Journal of Alzheimer's Disease xx (20xx) x–xx
DOI 10.3233/JAD-140576
IOS Press

Physical Exercise Improves Peripheral BDNF Levels and Cognitive Functions in Elderly Mild Cognitive Impairment Individuals with Different BDNF Val66Met Genotypes

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Accepted 26 May 2014

Abstract. The benefits of physical exercise on improvements in brain-derived neurotrophic factor (BDNF) levels and cognitive functioning have been reported in the literature. However, the variability of individual responses may be linked to genetic differences. BDNF is considered one of the most plausible factors involved in the cognitive benefits associated with physical activity practice. A single nucleotide polymorphism localized in the gene that codes BDNF results in a missense mutation that promotes an amino acid substitution (Val66Met) in the protein. This process has been associated with decreased levels of BDNF secretion, with corresponding impairments in specific cognitive functions. Therefore, the objective of this study was to analyze the effects of a multimodal physical exercise program on peripheral BDNF levels and cognitive functions in elderly individuals with mild cognitive impairment (MCI). The participants were genotyped for the BDNF Val66Met polymorphism. Cognitive functions were assessed by the Montreal Cognitive Assessment (MoCA) prior to and after the intervention. Forty-five participants were assigned to the control and trained groups. The trained group participated in a multimodal physical training for a 16-week period. The results showed a significant between-subjects interaction ($p < 0.05$), which indicates the beneficial contribution of training on cognitive functions independent of the BDNF genotype. However, only participants with wild-type genotypes ($BDNF^{-Met}$) exhibited significant improvements in peripheral BDNF levels. The BDNF genotype appears to modulate the effects of physical exercise on BDNF secretion, but it does not influence cognition. This is the first study that evaluated the influence of a BDNF polymorphism on physical activity and cognition performance in elderly MCI individuals.

Keywords: Brain-derived neurotrophic factor, cognition, genetic polymorphism, mild cognitive impairment, physical exercises

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INTRODUCTION

Age-related cognitive impairments in later life frequently generate a significant reduction in the quality of life of elderly individuals. Mild cognitive impairment (MCI) is a prodromal stage of dementia that is characterized by slight cognitive decline but the maintenance of daily living activities [1]. Elderly individuals who present MCI have a greater risk of developing dementia [2], with a conversion rate of 10–40%/year to Alzheimer's disease (AD) [3]. However, the presence of MCI is not a determinant condition for the development of dementia because multiple genetic and environmental factors interact to induce different phenotypes. Despite the irreversible characteristic of the dementia process, there is a potential to revert cognitive impairment to normal cognition [4].

The potential benefits of physical activity on mental health have been well documented. Some evidence supports the notion that individuals who are physically active during adulthood tend to present better levels of cognition during their aging process [5–7]. Moreover, it has been observed that physical exercise can improve cognition [8, 9], reduce brain tissue loss [10], and decrease the risk of developing dementia in late life [11, 12]. One of the most plausible, well documented, and accepted mechanisms that underlie these effects of regular physical activity involves the brain-derived neurotrophic factor (BDNF) pathway [13–16]. Despite the currently identified benefits, significant variability in the individual response to these interventions in multiple cognitive domains has been described. This variability has led to the hypothesis that genetic factors may control the benefits of physical activity on cognitive function [17].

BDNF is a protein involved in plasticity, brain health, and improvements of neuron survival and synapses [18, 19], and it is a critical factor for the formation of long-term potentiation, which has an important influence on memory and learning processes [16]. There is mounting evidence that this neurotrophin has also been linked to the protection of brain structures against neuronal damage promoted by multi factorial causes present in daily life that could lead to neuronal degeneration and AD [20].

The secretion of BDNF is genetically modulated. The human BDNF gene is composed of eleven exons and nine functional promoters, which are tissue and brain-region specific [21]. A single nucleotide polymorphism (SNP) at codon 66 in coding exon VIII of the BDNF gene at position 196 produces a missense mutation that results in an amino acid substitution (valine to methionine) in the pro-region of the protein. This SNP has been associated with decreased levels of BDNF-protein secretion, with corresponding impairments in specific cognitive functions [22, 23].

The human genotype for BDNF can be heterozygous (Val/Met), with at least one Met allele present, or homozygous (Met/Met or Val/Val). Lang et al. [24] demonstrated that wild-type individuals (Met/Met genotype) and heterozygous individuals (who carry at least one Met allele) had a significant decrease in peripheral blood BDNF concentrations [24]. A potential relationship between BDNF Met carriers and the increased risk of poorer cognitive performance has been suggested [22, 23].

A growing number of studies have shown that physical exercise can mediate the enhancement of BDNF concentrations [15, 16]. Evidence supports a significant role of different physical exercises on the increase of BDNF levels in humans [15]. Despite evidence regarding the ability of physical exercise to modulate peripheral levels of BDNF, few clinical trials have been conducted with elderly individuals to confirm this hypothesis [25]. Furthermore, it appears difficult to establish a consensus for the most effective type of physical exercise that could promote significant amelioration of cognitive and physical functions and still prevent neurodegenerative conditions.

Under these circumstances, this research had a two-fold objective. First, we aimed to determine if a multimodal physical exercise program could improve cognitive functions and peripheral levels of BDNF in MCI elderly individuals. Second, we analyzed if the BNDF Val66Met genotype profile influenced the expected changes.

METHODS

This prospective interventional research study was approved by the Ethics Committee of the UNESP – São Paulo State University (Protocol #7143). All participants signed the consent term. The target population consisted of adults aged 60 years or older who resided in a medium size city (Rio Claro) in Brazil. The participants were recruited through ads in newspapers, on television, and on radio to participate in the "Program of Physical Activity for Older People" (PROFIT) for this research. To be eligible for inclusion in data analysis, the participants were assessed by a neuropsychological screening conducted by a team of experts. The assessment included memory tests, an evaluation

of language, attention and executive functions, the absence of functional impairments (e.g., daily living and instrumental activity domains), and primary clinical outcomes. We followed Petersen's [26] criteria for the definition of MCI. All MCI subjects or their caregivers described cognitive deficits in the participants.

Participants with depressive symptoms, continuous use of benzodiazepines and anticholinergic drugs, a history of neurological or psychiatric disorders or other severe health issues, impairments in basic activities of daily living, and a score greater than 0.5 on the clinical dementia rating scale (CDR) [27] were excluded. To measure depressive symptoms, the participants were assessed by the validated version of the Geriatric Depression Scale-short form (GDS-15) for the Brazilian population [28, 29]. The patients who scored higher than 6 points were excluded because of the presence of depressive symptoms.

These criteria were chosen because the goals of the study were to examine MCI subjects who were followed longitudinally after the proposed multimodal physical exercise program. The trained group (TG) and control group (CG) were composed of 24 and 21 individuals, respectively. The CG did not attend a regular physical exercise program for at least one year prior to the beginning of this study or during the research period. To confirm the absence of regular physical activity at the beginning of the study, we applied the validated and adapted version of the Baecke Questionnaire of Physical Activity for elderly individuals [30]. Because of the lack of a cut-off point, we considered the lower quartile scores as sedentary and the higher quartiles as physically active individuals. For the data analyses, only individuals who scored in the lowest quartiles were included.

Main outcome measures

Data were collected during the first two weeks prior to the intervention and the first two weeks after the intervention occurred by the same evaluator (single-blinded for the group allocation).

Cognitive measures

The Montreal Cognitive Assessment (MoCA) [31] has a high sensitivity (81%) and specificity (77%) for detecting MCI in elderly Brazilian individuals, as determined by a score of <25 [32]. All participants were evaluated using the CDR [27]. The participants who scored 0 or more than 0.5 points were excluded from our data analysis. The Brazilian validated version of the Pfeffer Instrumental Activities Questionnaire (PIAQ) [33] was administered to assess functional deficits.

Blood collection and biochemical analyses

Blood samples were aseptically collected from a peripheral vein in the forearm by a nurse practitioner specialized in geriatric outpatients. A 10-h fasting period prior to the blood collection was instituted for all participants.

BDNF peripheral levels

Blood was collected in tubes that contained EDTA at baseline and after the 16-week training period. The samples were centrifuged at 5000× *g* at 4°C for 5 min and stored at –80°C until analyses. BDNF was measured using an anti-BDNF sandwich ELISA kit (Chemicon International, Temecula, CA) according to the manufacturer's instructions. The plasma was diluted 1:2 with sample buffer, and the analysis was performed in duplicate. BDNF levels were determined at an absorbance of 450 nm using optical density values based on the standard curve values. All samples were assayed in the same experiment. The coefficient of variation between the standards and duplicates was less than 5%.

BDNF genotyping

The analysis of the BDNF SNP (rs6265) was performed using a Real-Time PCR SNP genotyping system (*TaqMan*® Assays – Life Technologies, CA, USA) as follows: *TaqMan* PCR Mastermix (1x/μL), *TaqMan* SNP genotyping assay (1x/μL), genomic DNA (50 ng), and ultrapure water to complete a 7-μL volume were mixed in each well of an optical plate. Allelic discrimination was evaluated in a 7500 Real Time PCR system (Life Technologies, CA, USA) by comparing fluorescence levels prior to and after amplification (45 cycles of 15 s at 95°C and 1 min at 60°C).

HDL- and LDL-cholesterol levels

Blood samples were collected, and the serum was immediately separated and stored at −80°C until experimentation. The samples were then simultaneously analyzed. The levels of glucose, triglycerides, total cholesterol, LDL cholesterol, and HDL-cholesterol were determined using commercially available sandwich ELISA kits (LABORLAB®, Guarulhos, São Paulo/Brazil).

Table 1
Structure of the proposed Multimodal Physical Exercise program and each phase of the 16-week period

Warm up (5 min.) Gentle stretching Walking in different directions Main Component (45 min.) Physical function Phase	Muscular resistance	Aerobic fitness	Motor coordination/balance
Phase 1	Exercises performed with light-weight materials (i.e., rubber-bands; thera-band; batons; balls) to add a light overload to the series. Main and large muscle groups were chosen (i.e., Dorsal; Chest; Biceps; Triceps; Shoulders; Quadriceps; Hamstrings; Gastrocnemius and Tibialis Anterior);	*More intense walking* in different directions;	Rhythmic activities with upper and lower limbs simple movements (i.e., combination of a movement sequence);
	Series were performed in 3 sets for each group per session (the muscle groups were interpolates between the three weekly sessions) during 1' each set with 30" of rest.	*Walking* shifting support (heels, on tiptoe); *Marching* (raising knees-heels/legs apart-crossed/arms up/down);	Recreational activities that primarily stimulated the vestibular system.
Phase 2	Different materials were inserted (medicine balls; bobath balls and adjustments on rubber-band and thera-band overloads were made);	A greater intensity was added during walking;	More complex sequences of movements were added to the rhythmic activities with the upper and lower limbs;
	Series were performed in 3 sets with 15–20 RM with 30" of rest.	Cross lateral walking with greater velocity; Walking while touching the knees with their hands	Recreational activities that primarily stimulated the somatosensory system and proprioception
Phase 3	Intensity overload was applied. Ankles and Barbells were inserted to the training routine	Patients who were able were encouraged to engage in a short light run while maintaining the Maximal Heart Rate proposed (70%–80%)	Trunk movements were combined with upper and lower limb sequences of movements; Recreational activities that integrated visual, vestibular and somatosensory systems
Phase 4	Maintenance of the routine with specific and individual adjustments of overload when necessary.	Walking and light run while maintaining the Maximal Heart Rate proposed (70%–80%)	Circuits with walking across obstacles to transpose and to avoid (steps, hopes, hoops, trampolines (gym/mini trampolines) with and without visual information and with a variability of somatosensory stimulus (i.e., different surfaces))
Cool Down (10 min.) General Stretching Gentle walking			

Physical function evaluation

The American Alliance of Health and Physical Education, Recreation and Dance (AAHPERD) Battery for Elderly individuals [34] was utilized to assess the effects of the intervention program on the participants' functional fitness levels. A general functional fitness index (GFFI) was obtained for each patient following the standard procedure for the Brazilian population [35].

Intervention

The participants included in the TG performed a multimodal physical exercise program based on three one-hour sessions per week for six months (Table 1). The program aimed to stimulate aerobic metabolism (e.g., moderate intensity and over long duration tasks); however, different types of activities were developed to simultaneously benefit other components of functional capacity. All sessions were monitored by at least five physical education professionals who were familiarized with physical activity programs in elderly individuals and were previously trained specifically for the administration of this program. All participants exercised together as a group.

A heart rate monitor (chest pulsometer) was used to control the intensity of the training sessions. The participants were instructed to exercise at 60%–80% of their maximal heart rate, which was assessed by the means of the Karvonen's formula [36]. The age predicted maximal heart rate was estimated by the formula proposed by Tanaka et al. [37] for sedentary men and women. The program was distributed into four phases with twelve sessions per phase. After each phase, an overload was applied that considered the need to maintain stress levels and heart rate at the proposed percentages and the tolerance and safety of the participants.

The adherence to the program was calculated as the percentage of the total number of sessions that each participant attended during the intervention. The criterion for valid attendance was set to 75% [38, 39] to guarantee a minimum weekly practice. The participants who did not reach the established percentage were excluded from the data analysis. Pharmacological assistance was also controlled during the research period.

Statistical analysis

The statistical power and effect sizes were calculated with G*Power 3, version 3.1.4 [40]. The statistical sample power was 80% ($1–\beta = 0.80$) for the dependent variables. The distributions of all variables were tested for normality using the Shapiro–Wilk test.

Descriptive statistics were calculated to demonstrate the groups' baseline sociodemographic characteristics, and the differences between groups were tested using *one-way* ANOVA. For analyses composed of multiple factors (moment X groups), a general linear model for repeated-measures analysis of variance (ANOVA) was used to determine the group difference for the cognitive function and BDNF peripheral concentrations. Two time points were treated as the within-subjects factor (effect over time – pre and post multimodal program of physical exercise), and the differences between the TG and CG were treated as the between-subjects factor. When the repeated-measures ANOVA indicated that the group×time interaction was significant, tests of simple main effects were performed to determine which group or groups significantly differed across the intervention period.

A partial correlation was applied to analyze the relationship between the variables considering the genotype ($BDNF^{+Met}$/$BDNF^{-Met}$) as a variable held constant. A significance level was set at 5% for all statistical analyses, and all values in the text and tables are presented as the mean ± SD. The SPSS package version 20.0 was used for these analyses.

RESULTS

After analyzing the blood samples, 9 participants in the TG (37.5%) group and 10 participants in the CG (47.6%) group were identified as Met carriers (Met/Met, Val/Met genotype or $BDNF^{+Met}$). For data analysis, the participants were distributed into four groups according to two criteria: genotype and participation in the multimodal program of physical exercise. The participants with at least one Met allele were combined into one group with those carrying the homozygous genotype (Met/Met) because of the low incidence of the Met allele. There were no differences between the groups regarding the sociodemographic or clinical baseline variables (Table 2).

Significant increases in the peripheral concentrations of BDNF ($p = 0.013$) and cognitive functions ($p = 0.05$) were observed after the 16-week period of exercise for the TG. No significant changes were observed for these variables in the CG (Table 3).

When considering the group distribution regarding the BDNF genotype and the participation in the multimodal physical exercise program, significant increases

Table 2
Sociodemographic, clinical, and baseline cognitive characteristics of MCI participants in the trained and control groups separated by BDNF genotype ($BDNF^{-Met}$ and $BDNF^{+Met}$)

	Trained Group		Control Group		*one way*
	$BDNF^{-Met}$ $n=15$	$BDNF^{+Met}$ $n=9$	$BDNF^{-Met}$ $n=11$	$BDNF^{+Met}$ $n=10$	ANOVA F; p
Age, years	68.5 ± 5.8	66.7 ± 6.4	69.4 ± 4.9	65.5 ± 4.9	$F=0.51$; $p=0.67$
Education (years)	4.6 ± 3.4	6.6 ± 4.2	7.8 ± 6.2	7.3 ± 4.3	$F=1.72$; $p=0.17$
MoCA (points)	21.9 ± 3.4	22.6 ± 3.5	22.4 ± 3.7	21.8 ± 4.2	$F=1.91$; $p=0.13$
BDNF concentrations	2.47 ± 1.07	2.40 ± 0.72	2.61 ± 0.68	2.77 ± 1.24	$F=2.25$; $p=0.87$
Body Mass Index	26.7 ± 4.7	26.8 ± 4.5	27.4 ± 4.9	27.9 ± 4.13	$F=0.15$; $p=0.92$
GFFI	249.9 ± 98.7	265.1 ± 92.9	269.5 ± 73.6	270.7 ± 65.8	$F=0.32$; $p=0.75$
Gender Men/Women	5/10	2/7	4/7	2/8	$\chi w^2=1.02$; $p=0.79$
Diabetes Yes/No	2/13	1/8	1/10	0/10	$\chi w^2=1.39$; $p=0.70$
Hypertension, No. Yes/No	3/12	2/7	2/9	1/9	$\chi w^2=0.58$; $p=0.89$

Data are expressed as the mean ± SD; Body Mass Index, calculated as weight in kilograms divided by height in meters squared; MoCA, Montreal Cognitive Assessment; BDNF, Brain Derived Neurotrophic Factor; GFFI, General Functional Fitness Index.

Table 3
Peripheral BDNF concentrations and cognitive functions prior to and after a 16-week multimodal program of physical exercise in elderly MCI individuals

		Pre	Post	Between-subject factors for two-way ANOVA p value
BDNF pg/dL	Trained Group $n=24$	2.44 ± 0.98	**3.07 ± 1.06***	$F_{1,43}=6.78$;
	Control Group $n=21$	2.55 ± 0.94	2.4 ± 0.56	$p=0.013$
MoCA	Trained Group $n=24$	21.8 ± 3.8	**23.9 ± 3.5***	$F_{1,43}=4.14$;
	Control Group $n=21$	22.6 ± 3.1	22.8 ± 4.9	$p=0.05$

Values are expressed as the mean ± SD; *significant changes after a multimodal program of physical exercise.

in the BDNF peripheral concentrations were verified only for the homozygous variant individuals (Val/Val genotype) ($p=0.008$). We observed a significant reduction in BDNF concentrations in the CG for individuals carrying the Met allele ($p=0.03$). The between-subject factors for two-way ANOVA showed significant changes ($F_{3,41}=5.21$; $p=0.004$). Figure 1 displays the BDNF peripheral concentrations pre and post-multimodal exercise program for all evaluated groups.

Regarding cognitive functions, only the TG exhibited significant improvements ($F_{3,41}=4.59$; $p=0.007$). When each domain of the MoCA instrument was analyzed, we determined that the main changes were observed for executive functions ($F_{3,41}=4.29$; $p=0.01$) in the TG, independent of the genotype (Table 4).

Our program showed the effectiveness and feasibility of improving functional fitness, which indicates an adequate strategy of structure and overload control to promote beneficial changes in physical fitness domains. This result was demonstrated by significant improvements for both groups on the GFFI (Fig. 2). Both the Met carriers ($p=0.001$) and non-carriers ($p=0.03$) presented improvements in physical

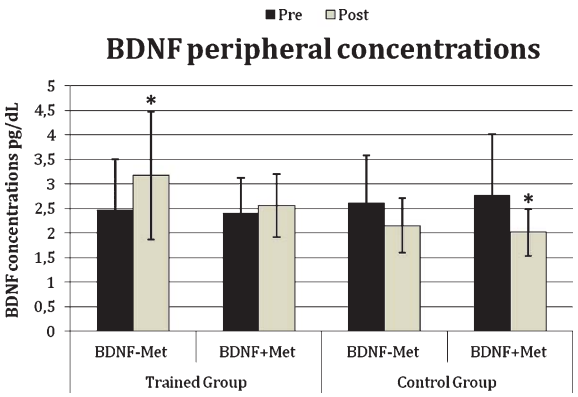

Fig. 1. Pre- and post-BDNF peripheral concentrations in the trained and control groups separated by different BDNF genotypes. BDNF, Brain-Derived Neurotrophic Factor; +Met, Met allele carriers; −Met, Met allele non-carriers. *Significant difference between pre- and post-16 weeks of a multimodal program of physical exercise.

Table 4
Global cognitive functions and the cognitive domain evaluated by the MoCA test for elderly MCI individuals with different BDNF genotypes demonstrated significant changes after the 16-week multimodal program of physical exercise

			Baseline Mean ± SD	Post Mean ± SD	Between-subject factors for two-way ANOVA p value
MoCA† (30 points)	Trained Group	$BDNF^{-Met}$	**22.4 ± 1.9**	**25.8 ± 2.4***	$F_{3,41} = 4.59$; $p = 0.007$
		$BDNF^{+Met}$	**21.8 ± 1.7**	**23.6 ± 2.1***	
	Control Group	$BDNF^{-Met}$	22.1 ± 2.5	22.6 ± 2.4	
		$BDNF^{+Met}$	21.8 ± 2.4	22.0 ± 3.4	
Executive functions †(5 points)	Trained Group	$BDNF^{-Met}$	**3.8 ± 0.34**	**4.3 ± 0.40***	$F_{3,41} = 4.29$; $p = 0.01$
		$BDNF^{+Met}$	**3.6 ± 0.55**	**4.1 ± 0.63***	
	Control Group	$BDNF^{-Met}$	3.7 ± 0.44	3.7 ± 0.39	
		$BDNF^{+Met}$	3.7 ± 0.60	3.6 ± 0.61	
	Trained Group	$BDNF^{-Met}$	4.4 ± 0.66	4.9 ± 0.68	$F_{3,41} = 3.24$; $p = 0.06$
		$BDNF^{+Met}$	3.9 ± 0.55	4.5 ± 0.73	

MoCA, Montréal Cognitive Assessment; BDNF, Brain-Derived Neurotrophic Factor; $^{+Met}$, Met allele carriers; $^{-Met}$, Met allele non-carriers. †Significant between-subjects factor for repeated-measures ANOVA ($p < 0.05$); *significant univariate differences considering pre and post-training moments for each group ($p < 0.05$).

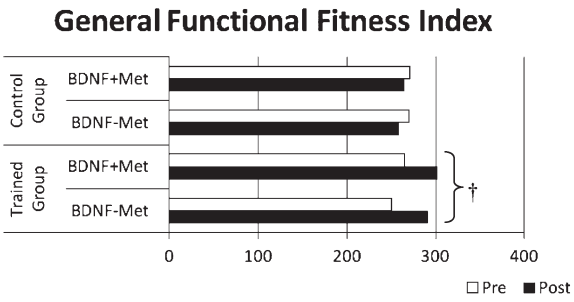

Fig. 2. Pre- and post-General Functional Fitness Index (GFFI) scores for the trained and control groups separated by different BDNF genotypes. BDNF, Brain-Derived Neurotrophic Factor; +Met, Met allele carriers; −Met, Met allele non-carriers. †Significant between-subjects factor for repeated-measures ANOVA ($F = 5.04$; $p = 0.04$) considering pre- and post-16 weeks of a multimodal physical exercise program.

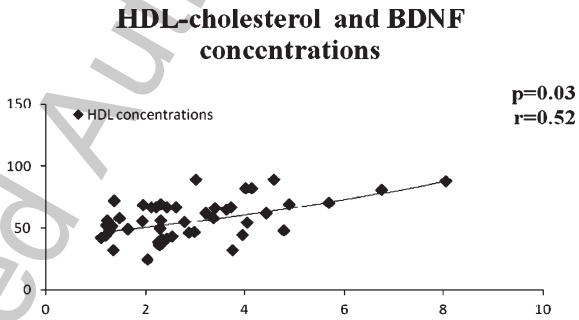

Fig. 3. Partial correlation coefficient demonstrated that higher levels of HDL-cholesterol were positively correlated with BDNF peripheral levels ($r = 0.52$; $p = 0.03$). BDNF, Brain-Derived Neurotrophic Factor; HDL-cholesterol, High-density lipoprotein.

domains. The between-subject factors for two-way ANOVA aimed to identify these changes ($F_{3,41} = 5.04$; $p = 0.04$).

We also observed a significant improvement in the HDL-cholesterol concentrations for the participants in the $BDNF^{+Met}$ TG. Regarding the LDL-cholesterol levels, no significant changes were observed as a training effect; however, the data indicated that the individuals in the $BDNF^{-Met}$ TG presented reduced levels of this dyslipidemic marker. Nevertheless, no changes were shown for this variable after the proposed multimodal physical exercise program. Table 5 displays the results of the HDL- and LDL-cholesterol pre and post 16-weeks of physical exercise training. Regarding the partial correlation conducted, we identified a significant association, which indicated that higher levels of peripheral BDNF were associated with higher concentrations of HDL-cholesterol in our sample (Fig. 3).

DISCUSSION

We determined that a 16-week multimodal physical exercise program improved peripheral BDNF levels and cognitive functions in MCI elderly individuals. It is important to note that in our study, we excluded 2 participants from the trained MCI group and 3 participants from the trained cognitive healthy group who exhibited poor adherence (below 75%). This exclusion, however, did not represent a significant impact on our findings, at least for the cognition data because a statistical analysis that included these subjects showed similar results to the analysis performed without the exclusion (data not shown).

Considering the BDNF Val66Met polymorphism, the analysis of cognitive functions showed

Table 5
HDL- and LDL-cholesterol for MCI elderly individuals with different BDNF genotypes prior to and after a 16-week multimodal program of physical exercise

			Baseline Mean ± SD	Post Mean ± SD	Between-subject factors for two-way ANOVA p value
HDL cholesterol	Trained Group	$BDNF^{-Met}$	50.8 ± 16.8	55.6 ± 16.9	$F_{3,41} = 0.71$; $p = 0.55$
		$BDNF^{+Met}$	**38.5 ± 12.3**‡	**48.2 ± 24.8***	
	Control Group	$BDNF^{-Met}$	52.9 ± 14.8	53.7 ± 13.4	
		$BDNF^{+Met}$	**40.6 ± 14.1**‡	43.2 ± 13.3	
LDL cholesterol	Trained Group	$BDNF^{-Met}$	**105.4 ± 24.5**‡	**101.3 ± 22.2**‡	$F_{3,41} = 1.87$; $p = 0.145$
		$BDNF^{+Met}$	117.5 ± 27.7	121.4 ± 34.6	
	Control Group	$BDNF^{-Met}$	115.1 ± 25.1	126.4 ± 26.6	
		$BDNF^{+Met}$	119.6 ± 25.1	124.1 ± 18.6	

HDL, High-density lipoprotein; LDL, Low-density protein; $^{+Met}$, Met allele carriers; $^{-Met}$, Met allele non-carriers. *Significant improvement in HDL-cholesterol ($p = 0.02$) considering pre and post-training for the $BDNF^{+Met}$ trained group. ‡Univariate differences between groups considering each condition (pre and post-tests).

improvements independent of the BDNF genotype. Nevertheless, the majority of findings in the literature have indicated that both young and old adult Met carriers ($BDNF^{+Met}$) exhibit more severe cognitive impairments [22, 41]. Our results could be related to the sample of MCI elderly individuals because it is a condition frequently linked to a neurodegenerative process that appears to be independent of the BDNF genotype. However, for the peripheral levels of BDNF, Met allele carriers did not present significant improvements in the serum concentrations of this neurotrophin after the training period. In the CG, peripheral BDNF levels were significantly reduced in the Met carriers compared with the Met allele non-carriers ($BDNF^{-Met}$). To explain this result, we hypothesized that after the 16-week period, the BDNF concentration decreased in the $BDNF^{+Met}$ carriers as a result of advances in the MCI condition. Therefore, the data indicated that this BDNF polymorphism may have an important role in the effects of physical exercise on BDNF peripheral concentrations but that it did not influence cognitive performance.

Our findings are consistent with previous reports that have shown that the regular practice of physical exercise facilitated improvements in cognitive functions, specifically in executive function domains [11, 42]. Previous studies have also shown the effectiveness of exercise training on cognition in elderly MCI individuals [43]. Physical exercise triggers molecular and cellular processes that promote, among several other effects, angiogenesis, neurogenesis, and brain synaptogenesis [44]. The underlying neurobiological mechanisms responsible for these beneficial effects include an increase in brain blood flow, which, in turn, results in a rise in the synthesis and use of neurotransmitters and an increase in the synthesis and release of BDNF [45]. Suzuki and co-workers [46] reported that in older adults with amnestic MCI (aMCI), an exercise intervention was beneficial for improving logical memory, maintaining general cognitive function and reducing whole brain cortical atrophy. The authors also observed that low total cholesterol and higher BDNF levels may predict improvements in cognitive functions in older adults with MCI.

To the best of our knowledge, there are no studies that have reported the influence of BDNF genotype and exercise on cognition in MCI elderly individuals. However, regarding the BDNF genotype, some studies have investigated the effects of the Val66Met polymorphism on cognitive functions in elderly MCI individuals. Forlenza and co-workers [47] reported that serum BDNF levels were reduced in patients with MCI and AD compared with controls. Baseline serum BDNF levels were not associated with the progression of cognitive impairment at follow-up in patients with MCI or with the conversion to AD. Although the Val66Met polymorphism was not associated with the cross-sectional diagnoses of MCI or AD, the presence of the Met allele has been associated with a higher risk of disease-progression in patients with MCI.

Hopkins and colleagues [48] found that a 4-week program of physical exercise could produce a positive impact on cognition in young adults, primarily in $BDNF^{Met-}$ subjects. Erickson and co-workers [49] determined that the BDNF Val66Met polymorphism may primarily affect executive functions, which suggests the potential role of this genotype on the trajectory of cognitive decline in old age.

Our findings demonstrated that improvements in the executive function domain were independent of the BDNF genotype. In our study, the participants were encouraged to perform multimodal exercises that aimed to stimulate multiple physical domains (e.g., aerobic training combined with strength, motor

coordination, stretching, and balance tasks). To perform the proposed sessions, the participants should have recruited multiple cognitive domains. The process of planning and learning more complex sequences of movements required the stimulation of prefrontal areas, helping to explain our findings. Li et al. [50] recently demonstrated that multimodal interventions could improve the activity in the prefrontal cortex and in the medial temporal regions, which play important roles in preserving the brain and cognition during old age.

Lim and colleagues [51] demonstrated that in individuals with aMCI and high Aβ, the Met carriers showed a significant and large decline in episodic memory and hippocampal volume; thus, the authors concluded that high Aβ levels and Met carriage may be biomarkers of the decline in episodic memory and may promote reductions in hippocampal volume in aMCI. Our results are in disagreement with this study because there was no significant difference in the baseline levels of plasma BDNF in the Met carriers and non-carriers. However, in our study, physical exercise improved BDNF levels only in the $BDNF^{Met-}$ group. It has been suggested that the Val66Met polymorphism leads to the decreased availability of BDNF in the brain [22]. Therefore, according to our results, MCI elderly individuals who carry the wild-type genotype would respond better to the exercise program.

In our study, Met allele carriers did not exhibit significant improvements in the peripheral BDNF levels after the proposed program of physical exercise. Kim et al. [52] demonstrated that the BDNF Val66Met polymorphism was not associated with cognitive outcome; however, in agreement with our findings, the study suggested that the Met allele was associated with a lower activity-dependent secretion of BDNF in physically active elderly individuals. In a cross-sectional investigation, Erickson et al. [53] verified that greater levels of physical activity compensated the deleterious effects of the Met allele on some aspects of cognitive function performance. These findings are in agreement with our results, which indicated that despite the lack of improvements in the BDNF levels, the subjects who participated in the physical exercise program improved their cognitive performance independent of the BDNF genotype.

Regarding the relationships between the peripheral BNDF levels and the markers of the dyslipidemic profile, we found that Met allele carriers in both groups presented lower levels of HDL at baseline compared with the non-carrier participants. Moreover, only the Met allele carriers ($BDNF^{+Met}$) in the TG presented significant improvements in these levels after the 16-weeks of proposed training. Physical exercise plays a major role in metabolic profile changes, reducing cardiovascular and other chronic disease incidence rates [54] and providing protection from the irreversible damage of reactive oxygen species by increasing the concentrations of antioxidants [55]. Interestingly, physical exercise appears to exert greater protective effects in carrier individuals compared with non-carriers. Higher levels of HDL-cholesterol were also associated with high BDNF serum levels at baseline in the MCI subjects. Previous studies have demonstrated that serum lipoprotein levels may be a common and potentially modifiable risk factor for AD [56]. A recent research study suggested that demented patients with cardiovascular diseases presented lower levels of HDL-cholesterol [57]. Additionally, a prospective study reported that lower serum HDL levels were an independent risk marker for subsequent cognitive decline in a population of older adults and may be useful in early clinical screening [58]. Our findings extend the knowledge regarding the relationships between lipid markers and cognitive function in different BDNF genotypes in MCI elderly individuals.

Considering the levels of LDL-cholesterol, only the $BDNF^{-Met}$ TG presented lower levels compared with the other three groups. It is important to highlight that only these participants presented concentrations closer to the recommended levels (<100 mg/dl), whereas the other participants were slightly above the optimal levels (100 to 129 mg/dl). No significant relationship was observed for this variable and peripheral BDNF levels. A body of evidence has previously linked the dyslipidemic profile in middle-age with cognitive impairments in late life. However, the brain changes underlying this link are unknown. Abnormal lipid metabolism appears to contribute to the degenerative process on cognitive functions. Physical exercise is also a valid and feasible approach to manage the lipid profile, thereby preventing cognitive decline in elderly individuals [59].

CONCLUSION

Based on our results, we can conclude that 1) a 16-week multimodal physical exercise program improved the peripheral levels of BDNF and cognitive function in MCI older adults; 2) BDNF Met allele non-carriers ($BDNF^{-Met}$) showed more pronounced and significant improvements in the peripheral BDNF levels after 16 weeks of the exercise program compared with the

BDNF Met allele carriers ($BDNF^{+Met}$); 3) Cognitive improvements were independent of the genotype; and 4) 16 weeks of an exercise program induced significant improvements in the lipid profile for both carriers and non-carriers of the BDNF Met allele.

ACKNOWLEDGMENTS

CAPES, LAFE, PROFIT and LIN-27 (IPq – USP/SP).

Authors' disclosures available online (http://www.j-alz.com/disclosures/view.php?id=2355).

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