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Physical exercise improves memory in sedentary middle-aged adults: Are these exercise-induced benefits associated with S-Klotho and 1,25-dihydroxivitamin D? The FIT-AGEING randomized controlled trial

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Abstract

We aimed to investigate the effect of three types of exercise interventions on memory (i.e., immediate memory (IM), long-term memory (LTM), and recognition). We also investigated whether exercise-induced changes in circulating S-Klotho and 1,25-dihydroxivitamin D ($1,25(OH)_2D$) levels were related to those observed in memory in healthy middle-aged sedentary adults. A 12-week randomized controlled trial was performed with a parallel-group design. Seventy-four participants (45–65 years old: 53% women) were randomly assigned to (1) no exercise (control) group, (2) concurrent training based on the international physical activity recommendations (PAR) group, (3) high-intensity interval training (HIIT) group, or (4) HIIT plus whole-body electromyostimulation (HIIT-EMS) group. Memory outcomes were assessed using the Wechsler Memory Scale-third edition. S-Klotho plasma levels were determined according to a solid-phase sandwich enzyme-linked immunosorbent assay kit while $1,25(OH)_2D$ plasma levels were measured using a DiaSorin-Liaison immunochemiluminometric analyzer. IM-Verbal Paired Associates (IM-VPA) and IM-Logical Memory (IM-LM) were improved in both the HIIT and HIIT-EMS groups compared with the control group (all $p \leq 0.045$). Exercise-induced changes in S-Klotho plasma levels were positively associated with those observed in IM, LTM, and recognition (all $p \leq 0.007$), whereas exercise-induced changes in $1,25(OH)_2D$ plasma levels were directly related to changes in IM and LTM (all $p \leq 0.048$). In conclusion, a 12-week HIIT intervention with or without WB-EMS seems to be the most effective exercise program to improve IM. The significant and positive associations between exercise-induced changes in S-Klotho and $1,25(OH)_2D$ levels with those observed in memory outcomes suggest that these factors may be potentially related to exercise-induced improvements of memory in middle-aged adults.

KEYWORDS
1,25-dihydroxivitamin D, long-term memory, physical exercise, short-term memory, S-Klotho

1 | INTRODUCTION

Aging is associated with a progressive deterioration of mental and physical health,[1] which usually reach a symptomatic stage during the middle-aged adulthood.[2] Regular physical activity has been positioned as an effective strategy to maintain brain health[3] by preventing cognitive decline in aged adults.[4–6] Several studies have employed physical exercise as a useful strategy for enhancing cognitive and mental health in older populations,[7–9] but it is still unknown the most effective type of physical exercise for that purpose[10] (i.e., intensity, type of exercise, or duration).[5] Previous evidence in this field of research has shown an exercise-induced increased size of the hippocampus in humans,[11] improved synaptic plasticity in rats,[12] and facilitated growth, development, function, and survival of neurons in response to physical exercise interventions in old adults.[13]

The physical activity recommendations provided by the World Health Organization (WHO) (i.e., combining endurance, >150 min/week at moderate intensity; and resistance training, >2 sessions/week) are associated to multiple health benefits, including those related with cognitive and mental health.[14,15] WHO recommendations are normally implemented for general health promotion but, considering that their adherence rates are relatively low at the present time,[16] new training tendencies are emerging as potential alternatives. In this context, high-intensity interval training (HIIT) has been also proposed as an efficient intervention to enhance cardiometabolic and cognitive health.[17] Nevertheless, it is unknown which of those two types of physical exercise interventions (i.e., concurrent exercise at moderate vs. HIIT) is more effective at improving cognitive health.[14,15]

Whole-body electromyostimulation (WB-EMS) training can be combined with the HIIT methodology allowing an extra activation in all the main muscle groups.[18] Although previous studies have reported that WB-EMS notoriously improved different health biomarkers,[19,20] it is not clear whether this methodology offers an additional advantage over HIIT alone,[21] since it can exert important neuromuscular adaptations through central and peripheral nervous systems' activation potentially resulting in additional cognitive benefits.

Klotho protein has been associated with a reduced aging-associated cognitive decline.[22] Klotho can be found as a transmembrane protein or in a soluble form (S-Klotho) formed by proteolytic cleavage of transmembrane Klotho.[23] Numerous physiological functions have been attributed to S-Klotho protein including a reduction of inflammatory processes, a decrease in cellular oxidative stress,[24] and a neuroprotective role in humans through the regulation of cognitive functions promoting the oligodendrocyte maturation and myelination, thus preventing neurodegeneration.[25] Vitamin D deficiency has become an important public health concern being especially important in the elderly.[26] Calcitriol or 1,25-dihydroxivitamin D ($1,25(OH)_2D$), the active form of vitamin D, presents neuroprotective properties since the vitamin D receptor (VDR) is widely disseminated in the nervous system.[27] We have previously demonstrated that a 12-week exercise intervention improves both circulating S-Klotho and $1,25(OH)_2D$ levels in sedentary middle-aged adults (i.e., 45–65 years old).[16,28] Therefore, we hypothesized that potential higher differences in cognitive health through physical exercise interventions could be associated with changes in S-Klotho and $1,25(OH)_2D$ levels.[29,30]

Although several studies have suggested the capacity of physical exercise to improve memory,[4,7,13] there is little evidence evaluating the effects of different training modalities on immediate memory (IM), long-term memory (LTM), and recognition. Moreover, to the best of our knowledge, there is no chronic exercise study focused on middle-aged adults who have not been clinically diagnosed with cognitive impairment. Furthermore, it remains elusive whether exercise-induced changes in S-Klotho and $1,25(OH)_2D$ levels can be potentially related to the above-mentioned changes in memory outcomes in sedentary healthy middle-aged populations. The aim of this study was to investigate the effects of three types of exercise interventions (i.e., physical activity recommendations provided by the WHO, HIIT, and HIIT combined with WB-EMS) on IM, LTM, and recognition. Moreover, we also investigated their association with exercise-induced changes in S-Klotho and $1,25(OH)_2D$ levels in healthy middle-aged sedentary adults. We hypothesized that the HIIT combined with WB-EMS program would show higher differences in the memory outcomes, and that changes in these parameters could be partially associated with changes in circulating S-Klotho and $1,25(OH)_2D$ levels.

2 | MATERIALS AND METHODS

2.1 | Participants

A total of 74 (53% women) middle-aged sedentary adults aged 45–65 years old were included in this study. These participants were enrolled in the FIT-AGING study[31] (clinicaltrial.gov: ID: NCT03334357), a randomized controlled trial which aimed to quantify the effects of different training modalities on health-related parameters in sedentary healthy adults. Details concerning the inclusion and exclusion criteria can be seen elsewhere.[31] Briefly, they reported (I) to be non-physically active (i.e., less than 20 min of physical activity on less than 3 days/week), (II) to have stable weight (weight changes <3 kg) in the past 12 weeks, (III) to be free of disease, (IV) not to be pregnant or breastfeeding, (V) to be a non-smoker, and (VI) not taking any medication. An extensive medical examination was performed before the beginning of the study. The study was approved by the Human Research Ethics Committee of the "Junta de Andalucía" [0838-N-2017], and the participants provided oral and written informed consent. The study protocols were applied in consonance with the last revised ethical guidelines of the Declaration of Helsinki.[32]

2.2 | Study design

The present study was designed as a 12-week randomized controlled trial following the Consolidated Standards of Reporting Trials (CONSORT) guidelines (available at EQUATOR Network: http://www.equator-network.org/reporting-guidelines/consort/; Table S1).[33] For practical reasons, the study was conducted in two waves with a maximum of 45 participants in each wave. Accordingly, blood samples were taken at baseline (i.e., September–October of either 2016 or 2017) with follow-up in either December 2016 or 2017 (all at the San Cecilio University Hospital of Granada); all other baseline and follow-up examinations were performed in the same wave periods at the Sport and Health University Research Institute (iMUDS, University of Granada). After the baseline evaluation, the participants were randomly allocated to one of four exercise programs using a computer-generated simple randomization procedure[34]: (1) no exercise (control group), (2) an intervention based on the physical activity recommendations for adults proposed by the World Health Organization (PAR group), (3) a HIIT group, and (4) a HIIT combined with WB-EMS (HIIT-EMS group). The participant's randomization allocation was blinded to the assessment staff (Figure S1). All participants were requested not to modify their dietary and physical activity habits the same as before the study, except for those in the exercise group, who were instructed not to do additional exercise as per their intervention programs.[31] Both age and educational level were self-reported by all the participants.

2.3 | Training modalities

A detailed description of the three exercise training programs is provided elsewhere.[31] The descriptions of the followed exercise training programs adhere to the Consensus on Exercise Reporting Template (CERT; ESM Table S2),[35] improving the transparency and replicability of the present study. Attendance of at least 90% of the planned sessions was required to be included in the final analyses. Participants who missed a session were asked the reason for their absence and requested to make up for it on another day in the same week (or, alternatively, in the next week). All training sessions were conducted in groups of 2–6 participants and were always supervised by an exercise science graduate. A gradual progression in intensity was also scheduled to ensure a proper adherence to each intervention group.

The participants assigned to the control group were provided with general advice on a healthy lifestyle, including nutritional information and physical activity guidelines.

The PAR group performed a concurrent training intervention (i.e., combining aerobic and resistance training) based on the international physical activity recommendations.[14,15] The participants trained 3 days/week for 12 weeks. The training volume was 150 min/week at 60%–65% of the heart rate reserve (i.e., ~70% of the maximum heart rate) for aerobic training. A treadmill, cycle ergometer, or elliptical ergometer were used to perform aerobic training. For the resistance training section, weight-bearing, and guided pneumatic machines were used (i.e., squat, bench press, deadlift, or lateral pull down). In addition, complementary exercises were performed (i.e., core stability, flexibility, and stabilizer muscles) to minimize risk of injuries and to promote training.

The HIIT group performed a high-intensity interval program characterized by short and intermittent efforts of vigorous activity, interspersed with rest periods of passive or low-intensity exercise. The participants exercised 2 days/week for 12 weeks with a recovery period between sessions of at least 72 h following two different complementary protocols alternatively[36]: a Long Interval Protocol (LIP) and a Short Interval Protocol (SIP). Training volume was 40–65 min/week at >95% of the maximum oxygen uptake in LIP session and >120% of the maximum oxygen uptake in SIP sessions. The exercise programmed for the LIP session was treadmill with a personalized slope;

SIP sessions were eight weight-bearing exercises in circuit form (i.e., squat, deadlift, high knees up, high heels up, push up, horizontal row, lateral plank, and frontal plank). Participants were not immediately capable of meeting the volume and intensity doses required; therefore, we conducted a training periodization divided into three phases (i.e., HIIT familiarization phase, HIIT phase I, and HIIT phase II).[31]

WB-EMS technology is able to simultaneously stimulate a total of 18 muscle regions covering a total area of 2800 cm^2.[37] The HIIT-EMS group performed a training program with the same structure as the HIIT group (i.e., volume, intensity, training frequency, type of exercise, and training sessions), but with the inclusion of electrical impulses. Since the participants had never been exposed to WB-EMS, this stimulation involved bipolar, symmetrical, and rectangular electric pulses with: (I) a frequency of 15–20 Hz in LIP sessions and 35–75 Hz in SIP sessions, (II) an intensity of 100 mA in LIP sessions and 80 mA in SIP sessions, (III) an impulse breadth of 200–400 μs, and (IV) a duty cycle (ratio of on-time to the total cycle time: % duty cycle = 100/[total time/on-time]) of 99% in LIP sessions, and 50%–63% in SIP sessions. The WB-EMS device manufactured by Wiemspro® was used.

All sessions started with a dynamic standardized warm-up (which included general mobility exercises) and ended with a cooling-down protocol (i.e., active global stretching), which alternated five posterior chain exercises with five anterior chain exercises.[31] An extra effort was made to promote maximum attendance by all participants, rescheduling sessions when anyone was unable to attend for work, family, or illness reasons. Participants were provided with strong verbal encouragement throughout each training session and were instructed to reach the specific target intensity. Heart rate was continuously monitored during exercise at 5 s intervals using a Polar RS300 pulsometer (Polar).

2.4 | Memory assessment

The Wechsler Memory Scale-Third Edition (WMS-III) is the most commonly used test for memory functions.[38] We used the logical memory (LM) and the verbal paired associates' (VPA) subtests. The LM subtest measures item-specific encoding and focuses on properties that are distinctive or unique to a particular item/information. This subtest assesses narrative memory under two different conditions: immediate and delayed free recall. Two brief stories were presented orally, and participants were asked to recall the stories both immediately and after a 25–35 min delay. They were informed that they would be asked to recall the stories after the delay. The VPA subtest measures relational memory and involves focusing on similarities or shared themes among disparate pieces of information. This subtest requires the presentation of eight pairs of unrelated words over a series of four trials. Recall of the pairs was assessed after each individual trial and after a 25–35 min delay. Recognition was also assessed after delayed recall: (I) by asking a series of "yes or no" questions in which the participants had to recognize whether each piece of information had been presented in the story and (II) by presenting pairs of words to which participants had to indicate whether they had seen the pair before or not.

The dependent variables for the immediate recall trial were total story units recalled from each story (IM-LM) and total units recalled of paired words (IM-VPA). The dependent variables for the delayed recall trial were total story units recalled from each story after the delay interval (LTM-LM) and total paired words recalled (LTM-VPA). The dependent variables for the recognition trial were total story units recognized from each story after the delay interval (recognition-LM) and total paired words recognized (recognition-VPA). Total rates were reported for the three standardized primary indices: IM (IM-VPA + IM-LM), LTM (LTM-VPA + LTM-LM), and recognition (recognition-VPA + recognition-LM).

2.5 | Anthropometry analysis

A pre-validated Seca model 799 scale and stadiometer (Seca) was used to measure body weight and height with light clothing and without shoes. Body mass index (BMI) was subsequently calculated as weight (kg)/ height (m^2).[39]

2.6 | Blood samples assessments

A 10 mL peripheral blood samples were collected using the Vacutainer SST system (Becton Dickinson). All participants were previously requested to abstain from drugs, alcohol, and/or caffeine, to eat a standardized dinner, and to avoid any physical activity of moderate (24 h before) and/or vigorous intensity (48 h before).[31] Blood samples were collected in prechilled ethylenediaminetetraacetic acid-containing tubes (Vacutainer, SST, Becton Dickinson), and were centrifuged at 4000 revolutions per min for 7 min at 4°C and stored at −80°C. S-Klotho plasma levels were determined according to a solid-phase sandwich enzyme-linked immunosorbent assay kit (Demeditec) strictly following the manufacturer's recommendations. The kit uses two types of highly specific antibodies (i.e., purified mouse anti-human Klotho IgG). The optical density was measured at a wavelength of 450–2 nm and a standard

curve was generated using known antigen concentrations. The $1,25(OH)_2D$ plasma levels were measured using a DiaSorin-Liaison immunochemiluminometric analyzer (DiaSorin Ltd.) according to the manufacturer's instructions and expressed in pg/mL.

2.7 | Statistical analysis

Visual check of histograms, Shapiro–Wilk test, box plots, and Q–Q plots were performed to assess the normal distribution of the study variables. Data presenting non-normal distribution (i.e., memory outcomes) were transformed by using the Z-score before further analyses. Continuous variables were reported as mean ± standard deviation (SD). Categorical parameters (i.e., age and educational level) were represented as frequencies (n) and percentages (%).

A chi-square test was used to compare intergroups when referring to qualitative variables. A univariate analysis of variance (ANOVA) was conducted to compare baseline parameters and changes in memory variables (IM, LTM, and recognition) among the four intervention groups and between the control group and exercise group. A post hoc Bonferroni-corrected t-test was used in the intergroup analysis. The effect size was measured by partial eta squared (η^2) and classified as small, medium, or large (<0.06, 0.06–0.14, and >0.14, respectively), following established guidelines.[40] We additionally performed a sensitive analysis to explore differences over time and intergroup based on the memory variables subtests (VPA and LM). In addition, association of changes in S-Klotho and $1,25(OH)_2D$ over time with changes in memory outcomes were examined by repeated measures correlation analysis; a statistical technique used to determine the within-individual association for paired measures assessed on two or more occasions for multiple participants.[41]

The statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS version 24, Inc.), R version 4.0.3 (R Project for Statistical Computing) and GraphPad Prism 5 (GraphPad Software). The level of significance was set at $p < 0.05$.

3 | RESULTS

Table 1 describes the baseline and follow-up characteristics of the study's participants together and by groups. No significant differences between groups on any baseline characteristic were observed (all $p > 0.05$). There were a nearly equal number of men and women in each group.

Figure 1 shows exercise-induced changes in memory variables (i.e., IM (Figure 1A), IM-VPA (Figure 1B), IM-LM (Figure 1C), LTM (Figure 1D), LTM-VPA (Figure 1E), LTM-LM (Figure 1F), recognition (Figure 1G), recognition-VPA (Figure 1H), and recognition LM (Figure 1I)) after the intervention among the four groups. Compared to the control group, IM-VPA (medium effect size; Figure 1B) exhibited higher differences in the HIIT-EMS group ($p = 0.045$), whereas, IM-LM (large effect size; Figure 1C) showed higher differences in the HIIT group ($p = 0.016$). No significant differences in the rest of memory parameters were observed (all $p > 0.053$). Moreover, we did not find significant differences for any of the memory outcomes analyzed between the exercise interventions (all $p > 0.050$). Similarly, higher differences were noted in IM, IM-VPA, and IM-LM when comparing the memory variables between the control group and the exercise group (all $p \leq 0.021$; (Figure S2)).

The relationships between exercise-induced changes in S-Klotho and $1,25(OH)_2D$, and those obtained in memory outcomes are presented in Table 2. Exercise-induced changes in S-Klotho were positively related to changes produced by exercise in all memory parameters (i.e., IM, IM-VPA, IM-LM, LTM, LTM-VPA, LTM-LM, recognition, and recognition-LM: all $r \geq 0.32$, $p \leq 0.007$; Table 2, Figure 2A–G,I), whereas no relationship was observed for recognition-VPA ($r = 0.01$, $p \leq 0.989$; Table 2, Figure 2H). Exercise-induced changes in $1,25(OH)_2D$ were positively associated with IM (Figure 3A), IM-LM (Figure 3C), LTM (Figure 3D), and LTM-LM (Figure 3F), (all $r > 0.24$, $p \leq 0.048$; Table 2); whereas, no significant associations were observed for IM-VPA (Figure 3B), LTM-VPA (Figure 3E), recognition (Figure 3G), recognition-VPA (Figure 3H), and recognition-LM (Figure 3I) (all $r < 0.06$, $p > 0.07$; Table 2).

4 | DISCUSSION

To the best of our knowledge, this is the first study investigating the effects of different exercise interventions on different memory outcomes (i.e., IM, LTM, and recognition) and whether changes in S-Klotho and $1,25(OH)_2D$ levels may be associated with those potentially exercise-related benefits in memory in middle-aged sedentary adults. Interestingly, HIIT and HIIT-EMS—compared with a control group—had beneficial effects on IM in our study. Moreover, exercise-induced changes in S-Klotho were related to those noted in IM, LTM, and recognition; whereas, exercise-induced changes in $1,25(OH)_2D$ were only positively associated with IM and LTM in the item-specific encoding condition measured by LM. We therefore suggest the promotion of physical exercise programs—especially in a high-intensity modality (i.e., HIIT)—as a useful non-pharmacological intervention which could improve

TABLE 1 Baseline and post-intervention parameters.

	All ($n=74$)		Control ($n=20$)		PAR ($n=17$)		HIIT ($n=18$)		HIIT-EMS ($n=19$)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Sex *n* (%)										
Men	35 (47.3)		8 (40.0)		8 (47.1)		9 (50.0)		10 (52.6)	
Women	39 (52.7)		12 (60.0)		9 (52.9)		9 (50.0)		9 (47.4)	
Educational level *n* (%)										
No studies	6 (8.0)		3 (15.0)		0 (0.0)		3 (16.6)		0 (0.0)	
Graduated	17 (23.0)		6 (30.0)		3 (17.6)		4 (22.2)		4 (21.0)	
Baccalaureate	21 (28.4)		5 (25.0)		8 (47.1)		2 (11.2)		6 (31.6)	
University degree medium degree	16 (21.6)		5 (25.0)		4 (23.5)		4 (22.2)		3 (15.8)	
University studies higher degree	14 (19.0)		1 (5.0)		2 (11.8)		5 (27.8)		6 (31.6)	
General outcomes										
Age (years)	53.6 ± 5.1		53.2 ± 5.3		54.9 ± 4.5		53.1 ± 5.6		53.5 ± 5.2	
Weight (Kg)	75.7 ± 14.9	74.8 ± 14.6	71.9 ± 13.6	71.6 ± 13.7	72.6 ± 11.3	70.9 ± 10.1	78.2 ± 17.7	77.5 ± 16.8	80.2 ± 15.9	79.3 ± 15.8
Height (m)	167.8 ± 9.8	168.1 ± 9.7	164.9 ± 8.9	166.2 ± 9.2	168.8 ± 9.4	168.6 ± 9.4	170.8 ± 11.4	170.3 ± 11.4	167.1 ± 9.1	166.7 ± 9.2
BMI (Kg/m^2)	26.7 ± 3.7	26.5 ± 3.7	26.3 ± 3.6	26.2 ± 3.6	25.4 ± 2.8	24.9 ± 2.4	26.4 ± 3.1	26.4 ± 2.9	28.6 ± 4.6	28.4 ± 4.6
S-Klotho (pg/dL)	775.3 ± 363.7	1070.4 ± 460.3	784.3 ± 350.8	862.8 ± 364.7	714.3 ± 294.5	1055.3 ± 435.8	788.5 ± 276.8	1057.1 ± 273.3	808.5 ± 499.0	1259.7 ± 613.1
$1,25(OH)_2D$ (pg/mL)	40.3 ± 14.1	44.5 ± 14.5	42.8 ± 14.3	39.6 ± 11.6	42.5 ± 11.5	48.1 ± 15.6	40.9 ± 9.4	46.8 ± 12.6	35.0 ± 18.5	42.9 ± 17.0
Memory outcomes										
IM	19.7 ± 5.4	23.4 ± 4.5	21.6 ± 5.7	22.4 ± 4.7	19.7 ± 7.1	24.2 ± 4.3	18.2 ± 4.2	22.5 ± 4.7	19.7 ± 4.5	24.4 ± 4.5
VPA	19.9 ± 6.9	23.5 ± 5.9	22.6 ± 6.1	23.3 ± 5.6	21.6 ± 6.1	24.5 ± 6.4	17.7 ± 1.7	22.2 ± 6.8	18.4 ± 7.4	24.2 ± 4.9
LM	32.4 ± 9.6	39.1 ± 8.7	32.5 ± 11.9	34.1 ± 9.3	33.2 ± 10.1	39.2 ± 6.9	29.5 ± 9.2	40.2 ± 8.0	34.5 ± 7.7	41.4 ± 9.4
LTM	9.9 ± 2.5	11.0 ± 2.5	10.8 ± 2.9	11.4 ± 3.2	9.3 ± 2.8	10.7 ± 2.2	9.1 ± 1.4	11.2 ± 2.5	10.4 ± 2.3	10.8 ± 2.5
VPA	6.2 ± 1.7	6.7 ± 1.6	6.9 ± 1.5	6.7 ± 1.7	6.9 ± 1.2	7.2 ± 1.2	5.2 ± 2.1	6.3 ± 2.1	6.1 ± 1.4	6.7 ± 1.3
LM	19.8 ± 7.3	25.4 ± 6.5	19.6 ± 9.4	23.1 ± 6.7	20.9 ± 6.7	26.2 ± 6.5	17.7 ± 6.7	25.5 ± 5.9	21.2 ± 6.8	26.5 ± 6.9
Recognition	20.8 ± 23.6	23.6 ± 3.8	22.6 ± 5.5	23.7 ± 3.9	21.3 ± 6.6	24.7 ± 3.3	18.7 ± 3.9	22.5 ± 3.8	21.0 ± 3.1	23.5 ± 4.1
VPA	23.1 ± 4.5	23.1 ± 4.5	24.0 ± 0.0	24.0 ± 0.0	24.0 ± 0.0	24.0 ± 0.0	23.7 ± 0.4	23.9 ± 0.3	23.9 ± 0.2	23.8 ± 0.4
LM	22.9 ± 5.3	24.0 ± 5.6	24.3 ± 2.9	24.7 ± 3.5	23.8 ± 2.1	24.6 ± 2.5	23.3 ± 1.9	25.4 ± 2.7	24.0 ± 2.9	25.3 ± 2.4

Note: Quantitative data are shown as means ± standard deviation. Qualitative data are shown as frequencies (*n*) and percentages (%). Univariate Analysis of Variance (ANOVA) with post-hoc Bonferroni-corrected *t*-test was used in the inter-groups analysis of the quantitative variables at baseline. Chi-square test was used to compare inter-groups when referring to qualitative variables. A p value of <0.05 was considered statistically significant.

Abbreviations: BMI, body mass index; HIIT, High Intensity Interval Training group; HIIT-EMS, High Intensity Interval Training adding Whole-Body Electromyostimulation group; IM, Immediate Memory; LM, Logical Memory; LTM, Long-Term Memory; PAR, physical activity recommendations for adults proposed by the World Health Organization group; VPA, Verbal Paired Associates.

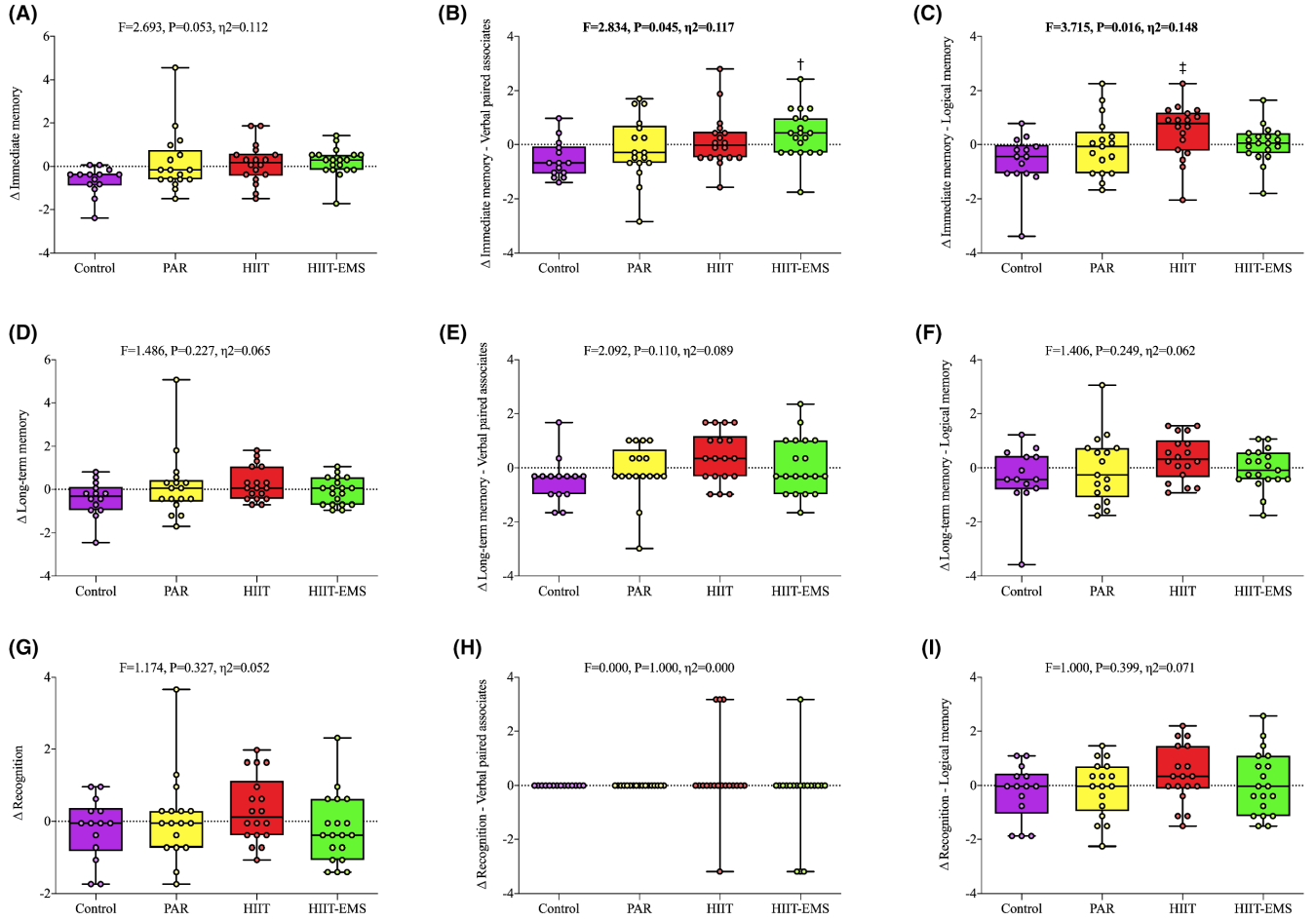

FIGURE 1 Changes in memory variables after the intervention study among the four intervened groups. (A) Changes in immediate memory by groups. (B) Changes in immediate memory—verbal paired associates (VPM) by groups. (C) Changes in immediate memory—logical memory by groups. (D) Changes in long-term memory by groups. (E) Changes in long-term memory—VPM by groups. (F) Changes in long-term memory—logical memory by groups. (G) Changes in recognition by groups. (H) Changes in recognition—VPM by groups. (I) Changes in recognition—logical memory by groups. Changes in memory outcomes were Z-scored before further analyses. Univariate Analysis of Variance (ANOVA) with post-hoc Bonferroni-corrected t test for inter-groups analysis. † = HIIT-EMS vs control group. ‡ = HIIT vs control group. A p value <0.05 was considered statistically significant. Δ represents changes in memory variables after intervention. PAR, physical activity recommendations for adults proposed by the World Health Organization group; HIIT, High Intensity Interval Training group; HIIT-EMS, High Intensity Interval Training adding Whole-Body Electromyostimulation group.

memory-related parameters, especially those involved in IM, via enhancing S-Klotho and 1,25$(OH)_2$D levels in middle-aged healthy individuals.

Among the three physical exercise interventions used in our study, HIIT and HIIT-EMS seem to be the most effective protocols to exhibit higher differences in memory (i.e., IM-VPA and IM-LM, respectively) in middle-aged sedentary adults. A previous study has shown that an aerobic training intervention can enhance immediate and delayed memory in older adults (mean age of 72 years), especially in healthy subjects without memory decline, whereas resistance training did not show any relevant effect.[5] In this line, a recent study investigating the exercise-induced changes of HIIT versus moderate-intensity continuous training (MICT) versus a control group (i.e., following national physical activity recommendations guidelines) on memory in older adults aged 72–77 years, demonstrated no significant inter-groups changes on cognition (learning and delayed memory).[42] Interestingly, a recent study comparing MICT versus HIIT exercise-induced changes on LTM concluded that MICT may be considered the best strategy for the consolidation of LTM—although both were effective at improving LTM.[43] Therefore, HIIT, combined or not with WB-EMS, seems to be useful and an effective approach for improving memory, especially IM, as observed in our results. These enhancements may be explained by a decrement of neuroinflammation,[25] an improved body composition status,[44] and a reduced neuronal damage.[45] The lack of effects observed in response to PAR on any memory variables analyzed in our study, together with the absence of studies evaluating this research question, remain unclear the role of PAR upon memory, and

Outcomes	S-Klotho			$1,25(OH)_2D$		
	r	95% CI	*p*-value	*r*	95% CI	*p*-value
IM	0.45	0.23–0.62	**<0.001**	0.33	0.10–0.54	**0.006**
VPA	0.46	0.24–0.63	**<0.001**	0.23	−0.02–0.45	0.069
LM	0.41	0.18–0.59	**<0.001**	0.43	0.21–0.61	**<0.001**
LTM	0.42	0.20–0.60	**<0.001**	0.24	−0.002–0.46	**0.048**
VPA	0.32	0.09–0.53	**0.007**	0.06	−0.19–0.30	0.625
LM	0.42	0.20–0.60	**<0.001**	0.38	0.14–0.57	**0.002**
Recognition	0.32	0.09–0.52	**0.007**	0.11	−0.14–0.35	0.375
VPA	0.01	−0.24–0.24	0.989	−0.11	−0.35–0.13	0.363
LM	0.38	0.15–0.57	**0.001**	0.15	−0.09–0.38	0.223

TABLE 2 Repeated measures correlation analyses examining association of changes in S-Klotho and $1,25(OH)_2D$ over time with changes in memory outcomes.

Note: Changes in memory outcomes were Z-scored before further analyses. Boldface values indicate significance differences ($p < 0.05$).

Abbreviations: CI, confidence interval; IM, immediate memory; LM, Logical Memory; LTM, Long-Term Memory; VPA, Verbal Paired Associates.

therefore, future interventions are needed to confirm our results.

The present results unveil a relationship of exercise-induces changes in S-Klotho levels with those obtained in different memory domains (i.e., IM, LTM, and recognition) in our study' cohort. It has been previously reported that S-Klotho levels are decreased in patients with aging-related chronic diseases associated with cognitive impairments.[29] Interestingly, S-Klotho is highly expressed in the mature neurons from the dentate gyrus of the hippocampus,[46] and the elevation of brain circulating levels of S-Klotho has been proposed as a potential mechanism to reverse cognitive impairments via the optimization of synaptic N-methyl-D-aspartate receptor function in the hippocampus and frontal cortex.[47] Mice with S-Klotho deficiency have shown impaired LTM, whereas those presenting overexpression of S-Klotho have exhibited significantly better memory performance through augmentation of circulating synaptic GluN2B unit levels in the hippocampus and cortex.[48] Moreover, previous studies have suggested that memory impairment in Klotho mutant mice occurs in an age-dependent way (i.e., the consolidation or retention deficit in LTM, whereas IM retention and/or memory acquisition is preserved).[49] Following these lines of evidence, our results suggest that the improvements in memory in response to exercise programs could be partially attributed to exercise-induced changes in S-Klotho levels.

In our study, exercise-induced changes in $1,25(OH)_2D$ levels were associated with IM and LTM during the item-specific encoding condition (measured by LM subtest). Circulating $1,25(OH)_2D$ is able to cross the blood–brain barrier and the brain itself can synthesize $1,25(OH)_2D$.[50] Since the brain expresses VDR, $1,25(OH)_2D$ can be considered a neuro-hormone.[51] Recent studies have demonstrated that $1,25(OH)_2D$ has a neuroprotective effect through the regulation of target gene expression in the brain, thus controlling neurotrophic factors' expression, regulating brain immune processes, and preventing neuronal damage.[30,52] In the adult dentate gyrus, vitamin D stimulates the synthesis of nerve growth factor in the hippocampus, improving neurite outgrowth, whereas deficient $1,25(OH)_2D$ levels raises the proliferation of neuroblasts in the hippocampus and alters neuronal differentiation in a mouse model, thus moderating neurogenesis.[53] When supplementing with vitamin D, the abovementioned beneficial effects can be potentiated ameliorating learning and memory impairments, therefore improving neurological function via moderating the morphological defects in the rat hippocampus.[54] We thus suggest that strategies aimed at promoting physical exercise at ages when cognitive decline begins could improve memory-related disturbances via improving $1,25(OH)_2D$ through exercise-induced changes.

Relevant studies evaluating the synergistic relationship between the role of the $1,25(OH)_2D$/S-Klotho binomial and cognitive development are currently scarce. A recent review has proposed that S-Klotho forms a complex with fibroblast growth factor 23 (FGF23) able to directly regulate $1,25(OH)_2D$ metabolism, thus preserving brain development and function through neurons' growth, survival, and proliferation.[55] In this sense, S-Klotho tends to balance $1,25(OH)_2D$ concentrations (i.e., upregulate when are underexpressed and downregulate when are overexpressed) favoring their benefits upon cognitive health.[25] Vitamin D supplementation and resistance exercise training are both positive stimuli to increase FGF23, which is able to cross the blood brain barrier interacting with the brain where, in association with the co-receptor of Klotho protein, moderates neuroprotection.[56]

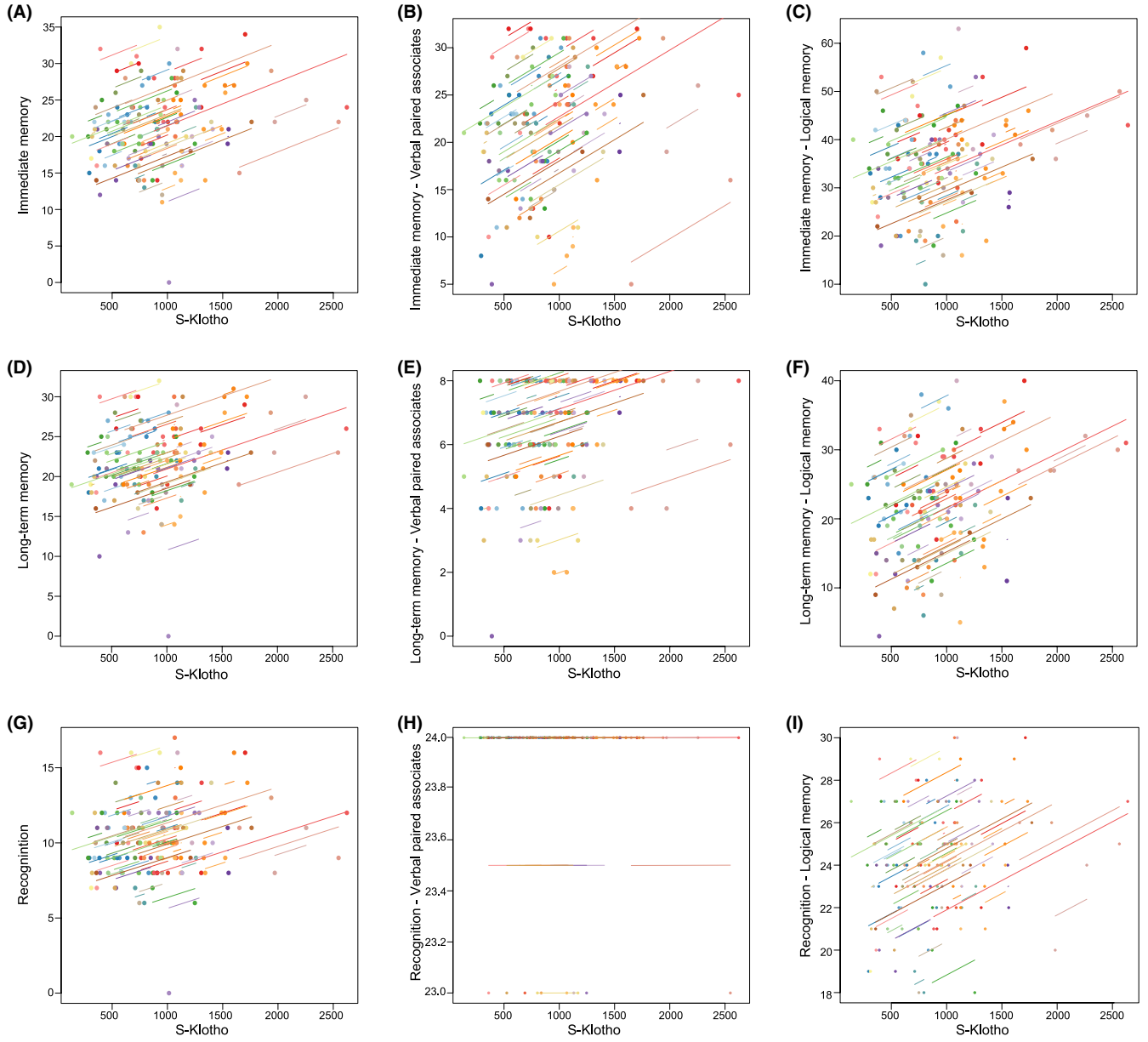

FIGURE 2 Association of changes in S-Klotho over time with changes in memory outcomes. (A) Changes in S-Klotho with immediate memory. (B) Changes in S-Klotho with immediate memory—verbal paired associates (VPM). (C) Changes in S-Klotho with immediate memory—logical memory. (D) Changes in S-Klotho with long-term memory. (E) Changes in S-Klotho with long-term memory—VPM. (F) Changes in S-Klotho with long-term memory—logical memory. (G) Changes in S-Klotho with recognition. (H) Changes in S-Klotho with recognition—VPM. (I) Changes in S-Klotho with recognition—logical memory. Changes in memory outcomes were Z-scored before further analyses. Each dot represents one of two separate observations (baseline, postintervention) of S-Klotho and memory outcomes for a participant. Observations from the same participant are given the same color, with corresponding lines to show the repeated measures correlation fit for each participant.

Our study has some limitations: (a) we only included healthy sedentary adults (i.e., 45–65 years old), the generalization of our findings being therefore limited to this population; (b) other possible parameters which could modulate memory via physical exercise (e.g., lactate and brain-derived neurotrophic factor) were not measured; (c) the assessment of 25-hydroxivitamin D levels were not determined and, thus, further studies are needed to better understand the role of exercise on vitamin D metabolism; and (d) the sample size of the study was relatively small, so the data should be interpreted with caution. In contrast, it should be noted that this study is the first randomized controlled trial investigating not only the influence of different types of exercise interventions on IM, LTM, and recognition, but also their association with exercise-induced changes in

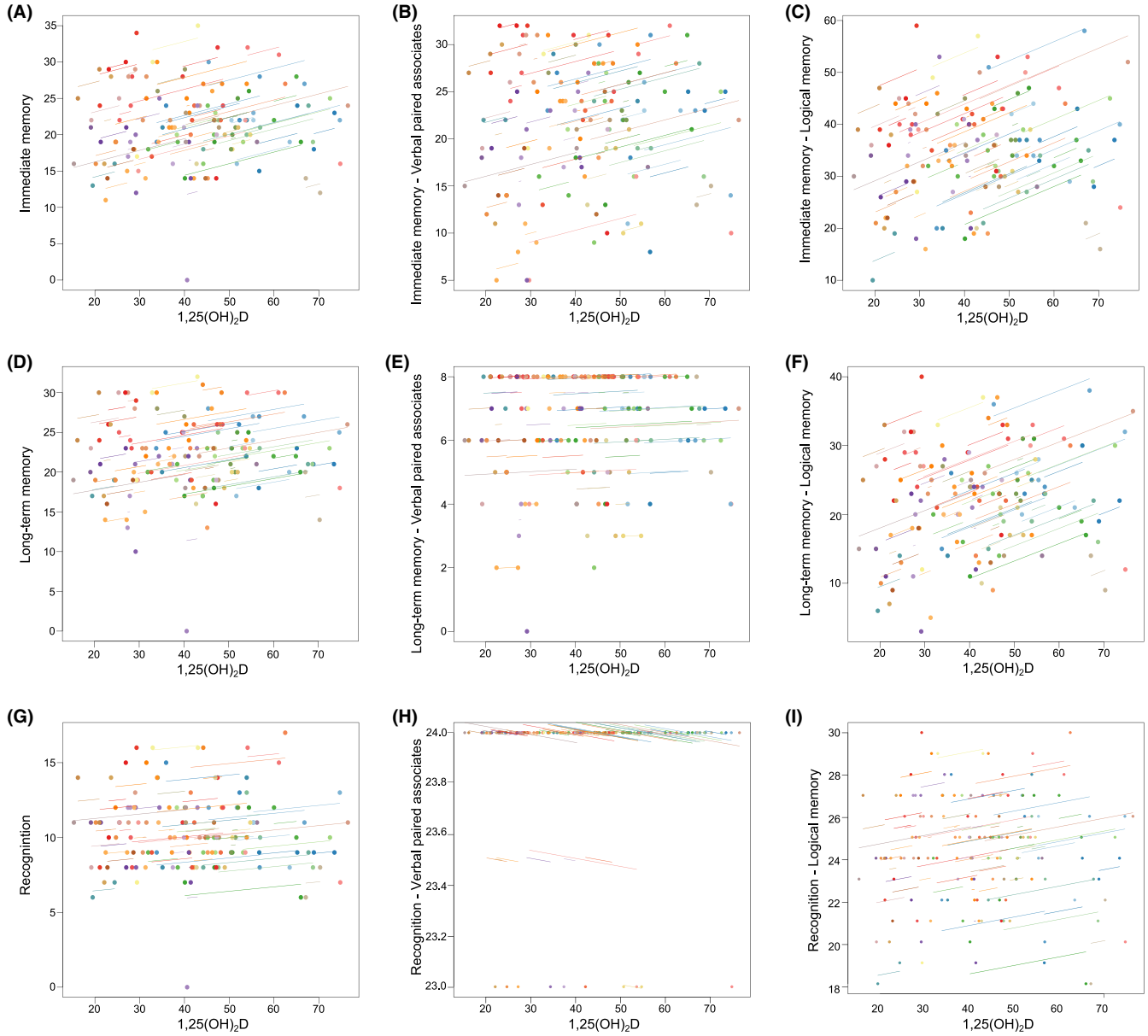

FIGURE 3 Association of changes in $1,25(OH)_2D$ over time with changes in memory outcomes. (A) Changes in $1,25(OH)_2D$ with immediate memory. (B) Changes in $1,25(OH)_2D$ with immediate memory—verbal paired associates (VPM). (C) Changes in $1,25(OH)_2D$ with immediate memory—logical memory. (D) Changes in $1,25(OH)_2D$ with long-term memory. (E) Changes in $1,25(OH)_2D$ with long-term memory—VPM. (F) Changes in $1,25(OH)_2D$ with long-term memory—logical memory. (G) Changes in $1,25(OH)_2D$ with recognition. (H) Changes in $1,25(OH)_2D$ with recognition—VPM. (I) Changes in $1,25(OH)_2D$ with recognition—logical memory. Changes in memory outcomes were Z-scored before further analyses. Each dot represents one of two separate observations (baseline, postintervention) of $1,25(OH)_2D$ and memory outcomes for a participant. Observations from the same participant are given the same color, with corresponding lines to show the repeated measures correlation fit for each participant.

S-Klotho and $1,25(OH)_2D$ levels in middle-aged healthy adults.

4.1 | Perspective

The present study provides relevant information about the efficacy of different physical exercise interventions on memory-related outcomes. Specifically, physical exercise protocols based on HIIT practice are the ones that would have a better effect, compared to the others 12-week exercise interventions. Furthermore, such possible higher differences in IM could be partially explained by an increase in S-Klotho protein and $1,25(OH)_2D$ levels induced from physical exercise. Moreover, our results pointed that higher exercise-related benefits on memory when using

relational and item-specific encodings occur since may provide a more useful role than reporting only composite scores. Further studies are needed to demonstrate the efficacy of HIIT—combined or not with WB-EMS—on memory in populations at risk of cognitive impairment.

5 | CONCLUSIONS

In conclusion, a 12-week HIIT intervention with or without WB-EMS seems to be the most effective exercise program to improve IM. The significant and positive associations between exercise-induced changes in S-Klotho and $1,25(OH)_2D$ levels with those observed in memory outcomes suggest that these factors may be potentially related to exercise-induced improvements of memory in middle-aged adults. Therefore, we suggest physical exercise interventions—with special emphasis to those focused on HIIT—as a potential strategy to enhance memory in populations at risk of cognitive-related disturbances.

AUTHOR CONTRIBUTIONS

Héctor Vázquez-Lorente: data curation, formal analysis, validation, visualization, investigation, roles/writing–original draft and writing–review and editing; Alejandro De-la-O: data curation, investigation, formal analysis, methodology, investigation, software and writing–review and editing; Almudena Carneiro-Barrera: data curation, investigation, formal analysis, methodology, investigation, software and writing–review and editing; Cristina Molina-Hidalgo: data curation, investigation, formal analysis, methodology, investigation, software and writing–review and editing; Manuel J. Castillo-Garzón: conceptualization, funding acquisition, project administration and resource and review and editing; Francisco J. Amaro-Gahete: conceptualization, data curation, formal analysis, investigation, funding acquisition, project administration, resources, validation and writing–review and editing. All authors approved the final draft of the manuscript for publication. The authors declare that all data were generated in-house and that no paper mill was used.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

This data has not been previously presented anywhere and will be shared upon reasonable request to the corresponding author. Francisco J. Amaro-Gahete (email: amarof@ugr.es).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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