-time users, the PTA-4 ranged from 15–64 dB HL and IQR from 10 to 12.5 dB HL (Table 3).

Another important difference between previous studies and the current study is that the HA technology used in the previous studies was at least one developmental epoch older than the current study, and the technology advances could therefore be expected to be more evident in the premium devices used in the current study. Hence, it is noticeable, that even with greater contrast in technology, the IOI-HA total score along with the Factor 1 and Factor 2 scores failed to show a significant difference between premium and basic level HAs. This suggests that the perceived effectiveness using premium and basic technologies were similar. However, as the IOI-HA addresses the overall effectiveness with HAs and the SSQ addresses the hearing (dis)abilities in different specific listening situations, we speculate if the IOI-HA items were not specific enough to detect perceived differences between the two technology levels. Thus, the SSQ-12 appears to be a more sensitive outcome measure to uncover small differences in technology levels or feature settings.

One explanation for premium HAs yielded better perceived hearing abilities in the SSQ speech domain could be that the more advanced features in the premium HAs can be better at distinguishing speech signals from noise than basic-feature HAs. The high-frequency boost feature and the speech-enhancer feature in premium HAs (Table 1) could contribute to better reported hearing abilities by amplifying speech signals and improving speech understanding in noisy environments. Although premium users reported better hearing abilities, only small outcome differences were observed between premium and basic devices. It could be that the listening environments in their daily lives were mostly quiet, so that the directional microphones or noise reduction algorithms were not activated most of the time, and because the advanced features in premium devices are only beneficial in more demanding listening environments, the benefits of using premium technology may go unnoticed (Wu et al., 2019). The occupational status revealed that the majority of HA users in the two study groups were retired (81%–84%, respectively), which could indicate less demanding sound environments in their daily life. This is line with results from Wu et al. (2019) that found only 10.9% of the self-reports were conducted in noise, which led the authors to the same conclusion. Nevertheless, because the logging data from the six HAs differ significantly across brands, except from use-time that was extracted, the comparability of these data is limited. The slightly better reported SSQ outcomes using premium HAs could also be related to non-signal-processing factors, such as connectivity and having access to smartphone user-controlled settings. Saleh et al. (2021) investigated drivers of user preference between premium and entry-level HAs using group concept mapping approach and found that these non-signal-processing factors significantly influenced the preference of premium HAs. This underlines the importance of including non-audiological features, such a connectivity, in modern HAs and could have a significant impact on the reported outcomes using premium HAs.

Female HA users reported significantly poorer hearing abilities than men in the space domain related to questions on directional and distance hearing. Previous studies have shown that females reported poorer outcomes than men using the IOI-HA which was related to Factor 2 scores (communication with others) (Arlinger, Nordqvist and Öberg, 2017; Houmøller et al., 2021), and they suggested this might be due to women being more socially active than men and consequently exhibit higher expectations towards the HAs.

Insertion gain levels at first-fit measured with REM showed that premium HAs prescribed more high-frequency gain than basic HAs for company A, but the opposite was found for company B (Figure 4). The gain differences within companies A, B, and C might reflect different fitting strategies, and the change in fitting strategy for

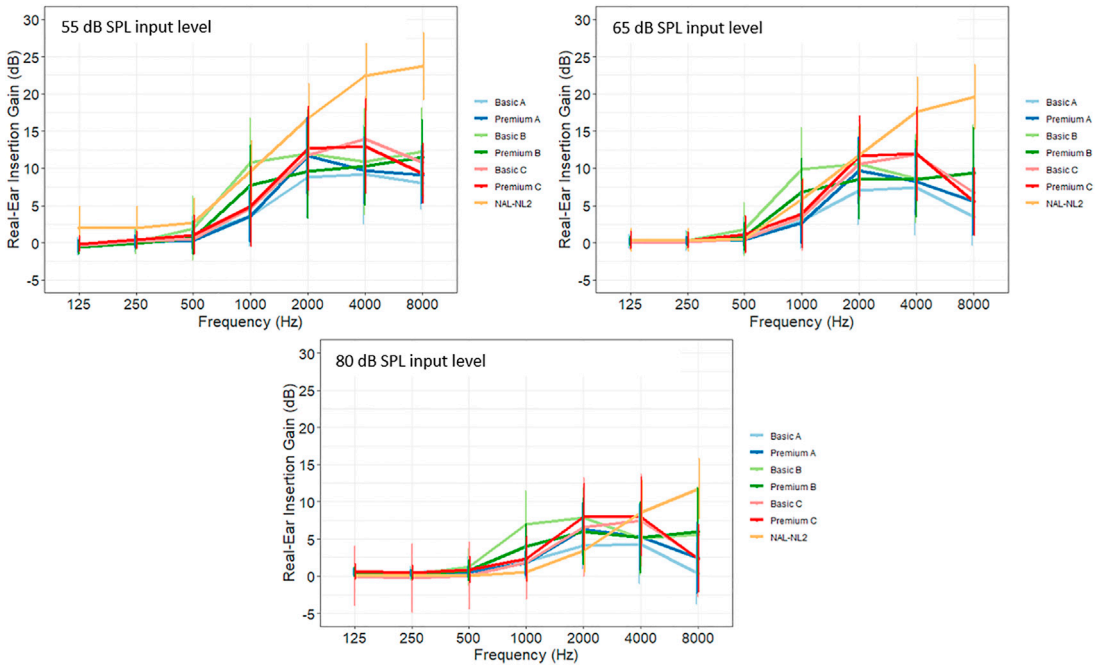

FIGURE 4
Average insertion gain at 55, 65, and 80 dB SPL input level by level of device technology and companies **(A-C)**. NAL-NL2 is shown as a reference target (n = 162).

company B, reported in Smeds et al. (2016), could explain why more gain is prescribed at 1 kHz for the basic-feature HA compared to the premium HA (seen in Table 5). Nevertheless, the insertion gain differences did not explain the small differences in reported SSQ outcomes as shown in Table 4, but it was striking to find that different gain levels are prescribed for highly similar types of presbycusis hearing losses. This finding is consistent with other studies that also showed extensive differences in amplification characteristics between different manufacturers' proprietary fitting algorithms for the same type of hearing loss (Keidser, Brew and Peck, 2003; Sanders et al., 2015). A substantial gain deviation from NAL-NL2 target was found in the high-frequencies (at 4 kHz) for all six HAs (Figure 4; Table 5) and could be explained by the intervention in this study followed standard practice in Denmark in which the adjustments at the fitting session are conducted in a non-systematic way. It is common practice in clinic to initially lower the gain in the high-frequencies, especially at 4 kHz, as part of an acclimatization. Thus, the proprietary initial fit might have prescribed more high-frequency gain than measured at the follow-up, but it is important to remember that all HAs were fitted using the proprietary fitting algorithm, which differs from the NAL-NL2 target. Even though Bell (2009) states that the quality of match to target is limited by the number of channels in the HA, and that the premium devices used in this study did include more compression channels, modern HAs–both premium and basic–in general have many compression channels, which are expected to allow for an accurate fit. The results from this study confirm this statement.

Although most previous studies did not find real-world differences between premium and basic devices, individual characteristics such as noise acceptance and listening demands along with satisfaction in large groups showed to have an impact on the perceived benefit of different HA technologies (Plyler et al., 2021). Those in more demanding listening environments reported significant improvement with premium HAs compared to those in less demanding sound environments. Also, the preference of using premium technology was found to be a factor in Cox et al. (2016), Plyler et al. (2021), and Saleh et al. (2021). These results indicate that candidacy for premium technologies and auditory ecology are highly relevant aspects that should be included in the HA provision process to improve current clinical procedures.

One of the aims of the BEAR project is to evaluate and improve the current hearing rehabilitation, including the HA prescription. Thus, to improve the clinical procedures in the HA provision process and achieve a personalized HA fitting, it is important that clinicians perform a thorough candidacy evaluation and consider the patients' individual listening goals and auditory ecology, including the listening needs in their everyday life acoustic environments (Gatehouse et al., 1999; Jensen & Nielsen, 2005).

This study aimed to investigate if arguments for prescribing a more costly premium HA for older adults with presbycusis could be found, thereby providing clinical guidance in HA prescription. We found only small differences on a subset of the self-reported outcomes between premium and basic level HAs. When HA cost is not covered, studies have shown that patients perform a cost-benefit analysis to decide if the premium HAs provide sufficient additional value to justify the extra cost. In this case, the willingness and ability to pay are important factors during the decision process. In countries were the HAs are free of charge as part of the public healthcare system, it is up to the clinician to decide if premium

technologies are worth the extra cost, and therefore, the decision is based on the individual patient-clinician relationship. A further analysis of the cost-effectiveness of the HA treatment would be interesting but was beyond the scope of the present study.

4.1 Strengths and limitations

The main strength of the current study lies in its study design, which allows for a highly homogenous study population including only patients with symmetric presbycusis. This minimized the variation in patient demographics excludes the risk of different types of hearing loss to affect the results and is a clear advantage compared to other similar studies. In contrast to previous studies, the current study only included first-time HA users to exclude bias towards a certain HA model that would have been a potential risk using experienced users. In addition, this study investigated a larger study population, including 190 patients compared to 24, 25, or 45 subjects, respectively, included in previous studies (Cox, 2014; Cox, 2016; Johnson, 2016; Johnson, 2017; Plyler et al., 2021). However, it is important to note that the pathology behind the presbycusis hearing loss can be different for the patient cohort in the current study which yields different audiometric phenotypes according to Dubno et al. (2013). Thus, differences in phenotype might affect the reported hearing ability. The fact that the HAs used in the current study were free of charge can be considered as a strength, as this removes the economic bias that is found to be an important factor for perceived HA benefit. Further, it was also a strength that the patients in the current study were treated as a part of a much larger study group (the BEAR group), and therefore no special attention was given towards the fitting of these patients. The researchers were not actively involved in collecting data before the follow-up, and they were blinded towards the level of technology for the analysis, hence, blinding was carefully controlled to limit researcher bias.

An important limitation to consider is that the outcome measures used in this study reflect the patients' perspective and might not be sensitive to the actual differences observed in their daily lives. Using self-reported measures such as the SSQ-12, we do not know if the addressed listening conditions were relevant or important to each HA user, or whether other relevant or important conditions were not addressed. A sentence-based test like HINT (Nielsen and Dau, 2011) or other Matrix tests (Kollmeier and Wesselkamp, 1997; Wagener, 2004; Gazia et al., 2022) could have been a more accurate measure to reveal the differences between premium and basic HA technology. The lack of user-environment logging data is a limitation of the study that could have provided insights to potential differences in their daily life sound environments. Another important aspect to consider is that the "wow-effect" among first-time users may dilute the perceived differences between device technologies and therefore less likely to detect differences between premium and basic technologies. However, as this study compares two groups of first-time users using only one technology level, this risk limited. Although patients reported no prior HA experience, they might have been previously fitted with HAs from a private vendor for a shorter trial period, but this was not included in the referral letter from the ENTs. The HA fitting in this study followed the current protocol in Denmark in which the adjustment of HAs are managed in a non-systematic way. We acknowledge that it might be a potential limitation of the current study, but we tried to control for this by including mean gain deviations in the regression analysis. The maximum power output (MPO) of each individual fitting was not registered, so we are unaware of potential differences in MPO between the premium and basic HA models. The counselling part was also non-systematic which might bias the results, but due to the randomized trial design, this potential bias should be equally representative across the two groups. At the time of inclusion, it was not known if patients were still employed full-time, or if they had unusually high leisure activity levels, so that the randomization of HA technology could not be based on these factors. However, as reported in Table 1, the vast majority of patients were retired. One could speculate that the results would be affected by including more patients in full-time employment because it might increase the need for more advanced feature settings in their HAs due to more difficult listening environments or higher listening demands. Patients' personalized listening goals were not addressed as suggested in the best-practice guidelines, which are considered important for the perception of the HA fitting. Also, the fact that patients were not completely blinded in regards to the selected type of HA could have introduced some bias into the results. If patients knew they were fitted with a more advanced HA model, it might have affected the reported outcome measures. Finally, the generalizability of the current study is limited to community-dwelling older adults with presbycusis and without previous HA experience.

5 Conclusion

The current study aimed to provide clinical guidance in HA prescription, to improve current clinical procedures for older adults with presbycusis. Premium HAs yielded slightly better self-reported hearing abilities in older adults with symmetrical presbycusis without previous HA experience, but this does not necessarily apply to other types of hearing loss, and the clinical relevance of the results is limited. Hence, there is limited evidence to support the choice of more costly premium technologies, and clinicians should therefore be careful not simply to conclude that more advanced technologies always produce a better outcome. Differences in real-ear insertion gain at first-fit did not explain the differences in reported outcome. Hearing care providers should continue to insist on evidence to support the choice of more costly premium technologies and include aspects as candidacy and auditory ecology in the HA provision to achieve a more personalized fitting and improve rehabilitation outcome.

Data availability statement

The raw data supporting the conclusion 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 Regional Committees on Health Research Ethics for Southern Denmark (S-20162000-64). The patients/participants provided their written informed consent to participate in this study.

Author contributions

SH, JS, DH, and CG contributed to the study concept and design. SH, AW, JS, DH, and MG recruited subjects and SH and AW was responsible for the survey. SH, L-TT, JS, TN, DH, and SN contributed to the analysis and interpretation of data. SH, L-TT, and JS prepared 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.

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Loneliness and older adults: psychological resilience and technology use during the COVID-19 pandemic—a cross sectional study

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Introduction: This study investigated how psychological resilience influenced greater technology use among older adults, and whether they moderated the impact of social isolation on loneliness during the COVID-19 pandemic. We also explored whether technology mediates the impact of psychological resilience on loneliness. To explain the relationship between variables, the research drew upon the socio-emotional selective theory, which posits the notion that older adults are more focused on current and emotionally important relationships and goals concerning emotional regulation goals such as psychological well-being.

Methods: Using a cross-sectional observational design, data were collected from 92 residents aged 65 to 89 in England from March 2020 to June 2021. Participants completed the Connor–Davidson Resilience Scale, Technology Experience Questionnaire, UCLA Loneliness Scale, and Lubben Social Network Index. Pearson correlation, mediation and moderation analyses were conducted to investigate the hypotheses.

Results: Most participants experienced moderate to severe levels of loneliness, displaying higher levels than pre-pandemic. Psychological resilience predicted greater technology use, and lower levels of loneliness. Technology was found to mediate the relationship between psychological resilience and loneliness. Neither technology use, nor psychological resilience was found to moderate the impact of social isolation on loneliness.

Discussion: Findings suggested that strategies directed towards screening older adults for psychological resilience levels and low technology experience may help identify those most at risk for adapting poorly when exposed to stressors in situations like the Covid-19 pandemic. Early interventions can be initiated to increase psychological resilience and technology use, including empirical interventions, that may help decrease loneliness, especially in times of elevated risks for loneliness.

KEYWORDS

loneliness, psychological resilience, older adults, cross-sectional study, technology

1 Introduction

Loneliness is subjective distress resulting from a discrepancy between desired and perceived social relationships (e.g., Perlman and Peplau, 1981) and is associated with depression, anxiety, functional disability and physical symptoms such as pain (Hoogendijk et al., 2020). Globally, older adults were already experiencing high levels of loneliness and social isolation before the pandemic (Berg-Weger and Morley, 2020), with loneliness predicting a range of common health risks, including increased systolic blood pressure (Hawkley and Cacioppo, 2010), infection (Pressman et al., 2005), impaired cognitive function (Wilson et al., 2007), depression (Cacioppo et al., 2010), diminished immunity (Kiecolt-Glaser et al., 1984) and mortality (Brummett et al., 2001). Loneliness is also associated with an increased risk of cognitive decline (Hawkley and Cacioppo, 2010; Ellwardt et al., 2013), dementia (Sutin et al., 2020) and the progression of Alzheimer's disease (Wilson et al., 2007). It is also known to increase the chances of premature death by 14% (Caballero et al., 2018). Holt-Lunstad et al.'s (2015) observation that social isolation and loneliness is a health risk factor comparable to smoking has been a significantly important message for policy makers and service providers long before the start of the pandemic. Although social isolation and loneliness can exist separately, it is not uncommon for them to coexist, and for social isolation to predict loneliness (Stepanikova et al., 2010).

During the pandemic, the increased risk of older adults contracting COVID-19 and having it progress to a life-threatening state (Ritchie et al., 2020) increased their vulnerability to the disease. Although reducing COVID-19 risk, government mandated social distancing measures potentially worsened the burgeoning problem of social isolation in older adults (Carmen, 2020; Groarke et al., 2020; Van Tilburg et al., 2021; Balki et al., 2022b) and with it, accompanying potential negative outcomes including loneliness. Groarke et al. (2020), whilst studying individuals aged between 18 and 87 between March 23rd and 24 April 2020, showed that disease-containment policies that increase social isolation placed individuals at higher risk of loneliness and continue to do so. In another study by Van Tilburg et al. (2021) on 1,679 Dutch community-dwelling participants aged 65–102 years found that pandemic had increased loneliness. Personal losses, worries about the pandemic, and a decline in trust in societal institutions were associated with increased mental health problems and loneliness (Van Tilburg et al., 2021). Substantial evidence pointed towards an increase in loneliness and its impacts amongst older adults during the pandemic (Emerson, 2020; Kotwal et al., 2021; Krendl & Perry, 2021). This view was mirrored in other studies which found that as physical distancing rules have tightened, rates of loneliness have risen, which may have exacerbated pre-existing mental health conditions (Groarke et al., 2020; Hwang et al., 2020). At its baseline loneliness is associated with worse physical and mental health (Beutal et al., 2017) and increases mortality risk (Holt-Lunstad et al., 2015; Rico-Uribe et al., 2018). Despite the well-established correlations between social isolation, loneliness, disease and mortality and their exacerbation being confirmed during the pandemic, there is a dearth of studies that look at the impact of personal resources the older adults may have used to mitigate the impact of social distancing.

One such personal resource is psychological resilience. Psychological resilience is defined as the process of adapting well in the face of adversity, trauma, tragedy, threats, or significant sources of stress (Sisto et al., 2019). As much as resilience involves "bouncing back" from these difficult experiences, it can also involve profound personal growth (Netuveli et al., 2008). Studies have suggested that although the existence of psychological resilience is universal, it can be thought as a continuum with some people having more resilience than others and it also increasing or decreasing in tandem with situational circumstances (Fletcher and Sarkar, 2013). Psychological resilience can also be considered as either a trait or a process/outcome. As a process, researchers have referred to it as a dynamic process encompassing positive adaptation when facing significant adversity (Luthar et al., 2000). As a trait, psychological resilience represents a constellation of characteristics that enable individuals to adapt to the circumstances they encounter (Connor and Davidson, 2003). During the pandemic, resilience may have had protective effects on the physical and mental status of individuals experiencing or facing adversity and could have impacted loneliness positively (Wortzel et al., 2021). Studies examining resilience in older adults during the pandemic have generally found it to be higher in older adults with Vannini et al. (2021) reporting mean total score for resilience on the CD-RISC-10 questionnaire being 29.5 (based on 141 participants with mean age of 74.4). Scores above 25 are considered to be associated with high average resilience.

The positive impact of psychological resilience on loneliness has been documented in earlier studies (Jakobsen et al., 2020). For example, Gerino et al. (2017) linked loneliness with resilience, mental health, and quality of life in older adults, finding that a high degree of resilience contributed to heighten perceived life quality at the physical and psychological levels and reduced anxiety, depressive symptoms, and loneliness. Although some studies during the pandemic have examined the impact of resilience on loneliness for younger adults (Labrague and Santos, 2020; Marchini et al., 2021), the nexus remains largely unexplored in older adults.

However, other studies during the pandemic found a connection between stress and anxiety and increasing feelings of isolation and loneliness (Balki et al., 2023). Gonçalves et al. (2020) found that when older adults feel worried, particularly about COVID-19, the detrimental effects of social isolation can be amplified on loneliness. Inversely, a sense of being able to successfully adapt to challenging experiences (resilience), can emerge as a potential buffering factor on the impact of social isolation on loneliness (Grossman et al., 2021). These processes and characteristics may have created a defence mechanism in the shape of psychological resilience and against increased social isolation thereby moderating its impact amongst older adults during the pandemic (Patel and Clark-Ginsberg, 2020). Thus, it is clear that its important to take into consideration the impact of resilience on loneliness and social isolation especially in times of elevated stressful conditions such as those imposed as a result of the pandemic.

Another personal resource that older adults could have employed to combat social isolation during the pandemic was technology use. Social support via technology is known to mediate the effects of life stress and loneliness (Pawar and Rathod, 2007; Sippel et al., 2015), but also supports the

development of psychological resilience during crisis, and is linked with a reduction in depression, loneliness and an increase in self-esteem (Zautra et al., 2010). If the problem during the pandemic-imposed social distancing measures resulted in deprivation or reduction in social contact, the impact may have also been lessened through use of digital communication technologies (DCT). Videoconferencing apps (such as Teams), instant messaging apps (like WhatsApp) and services (such as Zoom) have grown in popularity during the COVID-19 pandemic, for both business and social activities. It was possible that the use of technology might have mitigated the impact of social isolation, with technologies being used in place of previous in-person visits from friends, family and volunteers. Online group meetings were being orchestrated through videoconferencing programs, as well as religious gatherings like Sunday church gatherings, yoga (Belam, 2020), playing an online game (Nguyen et al., 2017) or new music technology (Court-Jackson, 2011), which all may have decreased feelings of loneliness. Technology use is therefore a logical avenue to investigate as a potential mitigating factor for the impacts of social isolation on older adults but may also have had a potential impact in increasing psychological resilience and through the ability to expand the depth and extent of connectivity (Jurgens and Helsloot, 2018).

It remains possible that technological skills acquired prior to the pandemic were used even more as older adults sought out pathways to remain socially connected through DCT. This investigation aims to explore the relationship between psychological resilience and technology use amongst this demographic and their impact on loneliness levels.

1.1 Conceptual framework

To conceptually explain how psychological resilience and technology use could have impacted older adults during the pandemic we drew upon the socio-emotional selectivity theory (SST) and resilience theory. SST argues that as older adult perceive time as more limited (a point that may have been further reinforced by the effect of the pandemic), older adults will value meaningful goals and relationships more than other goals (Kircanski et al., 2016); see also Galindo-Martin et al., 2020). This could have activated mood enhancing goals, as well as making older adults more willing to accept temporary negative experiences such as social distancing, for long term benefits leading to higher psychological resilience to adverse effects. Scheibe and Carstensen (2010), reported similar behaviorism in older adults relating to a shift occurring with age towards more positive disposition. Equally, these factors could have also made older adults seek activities that require technology whilst being socially isolated, like maintaining a connection with loved ones, or a technology enabled mood enhancing activity.

The pandemic can be partly thought of being similar to a natural disaster and may have had similar consequences on older adults. Older adults have been shown to have exhibited higher resilience linked to SST during natural disasters and have more positive disposition than other age groups (Eshel et al., 2016; Rafiey et al., 2016). Other studies have shown that older adults were showing high levels of resilience and coping well during the pandemic strengthening this argument (Fuller and Huseth-Zosel, 2020; Vannini et al., 2021).

Our study aims to provide further empirical support for SST supposition that during the pandemic older adults had high resilience, maintained established relationships especially using technology, and may have provided a degree of protection from the impact of social isolation on loneliness. We use the SST to explain how psychological resilience could have further mediated the relationship between technology and loneliness.

1.2 Hypotheses

The following hypotheses provide a basis to investigate these factors:

H_1. Higher psychological resilience will negatively predict loneliness.

H_2. Higher psychological resilience will be correlated with greater use of technology.

H_3. Greater use of technology will reduce loneliness after controlling for the impact of social isolation.

H_4. Technology will mediate the relationship between psychological resilience and loneliness.

H_5. Higher psychological resilience and technology will moderate the impact of social isolation on loneliness.

2 Material and methods

2.1 Study design and setting

This quantitative cross-sectional observational research employed the STROBE checklist (Strengthening the Reporting of Observational Studies in Epidemiology) von Elm et al. (2007). The study was conducted in England starting in 16 March 2020, to 21 June 2021, during the height of the government-mandated COVID-19 social distancing period.

2.2 Participants and sampling

A large majority of recruited participants (>80%) were located in the Northwest. The inclusion criteria were older adults (>65) (age inclusion criterion specified by American Psychological Association, 2002); proficient in the English language; and living in their own homes. Older adults living in nursing or care homes, with a history of mental health issues, and who did not speak English, were considered ineligible for this study, due to the variation in ability to participate in this research. Recruitment was conducted through advertisements in senior citizen resource centers, housing associations, third sector organizations, social activity clubs, and local senior groups, via personal approach, and word-of-mouth recommendation. Prospective volunteers telephoned and left a voicemail or sent an email to the researcher, after which a callback determined eligibility.

To determine the minimum size of the research sample necessary for the empirical verification of the tested moderation model, G*Power software (Faul et al., 2009) was used with effect size f2 = 0.15, power = 0.80, and 3 predictors options using multiple regression

for the sample size analyses. The total sample size was determined to be 87. A total of 110 volunteers signed up; however, 18 did not complete the questionnaires in their entirety and were excluded. The achieved sample was 92 volunteers aged 65 to 92 (M = 74.6 years, SD = 7.23). All participants identified as either male or female, with more women (n = 55/92, 60%) than men. More than 89% of the participants were White, with less than 11% representing ethnic minorities (n = 7, British Asian, n = 3, British Black). In the Northwest of England, less than 1.4% of the over 65 population is British Black, and less than 6.2% is British Asian (Kings Fund, 2006), and therefore our sample seemed to be representative of areas participants were recruited from.

Sampling ensured a diverse statistically significant representation of the older adult population in England. Due to the difficulties encountered in recruitment during the pandemic and the shrinking time span (to capture maximum effects), we focused on periods when life-space mobility was most restricted.

2.3 Variables and measures

Participants first completed a background questionnaire that was developed based on SAGE Encyclopedia of Communication Research Methods (Allen, 2017) as part of a larger study and the variables used in this study included age and ethnicity.

Loneliness was measured using the 20-item UCLA Loneliness Scale (Russell, 1996) with scores ranging from 20 to 80. Higher scores reflected higher loneliness (Cronbach's alpha = .88).

Technology use was measured using the Technology Experience Questionnaire (Czaja et al., 2006). Participants were presented with a list of technologies (representing communication technology, computer technology, everyday technology, health technology, recreational technology and transportation technology) and were asked to indicate their familiarity with each on a 5-point scale. Scores ranged from 0 to 180 with higher scores indicating greater use and familiarity with technology (Cronbach's alpha = .84).

Resilience was assessed by the 10-item Connor–Davidson Resilience Scale (CD-RISC-10; Connor and Davidson, 2003), where items were rated on a 5-item scale ranging from 1 (*strongly disagree*) to 5 (*strongly agree*). Questions were described in clear language, for example, "I believe I can achieve goals despite obstacles", with the surveyor explaining questions where they may not have been understood. The possible scores ranged from 0 to 60 with higher scores reflecting greater resilience (Cronbach's alpha = .84)

The 12-item Lubben Social Network Index (LSNS-12; Lubben et al., 2006) measured social network size and support, reporting on social isolation levels. The possible score range was 0–60 with a higher score indicating more social engagement and greater social connectedness (Cronbach's alpha = .88).

2.4 Ethics

Ethical procedures aligned with the British Psychological Society guidelines. The study received ethical approval from the University Faculty Research Ethics Committee (Ref: FHMREC19121). Participants were provided with an information sheet and allowed to ask any questions. They were informed of their rights to withdraw at any point in the research and advice about anonymity. Their consent was given either via email or read over the telephone. Data were captured over the phone after the identity of the participant was confirmed, recorded in spreadsheets and anonymized thereafter.

2.5 Procedure

Telephone surveys collected information on loneliness, social isolation, technology use and psychological resilience in addition to basic demographic information. Google Analytics was used to record and tabulate the data, with further analysis done using IBM SPSS Ver 28. Participants completed the assessments across 14 months, spanning various levels of COVID-19-related lockdown measures.

2.6 Statistical methods

All analyses were carried out using 95% probability. There were no missing data identified among the observations obtained. The variables of loneliness, technology use, social isolation and psychological resilience were screened for skewness and kurtosis to assess the deviation of their distributions from normality using a histogram with simulated overlapping normal curves. The homoscedasticity of the residuals was checked using a standardized residual versus a standardized predicted plot. Mahalanobis ($p < 0.001$) and Cook's distance were used to check for a linear relationship between dependent and each independent variable using a scatterplot matrix of dependent and continuous independent variables to establish if there were any high leverage points, significant outliers, or highly influential points. Before removing any significant outliers, a linear regression was performed to check the variance caused by the data point included and if it needed to be removed from the dataset. The criteria for discarding observations were the inability to meet two of the three gauges of the distance measures used. However, no outliers were found that would significantly impact the findings, and thus, none were removed. Confirmation of independence of observations and the assumption of no autocorrelation in residuals was checked using the Durbin-Watson d-statistic.

Initial descriptive analyses included frequencies, means and standard deviations. Pearson product-moment correlation coefficients were calculated to determine if there was an association between dependent and continuous variables, whether higher psychological resilience predicted lower loneliness (Hypothesis 1) and greater use of technology (Hypothesis 2).

Multiple linear regression models were built to evaluate whether greater technology use predicts reduced loneliness after controlling for social isolation to examine Hypothesis 3. The associated predictor variables were entered into the model and a backward elimination approach was used, removing any variable with $\alpha > 0.15$ (here α is defined as the critical p-value).

To test whether psychological resilience mediates the relationship between technology use and loneliness (Hypothesis 4), we used Hayes' PROCESS macro (v3.2) (Hayes, 2017) Model 4, which allows testing the mediating relationship with bootstrap

TABLE 1 Descriptive statistics (*N* = 92).

Scale	Minimum	Maximum	*M*	*SD*
UCLA-Loneliness Score	20	80	47.49	17.814
LSNS-12	1	49	26.91	15.304
CD-RISC-10	5	36	21.76	10.543
Technology Experience	48	175	116.87	40.951

confidence intervals (CIs) for an indirect effect. We applied a bootstrapping approach to determine the indirect effect for each of the bootstrapped 5000 sample items from the original dataset using stochastic sampling with replacement. As a nonparametric resampling procedure, bootstrapping is considered the most powerful method for small samples because it is the least vulnerable to Type I errors. If the CIs did not include zero, then the effects were significant ($p < .05$).

Hayes's (2017) PROCESS macro for SPSS with Model 1 was applied to investigate the moderating effects of psychological resilience and technology use on social isolation for loneliness as per Hypothesis 5. Moderation effects were examined by comparing the stratified models using Z-scores. If the standardized coefficients of the interaction terms were significant ($p < .05$) or marginally significant ($p < .09$), we conducted a simple slope test to examine the interaction effect at different levels to explain the moderating effect further.

3 Results

Table 1 shows the results for calculated means and standard deviations, maximum and minimum as basic descriptive statistics.

Participants demonstrated moderate to high levels of loneliness with 44% of older adults demonstrating loneliness scores of above 50 (Russell, 1996). The Lubbens Social Network Scale (LSNS-12) indicated that the majority of participants reported good levels of social connectedness with 82% scoring above 25. For psychological resilience (CD-RISC-10), most participants (>57%) scored above 25. As far as technology use was concerned, most participants scored above 125 (56%), demonstrating high use and familiarity with technology in general (Czaja et al., 2006). However, we did find that a significant number of participants (32%) scored below 120, which indicated low familiarity and use of technology (Czaja et al., 2006) and a binormal distribution.

Pearson correlation coefficients were conducted to establish the relationship between loneliness, technology, and social isolation. A correlation matrix of the variables was examined to investigate hypotheses 1 and 2. Table 2 presents the results from the correlational analysis.

Hypothesis 1: The correlational relationship between psychological resilience and loneliness

Table 2 shows that the correlation between psychological resilience and loneliness score was statistically significant ($r = -0.885$, $p < 0.001$) and negatively correlated. This meant that higher psychological resilience correlated with lower loneliness scores, thus supporting our first hypothesis.

Hypothesis 2: Higher psychological resilience is correlated to greater technology experience

Table 2 shows that the correlation between psychological resilience and technology experience was statistically significant ($r = .610$, $p < 0.001$) and positively correlated. This meant that higher psychological resilience was correlated with higher levels of technology use during the pandemic, thus supporting our fourth hypothesis.

Hypothesis 3: Greater use of technology predicts lower loneliness, after controlling for social isolation

Estimates regarding the residual of the hierarchical multiple regression model on loneliness were checked and found to follow a normal distribution. Analysis examining whether greater technology use level predicts lower loneliness after controlling for social isolation (Hypothesis 3) was conducted using hierarchical multiple linear regression analysis. Loneliness was set as the dependent variable, technology experience as independent variable and social isolation as the control variable. Table 3 shows the coefficient results of the multiple regression analysis.

Results showed that both technology experience ($b = -0.098$, $t = -3.645$, $p < 0.001$) and social isolation ($b = -0.847$, $t = -11.727$, $p < 0.001$) were significant negative predictors of loneliness, i.e., higher social connectedness and greater technology use was linked to lower loneliness scores. The results of the ANOVA test for the significance of the regression models showed that the combined effect for both predictors was significant ($F(2, 89) = 143.721$, $p < 0.001$). Adding social isolation to the multiple regression model changes the value of R^2 by 0.035 ($p < 0.001$). Therefore, technology experience significantly predicted loneliness score after controlling for social isolation, thus confirming our second hypothesis.

Hypothesis 4: The mediating role of technology use between psychological resilience and loneliness.

TABLE 2 Correlational analysis between variables (*N* = 92).

		UCLA- loneliness score	LSNS-12	CD-RISC-10	Technology experience
UCLA-Loneliness Score	Pearson correlation	1	−.853**	−.885**	−.631**
LSNS-12	Pearson correlation	−.853**	1	.866**	.557**
CD-RISC-10	Pearson correlation	−.885**	.866**	1	.610**
Technology Experience	Pearson correlation	−.631**	.557**	.610**	1

**$p < 0.01$ (two-tailed).

TABLE 3 Model output and coefficients of multiple linear regression model on loneliness.

Model	Regression equation		Overall fit				Significance of regression coefficient	
	Dependent variable	Independent variable	R	R^2	Δ R^2	F	B	t
Model 1	Loneliness		0.853	0.728		241.216		
		Intercept					74.224	37.533***
		Social Isolation					−0.993	−15.531***
Model 2	Loneliness		0.874	0.764	0.035	143.72		
		Intercept					81.778	29.403***
		Social Isolation					−0.847	−11.727***
		Technology Experience					−0.098	−3.645***

***$p < 0.001$.

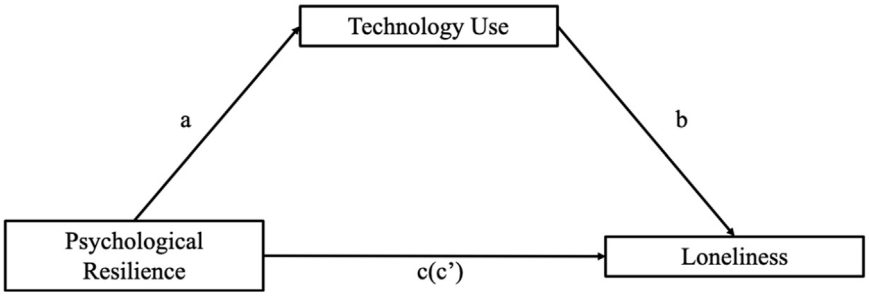

FIGURE 1
The mediating role of technology use between psychological resilience and loneliness. Here, a represents the effect of psychological resilience on technology use, b represents the effect technology use on loneliness, c represents the total effect of psychological resilience on loneliness. c' represents the direct effect of psychological resilience on loneliness.

Using Model 4 in SPSS's PROCESS macro40 compiled by Hayes (2012), we tested the mediating effect of technology use in the relationship between psychological resilience and loneliness, with the results summarized in Table 6 and seen in Figure 1.

Table 4 shows that psychological resilience had a significant predictive effect on loneliness (path c) (B = −0.88, t = −18.0254, $p < 0.001$), and when technology use (the intermediary variable) was put in, the direct predictive effect of psychological resilience on loneliness (path c') was still significant (B = −0.80, t = −13.1933, $p < 0.001$), indicating incomplete mediation. The positive predictive effects of psychological resilience on technology use (path a) (B = 0.61, t = 7.298, $p < 0.001$) and negative effects of technology use on loneliness (path b) (B = −0.15, t = −2.4139, $p < 0.05$) were also significant. Boot strap mediation was tested where Boot LLCI and Boot ULCI are 95% confidence limits. If the 95% confidence limit includes zero, the indirect effect test is not significant. The direct effect of psychological resilience on loneliness was established (upper and lower limits of bootstrap at the 95% confidence interval [−1.55 −1.14] did not contain 0), while the mediating effect of technology use was not significant (upper and lower limits of bootstrap at the 95% confidence interval [−0.42 0.04] contained 0); the bootstrapped mediation indicated that technology use only partially mediated the relationship between psychological resilience and loneliness as shown in Table 5.

Hypothesis 5: Psychological resilience and technology experience will moderate the impact of social isolation on loneliness.

We conducted the test of this hypothesis in two subsections. Model 2 was used in the PROCESS 4.0 macro for SPSS to examine the moderation effect of psychological resilience on social isolation for loneliness first, followed by moderation effect of technology experience on the relationship between social isolation and loneliness as proposed in Hypothesis 5 (Hayes 2018) and as presented in Figures 2A,B.

Here, all continuous variables were converted to Z-scores for use in the model. Z-scores describe the position of raw scores in terms of their distance from the mean, when measured in standard deviation units and standardize the distribution. Table 6 shows that the unconditional interaction (unconditional interaction looks at mean interaction variables) of social isolation and psychological resilience was not significant (β = 0.07, t = 0.8, $p > 0.05$).

We also saw that unconditional interaction of social isolation and technology use was not significant (β = −0.01, t = −0.67, $p > 0.05$) either. To check whether there was any conditional interaction between the variables, we carried out a simple slope test as can be seen in Figures 3A,B.

TABLE 4 Intermediary model test of technology use.

Regression equation		Overall fit			Significance of regression coefficient	
Dependent variable	Independent variable	*R*	R^2	F	B	t
Loneliness		0.88	0.78	324.9142		
	Psychological Resilience (c)				−0.8849	−18.0254***
Technology Use		0.61	0.37	53.2609		
	Psychological Resilience (a)				0.6097	7.2980***
Loneliness		0.89	0.80	174.084		
	Technology Use (b)				−0.1457	−2.4139*
	Psychological Resilience (c')				−0.7961	−13.1933***

*$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.
Note: All the variables in the model are standardised and brought into the regression equation.

TABLE 5 Decomposition table of total effect, direct effect, and indirect effect.

	Effect	Boot SE	Boot LLCI	Boot ULCI	Relative effect value (%)
Total effect	−1.4953	0.0830	−1.6601	−1.3305	
Direct effect	−1.3452	0.1020	−1.5478	−1.1426	89.96
Indirect effect	−0.1501	0.1193	−0.4187	0.0409	10.04

This intermediary effect accounted for 10.04% of the total effect.

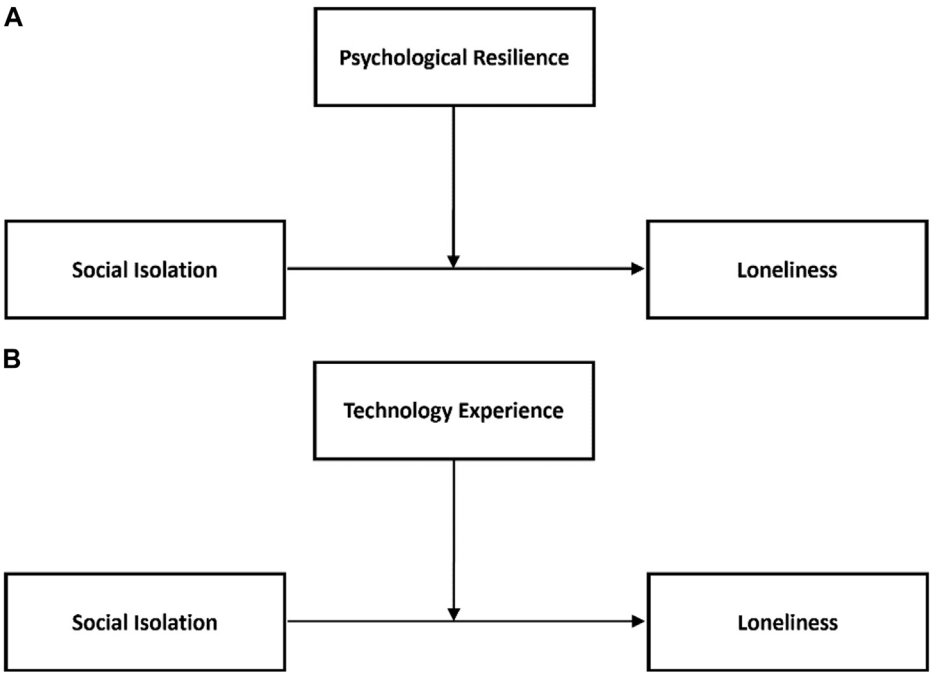

FIGURE 2
(A) The moderating role of psychological resilience on social isolation. **(B)** The moderating role of technology experience on social isolation.

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TABLE 6 Moderation analysis for the effect of social isolation on loneliness with technology use and psychological resilience as moderators.

	Overall fit indicators			Significance of standardized coefficient	
Independent variable	*R*	R^2	*F*	*β*	*T*
	0.91	0.83	81.60		
Social Isolation (ZSN1)				−0.36	−3.73***
Psychological Resilience (ZPR1)				−0.50	−4.67***
ZSN1*ZPR1				0.07	0.8
Technology Experience (ZTE1)				−0.11	−1.83
ZSN1*ZTE1				−0.01	−0.67

Note: *** $p < .001$.
Note: All the variables in the model are standardized and brought into the regression equation.

When psychological resilience was low, social isolation and loneliness were also not significantly correlated (βsimple(M-1SD) = 0.134, $p > 0.05$). Moreover, when the psychological resilience was high, social isolation and loneliness were not significantly correlated (βsimple(M+1SD) = 0.11, $p > 0.05$). This is visible in Figure 3A, with all three slopes parallel to each other. Next, when the technology experience was low, social isolation and loneliness were not found to be significantly correlated (βsimple(M-1SD) = 0.057, $p > 0.05$). Moreover, when the technology experience was high, social isolation and loneliness were again not significantly correlated (βsimple(M+1SD) = 0.046, $p > 0.05$). This can also be noted by the fact that all three slopes are parallel to each other in Figure 3B. Thus, we can conclude that higher psychological resilience and technology use do not significantly moderate the impact of social isolation on loneliness. Therefore, our fifth and final hypothesis was rejected.

4 Discussion

This study set out to examine the role played by psychological resilience and technology use on loneliness levels of older adults during the COVID-19 pandemic. We explored the mediating role played by technology in the relationship between psychological resilience and loneliness. We also explored whether psychological resilience was correlated with higher technology use and whether it played a moderating role in the effect of social isolation on loneliness. We also explored the relationship of technology experience with psychological resilience.

Our study found higher levels of loneliness during the height of the Covid-19 pandemic, especially when compared to pre-pandemic data. Victor and Yang. (2012) loneliness levels showed 30% on average, while Hawkley et al.'s (2020) comparison of loneliness across two continents found the prevalence to be around 25% in older adults compared to the 44% in this study using the UCLA loneliness scale. This confirms the heightened occurrence of loneliness prevalence during the pandemic and concurs with several studies that found a similar condition (Bu et al., 2020; Elran-Barak and Mozeikov, 2020; Emerson, 2020; Krendl and Perry, 2021).

The ability to use technology successfully to adapt to challenging experiences during lockdown emerged as a potential factor to reduce loneliness. Higher technology use was associated with lower loneliness. Having the ability to adapt to challenging experiences (psychological resilience) also predicted lower levels of loneliness. Psychological resilience was correlated to higher technology use, which seems to indicate that resilience may be playing role in increased use of technology during the pandemic. This is a notable finding and could potentially be because of perseverance and better coping attitudes associated with higher psychological resilience. The SST theory posited an explanation that older adults in times of stress could be seeking access to information that equipped their coping mechanisms better, using technological means, communicating with friends and family. This information could have also given them ability to cope with stressful situations but also potentially the ability to learn and persist with using technological tools, a point that has been alluded to in previous studies pointing to a bidirectional relationship (Bustinza et al., 2019). In a recent study by Savitsky et al. (2020), higher levels of resilience and positive coping skills related to decreased levels of pandemic related anxiety among participants during government mandated social distancing. The relationship between technology experience and psychological resilience appears to be complex, bidirectional and needs further in-depth research. It should be noted that the lack of pre-pandemic data on participants precludes any certainty that the justification for the results is valid.

Technology use was found to mediate the relationship only partially between psychological resilience and loneliness (Hypothesis 3). This was a notable observation as individually both psychological resilience and technology use were found to have an unconditional direct impact on loneliness levels. Technology could have an impact on helping older adults in finding new and effective pathways to connect with others and access information that would have mitigated thoughts that enhance loneliness. Systematic reviews across disciplines have conceptualized psychological resilience as encompassing multiple components including: a personal characteristic shaped by social contexts, a dynamic and agentic process of adapting to challenges or stressors, and an outcome that is favorable in spite of adversity or trauma (Hjemdal et al., 2006; Windle, 2011; Smith and Hayslip, 2012). However, technology could have also given access to negative

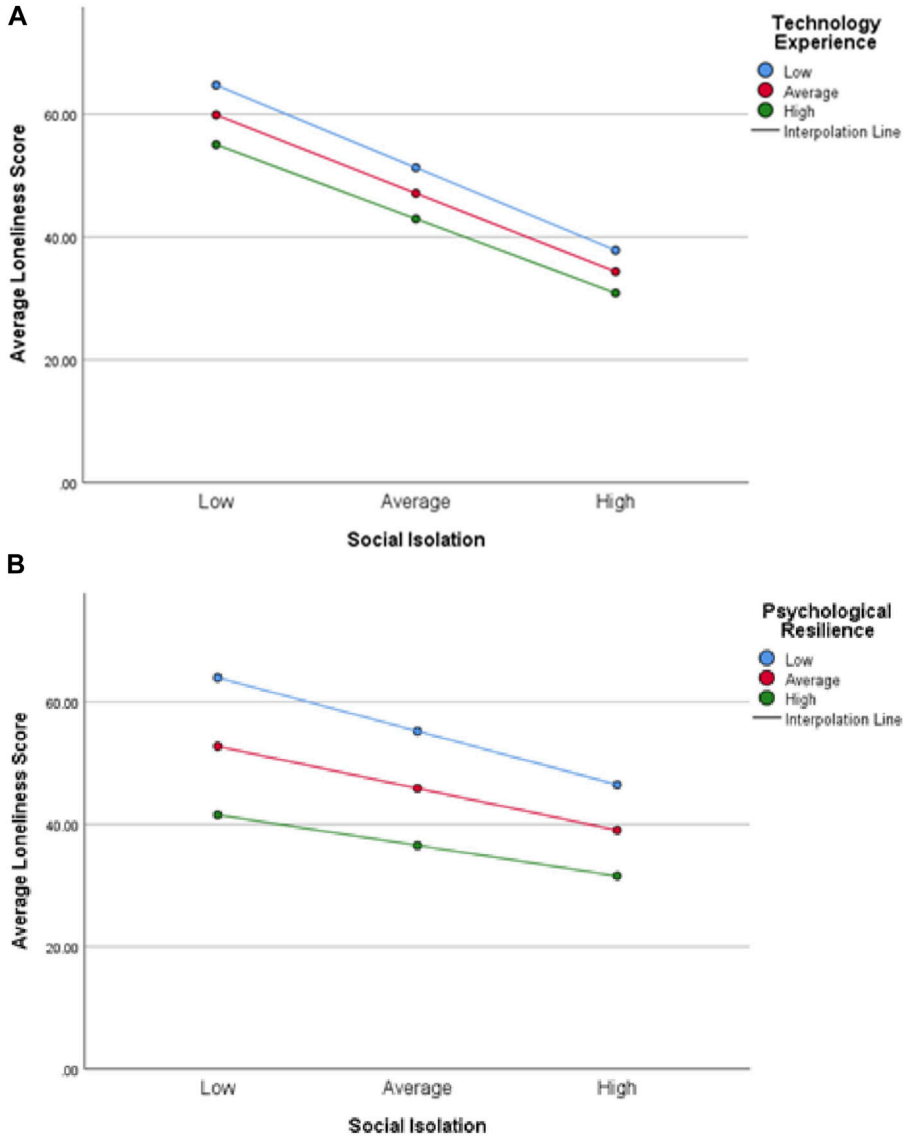

FIGURE 3
(A) Moderating effect of psychological resilience on social isolation. **(B)** Moderating effect of technology experience on social isolation.

information regarding the pandemic to older adults thereby neutralizing any mediation impact of technology.

Neither psychological resilience nor technology use moderated the impact of social isolation on loneliness. We can conjecture the reasons why this was possible, and this may be down to older adults having access to information via technology that perhaps led to more negative feelings towards being isolated, or perhaps worsened their isolation, increased anxiety and/or stress, or simply had little to no impact. Mustafa and Gold (2013) presented the concept of a sense of technology enabling a "perpetual contact" with a sense of being "chained," never being able to escape the demands imposed by a persons social network. It is more likely that the latter explanation was more plausible as the plethora of information that increased anxiety related to the Covid-19 pandemic as has been reported in recent studies (Drouin et al., 2020; Balki et al., 2022a; Balki et al., 2023), would have impacted psychological resilience negatively as stress and anxiety have been found to be negatively correlated to psychological resilience (Bitsika et al., 2013).

When linked to the socio-emotional selectivity theory, the pandemic may have influenced older adults to achieve their emotional goals (Carstensen, 1992), bringing them together in positive as well as negative ways. In coping with loneliness, older adults improving relationships through technology means investing in existing contacts and being pre-disposed to positive or negative outlooks.

Our results confirmed the correlation between technology use and lower levels of loneliness. Studies have previously highlighted the potential positive effects of technology use on individual wellbeing by decreasing the likelihood of social isolation due to the increase in connectivity, a sense of belonging, and a decrease in loneliness (Burke et al., 2010; Stepanikova et al., 2010). Technology can increase the potential of expression that is often limited in daily interactions (Rosenberg, 2019) and especially important in times of crisis (Chan, 2013). Technology can also

increase the likelihood of positive social support from social groups, family, friendships, and community that are especially important when someone is disconnected from the external environment, as experienced by older adults in our study in a COVID-19 lockdown situation (Dolev-Cohen and Barak, 2013). The concept of social support is especially pertinent because it mediates the effects of life stress on health and wellbeing (Sippel et al., 2015). Positive social support can protect against stress and facilitate the development of psychological resilience among individuals facing significant adversity (Zautra et al., 2010).

Older adults using technology for "social exchange" may communicate more frequently to exchange information and opinions, such as on the downsides of the pandemic. However, these prompt, spontaneous, and intentional or accidental increases of technology-mediated dialogue could potentially worsen social isolation. The motivation for this behavior occurs when individuals seek to increase and maintain high personal involvement in matters of significance as explained by the social contagion theory (Heath and Porter, 2017). The dual positive and negative effect of technology makes it a complex moderating or mediating variable, and its impact of it in conjunction with psychological resilience needs further investigation. For example, certain information-based technology application (such as SNS) may cause a negative impact, as opposed to others that are purely enable communication (such as videoconferencing).

Psychological resilience and adequate coping skills have been identified as vital personal resources to effectively manage and rebound from stressful situations such as disease outbreaks and disasters (Duncan, 2020). Earlier studies involving the general population have linked psychological resilience to reduced anxiety, stress and depression (Labrague and Santos, 2020) and improved mental and psychological health (Cooper et al., 2020). This study confirmed resilience as a protective factor against loneliness mitigating other negative effects of social distancing and lockdown measures during the pandemic (Groarke et al., 2020).

Technological advancements offer remarkable opportunities for older adults to maintain connections despite the need to stay physically separated. Further, although psychological resilience has a complex but important role to play in alleviating loneliness and greater technology use, there is a need to help people build psychological resilience. Beyond achieving mastery, the key to alleviating loneliness is to encourage a more positive outlook on technology use. Psychological resilience can be perceived as a dynamic, adaptive process that has important implications for cultivating and maintaining health and wellbeing in later life. Resilience can be taught and learned (Manning, 2013), and interventions that help individuals build resilience as a distal resource could have important, long-term effects.

5 Limitations

The study had several limitations, although these did not distract from the potential importance of the findings in relation to understanding how loneliness could be prevented in a time of adversity. First, the data produced through this research design cannot determine causality. Directionality may be important when examining loneliness in the context of resilience as earlier studies suggest that being in a restrained environment contributes to a resilient reaction (Sygit-Kowalkowska et al., 2017). Therefore, a longitudinal study would allow for generalizability, while a mixed-method approach would include the voices of the affected population. Second, the sampling bias was skewed toward populations with access to and literate in digital resources, or those who were more socially connected via virtual platforms. This is because most study participants were recruited through technology, including mobile phones and email. Their ability to manage the technology suggests that such participants may have experienced less loneliness.

Future studies might clarify this issue, as it may be possible to collect more detailed measures in order to receive more accurate data. In addition, the absence of pre-pandemic data precludes comparison with the pre-pandemic measures.

We also found that psychological resilience played a complex interactive role with technology and loneliness, that itself cannot be completely explained through a quantitative analysis. This calls for a qualitative or mixed method study that is able to dive deeper into the precise mechanisms behind these interactions.

6 Conclusion

The findings alluded to the possibility that improvement in loneliness levels is possible by promoting the use of DCT to counter loneliness, imposed by stay-at-home or social distancing orders associated with Covid-19, future pandemics or crises, or even to combat general isolation and loneliness in older adults. Tools such as DCT including the use of generative artificial intelligence (Generative AI) can be integrated into crisis communications, public health responses, and care programs to address loneliness among older adults, and helping them obtain information that would improve resilience in face of difficulties. Taking these elements into consideration will help decision-makers to develop a strong, effective approach. We saw that technology also mediated the relationship between psychological resilience and loneliness. However, psychological resilience and technology did not have a significant moderating impact on the relationship between social isolation and loneliness, hinting at other factors at play and a complex layered picture that needs further investigation.

The research infers that screening of older adults for psychological resilience levels and low technology experience may help identify those most at risk for adapting poorly when exposed to crises such as pandemics and wars. To this end, early interventions can help to build resilience among this demographic.

Data availability statement

The raw data supporting the conclusion 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 Lancaster University Faculty Health Research Ethics

Committee. The patients/participants provided their written informed consent to participate in this study.

Author contributions

EB conceived of the presented idea. EB developed the theory, recruited the participants and performed the computations and analysis. CH verified the analytical methods and reviewed research. CH encouraged EB to investigate impact of psychological resilience as an outcome variable and supervised the findings of this work. All authors (EB, NH, and CH) discussed the results and contributed to the final manuscript. All authors contributed to the article and approved the submitted version.

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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Loop diuretics association with Alzheimer's disease risk

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Objectives: To investigate whether exposure history to two common loop diuretics, bumetanide and furosemide, affects the risk of developing Alzheimer's disease (AD) after accounting for socioeconomic status and congestive heart failure.

Methods: Individuals exposed to bumetanide or furosemide were identified in the Stanford University electronic health record using the de-identified Observational Medical Outcomes Partnership platform. We matched the AD case cohort to a control cohort (1:20 case:control) on gender, race, ethnicity, and hypertension, and controlled for variables that could potentially be collinear with bumetanide exposure and/or AD diagnosis. Among individuals older than 65 years, 5,839 AD cases and 116,103 matched controls were included. A total of 1,759 patients (54 cases and 1,705 controls) were exposed to bumetanide.

Results: After adjusting for socioeconomic status and other confounders, the exposure of bumetanide and furosemide was significantly associated with reduced AD risk (respectively, bumetanide odds ratio [OR] = 0.23; 95% confidence interval [CI], 0.15–0.36; $p = 4.0 \times 10^{-11}$; furosemide OR = 0.42; 95% CI, 0.38–0.47; $p < 2.0 \times 10^{-16}$).

Discussion: Our study replicates in an independent sample that a history of bumetanide exposure is associated with reduced AD risk while also highlighting an association of the most common loop diuretic (furosemide) with reduced AD risk. These associations need to be additionally replicated, and the mechanism of action remains to be investigated.

KEYWORDS

Alzheimer's disease, bumetanide, electronic health record informatics, furosemide, quantitative pharmacology

Introduction

Medical systems generate massive amounts of electronic health record (EHR) data, and researchers have analyzed these data to derive new insights and improve healthcare (Rajkomar et al., 2019; Shah et al., 2019). Stanford University has established a novel and secure data platform: Observational Medical Outcomes Partnership (OMOP) Common Data Model (CDM). Alzheimer's disease (AD) is well suited for analysis with OMOP given its multifaceted complexity, prevalence, and the multitude of small sample size studies that claim benefit for certain interventions.

AD is a neurodegenerative disorder of uncertain cause and pathogenesis. In the United States, as many as one in nine people (10.7%) older than 65 years has AD

(Rajan et al., 2021). Recently, repurposing bumetanide as an AD medication was proposed based on data that showed bumetanide "reversed" *APOE* genotype-dependent transcriptomic signatures in mouse and cell culture models (Taubes et al., 2021). This finding was investigated in two EHR-based cohorts demonstrating that in individuals older than 65 years, bumetanide exposure was associated with lower AD prevalence (Taubes et al., 2021). This finding warrants further validation as bumetanide is more expensive than the commonly prescribed loop diuretic (furosemide), and thus, potential socioeconomic status (SES) confounding such as insurance coverage needs to be investigated. Both furosemide and bumetanide are indicated, and often interchangeably used, for patients with hypertension, congestive heart failure (CHF), and kidney disease. In this study, using Stanford's EHR data, we sought to replicate the bumetanide findings in an independent dataset accounting for SES, hypertension, and CHF, and additionally test the association of furosemide with AD risk.

Methods

Stanford's de-identified OMOP instance hosts multi-factor and multi-modal data, including Stanford's structured clinical data, clinical notes, meta-data on clinical notes, extracted concepts from clinical notes using natural language processing and other approaches, and radiological images. Participants or their caregivers provided written informed consent to store their data in OMOP. The Stanford University institutional review board granted the current study protocol an exemption because the analyses were carried out on de-identified data; therefore, additional informed consent was not required.

We have curated OMOP data for 656,683 patients older than 65 years at their last known visit. We focused on 5,872 patients with AD defined by ICD9 and ICD10 codes (ICD10: G30.1, G30.8, G30.9, and ICD9: 331.0, Table 1). We matched individual AD patients with up to 20 controls on age and exact match of sex, race, ethnicity, and hypertension (Table 2) using R package *optmatch* (Hansen and Olsen Klopfer, 2006). We excluded 937 matched controls with an age difference greater than 5 years and 373 matched controls that belonged to strata with fewer than 15 controls per case, resulting in 5,839 AD cases and 116,103 matched controls.

Statistical analysis

We scanned the data using the medication orders or medical history variable tables to identify those who had been exposed to bumetanide or, respectively, furosemide prior to AD diagnosis. We included any type of exposure to the drug, specifically oral or IV exposures, for any duration of time. We calculated the percent of AD cases and non-AD controls exposed to bumetanide and furosemide using a $\chi 2$ test. In addition, as *post hoc* sensitivity analyses, we calculated the odds ratio of AD diagnosis for those exposed to bumetanide (and separately furosemide) while adjusting for variables that could potentially be collinear with bumetanide exposure and/or AD diagnosis including diagnosis of CHF (defined by the ICD10 of I11, I13, and I50, Table 1), insurance type, and median income (defined by the patient's recorded zip code, derived from publicly available data from the United States Census Bureau), and explored the relationship of AD with the other commonly used loop diuretic, furosemide. Statistical analyses were performed using R (version 3.6.3). The results are shown in Tables 3A,B.

Results

A total of 1,732 patients (27 cases and 1,705 controls) were exposed to bumetanide during any of their visits (prior to their AD

TABLE 1 ICD9 and ICD10 code description.

ICD9/10 code	Description	Number of patients with this diagnosis code as their first Alzheimer's diagnosis	
		Male	Female
G30.1	Alzheimer disease with late onset	86	128
G30.8	Other Alzheimer's disease	76	148
G30.9	Alzheimer's disease, unspecified	705	1062
331	Alzheimer's disease	804	1380
I11.0	Hypertensive heart disease with heart failure	1223	1775
I13.0	Hypertensive heart and chronic kidney disease with heart failure and stage 1 through stage 4 chronic kidney disease, or unspecified chronic kidney disease	1406	1485
I13.2	Hypertensive heart and chronic kidney disease with heart failure and with stage 5 chronic kidney disease, or end-stage renal disease	129	144

ICD9 and ICD10 codes at first diagnosis.

TABLE 2 Demographics of participants by diagnosis status.

	AD case	Matched control	SMD
N	5,839	116,103	
Age at the last visit (median [IQR])	84.00 [79.00, 88.00]	83.00 [79.00, 87.00]	0.058
Gender (%)			0.005
Female, n (%)	3,583 (61.4)	70,969 (61.1)	
Male, n (%)	2,256 (38.6)	45,134 (38.9)	
Race (%)			0.013
White, n (%)	3,636 (62.3)	72,719 (62.6)	
Asian, n (%)	835 (14.3)	16,633 (14.3)	
Black or African American, n (%)	262 (4.5)	4,961 (4.3)	
Native Hawaiian or other Pacific Islander, n (%)	38 (0.7)	700 (0.6)	
American Indian or Alaska Native, n (%)	4 (0.1)	80 (0.1)	
Patient refused, n (%)	65 (1.1)	1271 (1.1)	
Other, n (%)	755 (12.9)	14,869 (12.8)	
Unknown, n (%)	205 (3.5)	4,100 (3.5)	
No matching concept, n (%)	39 (0.7)	770 (0.7)	
Ethnicity (%)			0.001
Not Hispanic or Latino, n (%)	5,022 (86.0)	99,824 (86.0)	
Hispanic or Latino, n (%)	437 (7.5)	8,717 (7.5)	
No matching concept, n (%)	380 (6.5)	7,562 (6.5)	
Hypertension (%)	3,667 (62.8)	72,663 (62.6)	0.004
Congestive heart failure (%)	414 (7.1)	5,627 (4.8)	0.095
Median household income (by zip code) (median [IQR])	63088.30 [48073.11, 76242.65]	55136.25 [40246.02, 74796.61]	0.219
Ever Medicare/Medicaid/VA insurance	5,415 (98.7)	95,593 (97.4)	0.092
Ever private insurance	1,414 (25.8)	24,240 (24.7)	0.025

Each AD patient was matched with up to 20 controls on age and exact match of sex, race, ethnicity, and hypertension.

diagnosis for cases). For the full-matched cohort, among AD cases, 0.5% (27/5839) of patients were exposed to bumetanide prior to diagnosis compared to 1.5% (1705/116103) of controls exposed to bumetanide, suggesting patients exposed to bumetanide are less likely to develop AD. The unadjusted odds ratio (OR) for AD diagnosis among bumetanide exposed was 0.31 (95% CI, 0.21–0.46; $p = 2.4 \times 10^{-9}$).

To adjust SES variables, we included insurance information (if patients ever had Medicare/Medicaid/VA and if patients ever had private insurance) and median household income by the zip code. However, we did have some missingness in our data—insurance information was available for 94.0% of cases and 84.5% of controls. In addition, for the zip code data informing the median income component, we focused on patients from California, and due to de-identification reasons, data were only available for 76.8% of cases and 74.1% of controls. Because SES estimates were not easily imputable from our data, a sensitivity analysis was performed as a complete case analysis. In this sensitivity analysis, we restricted the cohort to patients with complete insurance and median income data (N = 77,688) and repeated the unadjusted analysis prior to fitting a multivariable model adjusted for CHF, insurance, and median income. In the complete case-restricted cohort, 21/4238 (0.50%) AD cases and 1,321/73,450 (1.8%) matched controls were exposed to bumetanide. The unadjusted OR remained similar to that in our primary analysis (OR = 0.27; 95% CI, 0.18–0.42; $p = 3.7 \times 10^{-9}$). After adjusting for CHF, insurance, and median income the estimated OR for AD diagnosis among bumetanide exposed was 0.23 (95% CI, 0.15–0.36; $p = 4.0 \times 10^{-11}$).

For the full-matched cohort, among AD cases, 10.8% (633/5839) of patients were exposed to furosemide prior to diagnosis compared to 17.2% (20023/116103) of controls exposed to furosemide, suggesting patients exposed to bumetanide are less likely to develop AD. The unadjusted OR for AD diagnosis among furosemide exposed was 0.58 (95% CI, 0.54–0.63; $p < 2.0 \times 10^{-16}$). In the same sensitivity analysis performed for bumetanide exposure, the unadjusted OR for the complete case-restricted cohort remained similar to that in our primary analysis (OR = 0.53; 95% CI, 0.48–0.58; $p < 2.0 \times 10^{-16}$); this protective effect was replicated after

TABLE 3 (AB) Bumetanide and furosemide exposure details of exposed participants by diagnosis status (A). Bumetanide exposures for full matched cohort (B) and furosemide exposures for full matched cohort.

Characteristic	Case, N = 27	Control, N = 1,705
Duration of bumetanide exposure (days), median (IQR)	115 (6, 682)	21 (2, 334)
Days from first bumetanide exposure to first AD diagnosis, median (IQR)	225 (1, 978)	NA (NA, NA)
Ever exposed to bumetanide via oral route, n (%)	25 (93)	1,405 (82)
Ever exposed to bumetanide via oral route, n (%)	11 (41)	776 (46)
Ever exposed to 0.25 mg/mL dosage injectable solution, n (%)	11 (41)	768 (45)
Ever exposed to 0.5 mg dosage oral tablet, n (%)	10 (37)	302 (18)
Ever exposed to 1 mg dosage oral tablet, n (%)	22 (81)	1,079 (63)
Ever exposed to 2 mg dosage oral tablet, n (%)	11 (41)	587 (34)
Characteristic	**Case, N = 633**	**Control, N = 20,017**
Duration of furosemide exposure (days), median (IQR)	94 (2, 909)	25 (1, 664)
Days from first furosemide exposure to first AD diagnosis, median (IQR)	351 (12, 1,153)	NA (NA, NA)
Ever exposed to furosemide via oral route, n (%)	471 (74)	14,718 (74)
Ever exposed to furosemide via IV route, n (%)	394 (62)	12,433 (62)
Ever exposed to 8 mg/ml dosage injectable or oral solution, n (%)	5 (0.8)	128 (0.6)
Ever exposed to 10 mg/ml dosage injectable or oral solution, n (%)	394 (62)	12,449 (62)
Ever exposed to 20 mg dosage oral tablet, n (%)	397 (63)	11,786 (59)
Ever exposed to 40 mg dosage oral tablet, n (%)	204 (32)	6,775 (34)
Ever exposed to 80 mg dosage oral tablet, n (%)	26 (4.1)	980 (4.9)

adjusting for CHF, insurance, and median income (OR = 0.42; 95% CI, 0.38–0.47; $p < 2.0 \times 10^{-16}$).

Discussion

Most clinical trials in AD suffer from an inherent shortfall regarding primary prevention as they do not give insights on whether a compound reduces the incidence of AD as medications are tested after disease onset. Studying EHR using OMOP allows us to derive insight into possible primary prevention of AD (Datta et al., 2020).

In an independent dataset, our results replicate those of the original study that found a protective effect of bumetanide exposure on AD risk (Taubes et al., 2021). We further investigated whether this effect is generalizable to the more commonly used and less expensive medication in the same class and adjusted for potentially confounding variables such as SES and CHF. In our study, we calculated the odds ratio of AD diagnosis for those exposed to bumetanide (and separately furosemide) and found that the exposure of both bumetanide and furosemide was associated with reduced future AD diagnosis.

Both medications have similar indications and mechanisms of action: potential protective molecular modulation of neuronal transmembrane chloride gradients by blocking NKCC1 in the central nervous system (Kharod et al., 2019), which is the mechanism that led to proposed investigations to treat autism (Lemonnier et al., 2012), schizophrenia (Rahmanzadeh et al., 2017), and epilepsy (Eftekhari et al., 2013; Rahmanzadeh et al., 2017). Both bumetanide and furosemide have been shown to penetrate the blood–brain barrier, albeit at low concentrations (Javaheri et al., 1994; Töllner et al., 2015). The brain bumetanide concentrations following systemic administration are below those required for effective NKCC1 inhibition (Johanson et al., 1992; Holtkamp et al., 2003; Römermann et al., 2017; Brandt et al., 2018). However, other potential explanations for the protective effects including unique effects on the *APOE* genotype-dependent transcriptomic signature (Taubes et al., 2021), potent diuretic effects, and off-target metabolic, cardiorespiratory, and hormonal alterations that may be indirectly linked to reducing the risk of AD are also a possibility (Brater, 1991; Puskarjov et al., 2014). Our OMOP EHR dataset analysis demonstrated a potential inverse association between past bumetanide and furosemide exposures (Table 4) and AD onset. These associations remained significant even after correcting for SES and CHF, indicating that the results are not driven by differences in SES or severity of cardiac disease.

These results should be treated cautiously as they are based on retrospective data. Bumetanide and furosemide are potent loop diuretics that if given excessively, can lead to a profound diuresis with water and electrolyte depletion, which is particularly problematic in the elderly population. In addition, insurance and income were modeled through proxies available in OMOP and may not fully account for differences in SES. Last, additional functional studies are warranted to investigate the biological mechanism through which bumetanide and furosemide exposures are associated with reduced AD risk. The current findings do not

TABLE 4 Odds ratio for AD diagnosis among bumetanide- and furosemide-exposed participants.

Bumetanide analyses	Or (95% CI)	*p*-value
Unadjusted matched cohort bumetanide exposure prior to AD diagnosis	0.31 (0.21, 0.46)	2.4×10^{-9}
CHF-adjusted matched cohort bumetanide exposure prior to AD diagnosis	0.25 (0.17, 0.37)	2.59×10^{-12}
Unadjusted complete case cohort bumetanide exposure prior to AD diagnosis	0.27 (0.18, 0.42)	3.72×10^{-9}
CHF-adjusted complete case cohort bumetanide exposure prior to AD diagnosis	0.23 (0.15, 0.35)	2.11×10^{-11}
CHF- and SES-adjusted complete case cohort bumetanide exposure prior to AD diagnosis	0.23 (0.15, 0.36)	4.0×10^{-11}
Furosemide analyses		
Unadjusted matched cohort furosemide exposure prior to AD diagnosis	0.58 (0.54, 0.63)	$<2.0 \times 10^{-16}$
CHF-adjusted matched cohort furosemide exposure prior to AD diagnosis	0.48 (0.44, 0.52)	$<2.0 \times 10^{-16}$
Unadjusted complete case cohort furosemide exposure prior to AD diagnosis	0.53 (0.48, 0.58)	$<2.0 \times 10^{-16}$
CHF-adjusted complete case cohort furosemide exposure prior to AD diagnosis	0.43 (0.39, 0.48)	$<2.0 \times 10^{-16}$
CHF- and SES-adjusted complete case cohort furosemide exposure prior to AD diagnosis	0.42 (0.38, 0.47)	$<2.0 \times 10^{-16}$

Individuals were older than 65 years, and the odds ratio was adjusted for age, sex, race, ethnicity, hypertension, and with and without adjusting CHF and SES (insurance and median income).
AD, Alzheimer's disease; CHF, congestive heart failure; SES, socioeconomic status; OR, odds ratio.

support the use of bumetanide for the prevention or treatment of AD. There is a need for prospective, randomized, double-blinded, and placebo-controlled clinical trials to confirm the findings in patients without comorbidities and determine the lowest effective dose that may reduce the risk of AD without causing intolerable side effects.

Data availability statement

The data analyzed in this study are subject to the following licenses/restrictions: Electronic Health Record from Stanford University. Requests to access these datasets should be directed to zihuai@stanford.edu.

Ethics statement

The requirement of ethical approval was waived by the Stanford University institutional review board for the studies involving humans because the analyses were carried out on de-identified data; therefore, additional informed consent was not required. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board also waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because the analyses were carried out on de-identified data; therefore, additional informed consent was not required.

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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Anodal transcranial direct current stimulation (atDCS) and functional transcranial Doppler sonography (fTCD) in healthy elderly and patients with MCI: modulation of age-related changes in word fluency and language lateralization

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Introduction: In addition to age-related changes in language, hemispheric lateralization of language functions steadily declines with age. Also, performance on word fluency tasks declines and is sensitive to the expression of dementia-related changes. The aim of this study is to evaluate the effect of anodal tDCS combined with a word fluency training on language lateralization and word fluency performance in healthy elderly subjects and in persons with mild cognitive impairment (MCI).

Methods: The effect of anodal tDCS over the left inferio frontal gyrus (IFG) was measured in a group of healthy elderly up to the age of 67 years (YG, Ø = 63.9 ± 3.02), a group of healthy elderly aged 68 years and older (OG, Ø = 78.1, ± 4.85), and a group of patients with MCI (Ø = 81.18, ± 7.35) by comparing performance in phonological and semantic word fluency tasks before and after 3 days of tDCS. Half of the experimental participants received sham stimulation. In addition, language lateralization was determined using a lateralization index (LI) measured with functional transcranial Doppler sonography (fTCD) before and after the stimulation period.

Results: Anodal tDCS was associated with significantly higher scores in phonological but not semantic word fluency in both YG and OG. In MCI patients, no difference was measured between the tDCS and sham groups in either word fluency task. fTCD showed significantly increased left lateralization in all three groups after the training phase. However, this effect was independent of tDCS and the degree of lateralization could not be predicted by word fluency performance in any of the groups.

Discussion: Phonological word fluency can be increased with atDCS in healthy elderly people by stimulating the IFG in a 3-day training. When cognitive decline has reached a certain stage, as is the case with MCI, this paradigm does not seem to be effective enough.

KEYWORDS

transcranial direct current stimulation (tDCS), mild cognitive impairment (MCI), language lateralization, word fluency, functional transcranial Doppler sonography (FTCD)

1 Introduction

In normal aging, many higher cognitive functions like language processing and speech can be affected by structural and functional deterioration of the brain. For example, both gray and white matter volume and its integrity decrease. Besides other processes, this leads to a decrease in inhibitory processes in the brain, which can also affect processing speed (Fraundorf et al., 2019). For instance, difficulties in naming objects can occur, or word retrieval in general may be impaired when producing sentences (Taler and Phillips, 2008; Burke and Shafto, 2011; Rinehardt et al., 2014). More complex language functions, such as those required in phonological and semantic word fluency (WF) tasks, involve fast and intact long-range neuronal networks. In phonological WF tasks, participants are asked to find as many words as possible that begin with a presented letter within a given time span. In semantic WF tasks, a semantic category (e.g., animals) is presented and participants have to find as many words as possible that correspond to this category. Both word fluency tasks place high demands on participants' executive processes. On the one hand, they have to retrieve the appropriate items from memory and on the other hand, responses have to be initiated, previous responses have to be controlled, and inappropriate items have to be inhibited (Henry et al., 2004). In addition, these two tasks differ in terms of the strategies used to retrieve the words. Phonological WF requires the activation of lexical representations, whereas semantic WF relies on the retrieval of items that correspond to a higher-level concept. Semantic associations within the lexicon must be intact for the task to be completed successfully. These task differences are also reflected in the involvement of different brain regions. Recent fMRI findings suggest that phonological WF primarily activates (left) frontal brain regions, whereas semantic WF shows extensive activation in temporal and parietal networks (e.g., Baciu et al., 2016).

To compensate for declining performance in word fluency tasks, as well as in other cognitive tasks, certain compensatory mechanisms can be observed in older individuals compared to younger ones. One of these mechanisms discussed is *hemispheric asymmetry reduction in older adults* (HAROLD; Cabeza, 2002), which describes the change of formerly lateralized cognitive functions towards a more bihemispheric activity pattern. This correlates with a better performance of older persons in verbal-cognitive tasks. However, age-related functional changes in the healthy brain are exacerbated by neurodegenerative diseases such as Alzheimer's dementia (AD) and compensatory mechanisms are reduced. Cognitive-linguistic impairments are already evident in the preliminary stages of dementia, referred to as mild cognitive impairment (MCI). Patients with MCI may show impairments in one or more different cognitive domains, such as episodic memory, verbal functions, visuospatial abilities, perceptual speed, and executive functions (Taler and Phillips, 2008; Petersen, 2016). Various cognitive tests are used to diagnose MCI (e.g., trail-making test, visuospatial tasks, word fluency tasks, naming and learning tasks). Among other things, a slowed reaction time and an increased error rate can be observed. In the domain of language, MCI patients often show problems in accessing semantic information, which manifests in word finding difficulties (Taler and Phillips, 2008).

An effective and widely used method for clinical detection of cognitive changes associated with MCI and dementia is the use of WF tasks described above. In general, patients with MCI perform better on these tasks than AD patients but worse than healthy controls (Murphy et al., 2006; Nutter-Upham et al., 2008; Weakley et al., 2013; Meinzer et al., 2015; Rajji, 2021). Scores on both phonological and semantic WF tasks can significantly predict the severity and mortality of later AD already at the MCI stage (Cerhan et al., 2002; Cosentino et al., 2006). In MCI, word fluency difficulties are more evident at the semantic level than at the phonological level (Chasles et al., 2020).

One reason for the differential importance of these word fluency tasks in diagnosis could be the involvement of different brain areas, as indicated by the underlying patterns of cerebral blood flow (CBF, Marcolini et al., 2022). CBF emerged as the most important predictor of performance on the word fluency tasks, with mean flow across all vessels important for semantic fluency, and left frontal flow the most important predictor of performance on phonological fluency (Keilp et al., 1999; Nutter-Upham et al., 2008; Vonk et al., 2020). The stronger association of semantic fluency with the most typical CBF deficits in AD, associated mainly with structural changes in temporal and parietal brain regions, argues for the higher sensitivity of this task in this disease. The deficits in phonological fluency, on the other hand, provide important information about the extent to which perfusion deficits have spread to the frontal cortex (Keilp et al., 1999; Vonk et al., 2020).

In the vast majority of people, language functions such as word retrieval in word fluency tasks are lateralized to one of the two hemispheres, mainly the left (Knecht et al., 2000). This can be observed with fMRI, but also with functional transcranial Doppler sonography (fTCD, Gutierrez-Sigut et al., 2015). The fTCD shows high correlations with lateralization patterns in fMRI (Deppe et al., 2004; Jansen et al., 2004; Hattemer et al., 2011; Somers et al., 2011) or the Wada test (Knecht et al., 1997). The advantage of this method is that it is well suited for larger samples or study designs with multiple sessions (Heimann et al., 2022). It is also particularly suitable for studying lateralization patterns in adults (Woodhead et al., 2019) regarding language functions and other cognitive functions, e.g., arithmetic (Connaughton et al., 2017; Woodhead et al., 2019) or spatial skills (Rosch et al., 2012). Its use in clinical populations, e.g., patients with epilepsy (Conradi et al., 2019) or Parkinson's disease

(Gutteridge et al., 2020), is also well studied. Since the mobile use of fTCD allows for free head movement and speaking, it is of even greater benefit in uncooperative patients, including patients suffering from dementia (Deppe et al., 2004).

The current study aims to detect the benefit of anodal tDCS over the left inferior frontal gyrus (IFG) when performing phonological and semantic WF tasks in patients with MCI and two groups of healthy controls. In recent decades, tDCS has been shown to be effective in research in healthy elderly (Meinzer et al., 2009; 2014; Cattaneo et al., 2011; Jeon and Han, 2012) and in the rehabilitation of patients with language and memory impairments in various neurological disorders (Fregni and Pascual-Leone, 2007). In healthy older adults, one session of anodal tDCS over the left ventral inferior frontal gyrus was associated with significant improvement in a semantic WF task (Birba et al., 2017). Participants even reached the level of younger controls, suggesting that a single session of anodal tDCS can temporarily reverse the nonbeneficial effects of aging on cognition, brain activity, and connectivity. Moreover, anodal tDCS over the left IFG significantly improved word retrieval performance in patients with MCI to the level of healthy controls (Birba et al., 2017). In other clinical studies, tDCS was associated with improved word retrieval performance in patients with aphasia (Meinzer et al., 2016), in patients with Parkinson's disease (Manenti et al., 2016) and in neurological rehabilitation in patients with dementia (Holczer et al., 2020). Additionally, anodal tDCS intervention over the left frontotemporal cortex slowed down the progression of dementia symptoms and resulted in more physiological EEG patterns in AD (Gangemi et al., 2021). Other authors found that in patients with AD, abnormal patterns of EEG activity during memory processing were partially reversed by anodal tDCS and that this reversal correlated with an improvement in word recognition (Marceglia et al., 2016). Faster word recognition was found after one session of atDCS of the temporal cortex in elderly with MCI (Balduin-Philipps et al., 2021). Overall, however, conflicting or insufficient evidence was found for the efficacy of tDCS in dementia (Flöel, 2014; Chang et al., 2018; Buss et al., 2019), largely due to differences in study designs and stimulation parameters.

The aim of this study was to investigate phonological and semantic WF in healthy elderly individuals and individuals with MCI. This involved measuring language lateralization using fTCD before and after 3 days of WF tasks and concurrent atDCS/sham stimulation. Since most relevant studies have been based only on homogenous groups (e.g., only patients with specific diagnoses or groups of healthy elderly), we used this multi-dimensional approach. We compared three groups, including two healthy elderly groups (YG and OG) of two different age classes and one group of participants diagnosed with memory deficits (MCI). During the 3-day training period, participants received either anodal tDCS over the left IFG or sham stimulation. To our knowledge, no previous study has focused on the effect of tDCS on WF task performance and associated CBF changes over multiple days.

Therefore, in this study, we aimed to investigate the following questions:

1) Does anodal tDCS over the left IFG result in significant increase in phonological and/or semantic WF performance? Do the three groups benefit similarly?
2) Is there a difference in language lateralization during phonological and semantic WF tasks as measured by a language lateralization index (LI) using fTCD. Are there differences between groups?
3) Does the LI differ depending on whether participants were in the atDCS or sham group?
4) Are increases in WF performance associated with increases in CBF lateralization?

2 Materials and methods

2.1 Participants

31 native German speakers participated in the study (14 f, age range 60–100 years, Ø ±81.83, SD ± 10.25). The participants were all right-handed according to a modified version of the Edinburgh Handedness Inventory (Oldfield, 1971), while one participant reported a tendency towards left-handedness in early childhood but had been trained on the right hand. All had normal or corrected-to-normal hearing and vision. None of the participants had contraindications to tDCS according to the recommendations of Antal et al. (2017). Four participants had been taking plant-based medication for a diagnosed form of memory decline for more than 3 months without complaints, while all other participants were not taking any neuroactive medication.

The participants were either recruited through a study program for people over 60 at Bielefeld University, were volunteers who had read about the study in the local newspaper or in case of the MCI group, participants received a recommendation about the study from their treating neurologist. They came to the laboratory of Bielefeld University for the 5-day study or underwent the study at home for reasons of limited mobility. The participants were informed about the study orally as well as in written form and gave their written consent for participation and usage of data. After the diagnostic assessments in session 1, participants were ascribed to either the younger group of healthy participants (YG), the older group of healthy participants (OG), or the group of participants with MCI. The YG and OG each consisted of ten participants (5 atDCS, 5 sham), and the MCI group comprised 11 participants (6 atDCS, 5 sham).

After the last atDCS session, participants received an expense allowance for their participation. The study was approved by the Ethics Committee of Bielefeld University (Ethics Approval No. 2021-028) and was conducted according to the Declaration of Helsinki.

2.2 Experimental stimuli

The letters for the phonological WF task were derived from the ten most frequent German initial letters (defined by total amount of words beginning with the letters in question) according to the Wahrig-Brockhaus lexicon of the German Language (Wahrig-Burfeind et al., 2012). The five most frequent letters (A, K, H, B, S) were used for the diagnosis (session1) and the evaluation (session 5), and the remaining five (G, E, P, F, M) for the stimulation sessions. The items for the semantic WF task were chosen from a collection of semantic categories commonly used in German aphasia therapy. We used the categories *furniture*, *drinks*, *animals*, *electronic gadgets* and

diseases for the diagnosis (session1) and the evaluation (session 5) and the categories *clothes*, *fruit*, *professions*, *vehicles,* and *sports* for the stimulation sessions. All semantic categories had to meet the criterion that a possible member of a category could not be assigned to any of the other categories.

2.3 Diagnostic session (session 1)

In order to compare healthy individuals and individuals with memory impairments, we first tested the participants for their cognitive ability with the DemTect (version A, Kalbe et al., 2004). We then grouped participants by age and memory performance. Those participants who scored less than the age-matched cut-off value of 12 on the DemTect and/or had a medically diagnosed memory/cognitive decline were ascribed to the MCI group (Ø = 81.2 years ± 7.8; EHI = 80.5 ± 19.6; DemTect = 10.6 ± 3.5); participants with a score higher than the age-matched cut-off value of 12 were ascribed to the healthy groups. Since the group of participants with memory complaints was older than the overall group of participants, we divided the group without memory complaints into a younger group of healthy adults (YG) with a maximum age of 67 years (Ø = 63.9 years ±3.0; EHI = 86.7 ± 20.5; DemTect = 17.7 ± 0.5) and an older group of healthy adults (OG) aged 68 years or older (Ø = 78.1 years ±5.4; EHI = 92.4 ± 8.7; DemTect = 16.6 ± 1.7). These three groups were compared for age, handedness and memory performance. In an ANOVA, the groups differed significantly in age (F (2,28) = 25.77, $p \leq .001$) but not in handedness (F (2,28) = 1.3, $p = .288$). In a pairwise comparison using t-tests, only the younger group differed significantly in age from the MCI group (t (28) = −6.80, $p \leq 0.001$) and the OG (t (28) = −5.46, $p \leq 0.001$), while none of the older groups did (t (28) = −1.21, $p = 0.46$). The three groups differed significantly in their memory performance regarding their mean score on the DemTect (F (2,28) = 87.70, $p \leq 0.001$). As expected, the group that scored less than the age norm on the memory test (MCI) had a significantly reduced memory performance than the group of healthy older participants (OG, >68 years) (t (28) = 10.31, $p \leq 0.001$) and the healthy younger group (YG, 60–67 years) (t (28) = 12.22, $p \leq 0.001$). There was no significant difference in memory performance between OG and YG (t (28) = 1.86, $p = 0.17$) although the younger group showed a tendency to perform better in the DemTect.

When assessing, participants had to perform word fluency (WF) tasks, in which they had to complete five phonological and five semantic WF trials. Meanwhile, functional transcranial Doppler sonography (fTCD) was performed (see below). All items were presented in randomized order. Participants had 30 s for each of the five items in the WF tasks. The tasks were performed in an overt setting. Further, participants had to perform the subtest *connecting numbers* (CN) of the *Nuremberg Age Inventory* (NAI, Nürnberger Altersinventar, Oswald & Fleischmann, 1999). The participants had to connect numbers from 1 to 30 in the correct order as fast as possible. The numbers were arranged arbitrarily. The time required was measured with a stopwatch. The third diagnostic tool was the subtest *figure test* (FT) of the NAI in which participants had to memorize 12 differently shaped figures and recognize them in a second step. This test assessed their non-verbal memory functions. The fourth diagnostic part consisted of assessing the participants' mood with a multidimensional questionnaire on wellbeing (MDBF, *Mehrdimensionaler Befindlichkeits-Fragebogen*, Steyer et al., 1997). All participants completed a questionnaire assessing current mood in which they rated adjectives on a 5-point Likert scale that could later be assigned to three dimensions (good-bad, awake-tired, calm-agitated). If participants had difficulty assessing their current mood and/or answering a particular item, the experimenter helped determine the most appropriate score.

The results of the diagnostic tests underline the assumption that the third group consists of people with cognitive deficits who can be assigned to the MCI group. These are described and analyzed in the results section.

2.4 Functional transcranial Doppler sonography (fTCD)

Functional transcranial Doppler sonography (fTCD) was performed during the phonological and semantic word fluency tasks described above to measure the participants' patterns of language lateralization as assessed by the lateralization index (LI). This was done in session 1 as well as in session 5. Each trial comprised an initial baseline interval (Figure 1A) ranging from −15 to −5 s before the cueing tone and item presentation for later analysis, followed by a 30- second interval in which the participant generated words according to the letter or semantic category displayed on the screen (Figure 1B).

The period of interest (Figure 1C) was set to begin 5 s after item presentation to consider the process of neuro-metabolic coupling and to lasts up to 30 s relative to item presentation. The activation window (Figure 1D) itself, is the time interval with the largest event-related changes in cerebral blood flow velocities (CBFV) in the left and right middle cerebral arteries (MCAs) and includes the time point of LI calculation (Figure 1E; vertical red line), which marks the largest difference in CBFV between the left and right hemisphere after normalization. It was set to 2 s, where the LI calculation is performed by the fTCD analysis software by comparing the mean blood flow velocities in both MCAs in this time window. During the subsequent 30-s resting period (Figure 1F), the participant was instructed to rest and relax. During the last part of this resting period, seconds 55 to 59 were used as the next baseline period for the following item presentation and word generation.

For assessment of language lateralization, recorded fTCD data were analyzed using dopStep Master, which evolved from dopOSCCI, a Matlab (Mathworks, Natick, MA, United States of America) based software package (Badcock et al., 2012b). Its programming is based on the software package AVERAGE (Deppe et al., 1997), can be used with various TCD devices, and allows subtle quantitative offline analysis of Doppler flow signals. First, the channels for the left and right MCA as well as the trigger channel were set. The latter contains markers, which must be stored in the data file in order to time-lock the related activity. They are commonly sent via the parallel port before the presentation of each item. The electric trigger signals were sent from the PC to the fTCD-Computer (MultiDop T2, DWL, Sipplingen, Germany) via a customized cable connecting the PC's DB-25 parallel port to the fTCD-Computer's Av-in-port to mark the beginning of each trial. The computer then recorded the lateralization patterns extracted from the participants' blood flow patterns in the left and right MCA. To avoid interference from involuntary cardiac events when examining task-related signals, the activity within a single heart cycle was averaged, which resulted in a step-like summary of the activity as opposed to the natural variations in blood flow velocity during a heartbeat.

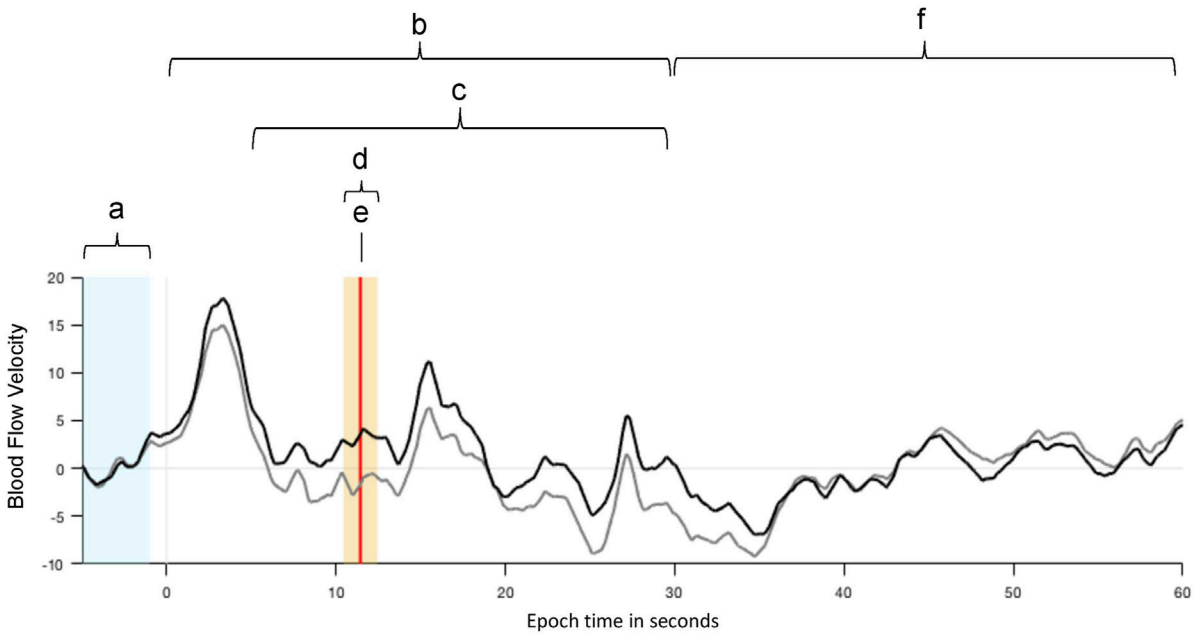

FIGURE 1
Example of mean cerebral blood flow velocities (CBFV) of the left (black) and right (grey) middle cerebral arteries (MCA) during the WF tasks. The velocity values were normalized and averaged over consecutive WF trials. **(A)** An interval of 10 s (from −15 to −5 s) before each item presentation is used as baseline (BL) for CBFV values. **(B)** The item presentation and overt word production start at 0 s and last for 30 s. **(C)** The period of interest, in which the process of neuro-metabolic coupling is considered, starts 5 s after the item presentation. **(D)** The highlighted interval of 2 s is the activation window in which the LI is calculated. **(E)** The vertical red line indicates the largest difference between the CBFV values of both MCAs. **(F)** After a subsequent relaxation phase of 30 s the next item is presented.

Additionally, the time span between two event markers (Figure 1, dashed line) was set to 60 s, and the range of blood flow was limited to 150 cm/s to exclude measurement of movement artifacts. Since the probe angles may differ between the two sides (Deppe et al., 2004), data from the left and right MCA are normalized to a mean of 100 using the following equation:

$$\frac{(100 x data)}{\emptyset(data)},$$

here data refer to the blood flow velocity values at a certain time of measurement.

2.5 Transcranial direct current stimulation

Transcranial direct current stimulation was administered via a battery-driven direct current stimulator (NeuroConn DC-Stimulator plus). Stimulation was delivered via two electrodes ($5 \times 7\ cm^2$) in saline-soaked sponges (0.9% saline solution) attached to participants' heads with rubber bands. The electrodes were placed on the head of the participants according to the international 10–20 system. The anode was placed over the crossing point Fp1–T3/Cz–F7 (part of the Broca's area, Homan, 1988) with the long side oriented vertically, and the cathode was placed on the participants' contralateral supraorbital region (Fp2) with the long side oriented horizontally. We applied 2 mA atDCS over the left inferior frontal gyrus (IFG) for 20 min with a fade-in and fade-out of 10 s. This resulted in a current density of $0.043\ mA/cm^2$ below each electrode. Since former studies in older participants have shown beneficial effects of online stimulation (Fertonani et al., 2014), we decided to use online stimulation while participants fulfilled the task. The sham procedure was identical to the atDCS, and because the study was double-blinded and randomized, neither participants nor the experimenter knew which stimulation condition they were in. In the sham condition, the current started but was automatically ramped down after 30 s. This procedure guaranteed the participants' blindness to the stimulation condition because it elicited a light tingling sensation on the participants' heads that was comparable to real tDCS but did not lead to neuronal enhancement (Nitsche et al., 2008).

2.6 Experimental procedure

The stimulation sessions took place at Bielefeld University or at participants' homes if this was necessary due to their immobility or mental status. All participants had the atDCS/sham sessions on similar days of the week and at a similar time of day. A schematic representation of the sequence of tasks and tests performed in each session is provided in Table 1. Session 1 was considered a diagnostic session, and session 5 was used to evaluate the intervention.

In diagnostic session 1, participants had an interview about their personal history, including medication use and general health. Furthermore, they were tested on their handedness (modified version of the Edinburgh Handedness Inventory, Oldfield, 1971) and asked about potential contraindications related to tDCS. The

TABLE 1 Sequence of tasks and diagnostic instruments performed in each of the five sessions.

Session 1 diagnostics	Session 2 atDCS/sham	Session 3 atDCS/sham	Session 4 atDCS/sham	Session 5 evaluation
Interview, Handedness, Contraindications	Phonological WF	Phonological WF	Phonological WF	
----------	Semantic WF	Semantic WF	Semantic WF	----------
DemTect				DemTect
----------				----------
MDBF				MDBF
fTCD incl. phon. and sem. WF				fTCD incl. phon. and sem.WF
CN (NAI)				CN (NAI)
FT (NAI)				FT (NAI)

Abbreviations: atDCS, anodal transcranial direct current stimulation; MDBF, multidimensional questionnaire on wellbeing; ftCD, functional transcranial Doppler sonography; WF, word fluency; CN, subtest *connecting numbers* (CN) of the Nuremberg Age Inventory (NAI); FT, subtest *figure test* (FT) of the Nuremberg Age Inventory (NAI).

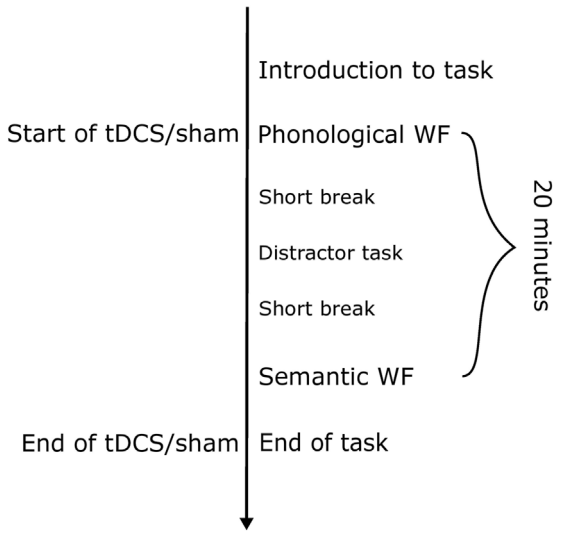

FIGURE 2
Sequence of a regular experimental session with either sham or anodal tDCS. The duration of the stimulation was 20 min. Between the two blocks of word fluency, a verbal memory task was introduced as a distractor task, to avoid having to perform the phonological and semantic WF in direct succession. This task was not evaluated. The total duration of an experimental session including the electrode application varied between 40 and 50 min.

diagnostic assessments were performed on the desk in front of the participant. The same diagnostic/assessment instruments were used in the first and last sessions. In sessions two to four, the atDCS/sham intervention was conducted during phonological and semantic word fluency tasks (Figure 2).

Each of the individual WF trials lasted 1 minute and was performed in an overt setting. All items were presented in randomized order for each participant. A distractor task was performed between the WF tasks so that the WF tasks did not have to be completed one right after the other. The stimuli were arranged and presented using Cogent 2000, a MATLAB-based toolbox, which was installed on a Dell-laptop PC (Windows XP). Participants sat in front of a computer monitor at a distance of 50 cm between their eyes and the screen. The letters and semantic categories for both WF tasks were visually presented in a light grey serif-free font (Helvetica size 40) on a black background on a 15″LCD monitor. The visual angle was 1,15°. Before the WF tasks were started, we conducted one exemplary phonological and semantic trial to ensure the participant understood the task. Before the experiment started, the participant was asked to rest for 1 min.

2.7 Statistical analysis

The data of 31 participants were included in the statistical analysis, performed with SPSS software (IBM, vers. 16) and the open-source program jamovi (The jamovi project, 2022; vers. 2.3). We grouped the participants according to age and memory performance, namely, the cut-off value of the DemTect dementia screening (18–13 = age-norm; ≤12 = less than the age norm).

To account for the differences in baseline diagnostic test scores and word fluency within and between the three groups (YG, OG, MCI), we calculated difference scores (DS) between session 5 and session 1 for diagnostic tests (DemTect, MDBF, both NAI subtests), as well as phonological and semantic word fluency. These difference scores were used as dependent variables. We calculated ANOVAs with the factors *group* (YG, OG, MCI) and *stimulation* (sham vs. atDCS). To measure changes in language lateralization index (LI) before and after the stimulation, we also calculated a difference score between session 5 and session 1 for each participant. To determine a possible relationship between WF performance, intervention, and degree of linguistic lateralization, we conducted a linear regression analysis with WF performance as the predictor and degree of language lateralization as dependent variable.

3 Results

3.1 Diagnostic session (session 1)

In the diagnostic session, the mean number of words produced in the phonological WF task was 56.8 for the YG (SD = 12.4), 52.7 for the OG (SD = 13.8), and 39.1 for the MCI group (SD = 11.3). ANOVA

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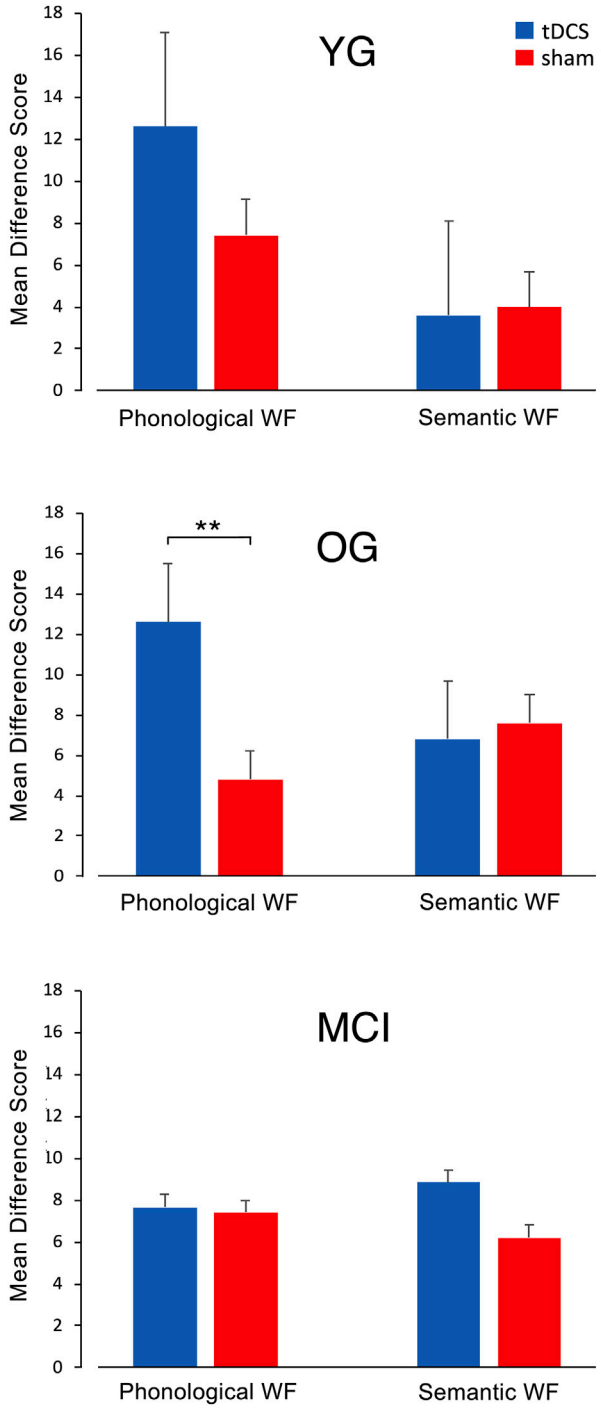

FIGURE 3
Mean difference scores (DS) for both WF tasks with atDCS and sham stimulation in the YG, OG, and MCI group. In the YG and OG, participants reached a higher learning score in the phonological WF task after anodal tDCS compared to sham stimulation, while the DS for the semantic WF task did not show much variation. In the MCI group, there were no differences in DS between stimulation conditions for the phonological WF task. For the semantic WF task, DS was higher for the anodal tDCS than for the sham treatment.

revealed a significant group effect in the phonological word fluency task (F (2,28) = 4.74, p = 0.017). Tukey *post hoc* tests showed that the YG produced significantly more words than the MCI group (t (28) = 2.92, p = 0.018). The OG, on the other hand, only tended to show better phonological WF than the MCI group (t (28) = 2.24, p = 0.082). The YG and OG did not differ significantly. In the semantic WF task the mean number of words produced was 62.5 for the YG (SD = 4.8), 50.3 for the OG (SD = 8.9), and 33.9 for the MCI group (SD = 8.8). Semantic word fluency also showed a significant group effect (F (2,28) = 28.4, $p \leq$ 0.001). Here, all groups differed significantly from each other, with the YG performing best and the MCI group performing worst. The YG was significantly different from the OG (t (28) = 2.99, p = 0.015) and the MCI (t (28) = 7.48, $p \leq$ 0.001). The OG also differed significantly from the MCI group (t (28) = 4.41, $p \leq$ 0.001).

The mean time required for the subtest *connecting numbers* (CN) of the *Nuremberg Age Inventory* (NAI, Nürnberger Altersinventar, Oswald and Fleischmann, 1999 was 20.2 s for the YG (SD = 18.2), 25.4 s for the OG (SD = 24.3), and 50.7 s for the MCI group (SD = 42.2). We found a significant group effect (F (2,28) = 5.8, p = 0.008). Tukey *post hoc* tests showed that the YG was significantly faster than the MCI group (t (28) = −3.16, p = 0.01). The OG also differed significantly from the MCI group (t (28) = −2.61, p = 0.037). The YG and OG did not differ significantly.

The mean number of memorized items in the figure test (FT) of the NAI was 10.4 for the YG (SD = 0.8), 9.7 for the OG (SD = 0.9), and 8.0 for the MCI group (SD = 1.6). This test also showed a significant group effect (F (2,28) = 9.17, $p \leq$ 0.001). Tukey *post hoc* tests showed that the YG recognized significantly more items than the MCI group (t (28) = 4.13, $p \leq$ 0.001). The OG also recognized significantly more items than the MCI group (t (28) = 2.97, p = 0.016). The YG and OG did not differ significantly.

For the mood assessment using the multidimensional questionnaire on wellbeing (MDBF, *Mehrdimensionaler Befindlichkeits-Fragebogen*, Steyer et al., 1997), none of the three dimensions differed significantly between the three groups for either of the three measured dimensions (s. 1.3 Diagnostic session (session 1).

The results of the above tests show that the MCI group had lower word fluency, lower processing speed in a trail making test for numbers, and lower recall performance in non-verbal memory compared to the other groups. All of these diagnostic tests described above were performed again in session 5 to evaluate the intervention (Table 1).

3.2 Word fluency and tDCS

When comparing the diagnostic session and the evaluation session, both phonological and semantic WF increased in all three groups and all conditions from session 1 to session 5 (Figure 3), suggesting a general learning effect. YG participants achieved a high difference score on the phonological WF task (12.6 ± 7.71 after atDCS and 7.4 ± 2.42 after sham stimulation), indicating a significant improvement in WF performance. The difference score for the semantic WF task did not differ noticeably between stimulation conditions and was slightly higher during sham (4 ± 6.2) compared to atDCS (3.6 ± 4.8) (Figure 3). In the OG also, phonological WF was higher in session 5 after atDCS (12.6 ± 4.5) compared with sham (4.8 ± 2.8). There were no major differences in DS in the semantic WF task in both atDCS (6.8 ± 4.2) and sham (7.6 ± 7.3) (Figure 3). In the MCI group, there was little difference in DS in the phonological WF task for atDCS (7.7 ± 6.3) and for sham (7.4 ± 6.0) but a higher difference score for atDCS (8.8 ± 5.2) compared to

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TABLE 2 Sum of words produced during the five phonological and semantic WF-tasks in the diagnostic session and the evaluation session for each participant.

Participant	Sex	Age	Stimulation condition	Phon. WF diagnostic session	Phon. WF evaluation session	Sem. WF diagnostic session	Sem. WF evaluation session
YG1	w	67	sham	53	58	63	62
YG2	w	66	sham	56	62	66	77
YG3	w	61	sham	61	68	68	78
YG4	w	65	sham	79	86	70	75
YG5	m	60	sham	41	53	67	62
YG6	w	64	tDCS	44	66	48	59
YG7	w	60	tDCS	50	69	61	64
YG8	w	63	tDCS	79	86	70	75
YG9	m	67	tDCS	57	58	54	50
YG10	m	65	tDCS	48	62	58	61
OG1	w	78	sham	83	84	67	89
OG2	w	77	sham	33	35	40	45
OG3	m	80	sham	51	59	51	53
OG4	m	76	sham	56	62	50	54
OG5	m	75	sham	61	68	56	61
OG6	w	91	tDCS	59	75	61	61
OG7	m	75	tDCS	38	51	42	50
OG8	m	70	tDCS	65	70	54	67
OG9	m	79	tDCS	37	48	35	42
OG10	m	80	tDCS	44	62	47	53
MCI1	w	75	sham	17	22	21	23
MCI2	m	85	sham	53	50	41	48
MCI3	m	85	sham	43	56	35	40
MCI4	m	78	sham	21	34	20	29
MCI5	m	79	sham	47	56	29	37
MCI6	w	100	tDCS	35	52	34	41
MCI7	w	88	tDCS	37	39	35	38
MCI8	w	74	tDCS	54	70	55	64
MCI9	m	76	tDCS	46	50	30	50
MCI10	m	75	tDCS	49	52	36	43
MCI11	m	78	tDCS	31	35	24	31

sham (6.2 ± 2.5) during the semantic WF task (Figure 3). The sum of words produced during the five phonological and semantic WF-tasks in the diagnostic session and the evaluation session on the final day is provided in Table 2.

An ANOVA for difference scores on WF performance showed a significant effect of atDCS (F (1,25) = 4.27, p = 0.05) during the phonological WF task but not during semantic WF (F (1,25) = 0.05, p = 0.82). There was no significant difference between *groups* or in a two-way *group* × *stimulation* interaction. Post-hoc tests revealed significantly increased WF performance for tDCS *versus* sham for the OG (t = −2.95; p = .009).

3.3 Word fluency and fTCD

The fTCD measurement showed left lateralization of blood flow in both word fluency tasks for all groups during the diagnostic session. The lateralization index (LI) did not differ significantly

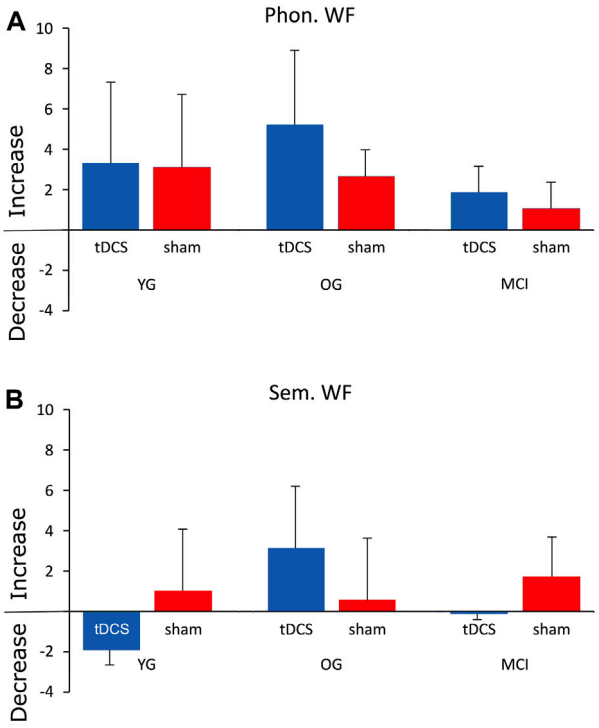

FIGURE 4
Mean difference scores of the lateralization index (subtracting the LI values of session 1 from those of session 5) for the phonological **(A)** and semantic **(B)** WF tasks.

between the three groups during either phonological or semantic word fluency before treatment. LI during phonological WF increased in all groups from session 1 to session 5. This increase was evident in both stimulation conditions (Figure 4A). The LI during the semantic WF task also showed an overall increase except for the YG and MCI groups, which had lower LI values in session 5 after atDCS (Figure 4B). A generalized linear model revealed that CBF was significantly more lateralized during phonological WF in the evaluation session than in the diagnostic session (ß = 2.90, z = 3.36, p = .002). However, this effect did not differ between groups and was not influenced by type of stimulation. LI during the semantic WF did not change significantly from session 1 to 5.

The next step of data analysis addressed the question of whether increases in phonological word fluency were related to or predicted increases in lateralization index. Linear regression showed that the difference scores of WF performance could not significantly predict changes in the degree of language lateralization as described by the LI for the YG group during phonological (R^2 = 0.104, p = 0.72) or semantic WF (R^2 = 0.30, p = 0.35), the OG during phonological (R^2 = 0.239, p = 0.52) or semantic WF (R^2 = 0.258, p = 0.52), and the MCI group during phonological (R^2 = 0.271, F (2,4) = 0.744, p = 0.53) or semantic WF (R^2 = 0.568, p = 0.19).

3.4 Diagnostic session vs. evaluation session

Another analysis addressed the question of whether DemTect scores changed from session 1 to session 5 also determined by difference scores. There were no statistically significant changes in DemTect scores for all groups, as shown by ANOVA analysis (F (2,25) = 1.802, p = 0.17). There was also no effect of the respective stimulation condition (F (1,25) = 0.002, p = 0.96). The mean DemTect scores for the three groups and stimulation conditions are shown in Table 3. Further analyses involved the NAI subtests *connecting numbers* (CN) and *figure test* (FT), and the multidimensional questionnaire on wellbeing (MDBF). All groups became faster on the subtest *connecting numbers* (CN) from session 1 to session 5 (Table 3). This suggests that training improved processing speed specifically in the MCI group, though not statistically significant. The change was independent of whether the group actually received tDCS or sham stimulation (F (1,25) = 0.18, p = 0.68). There was also no difference between groups (F (2,25) = 2.12, p = 0.14) and no interaction between *group* and *stimulation* (F (2,25) = 0.46, p = 0.64).

In addition, all groups showed improvement in nonverbal memory from session 1 to session 5, as shown in the *figure test* (Table 3). However, ANOVA revealed that this change was independent of whether the group actually received atDCS or sham stimulation (F (1,25) = 0.04, p = 0.84). There was also no difference between groups (F (2,25) = 2.89, p = 0.08) and no interaction between *group* and *stimulation* (F (2,25) = 0.50, p = 0.61).

ANOVAs of the three dimensions (good-bad, awake-tired, calm-excited) of the multidimensional wellbeing questionnaire (MDBF) showed no significant results of the good-bad and calm-agitated dimensions. However, there was a significant interaction for *group* x *stimulation* for the awake-tired dimension (F (2,25) = 5.62, $p \leq 0.01$), based on the fact that after atDCS the YG were less tired than the sham group; the opposite was true for the other two groups.

4 Discussion

4.1 Diagnostic session

In this study, three groups of individuals of different ages and cognitive abilities were examined. The younger healthy group (YG) differed significantly in age from the older healthy group (OG) and the individuals with MCI (MCI). On dementia screening (DemTect), the MCI group had a significantly lower score than the YG and OG. The latter two groups did not differ. Performance in phonological WF was significantly lower in the MCI group than in the YG, but only tended to be worse than in the OG. The YG and the OG did not differ. On semantic word fluency, however, the YG performed significantly better than the OG and MCI groups, and the OG was also significantly better than the MCI. On the *connecting numbers* test, both the YG and OG were faster than the MCI group, and the YG and OG did not differ. In the *figure* test, the YG and OG recognized significantly more figures than the MCI group; the YG and OG did not differ significantly. The test on wellbeing did not differ between groups.

In summary, before the atDCS/sham stimulation the MCI group differed from the other two groups mainly in lower memory performance, lower performance in nonverbal memory recognition, phonological and especially semantic WF. In the trail making test, they

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TABLE 3 Diagnostic instruments and scores for all three groups in session 1 and session 5 under the respective stimulation conditions (sham/atDCS).

a) Mean DemTect scores (max. 18). Scores below 12 indicate a moderate memory deficit					
Group	Intervention	DemTect session 1	SD	DemTect session 5	SD
YG	sham	17.6	0.5	18.0	0
	atDCS	17.8	0.4	18.0	0
OG	sham	16.8	1.5	17.6	0.5
	atDCS	16.4	1.6	16.8	1.6
MCI	sham	10.2	1.8	11.2	1.9
	atDCS	11.0	0.8	12.6	2.3
b) Subtest *connecting numbers* (CN) for session 1 and session 5, mean time in s					
Group	Intervention	CN Session 1	SD	CN Session 5	SD
YG	sham	20.7	3.8	18.6	2.4
	atDCS	19.9	4.0	17.7	3.6
OG	sham	27.8	7.5	27.4	7.7
	atDCS	22.9	4.1	21.1	2.6
MCI	sham	62.1	44.8	50.8	28.4
	atDCS	39.3	11.5	33.7	10.6
c) Mean scores (max. 12) for the *figure test (FT)* for session 1 and session 5					
Group	Intervention	FT Session 1	SD	FT Session 5	SD
YG	sham	10.0	0.9	10.8	0.8
	atDCS	10.8	0.8	11.0	0.6
OG	sham	10.2	0.8	10.8	0.8
	atDCS	9.2	1.0	10.0	0.6
MCI	sham	8.8	1.3	10.4	0.5
	atDCS	7.2	1.8	9.5	1.0

were significantly slower than the other two groups. The YG and OG differed only in semantic WF, with the YG retrieving significantly more items. These results fit very well with the criteria for the diagnosis of MCI according to Petersen (2016).

The fTCD measurement showed left lateralization of blood flow in both word fluency tasks for all groups during the diagnostic session which confirms frequent findings in the literature (Heinzel et al., 2013). Interestingly, the lateralization index (LI) did not differ significantly between the three groups in either task. This contradicts the assumption that in older individuals, better performance on cognitive tasks should be associated with increased bihemispheric activity compared to cognitively impaired individuals, as postulated in the HAROLD model (Cabeza, 2002), or with greater lateralization compared to MCI individuals, which has also been frequently shown in the neuroimaging literature (e.g., Yeung et al., 2016). Because of the significant differences within and between groups before the tDCS intervention, we calculated difference scores between the respective tasks and scores of the diagnostic session 1 and evaluation session 5, which were included as dependent variables in the statistics.

4.2 Word fluency and tDCS

The next outcome relates to the effect of tDCS/sham stimulation combined with 3 days of word fluency training on word fluency performance in evaluation session 5. Anodal tDCS (in contrast to sham stimulation) over the left inferior prefrontal cortex significantly improved WF performance on phonological but not on semantic WF tasks. This effect was present in both age groups of healthy elderly participants (YG and OG) but not in the MCI group. This is in line with Vannorsdall et al. (2016), who found better phonological WF performance after atDCS over the left Broca region, whereas semantic WF performance was better after sham stimulation. Although these differences were not statistically significant, they are hinting at the distinct neural networks activated during phonological and semantic WF, which is also supported by our results. Our results are also consistent with previous findings which showed that increased cortical perfusion in left frontal cortical regions is associated with corresponding neuronal activity during phonological WF tasks (Keilp et al., 1999; Birba et al., 2017), hence our application of atDCS over the left IFG might explain the significant increase in

phonological WF performance in both groups of healthy elderly participants. It should be noted, however, that our results differ from previous research that reported significant improvement in both phonological and semantic WF tasks after stimulation over the left DLPFC (Cattaneo et al., 2011; Pereira et al., 2013). The different position of the electrodes during tDCS in these studies might offer an explanation here, so Cattaneo et al. (2011) stimulated additional not primarily task-related networks. Other differences in study design could also offer an explanation for the different results (Cattaneo et al., 2016).

Additionally, a recent study by Vonk et al. (2020) even showed that anatomical thickness in frontal and left-frontal brain structures correlates with phonological WF performance and that corresponding anatomical differences in temporal and (para-)hippocampal structures correlate with varying semantic WF performance in healthy individuals and patients with MCI or AD. Since the reduction of cortical perfusion in relevant brain areas correlates with the stage of cognitive decline in MCI and AD (Chao et al., 2010), this functional interconnection could explain the significant improvement we found in phonological but not semantic WF. Semantic WF requires a higher cognitive load and relies on other, partly non-linguistic cognitive processes (i.e., use of mental images, semantic features) that occur in extensive neural networks of the temporal and parietal cortex (Keilp et al., 1999; Vonk et al., 2020). These were not directly stimulated by left inferior atDCS. However, results of previous studies also show a significant effect of atDCS over the left IFG on semantic WF performance in healthy elderly participants (Meinzer et al., 2013; 2015) and in patients with dementia-related cognitive decline (Penolazzi et al., 2013; Smirni et al., 2021), but their experimental task procedure and the associated word retrieval of the subjects were very different and not very comparable to our tasks.

In participants with MCI, atDCS did not induce an increase in WF compared with sham stimulation. One reason for this could be the selection of the position of the stimulation electrodes, which is a possible limitation of our study. It is likely that brain regions particularly affected by neurodegenerative processes have reduced overall neuronal activity and thus cognition can only be improved by interventions such as atDCS under certain conditions. It is well-known that tDCS is only effective when neurons in the stimulated brain regions are active. As a result, it is possible that the neural processes necessary to increase WF in persons with MCI cannot be modulated by stimulation lasting only 3 days. For instance, recent findings indicate that improvements in phonological and semantic WF were observed in MCI patients after 20 days of atDCS (Fileccia et al., 2019). Word recognition was also significantly accelerated in MCI patients after a single stimulation of the temporal cortex (Balduin-Phillipps et al., 2021). It is possible that stimulation of the temporal cortex could lead to better outcomes in MCI, as also suggested by Chen et al. (2022). Moreover, cathodal stimulation of the right DLPFC improved WF in mildly affected AD patients by supporting left hemisphere networks through short-term inhibition (Smirni et al., 2021). From this, one could assume that the same stimulation conditions do not apply to MCI patients and healthy elderly and therefore do not lead to comparable results. Consequently, stimulation conditions would need to be adapted to a person's neuronal and cognitive status. Possibly, this assumption is also supported by the fact that only in the MCI group semantic WF after tDCS is slightly increased than in the sham group. Thus, they react differently than the healthy subjects. Another limitation of our study could be that WF was not measured during tDCS, but in session 5, 1 day after the last stimulation. Improvements in WF may not last as long in MCI patients and thus can only be observed online during the task (Chen et al., 2022). In our approach, measuring WF performance in the last session 1 day after the last anodal stimulation might be too late to detect associated improvements compared to sham stimulation – especially with only 3 days of stimulation.

4.3 Word fluency and fTCD

A further finding concerned the language lateralization index (LI) during the phonological and the semantic WF tasks, which was measured by functional transcranial Doppler sonography (fTCD). The lateralization index (LI) was left lateralized in both word fluency tasks for all groups during the diagnostic session, but did not differ significantly among the three groups. In phonological WF, blood flow lateralization was significantly higher in session 5 than in session 1, in all groups. Although lateralization was higher overall, particularly in OG after atDCS, this result was also not significant. The reason for the lack of significance was, presumably, the high variance within groups due to difficulties in measuring LI in some subjects. Moreover, it is possible that a significant effect would have been found if LI had been measured immediately after stimulation. Since an increasing degree of language lateralization in phonological WF was observed in all groups, it could be speculated that WF training produced greater lateralization associated with better performance (Yeung et al., 2016). This was true for MCI group, although the increase in the degree of language lateralization was smaller here than in the YG or OG. For semantic word fluency, lateralization increased similarly only in the OG. The YG even showed a decrease in lateralization after tDCS. These changes were also not significant. The finding that there were even negative LI difference scores in the YG and the MCI group for the semantic WF task might be a result of the fact that 1) semantic WF performance could not be adequately targeted by anodal stimulation of the left frontal IFG and 2) an increase in semantic WF performance, contrary to phonological, results in a higher activation in posterior neural networks (Gourovitch et al., 2000; Kitabayashi et al., 2001; Birn et al., 2010).

Another question in this study was whether the increase in WF performance was related to language lateralization. Here, the question was whether the significant increase in phonological WF after tDCS correlated with the significant increase in LI, as postulated by Yeung et al. (2016), or with a more bihemispheric pattern, as postulated in the HAROLD model (Cabeza, 2002). Neither phonological nor semantic WF performance changes could predict the degree of left lateralization, consistent with the results of Lust et al. (2011). Whether this resulted from the small number of subjects and thus high variability in WF performance or LI, or from the difficulty in measuring CBF in some subjects, could not be clarified by this study. In any case, the number of subjects per group could be another limiting factor of the study.

Moreover, examining the correlation between a broader spectrum of cognitive functions (i.e., overall scores during dementia screenings) and the direction and degree of language lateralization might be useful for understanding neurophysiological mechanisms in neurodegenerative diseases.

4.4 Diagnostic session vs. evaluation session

The behavioral tests (DemTect, connecting numbers, figure test) did not show changes in any group as a function of atDCS between the first and the last session. However, scores in the DemTect increased for all groups, indicating a general learning effect. This was also true for the results of the NAI subtest *connecting numbers* (CN), which showed a general reduction in the time needed for the task in all three groups. However, the improvement was comparatively more pronounced in the MCI group than in the YG and OG groups (Table 3b).

Regarding participants' mood tested via MDBF, the only change as a function of atDCS was that the YG was significantly less tired than the sham group after the 3 days of stimulation; the opposite was true for the other two groups. A possible explanation for this finding might be that the YG are more physically active compared to OG and MCI, which is associated with better psychosocial wellbeing (Finkenzeller et al., 2019) and therefore might affect specific dimensions of mood assessment. It is possible that the intervention with atDCS enhanced these differences.

4.5 Conclusion

In summary, unlike phonological WF, semantic WF and all other cognitive tests showed no significant change after atDCS for three consecutive days in healthy elderly and elderly with MCI. This implies that stimulation of the IFG is specific for improving phonological WF, at least in healthy elderly. Left lateralization was not significantly affected by atDCS but showed significantly higher values after 3 days of training. In future studies, blood flow should be measured during atDCS to verify whether lateralization changes more online than offline. To improve semantic WF, a different electrode configuration and/or more frequent stimulation would probably need to be targeted. The lack of improvement in phonological and semantic WF in the MCI group suggests that experimental stimulation parameters likely need to be adjusted to a person's neuronal and cognitive status. Training of WF and other cognitive functions in MCI is certainly useful, but needs to be additionally supported by interventions such as individualized atDCS. Further research with larger samples (Minarik et al., 2016) and altered stimulation parameters is needed to investigate whether this can produce more successful results in MCI patients and patients with more severe dementias (e.g., Alzheimer's disease).

Data availability statement

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

Ethics statement

The studies involving humans were approved by the Ethics Committee of Bielefeld University (Ethics Approval No. 2021-028). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

All authors FH, SW, and HM contributed to the study conception and design. FH Conducted the fTCD measurements and tDCS application and drafted the manuscript. All authors FH, SW, and HM analyzed the data and revised the manuscript. All authors FH, SW, and HM read and approved the final 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.

Publisher's note

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