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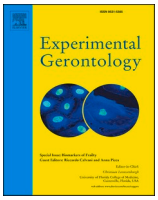

14-Week exercise training modifies the DNA methylation levels at gene sites in non-Alzheimer's disease women aged 50 to 70 years

Guilherme da Silva Rodrigues [a,1], Natália Yumi Noronha [b,*,1], Isabella Harumi Yonehara Noma [c], João Gabriel Ribeiro de Lima [a], Andressa Crystine da Silva Sobrinho [a], Marcela Augusta de Souza Pinhel [a], Mariana Luciano de Almeida [d], Lígia Moriguchi Watanabe [e], Carla Barbosa Nonino [e], Carlos Roberto Bueno Júnior [a,f]

[a] Department of Internal Medicine, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, São Paulo, Brazil
[b] Department of Gynecology and Obstetrics, University Medical Center Groningen, Groningen, the Netherlands
[c] Department of Clinical and Toxicological Analyses, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, Brazil
[d] University of São Paulo at Ribeirão Preto College of Nursing, Brazil
[e] Department of Health Sciences, Ribeirão Preto Medical School, Ribeirão Preto, São Paulo, Brazil
[f] School of Physical Education of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil

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ABSTRACT

Exercise training emerges as a key strategy in lifestyle modification, capable of reducing the risk of developing Alzheimer's disease (AD) due to risk factors such as age, family history, genetics and low level of education associated with AD. We aim to analyze the effect of a 14-week combined exercise training (CT) on the methylation of genes associated with AD in non-alzheimer's disease women. CT sessions lasted 60 min, occurring three times a week for 14 weeks. Forty non-Alzheimer's disease women aged 50 to 70 years (60.7 ± 4.1 years) with a mean height of 1.6 ± 0.1 m, mean weight of 73.12 ± 9.0 kg and a mean body mass index of 29.69 ± 3.5 kg/m2, underwent two physical assessments: pre and post the 14 weeks. DNA methylation assays utilized the EPIC Infinium Methylation BeadChip from Illumina. We observed that 14 weeks of CT led to reductions in systolic ($p = 0.001$) and diastolic ($p = 0.017$) blood pressure and improved motor skills post-intervention. Among 25 genes linked to AD, CT induced differentially methylated sites in 12 genes, predominantly showing hypomethylated sites (negative β values). Interestingly, despite hypomethylated sites, some genes exhibited hypermethylated sites (positive β values), such as *ABCA7*, *BDNF*, and *WWOX*. A 14-week CT regimen was adequate to induce differential methylation in 12 CE-related genes in healthy older women, alongside improvements in motor skills and blood pressure. In conclusion, this study suggest that combined training can be a strategy to improve physical fitness in older individuals, especially able to induce methylation alterations in genes sites related to development of AD. It is important to highlight that training should act as protective factor in older adults.

1. Introdution

Alzheimer's disease (AD) stands as the most prevalent form of dementia among the older population, representing 60 to 80 % of diagnosed cases in the world (Duthey, 2013). Global prevalence estimates exceed 45 million individuals. With an aging global population, a threefold rise in disease prevalence by 2050 is anticipated (Jakovljevic et al., 2021; El-Hayek et al., 2019). The economic impact of AD is substantial, evidenced by an estimated cost of $818 billion due to clinical and caregiving expenses (El-Hayek et al., 2019).

Therefore, it is essential to allocate efforts to research and find effective interventions for disease prevention. Exercise training emerges as a key strategy in lifestyle modification, capable of reducing the risk of developing AD arising from risk factors such as age, familial history, genetics, and low educational attainment associated with AD (Crous-Bou et al., 2017). Neurological and vascular adaptations during exercise

* Corresponding author at: Department of Gynecology and Obstetrics, University Medical Center Groningen, Groningen, the Netherlands.
E-mail address: n.y.noronha@umcg.nl (N.Y. Noronha).
[1] These authors have contributed equal to this work.

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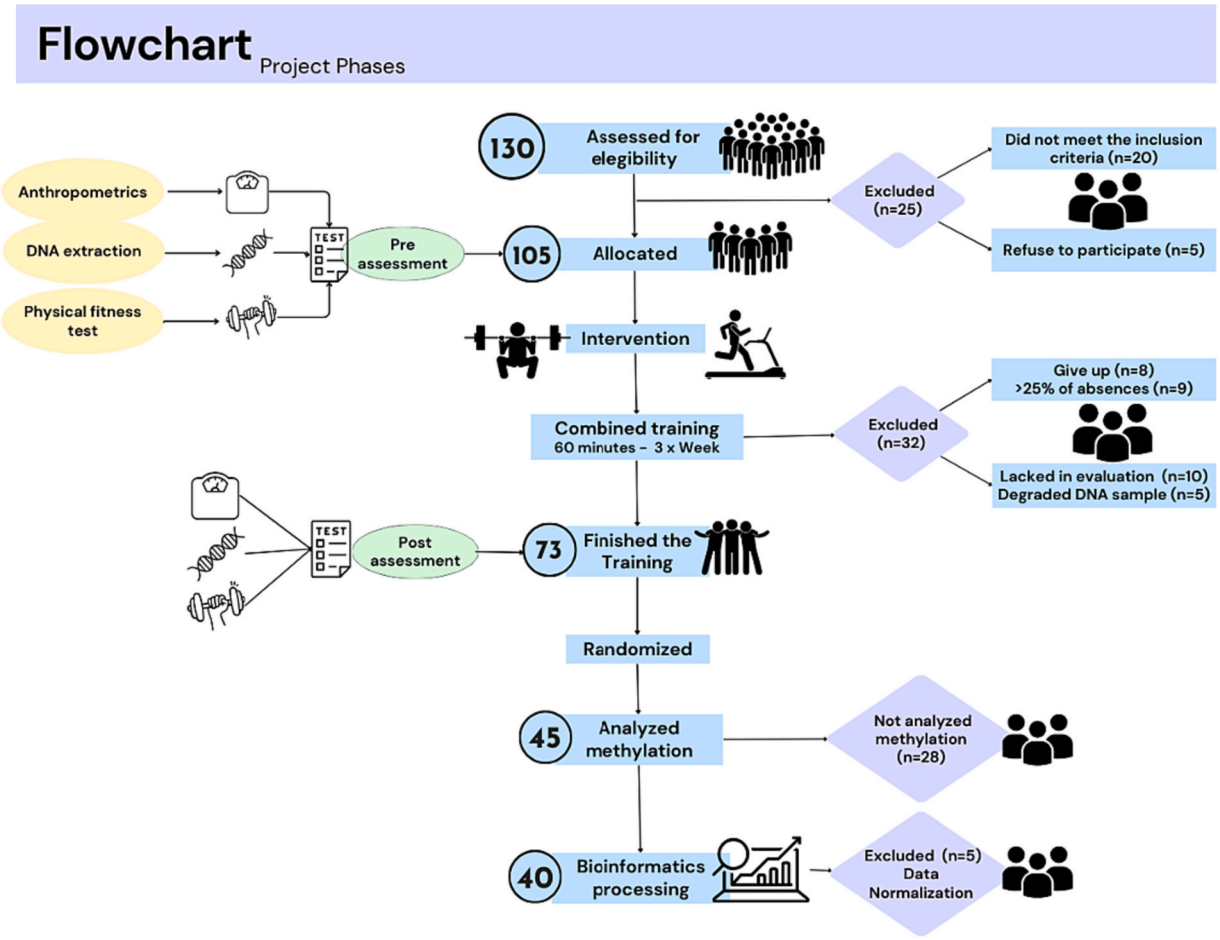

Fig. 1. Flowchart and experimental design.

training foster neurogenesis, and synaptic plasticity, diminish inflammatory processes, and mitigate cellular damage due to oxidative stress, important processes in prevention AD prevention (Foster et al., 2011).

A recent meta-analysis highlights that engaging in moderate-intensity aerobic and resistance exercises for at least 45 min, multiple times weekly, enhances cognition in adults aged 50 and above (Northey et al., 2018). Additionally, exercise training contributes to AD prevention even in cases of high genetic risk, an unmodifiable factor (Hamer and Chida, 2009). The meta-analysis that analyzed 16 studies (n = 160,000 participants) found a 45 % reduction in the risk of developing AD due to the regular practice of physical activity (Hamer and Chida, 2009). After 3.5 years of monitoring 716 older subjects, one study reported that individuals with low daily physical activity levels were 53 % more likely to suffer AD than those who reported more active lives (Buchman et al., 2012). It is still estimated, that 3 % of dementia cases could be prevented by increasing levels of free-living physical activity (Liang et al., 2020; Livingston et al., 2020).

It is well know that exercise training and others external factors such as dietary habits, smoking, or alcohol consumption interact with the genome and affect the phenotype via modifications in epigenetic mechanisms (DNA methylation, histone modifications, chromatin remodeling and non-coding RNA), which can suppress or increase gene expression (Wu et al., 2023).

Potentially, DNA methylation is the epigenetic mechanism that has the greatest impact on the regulation of gene expression, being related to the aging process, health parameters, and the increase or reduction in the risk of developing cardiovascular and neurological diseases, cancers, among other complications (Wu et al., 2023; Barrón-Cabrera et al., 2019). Current dementia research increasingly delves into the role of epigenetics in AD development, a crucial mechanism in memory formation and regulation (Higgins-Chen et al., 2021).

Recently, it was presented in the literature that eight weeks of combined exercise training showed changes in methylation patterns compared to baseline values, as well as rejuvenating the epigenetic clock by two years in older women (da Silva et al., 2023a). Conversely, a year-long aerobic training study in 320 healthy older women revealed no influence of exercise training on DNA methylation in specific breast cancer-related genes (Boyne et al., 2018). To date, evidence remains lacking regarding whether any form of exercise training can modify methylation levels in genes linked to AD among healthy seniors. A recent meta-analysis delineating AD risk loci implicated in Aβ, tau, immunity, and lipid processing identified 25 genes associated with AD development, emphasizing the need to elucidate how exercise training impacts the epigenetics of these genes to bolster AD prevention in conjunction with training (Verheijen and Sleegers, 2018).

It is important to investigate alternative ways of preventing AD. The literature shows that exercise can be used as a tool, but it is still not clear how exercise acts on DNA methylation in healthy older people when we observe genetic sites related to AD, being able to understand whether the exercise would actually act as a protective factor for the development of AD in healthy women aged 50 to 70 years.

Our investigation aims to examine the effects that combined exercise training carried out for 14 weeks has on DNA methylation in genes associated with AD in healthy non-Alzheimer's disease women aged 50 to 70 years, understanding how exercise would act in an epigenetic way to prevent AD.

2. Methods

The research project underwent review by the Research Ethics Committee involving Human Subjects at EEFERP-USP (CAAE: 79582817.0.0000.5659), adhering to Resolution 466/12 by the Ministry of Health, as regulated by the National Health Council. This study was registered in the Brazilian Clinical Trials Registry (REBEC) under registration number RBR-3g38dx.

2.1. Eligibility criteria

Women aged between 50 and 70 years old were included, with medical authorization to practice physical exercise and identified as physically inactive using the Modified Baecke Questionnaire (QBMI) for the Elderly. The QBMI measures the three domains of physical activity (domestic, sporting, and free time) usually carried out by the elderly, scores lower than 9.11 consider those assessed to be physically inactive (Florindo et al., 2003). Another criterion chosen for eligibility not to include people with cognitive impairment was achieving >26 points on the Montreal Cognitive Assessment (Wang et al., 2022), the minimum score for cognitively normal older adults in this cognitive test. This instrument was developed as a brief instrument for screening mild cognitive impairment. The Montreal Cognitive Assessment stands out for evaluating different cognitive domains such as: attention and concentration, executive function, memory, language, visual-constructive capacity, abstract summary, calculation, and orientation (Wang et al., 2022).

Participants were excluded based on criteria such as physical limitations that make it difficult to perform exercises or motor tests used in the study, current smokers or those who smoked in the last six months, degraded DNA at the time of bioinformatics processing, individuals who consumed caffeine within 24 h prior to blood collection and those diagnosed with Alzheimer's disease (Fig. 1).

2.2. Participants

The sample size required for the experiment was calculated using the GPower software. We used tests for independent samples to analyze the results of post-intervention changes. With an alpha error probability of 0.05 and power adjusted to 0.8 %, we estimated that a minimum of 35 participants would be necessary to detect a difference between the means.

We recruited more participants than necessary due to dropouts during the intervention process and exclusion of participants after normalization in the processing of methylation data, we calculated a sample with 28 % more, totaling 40 participants in the final group (Fig. 1).

2.3. Combined training protocol

The combined exercise training protocol consisted of sessions lasting 60 min, conducted three times a week over a period of 14 weeks. The training sessions were a combination of cardiovascular exercises (treadmill or bicycle) lasting 30 min and muscular strength exercises, also lasting 30 min (Rodrigues et al., 2023; da Silva et al., 2023b).

We adopted eight exercises for strength training, comprising five exercises for the upper limbs (inclined bench press, leg curl, leg extension, leg press 45, triceps bar, rower, arm curl, and lat pull-down) and three for the lower limbs (leg curl, leg extension, and leg press at 45°) (da Silva et al., 2023b). During the initial two weeks, participants performed two sets of 15 to 17 maximum repetitions for strength exercises and spent 30 min on a treadmill or stationary bicycle using 50 % of their heart rate reserve. From weeks 3 to 14, two sets of 10 to 12 maximum repetitions were completed, along with 30 min on a treadmill or stationary bicycle at 70 % of the heart rate reserve (Rodrigues et al., 2023). Training intensities were also assessed using the Borg Rating of Perceived Exertion (Borg and Noble, 1974). The Polar Team2 from Finland was utilized for heart rate measurements (Rodrigues et al., 2021).

2.4. Assessment

Participants underwent two rounds of physical assessments: before and after the 14 weeks of combined training. We assessed anthropometric variables (height (cm), weight (kg), body mass index (kg/m^2), waist circumference (cm) and hip circumference (cm)) (Rodrigues et al., 2023), and systolic and (SBP) diastolic blood (DBP) pressure pressures were measured using a previously calibrated automatic digital blood pressure gauge (OMRON®, Jundiaí, Brazil, model HEM-7113, SBH, 2010).

The motor variables evaluated included arm curl, chair stand, agility, 6-Min walk test (Rikli and Jones, 1999), maximum strength of the upper limbs measured by inclined bench press, and maximum strength of the lower limbs assessed by the leg press 45° (Izquierdo et al., 2002).

2.5. DNA extraction and methylation analysis

Peripheral blood samples were collected in tubes with EDTA at two different time points: before and after 14 weeks of training. Subsequently, genomic DNA was isolated from peripheral blood using the salting-out method (Shokrzadeh and Mohammadpour, 2018). The quality and integrity of the DNA were assessed by spectrophotometry (Bulla et al., 2016).

DNA methylation assays were conducted using the array technique, employing the EPIC Infinium Methylation BeadChip from Illumina. The fluorescence of the Infinium BeadChip was detected using the Illumina iScan system (Noronha et al., 2022). Intensities were converted into Beta values of methylation and exported as raw data. Subsequently, data processing was carried out using Rstudio software (R version 3.6.3) (Noronha et al., 2022), analyzing methylation patterns following the recommendations of the chAMP package from Bioconductor (Tian et al., 2017; Correia et al., 2023).

In the DMP function, we modified the command to pheno = myLoad $pd.$Sample_Group and conducted compare group to analyze the pre- and post-intervention moments. We adopted a significance threshold of $p < 0.05$ and subsequently filtered hypermethylation and hypomethylation values in the gene list, considering the beta values (Correia et al., 2023), as a method of adjusting the p value, the default = BH (Benjamini-Hochberg) recommended by ChAMP was adopted (Li and Barber, 2019).

2.6. Enrichment analysis (String)

We enriched our gene list using the STRING software. It's worth noting that the desired specificity cutoff for functional associations in STRING approximately corresponds to the annotation granularity of KEGG pathway maps (Kanehisa et al., 2017). We performed cytosine-phosphate-guanine enrichment through the epigenome-wide association study (EWAS), as it is becoming increasingly significant in identifying associations between epigenetic variations and the different biological characteristics that can be presented (Li et al., 2019).

2.7. Statistical analysis

The motor and anthropometric data collected were entered into an Excel file by two researchers and subsequently cross-checked to eliminate potential errors. We utilized GraphPad Prism 8 for statistical analyses, employing the Shapiro-Wilk test for normality assessment.

To compare values before and after the intervention, we used the Student's t-test for symmetrically distributed data. For asymmetrically distributed data, we employed the Mann-Whitney test. Across all statistical analyses, we set the significance level at $p < 0.05$.

Table 1
Means and standard deviations of the variables adopted for sample characterization ($n = 40$).

Variables	Pre	Post	*P*-value
YEARS OF EDUCATION (years)	10.6 ± 3.3		
AGE (years)	60.7 ± 4.1		
HEIGHT (m)	1.6 ± 0.1		
BW (Kg)	73.12 ± 9.0	73.46 ± 8.5	0.887
BMI (Kg/m2)	29.69 ± 3.5	29.35 ± 3.1	0.714
WC (cm)	94.56 ± 8.0	96.35 ± 9.0	0.467
HC (cm)	106.8 ± 7.5	106.1 ± 6.8	0.718
SBP (mmHg)	134.1 ± 14.7	121.0 ± 10.6	0.001*
DBP (mmHg)	80.15 ± 8.4	75.17 ± 5.5	0.017*

Note - BW: body weight; BMI: body mass index; WC: waist circumference; HC: hip circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; Normality test: Shapiro-Wilk; Student's t-test $p < 0.05$.

3. Results

3.1. Anthropometric data and blood pressure

Below are presented the results as mean and standard deviation of the 40 participants, regarding anthropometric comparisons and pre- and post-training blood pressure. We observed that 14 weeks of combined training lead to reductions in both SBP ($p = 0.001$) and DBP ($p = 0.017$) women with an average age of 60.7 ± 4.1 years, but no significant differences were found in anthropometric variables (Table 1).

3.2. Physical fitness tests

Physical improvements were observed after 14 weeks across all motor tests employed in this study. Significant enhancements and a considerable increase in strength were evident in the arm curl (Fig. 2A), chair stand (Fig. 2B), upper limb maximum repetition (Fig. 2E), and lower limb maximum repetition (Fig. 2F) tests when comparing the initial assessment to the final evaluation. The physical outcomes demonstrate that the combined training regimen yielded improvements in both strength tests and the assessment of aerobic fitness (6-min walk, Fig. 2D), as well as agility (Fig. 2C).

3.3. Genetic enrichment

We conducted enrichment analysis of the list of 25 genes used in this study to comprehend their potential relationships (Fig. 3). Following comparisons between the two assessment points, we observed differentially methylated sites in 12 genes (Fig. 4).

In the comparison of beta values, we observed that the vast majority of genes exhibited hypomethylated sites (negative β values). Despite these hypomethylated sites, some genes still displayed hypermethylated sites (positive β values), such as *ABCA7*, *BDNF*, and *WWOX* (Table 2).

We performed Cytosine-phosphate-Guanine enrichment of the 12 genes that were differentially methylated after 14 weeks of training to identify which gene ontology they were linked to (Fig. 5).

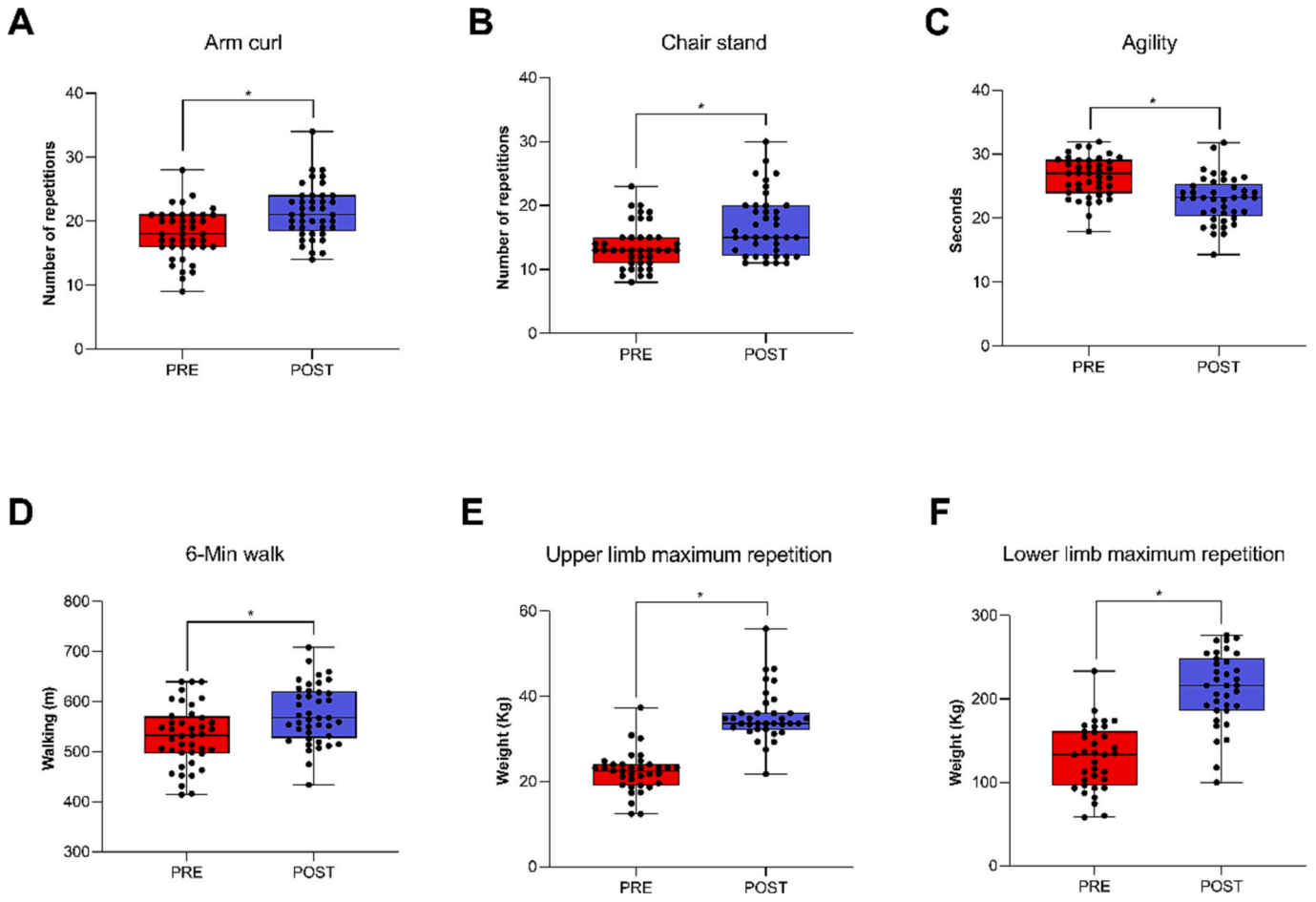

Fig. 2. Pre- and Post-Exercise Intervention Comparisons for Motor Variables ($n = 40$).
Note - Normality test: Shapiro-Wilk; Mann-Whitney test $p < 0.05$.

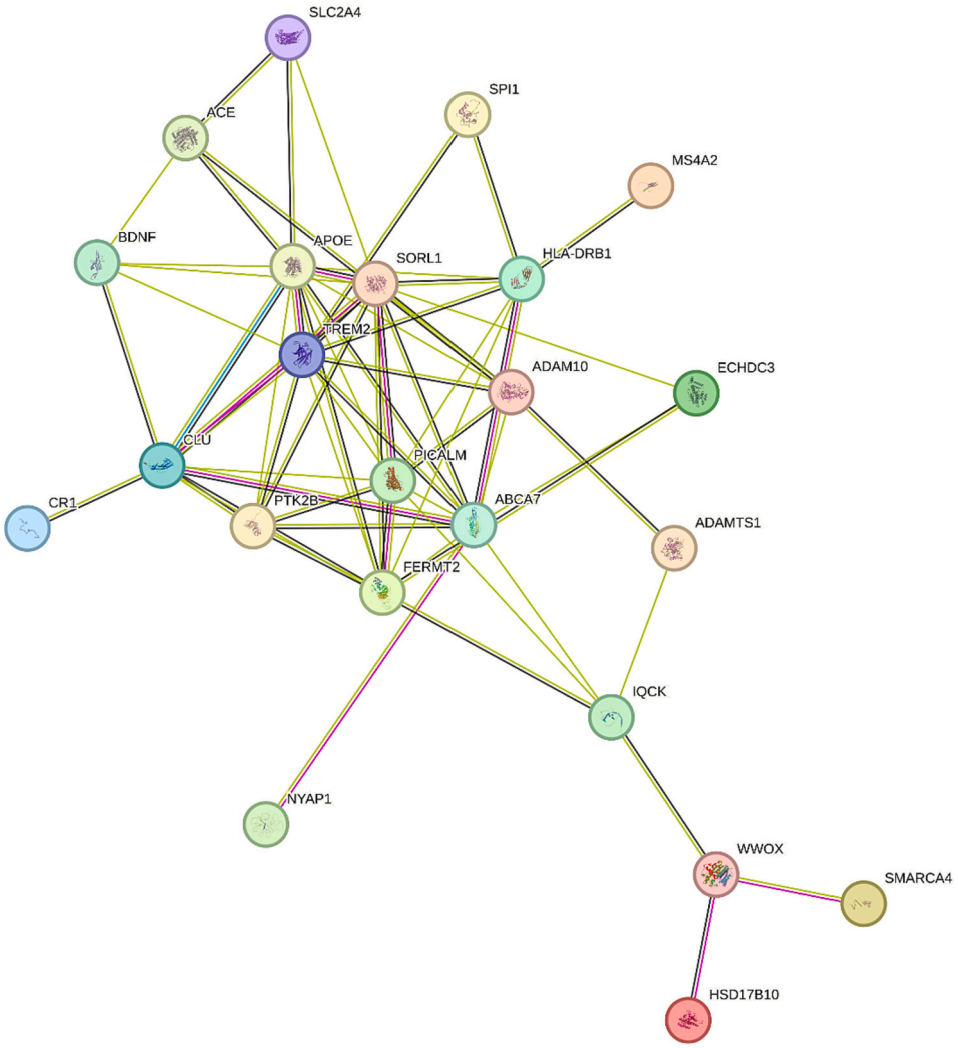

Fig. 3. Genetic enrichment of the list of genes adopted in the study.

4. Discussion

The present study aimed to investigate the effect that 14 weeks of combined training has on three distinct evaluation moments at sites of genes related to AD in women aged 50 to 70 years. Our data presented that 14 weeks of combined training were insufficient to obtain significant differences in anthropometric variables. The combined training is effective in improving health, however some recent studies have shown it may not be as effective in improving anthropometric data in older people (Rodrigues et al., 2023; da Silva et al., 2023b). These findings align with a 2020 study conducted by Brandão et al. (Brandao et al., 2020) that assessed the effects of an 8-week combined training program in overweight or obese older women. Although combined training was effective in improving cardiorespiratory fitness and muscular strength of participants, there was no significant change in body mass index (BMI) and waist circumference (Brandao et al., 2020).

The literature shows that similar training periods also did not result in changes in anthropometric data, such as the 2023 (Rodrigues et al., 2023) study comparing fourteen weeks of multicomponent training versus combined training in physically inactive older women, which did not show significant changes in anthropometric data like BMI and body fat percentage (Rodrigues et al., 2023). Therefore, other intervention strategies (such as nutritional ones) may be necessary in association to enhance the response magnitude of these data in older people (Amati et al., 2010).

According to our observations, combined training is an effective strategy to reduce systolic and diastolic blood pressure in older people, achieving significant results in 14 weeks of training in this study. When compared with the literature, we found a 2019 study by Leando et al. (Leandro et al., 2019) that assessed the effects of an eight-week combined training program on the blood pressure of hypertensive older women. The study showed that combined training significantly reduced systolic and diastolic blood pressure compared to a control group that did not undergo physical training. Furthermore, a 2019 study conducted by Ruangthai and colleagues (Ruangthai and Phoemsapthawee, 2019) evaluated the effects of a 12-week combined training program on the blood pressure of pre-hypertensive older women and men. The results indicated that the hypotensive and antioxidant effects of combined training were significantly high. The combined training is effective in reducing systolic and diastolic blood pressure, as well as providing other health benefits, similar to our findings. Our study had a longer training period than the studies presented above, which may have potentiated the results due to the broader range of stimuli.

There is an association between epigenetic modifications and the loss of phenotypic plasticity. Epigenetic patterns contribute to the etiology of Alzheimer's disease (AD) and other neurodegenerative disorders (Fuso, 2013). Various external factors, including stressors (physical and behavioral), nutritional elements, pollutants, pesticides, chemicals, metals, physical exercise, mental activity, and lifestyle, can exert effects that might contribute to the onset and progression of numerous diseases.

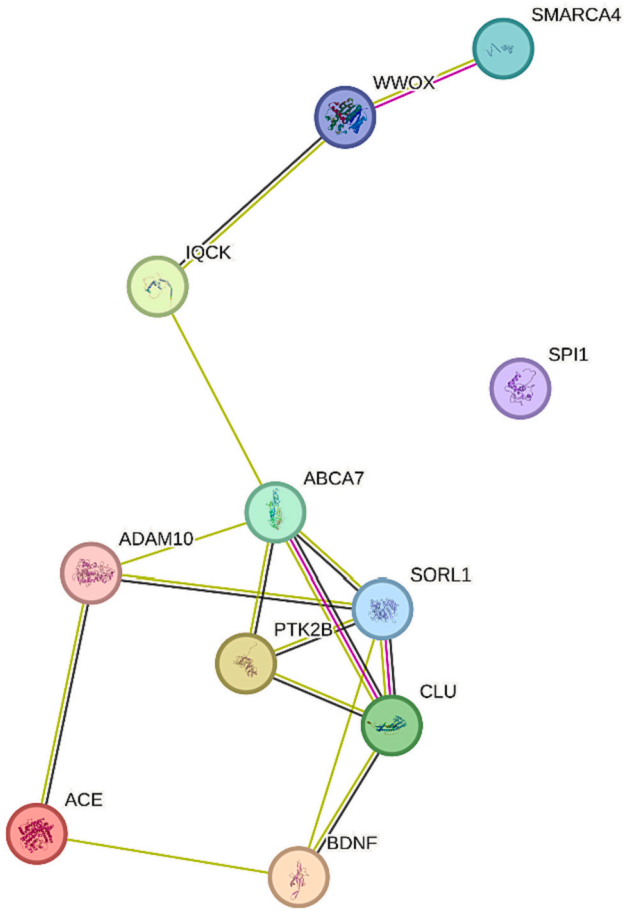

Fig. 4. Enrichment of genes with differentially methylated sites in training comparisons.

Physical exercise has been linked to a reduced risk and protective effects against mild cognitive impairment and AD (Nicolia et al., 2015). Studies have shown that moderate physical activity in animal models induces positive physiological and cognitive effects by altering transcriptional profiles through epigenetic modifications (Kaliman et al., 2011). A meta-analysis of 13 studies has confirmed the impact of physical activities on positive cognitive effects and their protective role against AD (Jia et al., 2019).

A literature review has indicated that physical activity, ranging from mild to moderate intensity, can decrease the risk of dementia and AD. This is in line with our findings since the studied population was healthy. Furthermore, potential benefits were observed for patients already diagnosed with AD (Cass, 2017), and physical activities may provide benefits for the treatment or prevention of AD.

The capacity of exercise to induce alterations in genes and proteins through epigenomic manifestations that regulate synaptic and cognitive plasticity is fundamental in controlling neurological disturbances (Fernandes et al., 2017). Shireby et al. (2022) conducted a DNA methylation profiling in cortical regions associated with AD neuropathology. They identified differentially methylated positions associated with AD, utilizing measures of neuropathology to identify epigenetic signatures of AD, underscoring the importance of characterizing the disease (Shireby et al., 2022). Despite several studies demonstrating the relationship between exercise and AD, our results showcased epigenetic modulation in crucial genes to prevent AD in healthy patients.

Epigenetic biomarkers could potentially serve as a helpful tool in patient screening in the future, as changes in methylation in specific genes have already been identified as biomarkers in AD using peripheral blood leukocytes (Hou et al., 2013). Additionally, an association has been observed between diet, exercise, and improved health in AD patients related to epigenetic modifications (Athanasopoulos et al., 2016).

After 14 weeks of exercise, modulation of gene methylation correlated with AD (*ABCA7*, *ACE*, *ADAM10*, *BDNF*, *CLU*, *INPP5D*, *IQCK*, *PTK2B*, *SMARCA4*, *SORL1*, *SPI1*, *WWOX*) was evident. Studies have demonstrated that methylation in ABCA7 is linked to AD, as decreased *ABCA7* levels are associated with the condition. Hence, increasing functional *ABCA7* expression levels could be a valuable therapeutic approach (De Roeck et al., 2019). Our results indicated hypomethylation in *ABCA7* after exercise, suggesting a potential preventive role against AD. The *WWOX* gene's loss of function has been associated with various central nervous system pathologies. Dysfunctional *WWOX* induces tau and Aβ aggregation, leading to apoptosis. Additionally, *WWOX*'s role in brain development and pathology, such as AD, is well-documented (Stevenson-Hoare et al., 2023). This gene plays a critical role in inflammation and is associated with AD progression. Therefore, dysfunctional *WWOX*, such as loss or excessive *WWOX*, contributes to neurodegenerative diseases (AD, Parkinson's disease, among others) (Hsu et al., 2021). Hence, in our results, this gene predominantly appeared hypomethylated at most sites after exercise, although some sites exhibited hypermethylation.

The primary strength of the current study was to analyze methylation patterns using a chip capable of examining 850 thousand CpG sites, providing a comprehensive assessment of the alterations that combined physical training may induce in the methylation patterns of genes related to AD. No methods were employed to assess dietary patterns, and it is known that diet constitutes important environmental factors that can modify methylation patterns. However, participants were instructed not to make lifestyle changes during their involvement in the study, in addition to completing a 24-h dietary recall at the pre-collection stage and adhering to the same record during the post-intervention collection.

Finally, it is plausible that alterations in the methylation profile might not solely underlie the mechanisms inducing adaptations in physical performance but could represent an additional adaptation to the exercise program in a more established trained condition. In future studies, determining the gene expression associated with hypomethylated and hypermethylated sites will be crucial.

5. Conclusion

The combined physical training conducted over a 14-week period induced alterations in the methylation patterns of gene sites related to the development of AD in middle-aged and older women unaffected by neurodegenerative diseases. This epigenetic evidence highlights that physical exercise for 14 weeks was adequate to cause varying levels of methylation in genes responsible for the development of AD in women aged 50 to 70 years, acting as a protective factor for the development of the disease. Moreover, it facilitated improvements in functional capacity, physical performance, and blood pressure among these women.

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CRediT authorship contribution statement

Guilherme da Silva Rodrigues: Writing – review & editing, Writing – original draft, Visualization, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **Natália Yumi Noronha:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **Isabella Harumi Yonehara Noma:** Writing – review & editing, Writing – original draft, Methodology,

Table 2
Differentially Methylated Sites Across the 14 Weeks of Training.

Genes	CpG	CHR	Gene Region	Type	rs	β1 I x III	p-value
ABCA7	cg02817925	19	Body	island	rs150971192	0.009	0.020
ABCA7	cg17993394	19	Body	shelf	rs59237564	−0.025	0.008
ABCA7	cg26970077	19	Body	shore	rs542467691	−0.038	0.015
ABCA7	cg12064401	19	Body	island	rs576278275	−0.024	0.017
ABCA7	cg15681366	19	Body	shelf	rs561581862	−0.017	0.027
ABCA7	cg18846541	19	ExonBnd	shore	rs149365975	−0.017	0.030
ABCA7	cg26263477	19	TSS1500	island	rs184510773	−0.008	0.042
ACE	cg24189328	17	TSS1500	shore		−0.029	0.006
ADAM10	cg16123305	15	Body	opensea	rs113109705	−0.016	0.035
BDNF	cg12021170	11	TSS1500	opensea	rs184293959	−0.029	0.039
BDNF	cg08760147	11	Body	opensea	rs184548784	−0.022	0.042
BDNF	cg11241206	11	TSS1500	shore	rs56195957	0.01	0.046
CLU	cg08594681	8	1stExon	shelf		0.018	0.030
INPP5D	cg04975586	2	ExonBnd	opensea	rs201252871	−0.021	0.016
INPP5D	cg18097144	2	TSS1500	shore	rs188579739	−0.028	0.015
INPP5D	cg16151789	2	Body	opensea		−0.027	0.033
INPP5D	cg06831850	2	Body	opensea	rs574989226	−0.017	0.038
IQCK	cg17440572	16	Body	opensea	rs114245109	−0.024	0.014
IQCK	cg17595464	16	5'UTR	island	rs530998132	−0.007	0.017
IQCK	cg10032375	16	Body	opensea	rs540939870	−0.019	0.040
PTK2B	cg12242935	8	5'UTR	opensea	rs191315642	−0.014	0.045
SMARCA4	cg03921155	19	Body	opensea	rs568094122	−0.03	0.044
SORL1	cg25236143	11	Body	opensea	rs544790047	−0.027	0.011
SORL1	cg04707711	11	Body	opensea	rs569947455	0.021	0.038
SPI1	cg24234141	11	3'UTR	shore	rs531943594	0.024	0.046
SPI1	cg01539849	11	3'UTR	island		0.011	0.045
WWOX	cg05475027	16	Body	opensea	rs550214854	−0.022	0.018
WWOX	cg03648054	16	Body	opensea	rs527659014	−0.021	0.019
WWOX	cg03295251	16	Body	opensea	rs567996965	0.012	0.020
WWOX	cg02236679	16	Body	opensea	rs563057433	−0.026	0.022
WWOX	cg02546607	16	Body	opensea	rs373052053	0.024	0.022
WWOX	cg23054284	16	Body	opensea	rs148661660	−0.025	0.036
WWOX	cg14789524	16	Body	opensea	rs56073608	−0.027	0.037
WWOX	cg26229208	16	TSS1500	shore	rs141439937	−0.018	0.042
WWOX	cg02317358	16	Body	opensea	rs202052438	−0.019	0.045
WWOX	cg26581964	16	Body	opensea	rs145917497	−0.019	0.049

Note – CpG: Cytosine-phosphate-Guanine; CHR: Chromosome; TSS1500: Transcription Start Site 1500; TS200: Transcription Start Site 200; 5'UTR: 5' Untranslated Region; 3'UTR: 3' Unstranslated Region.

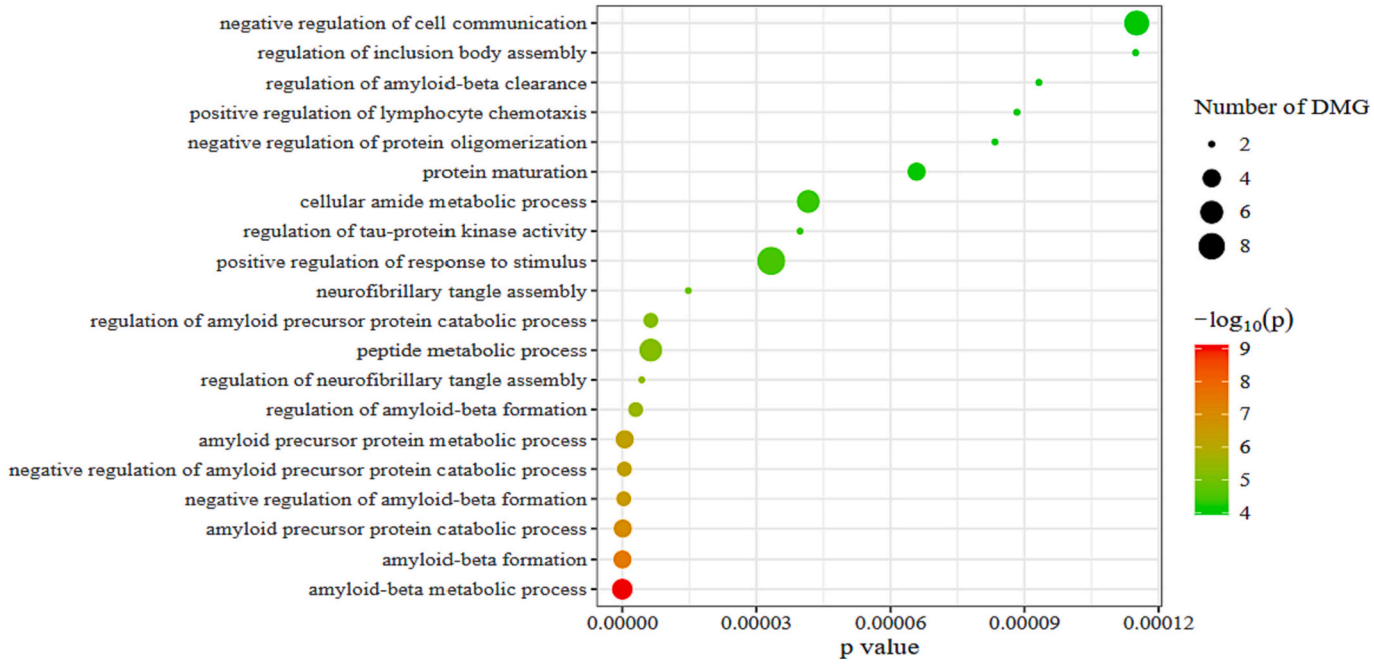

Fig. 5. Gene Ontology enrichment of probes that are differentially methylated after the 14 weeks of combined training.
Note - DMG: Number of Genes Differentially Methylated in the respective pathways.

Investigation, Formal analysis, Conceptualization. **João Gabriel Ribeiro de Lima:** Writing – review & editing, Writing – original draft, Validation, Conceptualization. **Andressa Crystine da Silva Sobrinho:** Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Conceptualization. **Marcela Augusta de Souza Pinhel:** Writing – review & editing, Writing – original draft, Validation, Conceptualization. **Mariana Luciano de Almeida:** Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Lígia Moriguchi Watanabe:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Conceptualization. **Carla Barbosa Nonino:** Writing – review & editing, Writing – original draft, Formal analysis, Conceptualization. **Carlos Roberto Bueno Júnior:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Data availability

The DNA methylation datasets presented in this study can be found online: https://doi.org/10.6084/m9.figshare.21763535. Other data are available upon request to the corresponding author.

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