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Research Articles

# The effect of physical exercise with cognitive training on inflammation and Alzheimer's disease biomarkers of Mild Cognitive Impairment patients

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ABSTRACT

Physical exercise (PE) was proven crucial for cognitive health of both demented and cognitively intact individuals. Simultaneous cognitive training may augment PE beneficial effects in demented patients. However, it is still debatable how PE and/or cognitive training reflect on mild cognitive impairment (MCI) and pathological factors related to Alzheimer's disease, namely inflammatory cytokines interleukin (IL) 1 beta and 6, tumor necrosis factor alpha (TNF-α), amyloid beta (Aβ) peptides, total tau protein (t-tau) and tau phosphorylated at threonine 181 (p-tau181). In the current study, 74 MCI patients were allocated to three groups: non-intervention (Control), PE, and PE with cognitive training (Mixed). Blood serum was received at the Baseline and after the intervention (at 3 months). Levels of IL-1β, IL-6, TNF-α, $A\beta_{42}$, $A\beta_{40}$, tau and p-tau181 were determined with enzyme-linked immunosorbent assay. PE and Mixed interventions reduced the levels of IL-1β, IL-6 and of p-tau181. Augmented levels of $A\beta_{42}$ and the ratio $A\beta_{42/40}$, and reduced ratio p-tau181/$A\beta_{42}$ were verified only in the PE group. Reduced levels of TNF-α, $A\beta_{40}$ and t-tau were verified only at the Mixed group. In addition, correlation analyses provided a significant interrelation between the alleviation of inflammation and p-tau181/$A\beta_{42}$ reduction in patients of the Mixed intervention. These results suggest the beneficiary effect of PE and cognitive training on the regulation of inflammation resulting in mitigation of AD-related blood biomarkers and urge for their employment as non-pharmacological alternatives for stalling the progression of dementia.

## 1. Introduction

Alzheimer's disease (AD) constitutes the most prominent form of dementia (Alzheimer's Association, 2021). Pathological hallmarks of the disease include extracellular plaques of amyloid beta peptide 1–42 ($A\beta_{42}$), and intracellular neurofibrillary tangles (NFTs) of the hyperphosphorylated tau protein (p-tau) (Hampel et al., 2008). The exact causes of the disease and holistic treatment for AD have not yet come to light. Common therapeutic strategies for AD include acetyl-cholinesterase inhibitors (rivastigmine, donepezil, and galantamine) and the N-methyl-d-aspartate receptor antagonist memantine, but these drugs cannot halt the progression of dementia and only lead to a slight slowdown in the loss of cognitive function (Godyń et al., 2016).

Mild cognitive impairment (MCI) is often characterized as the "in-

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between" of normal cognition and verified dementia, with an increased probability of evolving into AD. Many MCI patients present histological degenerations that resemble a very early AD pathology (Petersen and Negash, 2008). Aducanumab and Lecanemab are newly FDA-approved antibody-based drugs against Aβ, that proved promising during initial trials for early AD patients and especially MCI patients. However, none of these drugs have been approved abroad USA, and both are treated with caution, due to high cost, potentially serious adverse effects, and questionable effectiveness (Wu et al., 2023).

Staging and monitoring of AD progression is performed by tracking degeneration and amyloid and tau burden by imaging examinations (MRI, PET), and with behavioral-cognitive tests. However, biofluid biomarker analysis is imperative to increase the diagnosis's availability, sensitivity, and specificity, as suggested in the recent diagnostic criteria (Dubois et al., 2021). Recognized biomarkers for AD are low $A\beta_{42}$ and elevated tau (total and phosphorylated) in the cerebrospinal fluid (CSF), with increased CSF light neurofilament protein, and plasma total tau and glial fibrillary acidic protein also considered (Olsson et al., 2016; Pereira et al., 2021).

The big obstacle against AD is that when the full neuropsychological spectrum of the disease has emerged, it is too late for any intervention to be implemented (Mehta et al., 2017). Considering the concerns raised for the current therapies' cost and safety, there is an urge for non-pharmacological interventions for MCI and AD (Wu et al., 2023). Nonpharmacological treatments for MCI are increasingly incorporating combined physical and cognitive training programs as a standard approach for individuals exhibiting dementia symptoms (Couch et al., 2020; Wang et al., 2020; Song et al., 2018; Karssemeijer et al., 2017). Research indicates that physical and cognitive development are interconnected and mutually influential (Nathaniel-James, 2002; Hillman et al., 2008; Olazarán et al., 2004; Wulf and Prinz, 2001; Hertzog et al., 2008). Physical activities introduce new experiences that can lead to beneficial brain changes, aiding in cognitive improvement. Recent studies have demonstrated that cognitive performance improves more when both physical and cognitive activities are combined, compared to engaging in either activity alone (Hillman et al., 2008; Olazarán et al., 2004). Dual-task training has shown the potential to improve balance (Nathaniel-James, 2002), gait, and cognitive abilities in patients with neurological disorders, including MCI (Lauenroth et al., 2016). Early evidence also suggests that interventions targeting cognitive-motor interference, both directly and indirectly, can benefit older adults with neurodegenerative diseases (Kounti et al., 2011). Though findings showed that PE ameliorates cognitive decline and dementia (Blondell et al., 2014), the effect of Physical Exercise (PE) on AD hallmarks in human patients suffering from MCI or AD has not yet been examined in detail (Frederiksen et al., 2018; Huang et al., 2021; Jensen et al., 2015).

It has not yet been determined what is the exact contribution of inflammatory dyshomeostasis to the progression of AD (Bautmans et al., 2021; Shintouo et al., 2020; Franceschi and Campisi, 2014; Ashraf et al., 2019). Evidence associates Aβ formation with increased cytokine levels (Skaper et al., 2018). In addition, NFTs could initiate microglial activation and thus inflammatory responses, while conversely, cytokines can alter the functional and structural properties of intracellular tau (Zilka et al., 2012). Studies proved the direct correlation of PE with the downregulation of pro-inflammatory cytokines in the brain (Souza et al., 2013). MCI patients dedicated to PE appeared to have decreased pro-inflammatory cytokines (Segal et al., 2012; Nascimento et al., 2014a). Furthermore, increased incline has been detected in anti-inflammatory cytokines (Petersen and Pedersen, 2005; Pedersen and Bruunsgaard, 2003; Pedersen, 2000). However, studies regarding the effect of PE are scarce when looking at outcomes related to inflammation (Bautmans et al., 2021; Abd El-Kader and Al-Jiffri, 2019; Ferrer et al., 2018; Sellami et al., 2018). Additionally, PE in conjunction with cognitive training, presents a neuroprotective effect enhancing cognitive function in transgenic mice models of AD (Kim et al., 2019; Vasconcelos-Filho et al., 2021), in non-demented individuals and human patients suffering from MCI, AD, or other dementias (Rolland et al., 2010; Gallaway et al., 2017; Macpherson et al., 2017; Cammisuli et al., 2017; Ströhle et al., 2015; Du et al., 2018) and improves peripheral vascular function in AD patients, by simultaneously increasing vascular endothelial growth factor (Pedrinolla et al., 2020). Additionally, PE induces neurogenesis in AD animal models (Kohman et al., 2012; Barrientos et al., 2011; Tapia-Rojas et al., 2016; Rodríguez et al., 2015; Zhang et al., 2019).

The two main objectives of the present study are: (a) to determine the effect of moderate to high-intensity PE on inflammatory and AD biomarkers of MCI patients, and (b) to study the effect of a dual-task intervention (PE and cognitive training) in the aforementioned biomarkers' levels, in comparison with the single implementation of PE. Mixed performance included 45-min sessions twice/thrice weekly for 3 months, and cognitive training comprised of tasks of cognitive functions, language practice, and executive functions. The implementation of cognitive training alongside PE is postulated to bring better results regarding the reduction of AD-related biomarkers, thanks to augmented stimulation of brain functions. This study hopefully offers novel prospects in the non-pharmacological approach for coping with dementia specifically with MCI and AD pathology.

## 2. Material and methods

### 2.1. Study design, participants and COVID-19 precautions

This study was part of a grand research intervention evaluating the benefits of PE, with or without the assistance of virtual reality (VR) technology and cognitive training, on patients suffering from amnestic, multiple-domain MCI (see also Tzekaki et al., 2023). The present study aimed to evaluate the possible ameliorative effect of PE on blood serum biomarkers of inflammation and AD. A total number of 74 visitors (9 males and 65 females; mean age 72.5 (SD = 7.8)) of the Greek Association of Alzheimer's Disease and Related Disorders' Day Care Centre "Saint Helen" (Alzheimer Hellas) in Thessaloniki were enrolled in the study, from 2020 to 2021. Participants were randomly selected using SPSS software, version 25. Each participant was assigned a unique identifier and was allocated through the SPSS algorithm to the respective training groups. Due to the simple random selection process, the group sizes were uneven. The Fullerton Fitness Test was used which measures the physical-functional abilities of the elderly. The baseline measurements showed that there was no difference between the 3 groups before the start of the intervention.

Three separate groups were involved: (a) MCI patients who comprised the PE group, (b) MCI patients who comprised the PE and cognitive exercise group (i.e., the Mixed group), and (c) MCI patients who were the control group. Before starting the intervention, participants were assessed for blood glucose, lactic acid, weight, and height. In parallel, a comprehensive neuropsychological battery was administered. The participants were white, community-dwelling individuals and literate. MCI diagnosis was performed through neurological, neuropsychological, and neuropsychiatric evaluation, neuroimaging, and blood tests according to the criteria for Mild Neurocognitive Disorders (Petersen and Morris, 2005). Inclusion criteria: (1) a Mini-Mental State Examination (MMSE) score of 26–28, (2) a Clinical Dementia Rating score of 0.5 (stage 3 of the disease), (3) 1.5 standard deviations (SD) below the normal mean depending on education and Age, in at least one cognitive area following the neuropsychological testing that was administered (Petersen and Morris, 2005). Exclusion criteria: 1) diagnosis of dementia, 2) severe depression, 3) epilepsy, stroke, Parkinson's Disease, or hydrocephalus, 4) sensory deficits, 5) cardiovascular disease, 6) severe hypertension, and 7) severe other mental illness. All subjects gave written informed consent to the Declaration of Helsinki (World Medical Association, 1964). The study was approved by the Scientific and Ethics Committee of Alzheimer Hellas (Protocol Number: 40/16-05-2018). Participants in both intervention groups completed 32

sessions over 3 months and there were no dropouts.

During the study, participants were obliged to stay at home due to COVID-19 lockdown measures in Greece and besides the exercise performed due to the study, no other PE programs could be possible. For the participants' safety, it was decided, following the consultation of the Scientific and Ethics Committee of Alzheimer Hellas, that the intervention programs would be conducted via Skype or Messenger, and with the use and assistance of the electronic devices available to each participant (tablets, smartphones, laptops). Whenever technical guidance was needed, it was provided by telephone. As a result of these conditions, small subgroups were formed from each intervention group, where each subset had a different start time. Before the start of the intervention, the participants had to come to the Day Centre at a specific appointment in the morning hours (2 persons per 1 h) for blood collection. All the appropriate COVID-19 safety precautions were followed by both participants and staff during their arrival. Despite the COVID-19 restrictions, participants attended the sessions according to the training protocol, demonstrating their commitment to the study. The participants did not receive any vaccinations during the study, including COVID-19 vaccinations, as the vaccination process for the disease had not yet started in Greece.

### 2.2. Physical exercise and Mixed Intervention protocols

The PE group followed 32 training sessions of 45 min, 2 to 3 times per week for 3 months. Their physical exercise program was a combination of aerobic exercises followed by strengthening, flexibility, and balance exercises with progressively increasing intensity every 2 weeks. The aim was to be stabilized at the level of moderate to vigorous intensity in the last 3 weeks. Trainees learned to control intensity and assess themselves using Borg's subjective impairment scale (11 - mild to 14 - moderate to vigorous). Participants engaged in simple and complex exercises in a seated or standing position, following simple, rhythmic, verbal instructions. The routines included: (a) turning the head and neck while counting time and repetitions; (b) shoulder exercises (shrug both shoulders x 10 (counting each repetition either using digits or the alphabet letters); (c) hands and hips (dual-task) (raise the right foot slightly while making a fist, lower the foot and relax the hand, raise the left foot and stretch hands, lower the foot and relax hand x 10 (counting each repetition either using digits or the alphabet letters) a dual-task, hands, and hips exercise with counting). The instructor provided simple, rhythmic instructions. The exact words were used each time, and the exercise routine was presented in the same way each time. Guidance was given with verbal, visual, and manual cues. Verbal cues were simple, with a minimum of substantives and explanations. They were most effective when reinforced with visual cues. When instructions must be repeated, the exact words are used each time, using a calm, rhythmic intonation. This helped to reinforce learning and establish a pattern. Instructions faded out as soon as it was clear that the participants knew what they had to do.

The second group, the Mixed group, conducted 32 physical training 45-min sessions performed 2 or 3 times a week for 3 months. The PE program was the same as in the first group. The cognitive exercise in the mixed group was conducted separately from the physical exercise, at a different time of the same day, having the same duration as the physical exercise (45 min). Each cognitive training program practiced a broad area of cognitive functions such as attention (visual-auditory, sustained-divided), language (naming), and aspects of executive function (working memory, inhibition, shifting, cognitive flexibility).

Finally, patients in the control group were not taking part in any intervention program. In these patients, it was suggested to follow their everyday routine (non-active control). During the intervention and due to the special conditions of the COVID-19 pandemic, the experimenters made sure to be in frequent contact with the personnel from Alzheimer Hellas, for information regarding their routine and to partially simulate the contact achieved in the intervention groups during exercise activities.

### 2.3. Handling of blood serum samples

Blood samples were collected in the morning hours, after overnight fasting, before the onset of the study (Baseline), and after its completion (at 3 months). Before the blood draw, the participants were instructed not to do any vigorous activities. Serum separator tubes were employed, allowing samples to clot for 30 min at room temperature. Then, blood was centrifuged for 20 min at $1000\times g$, sera were collected, aliquoted, and stored at $-80^{\circ}$C until analysis. Necessary dilutions of the sera before the analyses were performed with commercial dilution buffer provided with the kits described below, just before the analyses.

### 2.4. Analyses of biomarkers levels by ELISA

All studied biomarkers in the sera of the study participants were detected by sandwich, horseradish peroxidase (HRP)-conjugated enzyme-linked immunosorbent assay (ELISA) kits provided by Assay Genie (Dublin, Ireland), as follows: Human IL-1 beta (IL-1β) PharmaGenie ELISA kit (#SBRS0740), Human interleukin-6 (IL-6) PharmaGenie ELISA kit (#HUDC0061), Human tumor necrosis factor alpha (TNF-α) PharmaGenie kit (#HUDC0073), Human $A\beta_{42}$ kit (#HUFI02245), Human amyloid beta 1–40 ($A\beta_{40}$) ELISA kit (#HUFI02244), Human total tau protein (t-tau) kit (#HUES02072), and Human Phospho-Tau at threonine 181 (tau-p181) kit (#HUFI03189). All analyses were run per the manufacturer's instructions in duplicates and the mean concentration value was included in the results. All duplicates' variations were non-significant different, and with a CV<5%, as proposed by the manufacturer of the kits. Baseline and post-intervention serum samples were analyzed by two different researchers, who were blind to the origin of the samples and the analyses performed by each other.

### 2.5. Statistical analyses

GraphPad Prism 8 (GraphPad Software Inc.) has been employed for statistical analyses and graphs' construction. Statistical analyses for differences in age, education, or Body Mass Index (BMI) were performed with the Kruskal-Walli's test, followed by Dunn's multiple comparisons post-hoc tests. In contrast, for gender, Chi-Squared analysis with Yates' correction was used.

Mixed two-way analysis of one-way variance (ANOVA) with Sidak's multiple comparisons tests was employed for studying possible significant differences of the studied biomarkers: a) between baseline and after the intervention, b) between after levels of each studied group. $p$ values for all the analyses are provided in the Supplementary Table S1.

Matrix correlation analysis with Spearman's test (confidence interval set at 95%) has been used for determining the relationships among analyzed variables, at baseline and after interventions.

For statistical significance to be reached, a value of $p<0.05$ has been demanded in all cases.

## 3. Results

### 3.1. Statistical analysis of subjects' demographics

The demographic characteristics of the current study participants and the received $p$ values of the statistical analysis are summarized in Table 1. In addition, a graph for the distribution of age, education, and BMI of the subjects is provided in Supplementary Fig. S1. No significant differences were verified for the demographics of the studied cohorts.

### 3.2. Alteration in inflammation status of MCI patients after Exercise and Mixed interventions

The levels of the examined cytokines are provided in Table 2 and the

**Table 1**
Demographics of MCI patients assigned to physical Exercise or Mixed physical-neuropsychological interventions, or to the Control group, from whom blood serum was received.

| Demographics | Blood Donor Demographics | | | |
|---|---|---|---|---|
| | Control | Exercise | Mixed | *p* value |
| Participants Number (N) | 23 | 22 | 29 | |
| Gender (Female/Male) | 21/2 | 18/4 | 26/3 | C-E: 0.5818 C-M: 0.8813 E-M: 0.6913 |
| Age (years) | 75.1 ± 7.2 | 72.8 ± 8.2 | 70.2 ± 7.6 | 0.0565 |
| Education (years) | 11.2 ± 3.8 | 11.4 ± 3.4 | 12.7 ± 3.2 | 0.2599 |
| Body Mass Index (BMI) (kg/m$^2$) | 28.0 ± 3.8 | 30.6 ± 9.4 | 27.9 ± 4.1 | 0.6810 |

All values are provided as Mean ± Standard Deviation (SD). Statistical analysis for differences between groups was performed with the usage of the Graph Pad Prism 8 statistical package. Kruskal-Wallis test, with Dunn's post-hoc test for multiple comparisons was employed for Age, Education and BMI. Chi-squared test with Yate's correction was employed for possible differences in Gender. *p* values of the statistical analyses are provided in table. *p* values for statistical differences between the groups: C-E: Control and Exercise; C-M: Control and Mixed; E-M: Exercise and Mixed. No statistically significant differences ($p>0.05$) were found between the demographics of the studied groups. MCI: Mild Cognitive Impairment.

distribution of those is depicted in Fig. 1. Patients undergoing PE or Mixed interventions had significantly lower IL-1β and IL-6 after the intervention compared with baseline and Control levels. Conversely, MCI patients not following any intervention had significantly increased levels of IL-6 after the study, while IL-1β and TNF-α of the control group were not differentiated significantly after the study. In addition, Mixed intervention led to a significant reduction in the titers of TNF-α, after the completion of the study, while no significant effect was verified after PE intervention. Also, the alleviating effect of Mixed intervention on TNF-α levels was found to be significant, when compared to After levels of both the control and the PE group. On the other hand, no significant differences were observed between PE and Mixed groups, regarding the after levels of cytokines IL-1 and IL-6.

Correlation analyses before and post-interventions indicated that IL-1β levels correlate significantly, positively, with both IL-6 and TNF-α levels after the end of the study, but not at Baseline, as presented in Table 3. On the other hand, IL-6 levels strongly correlate positively with the TNF-α level at both the baseline stage and after the end of the study.

### 3.3. Exercise and Mixed interventions lead to diverse changes in the amyloid pathology of MCI patients

The mean levels of peptides $A\beta_{42}$ and $A\beta_{40}$ and their ratio for every studied group, are provided in Table 2, and the distribution of those is depicted in Fig. 2. The ratio $A\beta_{42/40}$ was found to be significantly decreased in MCI patients of the control group after the study, while $A\beta_{40}$ levels were found increased. MCI patients following PE presented significantly increased levels of $A\beta_{42}$ and of the ratio $A\beta_{42/40}$ when compared with both their Baseline levels, and control MCI patients after the study. On the other hand, no improvement regarding $A\beta_{40}$ levels has been detected and there was no difference in comparison with the Control group.

Regarding Mixed intervention, no significant changes have been found after the intervention, at the levels of $A\beta_{42}$ and $A\beta_{42/40}$ ratio of the employed MCI patients. However, the $A\beta_{42/40}$ ratio of patients who followed the Mixed intervention were significantly higher than that of patients in the Control group after completion of the study (3 months),

**Table 2**
Levels of studied biomarkers of MCI patients assigned to physical Exercise or Mixed physical-neuropsychological interventions, or to the Control group, from whom blood serum was received at baseline, and after completion of the study.

| Biomarker | Biomarker Analysis of Study Groups | | | | | |
|---|---|---|---|---|---|---|
| | Control (n=23) | | Exercise (n=22) | | Mixed (n=29) | |
| | Baseline | After | Baseline | After | Baseline | After |
| IL-1β (pg/mL) | 37.32 ± 8.31 | 40.61 ± 8.31 | 40.51 ± 7.53 | 28.20 ± 8.55[a, b] | 39.52 ± 13.55 | 22.98 ± 21.22[a, b] |
| IL-6 (pg/mL) | 4.27 ± 2.50 | 5.48 ± 2.72[a] | 4.37 ± 1.40 | 2.19 ± 1.25[a, b] | 4.38 ± 1.75 | 2.56 ± 1.63[a, b] |
| TNF-α (pg/mL) | 17.83 ± 4.63 | 18.11 ± 6.90 | 17.60 ± 3.50 | 19.97 ± 4.02[a] | 17.08 ± 6.33 | 16.02 ± 6.46 |
| $A\beta_{42}$ (pg/mL) | 30.25 ± 6.38 | 24.69 ± 7.28[a] | 29.46 ± 7.64 | 44.95 ± 12.33[a, b, c] | 28.09 ± 5.43 | 26.93 ± 9.12 |
| $A\beta_{40}$ (pg/mL) | 94.36 ± 13.58 | 105.00 ± 14.72[a] | 94.75 ± 17.39 | 100.90 ± 18.41[a, c] | 94.12 ± 16.96 | 81.06 ± 18.77[a, b, c] |
| t-tau (pg/mL) | 0.18 ± 0.04 | 0.26 ± 0.06[a] | 0.20 ± 0.06 | 0.17 ± 0.04[a, b, c] | 0.19 ± 0.05 | 0.08 ± 0.03[a, b] |
| p-tau181 (pg/mL) | 0.61 ± 0.22 | 0.95 ± 0.24[a] | 0.60 ± 0.19 | 0.37 ± 0.11[a, b] | 0.56 ± 0.14 | 0.45 ± 0.23[a, b] |
| $A\beta_{42/40}$ | 0.32 ± 0.08 | 0.24 ± 0.08[a] | 0.31 ± 0.06 | 0.46 ± 0.15[a, b, c] | 0.30 ± 0.04 | 0.34 ± 0.12[a, b] |
| p-tau181/$A\beta_{42}$ | 0.020 ± 0.007 | 0.041 ± 0.011[a] | 0.021 ± 0.005 | 0.008 ± 0.002[a, b, c] | 0.020 ± 0.004 | 0.017 ± 0.006[a, b] |

Levels of IL-1β, IL-6, TNF-α, $A\beta_{42}$, $A\beta_{40}$, total tau protein (t-tau), and tau phosphorylated at threonine-181 (p-tau181), were determined with Sandwich, quantitative ELISA. Ratios of $A\beta_{42/40}$ and p-tau181/$A\beta_{42}$ protein were calculated afterward. All values are provided as Mean ± Standard Deviation (SD). Statistical analysis for differences between groups was performed with the usage of the Graph Pad Prism 8 statistical package. Mixed two-way ANOVA with Sidak's multiple correction tests were performed between different study groups. Statistically significant differences ($p<0.05$) [a] with "Baseline" of each group; [b] with "Control group After"; [c] Between "After Exercise" intervention and "After Mixed" intervention. MCI: Mild Cognitive Impairment.

showing that Mixed intervention can halt the progress of amyloid pathology. Additionally, patients following the Mixed intervention presented significantly lower levels of $A\beta_{40}$ in comparison with the Baseline, Control group, and PE group.

### 3.4. Correlation of amyloid species in patients following PE and mixed interventions

Correlation analysis estimated the direct, positive link between $A\beta_{42}$ and $A\beta_{40}$ peptides before and after the interventions (Table 4). Furthermore, the $A\beta_{42/40}$ ratio was also found to positively correlate to $A\beta_{42}$ strongly before and after interventions. In contrast, no significant correlation with $A\beta_{40}$ levels was found, implying that $A\beta_{42}$ levels are the most crucial factors for amyloid pathology in the studied MCI patients.

The results of the correlation analysis of amyloid species with inflammatory markers, namely IL-1β, IL-6, and TNF-α, are provided in Table 3. $A\beta_{42}$ levels positively correlate, in a significant way with IL-6 levels at the Baseline and with TNF-α at both Baseline and after the study. A more consistent, positive correlation was found between the studied inflammatory factors and $A\beta_{40}$, as with all correlations, but the one with baseline IL-1β was proven to be statistically significant. As regards the amyloid ratio $A\beta_{42/40}$, however, the only significant correlation that could be proved was a slight positive with baseline IL-6 levels.

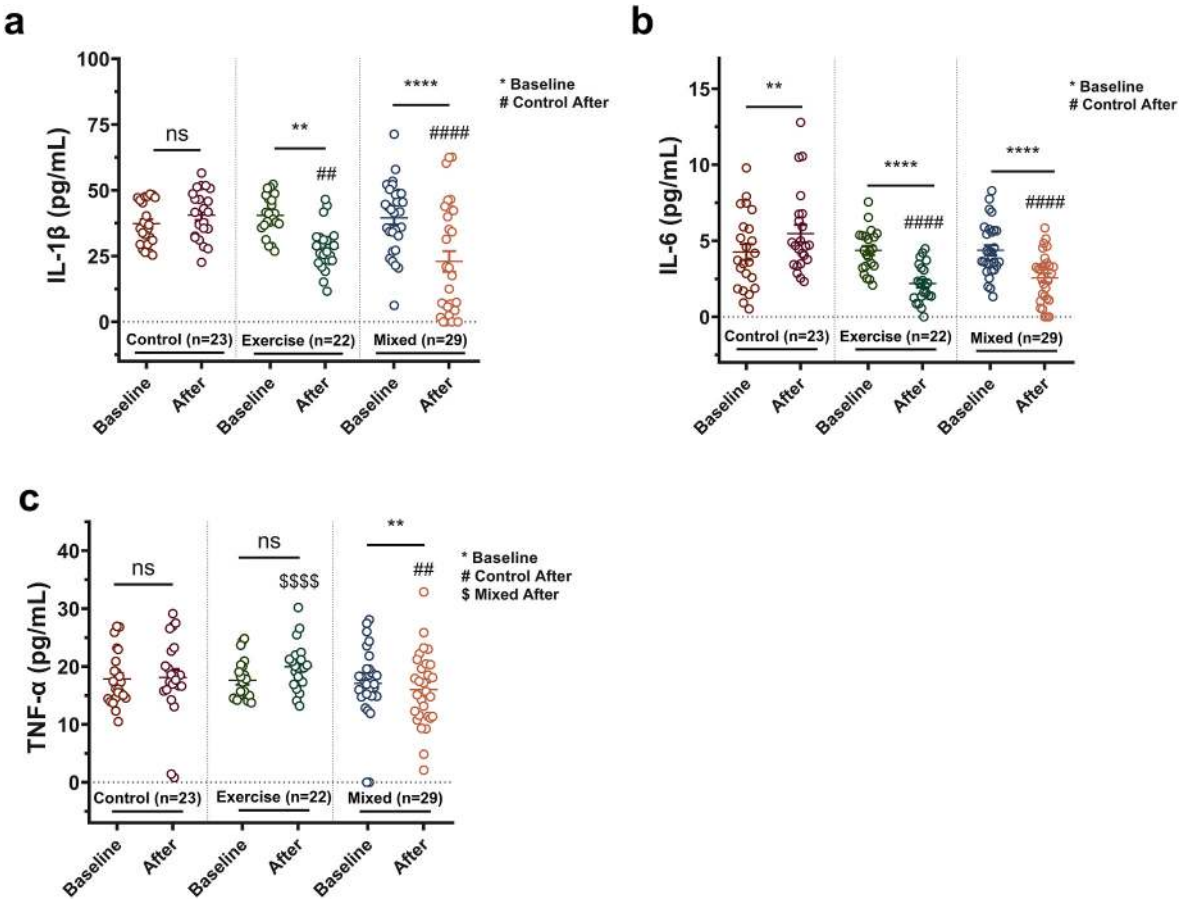


**Fig. 1. Blood serum levels of (a) IL-1β, (b) IL-6, and (c) TNF-α from MCI patients not-following any intervention (Controls) (n=23), MCI patients following a 3-month physical exercise program (Exercise) (n=22), and MCI patients following a 3-month physical exercise program, accompanied with training of cognitive skills (Mixed) (n=29).** Blood from patients was collected at the Baseline (t=0 months), and After the study (t=3 months), and the respected cytokines were analyzed by commercial ELISA kits. Results are provided with individual values scatter plots. Lines present mean values ± standard deviation (SD). All samples were analyzed at least in duplicates. Statistical analyses were performed with Graph Pad Prism 8.0 statistical software. Mixed two-way ANOVA Two-way Mixed ANOVA tests were employed to examine possible differences between groups. *Differences between After the study and respective baseline levels. #Differences between levels of the Control group and the Exercise or Mixed groups, After the study. $ Differences between levels of the Exercise or Mixed group, After the interventions. ns: non-significant, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, ****: $p<0.0001$.

**Table 3**
Correlation analysis of serum cytokines (IL-1β, IL-6, and TNF-α) with the analyzed biomarkers and demographics of the study.

| Biomarker | Correlation Analysis of Study Groups | | | | | |
|---|---|---|---|---|---|---|
| | IL-1β | | IL-6 | | TNFα | |
| | Baseline | After | Baseline | After | Baseline | After |
| IL-1β | | 0.0618 | 0.1822 | 0.5718[d] | 0.2048 | 0.3750[b] |
| IL-6 | 0.1822 | 0.5718[d] | | 0.3536 | 0.5367[d] | 0.4586[d] |
| TNF-α | 0.2048 | 0.3750[b] | 0.5367[d] | 0.4586[d] | | 0.4150 |
| $A\beta_{42}$ | 0.1651 | 0.0457 | 0.6073[d] | 0.0628 | 0.4214[c] | 0.3974[c] |
| $A\beta_{40}$ | 0.1496 | 0.4212[c] | 0.4631[d] | 0.4702[d] | 0.5275[d] | 0.5835[d] |
| T-tau | 0.1273 | 0.4862[d] | 0.4919[d] | 0.5959[d] | 0.4120[c] | 0.4510[d] |
| p181-tau | 0.2202 | 0.6345[d] | 0.5781[d] | 0.8354[d] | 0.5265[d] | 0.5017[d] |
| $A\beta_{42/40}$ | 0.0159 | −0.1768 | 0.2614[a] | −0.1823 | 0.0618 | 0.0948 |
| p181-Tau/$A\beta_{42}$ | 0.1309 | 0.4719[d] | 0.1347 | 0.6056[d] | 0.1819 | 0.1844 |
| Age | −0.0588 | 0.1588 | −0.1846 | 0.0948 | −0.1210 | −0.0826 |
| Education | −0.0211 | −0.0298 | 0.0364 | −0.0308 | −0.0104 | 0.0879 |
| BMI | 0.0772 | 0.0284 | −0.0689 | −0.0339 | −0.0653 | 0.1800 |
| Gender | −0.0697 | −0.0406 | −0.1345 | −0.0058 | −0.1297 | 0.0126 |

Correlation analysis has been performed with Graph Pad Prism 8.0 statistical package and rank correlation coefficients (r) and their corresponding $p$ values were evaluated using Spearman's test.

[a] $p<0.05$.
[b] $p<0.01$.
[c] $p<0.001$.
[d] $p<0.0001$.

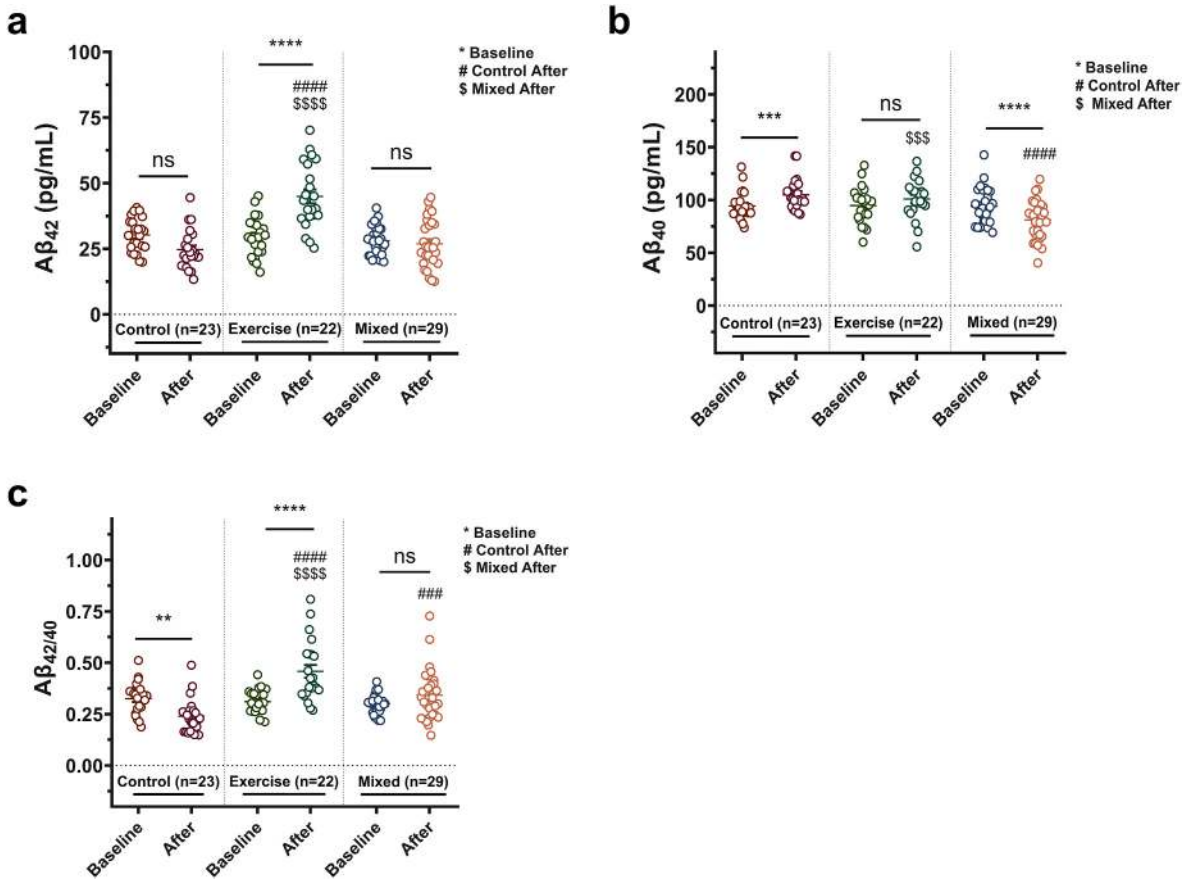


**Fig. 2. Blood serum levels of (a) amyloid peptide 1–42 ($A\beta_{42}$), (b) amyloid peptide 1–40 ($A\beta_{40}$), and (c) of the ratio $A\beta_{42}/A\beta_{40}$ ($A\beta_{42/40}$) from MCI patients not-following any intervention (Controls) (n=23), MCI patients following a 3-month physical exercise program (Exercise) (n=22), and MCI patients following a 3-month physical exercise program, accompanied with training of cognitive skills (Mixed) (n=29).** Blood from patients was collected at the Baseline (t=0 months), and After the study (t=3 months), and the respected biomarkers were analyzed by commercial ELISA kits. Results are provided with individual values scatter plots. Lines present mean values ± standard deviation (SD). All samples were analyzed at least in duplicates. Statistical analyses were performed with Graph Pad Prism 8.0 statistical software. Mixed two-way ANOVA tests were employed to examine possible differences between groups. *Differences between After the study and respective baseline levels. #Differences between levels of the Control group and the Exercise or Mixed groups, After the study. $ Differences between levels of the Exercise or Mixed group, After the interventions. ns: non-significant, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, ****: $p<0.0001$.

**Table 4**
Correlation analysis of serum amyloid ($A\beta_{42}$, $A\beta_{40}$, and the ratio $A\beta_{42/40}$) with the analyzed biomarkers and demographics of the study.

| Biomarker | Correlation Analysis of Study Groups | | | | | |
|---|---|---|---|---|---|---|
| | $A\beta_{42}$ | | $A\beta_{40}$ | | $A\beta_{42/40}$ | |
| | Baseline | After | Baseline | After | Baseline | After |
| $A\beta_{42}$ | | 0.2751 | 0.5292[c] | 0.2746[a] | 0.6902[c] | 0.8355[c] |
| $A\beta_{40}$ | 0.5292[c] | 0.2746[a] | | 0.5560 | −0.1896 | −0.2272 |
| t-tau | 0.4697[c] | 0.0662 | 0.4444[c] | 0.6132[c] | 0.1732 | −0.2410[a] |
| p-tau181 | 0.4895[c] | −0.0989 | 0.4291[b] | 0.5743[c] | 0.2040 | −0.3953[b] |
| $A\beta_{42/40}$ | 0.6902[c] | 0.8355[c] | −0.1896 | −0.2272 | | 0.0876 |
| p-tau181/$A\beta_{42}$ | −0.2347[a] | −0.6271[c] | 0.0704 | 0.2623[a] | −0.3312[a] | −0.7649[c] |
| Age | −0.0989 | −0.0629 | −0.0766 | 0.1223 | >0.0001 | −0.1539 |
| Education | 0.02479 | −0.0119 | 0.0441 | 0.0412 | 0.0092 | −0.0045 |
| BMI | −0.0148 | −0.1498 | −0.0866 | 0.0496 | −0.0058 | −0.2030 |
| Gender | −0.2371 | −0.1055 | −0.1384 | −0.0610 | −0.1906 | −0.0977 |

Correlation analysis has been performed with Graph Pad Prism 8.0 statistical package and rank correlation coefficients (r) and their corresponding *p* values were evaluated using Spearman's test.
[a] $p<0.05$.
[b] $p<0.001$.
[c] $p<0.0001$.

### *3.5. PE and mixed interventions led to improvement of tau pathology in MCI patients*

The levels of t-tau, p-tau181, and the ratio p-tau181/$A\beta_{42}$ ratio for every studied group, before or post-study, are provided in Table 2, and the distribution of those is depicted in Fig. 3. Patients not following any intervention presented significantly higher levels of t-tau, p-tau181, and of the ratio p-tau181/Aβ42 after the end of the study. On the other hand,

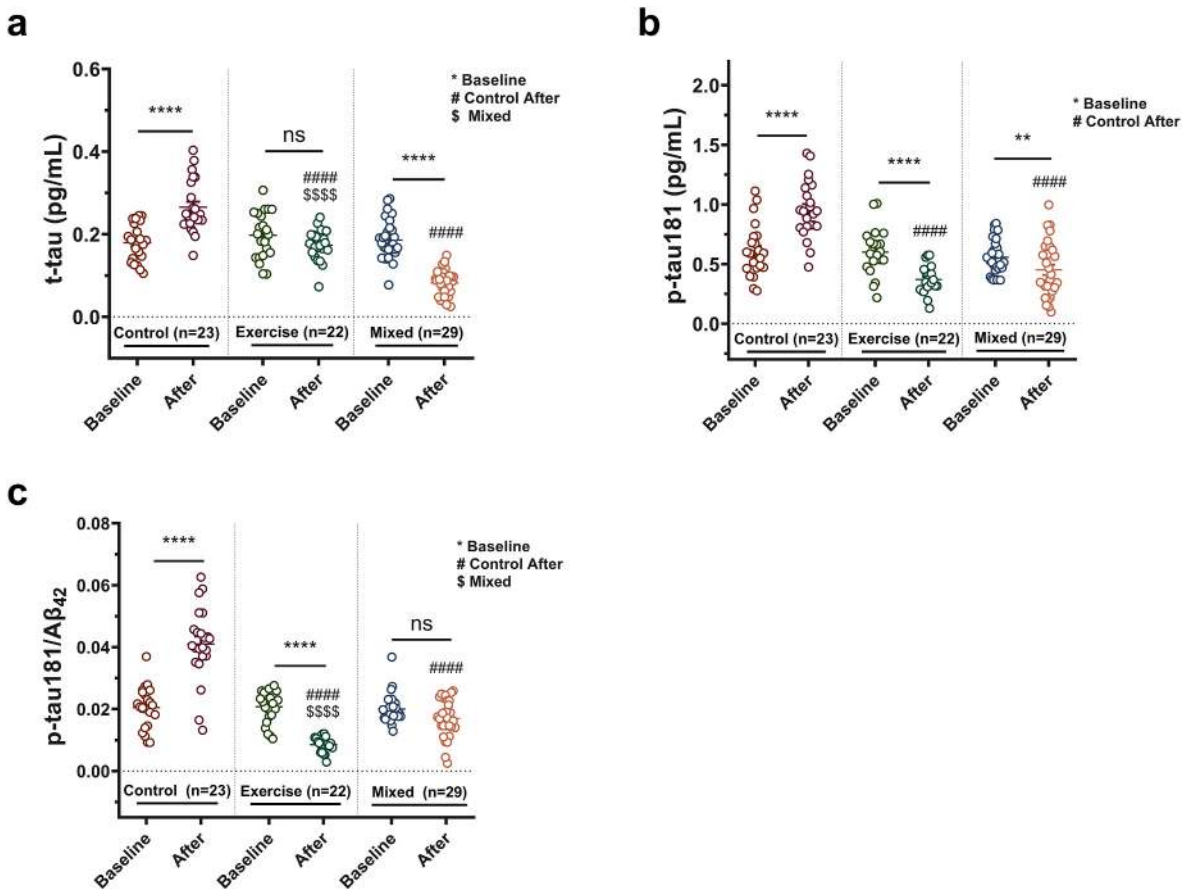


**Fig. 3. Blood serum levels of (a) total tau protein (t-tau), (b) tau protein phosphorylated at Threonine-181 (p-tau181), and (c) of the ratio p-tau181/Aβ$_{42}$ from MCI patients not-following any intervention (Controls) (n=23), MCI patients following a 3-month physical exercise program (Exercise) (n=22), and MCI patients following a 3-month physical exercise program, accompanied with training of cognitive skills (Mixed) (n=29).** Blood from patients was collected at the Baseline (t=0 months), and After the study (t=3 months), and the respected biomarkers were analyzed by commercial ELISA kits. Results are provided with individual values scatter plots. Lines present mean values ± standard deviation (SD). All samples were analyzed at least in duplicates. Statistical analyses were performed with Graph Pad Prism 8.0 statistical software. Mixed two-way ANOVA tests were employed to examine possible differences between groups. *Differences between After the study and respective baseline levels. #Differences between levels of the Control group and the Exercise or Mixed groups, After the study. $ Differences between levels of the Exercise or Mixed group, After the interventions. *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$, ****: $p<0.0001$.

participants in the Mixed group presented statistically significantly lower levels of t-tau and p-tau181 when compared with their respective Baseline and the patients of the Control group. In addition, MCI patietns of the Mixed group has lower levels of the ratio p-tau181/Aβ$_{42}$ after the end of the study, in comparison with MCI patients of the Control group. Levels of p-tau181 and of the ratio p-tau181/Aβ$_{42}$ were also decreased for patients following PE intervention, but no significant differences were verified for t-tau. However, t-tau levels after PE intervention were still significantly lower than the ones verified at the control group. Also, patients following the Mixed intervention presented significantly lower serum t-tau protein when compared with patients of the PE group.

**Table 5**
Correlation analysis of serum tau (t-tau, p-tau181, and the ratio p-tau18/Aβ$_{42}$) with the analyzed biomarkers and demographics of the study.

| Biomarker | Correlation Analysis of Study Groups | | | | | |
|---|---|---|---|---|---|---|
| | t-tau | | p-tau181 | | p-tau181/Aβ$_{42}$ | |
| | Baseline | After | Baseline | After | Baseline | After |
| t-tau | | 0.1348 | 0.4677[b] | 0.7220[b] | 0.2106 | 0.4756[b] |
| p-tau181 | 0.4677[b] | 0.7220[b] | | 0.3086[a] | 0.6559[b] | 0.8222[b] |
| p-tau181/Aβ$_{42}$ | 0.2106 | 0.4756[b] | 0.6559[b] | 0.8222[b] | | 0.0556 |
| Age | −0.2092 | 0.2151 | 0.0647 | 0.2007 | 0.1644 | 0.1785 |
| Education | 0.0152 | −0.0902 | 0.0868 | 0.0117 | 0.0210 | 0.0083 |
| BMI | −0.0023 | 0.0840 | −0.0264 | 0.0345 | 0.0311 | 0.1042 |
| Gender | −0.0300 | 0.0164 | −0.0629 | 0.0494 | 0.1490 | 0.1365 |

Correlation analysis has been performed with Graph Pad Prism 8.0 statistical package and rank correlation coefficients (r) and their corresponding $p$ values were evaluated using Spearman's test.
[a] $p<0.01$.
[b] $p<0.0001$.

### 3.6. Correlation of serum tau species in patients following PE or mixed interventions

Correlation analysis (Table 5) showed a significant, positive correlation between MCI patients' serum levels of t-tau and p-tau181, both at the Baseline but more strongly after the study. In addition, levels of p-tau181 were found to be positively correlated in a significant manner, before and after the interventions. T-tau and p-tau181 levels were also correlated positively with $A\beta_{42}$ levels (Table 4) only at the Baseline. Significant correlations have also been found between $A\beta_{40}$ levels and t-tau or p-tau181 levels (Table 4), both at the Baseline and after the study, with more robust Rho coefficients found post-intervention. Finally, the amyloid species' ratio $A\beta_{42/40}$ presented negative, significant correlations with both t-tau and p-tau181, but only after completion of the study.

Correlation analysis of tau species with inflammatory markers IL-1β, IL-6, and TNF-α is given in Table 3. Positive significant correlations were found almost for all analyses except for baseline IL-1β. The stronger correlations, in terms of the Rho coefficient, were found between p-tau181 and levels of IL-6 and IL-1β after the study.

### 3.7. Correlation analysis of ratio p-tau181/$A\beta_{42}$ in patients following PE or mixed interventions

To examine the differential interrelation of inflammation with amyloid and tau pathologies in MCI patients, multilinear regression analysis and correlation analysis of ratio p-tau181/$A\beta_{42}$ have been performed independently for each studied group. As shown in Fig. 4a and b, significant correlations of IL-1β and IL-6 with the ratio p-tau181/$A\beta_{42}$ were only proved in the Mixed group. Regarding TNF-α, a significant correlation with the p-tau181/$A\beta_{42}$ was found in the Control group and the Mixed group but not in the PE group. These results imply that for the MCI patients implicated in the Mixed intervention, there is a significant, positive interrelation between inflammation subduing and amelioration of tau/amyloid pathology. This is possibly attributed to the levels of TNF-α that were reduced only in the Mixed but not in the PE group. More aspects regarding the physiology of the ratio p-tau181/$A\beta_{42}$ in the current study were explored via multilinear regression analysis, presented in detail in the Supplementary Material.

## 4. Discussion

The discovery of non- or less-toxic therapeutic alternatives that could target dementia at its very early stages is imperative. Everyday PE activity could be a simple, beneficial alternative for halting cognitive decline, while also providing several benefits to the physical and psychological health of the trainees (Gallaway et al., 2017; Macpherson et al., 2017; Cammisuli et al., 2017; Du et al., 2018). The current study examined the effect of a 3-month PE engagement, with or without the simultaneous employment of neuropsychological and cognitive training, on inflammatory exacerbation, amyloid, and tau pathology. Additionally, the possible interrelation between these crucial hallmarks of MCI and AD due to the employed interventions was also assessed.

For MCI patients that followed no intervention, pro-inflammatory cytokine IL-6 was significantly increased after the study. Elevated titers of IL-6 are linked with chronic inflammation (Del Giudice and Gangestad, 2018), with its expression fueled by TNF-α (Cestari et al., 2016; Kim et al., 2017). Indeed, a significant correlation between serum levels of IL-6 and TNF-α was found. Several previous studies have verified increased levels of IL-6 in patients with MCI and AD (Kim et al., 2017; Teunissen et al., 2002; Licastro et al., 2000; Schuitemaker et al., 2009; Zheng et al., 2019) and found them to gradually increased from healthy controls to MCI and finally AD (Kim et al., 2017). In addition, elevated IL-6 levels were previously linked with higher MCI incidence (Zheng et al., 2019) and lower cognitive ability as determined by lower scores on the MMSE test (Kim et al., 2017). IL-6 levels were also found to be crucial determinants of inflammatory reactions of sporadic AD dementia, which concerns more than 95% of the patients (Dursun et al., 2015; McGeer and McGeer, 2013).

MCI patients engaged in PE and Mixed programs of the current study

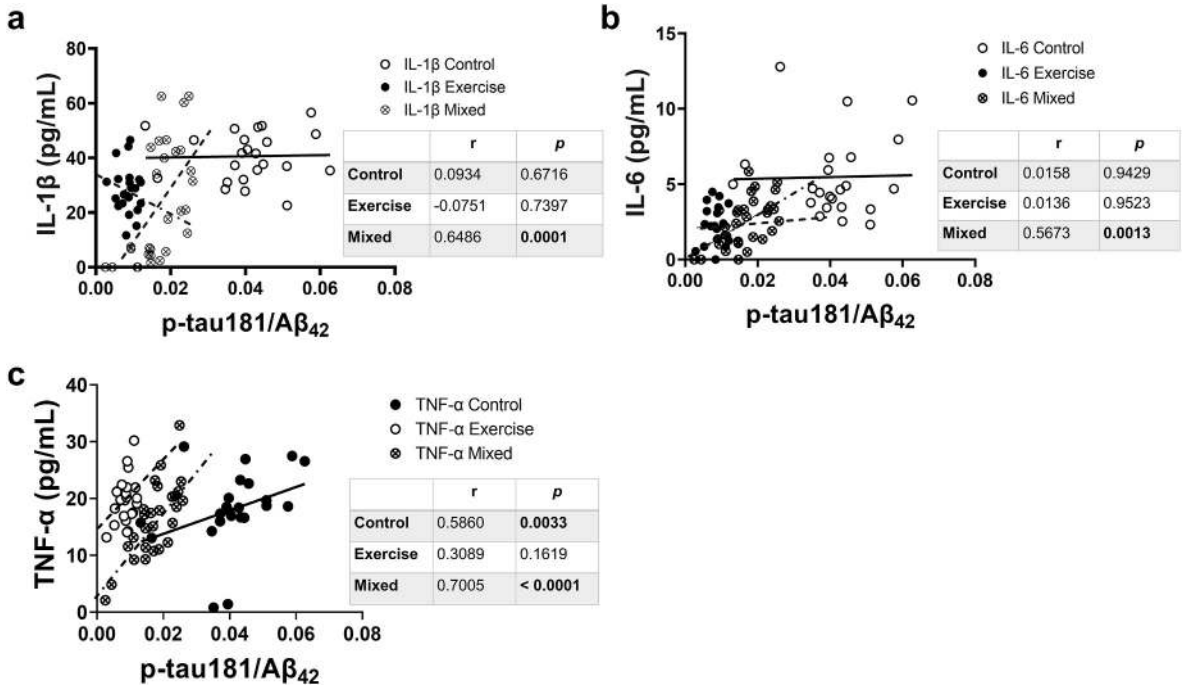


**Fig. 4. Correlation analysis of the ratio p-tau181/$A\beta_{42}$ against the levels of (a) IL-1β, (b) IL-6, and (c) TNF-α, in blood serum of MCI patients employed in the current study.** All biomarkers were analyzed with commercial ELISA kits. The correlations were evaluated using Spearman's rank correlation coefficients (r) and their corresponding *p* values. Analyses were run and graphed separately for each cohort of MCI patients employed in the current study. Scatter plot of p-tau181/$A\beta_{42}$ levels against predicted p-tau181/$A\beta_{42}$ levels. Statistical analyses were performed with Graph Pad Prism 8.0 statistical software.

presented attenuated levels of IL-1β concomitantly with IL-6, notably below the baseline levels of the control group. IL-6 is characterized as the "exercise cytokine"; it increases in response to exercise but declines in the post-exercise period (Pedersen and Fischer, 2007; Steensberg et al., 2003). IL-6 production due to PE seems to be anti-inflammatory, blocking endotoxin-induced TNF-α production in healthy subjects (Starkie et al., 2003). Specific reduction of IL-6 is a common finding in clinical trials employing PE intervention (Huang et al., 2021). For instance, a 4-month multimodal PE program reported reduced levels of IL-6 after the intervention completion and improved cognitive scores (Nascimento et al., 2014b). Additionally, IL-1 is centrally implicated in AD, as elevated hippocampal IL-1 was previously related to memory disturbances (Ben-Menachem-Zidon et al., 2014). In another study, chronic administration of an antibody that explicitly blocks the IL-1 receptor led to diminished neuroinflammation and neurobehavioral pathology in a transgenic animal model of AD (Kitazawa et al., 2011). Thus, restraining increased levels of IL-1β and IL-6 due to the PE interventions found in the current study could halt the progression from MCI to AD.

Acute exercise might be associated with increased levels of TNF-α, attributed to a stress reaction, but in the long term, exercise training was found to reduce cytokine levels (Huang et al., 2021; Golbidi and Laher, 2014; Bernecker et al., 2013). TNF-α is mainly produced by monocytes and macrophages and is a crucial factor for apoptosis and inflammation (Golbidi and Laher, 2014). Increased expression of the TNF-α gene is found in the adipose tissue of obese humans (Kern et al., 1995) and is related to insulin resistance (Hivert et al., 2008). On notice, the groups of this study had statistically similar mean BMIs, and no significant correlation between TNF-α levels and BMI was verified. Patients following the Mixed intervention had significantly lower post-intervention serum levels of TNF-α when compared with the respective baseline, but also with patients of the Control and Mixed groups after the study. This may imply that simultaneous cognitive training may help in the control of inflammatory processes. Previously, employment in a VR-assisted cycloergometric exercise provided attenuation of IL-1β titers and TNF-α levels, as measured after a 3-month exercise program (Tzekaki et al., 2023).

Crucial AD hallmarks, namely Aβ peptides, t-tau, and p-tau181, were rarely studied in clinical studies employing PE against progression to dementia (Huang et al., 2021). Though routinely blood plasma is utilized for amyloid and tau determination, platelets contain significant Aβ amounts, and this could linked with misleading results (Ritchie et al., 2010). In a previous study of our scientific group, amyloid, and tau species were successfully determined in the serum of MCI patients and found to be pathologically altered compared with non-demented control persons (Tzekaki et al., 2021). Thus, blood serum was preferred in the current study as an alternative and more "clean" blood product.

The "amyloid cascade hypothesis" is still the most accepted hypothesis for Aβ accumulation and AD induction. Through the amyloidogenic pathway, APP is cleaved to produce an abundance of amyloid-β peptides with the isoforms consisting of 42 and 40 amino acids being the most abundant. A reduction of the $A\beta_{42}$ is considered typical in AD's blood and CSF profile (Wilczyńska and Waszkiewicz, 2020; Spallazzi et al., 2019), suggesting ineffective cleanup and aggregation of amyloid (Spallazzi et al., 2019; Nakamura et al., 2018). However, the literature is limited regarding the specific role of $A\beta_{40}$. $A\beta_{40}$ is considered a general indicator of the amyloid processing pathway of APP, but its measurement presents interindividual variability, restricting its employment as an independent biomarker (Garcia Castro et al., 2022).

A less standard blood profile is found for $A\beta_{40}$ levels, as its concentration is either non-different, increased, or decreased in several studies with MCI patients (Wilczyńska and Waszkiewicz, 2020). On that note, $A\beta_{40}$ levels are not considered a good predictor for dementia while, on the other hand, the ratio $A\beta_{42/40}$ is highly associated with the development of AD dementia (Koyama et al., 2012). Indeed, several studies demonstrated that individuals with low plasma $A\beta_{42/40}$ ratio have a greater risk for increased brain amyloid, dementia development, and transition from MCI to AD (van Oijen et al., 2006; Graff-Radford et al., 2007; Fandos et al., 2017; Hanon et al., 2022; Campbell et al., 2021).

In the present study, MCI control patients presented a worsened profile of AD hallmarks. However, both PE interventions provide a significant ameliorative effect on amyloid and tau pathology, as reflected in serum levels. PE without cognitive training was the driving force to induce the clearance of Aβ, as proven by both the increased $A\beta_{42}$ levels and the ratio $A\beta_{42/40}$ in the serum of the participated MCI patients. The significantly increased ratio of $A\beta_{42/40}$ found only after PE intervention underlines the promising nature of this intervention and its possible superiority against the Mixed protocol regarding amyloid pathology. On the other hand, only MCI patients of the Mixed group presented decreased levels of $A\beta_{40}$. Previously, employment of MCI patients in cycloergometric exercise also stalled the amyloid and tau pathology but could not lead to increased amyloid and tau clearance, as determined by the determined amyloid and tau markers (Tzekaki et al., 2023), posing PE as a simple and effective protocol for battling the crucial MCI and AD hallmarks.

In AD transgenic mice, low-intensity running (speed values lower than 0.9 km/h) induces Aβ reduction through decrease of the amyloidogenic pathway factors (BACE-1 and presenilin 1) and concomitant increase of non-amyloidogenic pathway (Vasconcelos-Filho et al., 2021). Moreover, treadmill running could reduce Aβ conglomeration (Kang et al., 2013), Aβ hippocampal levels (Vasconcelos-Filho et al., 2021) and prevent cognitive dysfunction induced by Aβ in mice models of AD (Sun et al., 2018). Taken together, the differential results of amyloid levels of the studied groups of the research could be reflected in differential effects on amyloidogenic and non-amyloidogenic pathways, according to the program followed by each participant. A future study should verify the molecular events specifically employed in these processes.

Patients not following any PE intervention presented increased titers of both t-tau and p-tau181, as well as of the ratio p-tau181/$A\beta_{42}$. Conversely, both PE and Mixed interventions lead to diminished titers of tau species. P-tau181 was recently found to be positively correlated with cognitive decline in MCI patients, prodromal of AD (Thomas et al., 2022), with its levels increasing over time in an AD longitudinal study (Chen et al., 2021a). High p-tau181 levels are accompanied by amyloid deposition and faster cognitive decline (Chen et al., 2021b). The ratio of p-tau181/$A\beta_{42}$ was also found to be a valuable tool for discriminating the progression of the disease, having a higher percentage of agreement with normal or dysregulated amyloid PET classification. The higher the ratio, the greater the deleterious progression of the disease (Campbell et al., 2021). Thus, the beneficial results of PE interventions are promising for tau pathology amelioration.

An interplay between AD and inflammation is well proven (Ransohoff, 2016), though it has not been elucidated whether the inflammation is the driving force for Aβ deposition or if Aβ toxicity provokes inflammatory response (Bagyinszky et al., 2017). In a study involving old male C57BL/6J mice, involuntary running led to a reduction in the accumulation of Aβ deposits, accompanied by a significant attenuation of the inflammatory activation of microglia and astrocytes and an improvement in cognition (He et al., 2017). Here, correlation analysis proved that IL-1β and IL-6 levels, after the end of the study, correlated positively with p181-tau/$A\beta_{42}$. However, when groups were studied individually, only the Mixed group presented a significant, positive correlation between IL-1 and IL-6 levels and the ratio p-tau181/$A\beta_{42}$. Also, a significant positive correlation was found between TNF-α levels and the ratio p-tau181/$A\beta_{42}$ in the Control and Mixed cohorts. These results imply that the diminishing levels of both IL-1β and IL-6, as well as the restrained TNF-α levels, lead to a concomitant decrease at the titers of the ratio p-tau181/$A\beta_{42}$ in the Mixed intervention cohort. This effect is possibly attributed to the significant correlation between p-tau181 levels and cytokines levels after the interventions and not to amyloid pathology amelioration. On the other hand, the decreased ratio verified

in PE intervention patients is attributed to simultaneous amelioration of amyloid-tau pathology due to PE, possibly not linked to the attenuation of inflammatory processes.

Multilinear regression analysis for the Mixed intervention group proved that TNF-α levels were the most crucial determinants of the ratio p-tau181/A$\beta_{42}$. Together with correlation analysis, these results implicate TNF-α regulation with improvement in tau pathology in these MCI patients. Age was the other factor found to have a significant impact on the ratio p-tau181/A$\beta_{42}$. Therapies targeting TNF-α have been recently proposed to reduce microgliosis (Ou et al., 2021), delay or even invert amyloid-associated pathology (McAlpine et al., 2009; Shi et al., 2011), prevent tau phosphorylation (Ou et al., 2021; McAlpine et al., 2009; Shi et al., 2011), and stall cognitive deficits, and potentially the progressive loss of neurons (McAlpine et al., 2009) in AD transgenic mice.

Increased TNF-α titers in conjunction with senescence were previously reported to impair mitochondrial homeostasis and mitophagy, thus leading to oxidative stress, activation of the NOD-like receptor protein 3, and finally tau phosphorylation by glycogen synthase kinase-3β (Zhang et al., 2022; Moreira et al., 2017). PE was previously found to upregulate antioxidant enzymes (Tsukiyama et al., 2017; Bouzid et al., 2018) and induce mitophagy concomitantly with the biogenesis of novel mitochondria (Guan et al., 2019; Carter et al., 2015; Tam and Siu, 2014). Thus, the remedying effect found in this study could be attributed to a mechanism preventing TNF-α/oxidative stress pathology that finally reflects in reduced tau phosphorylation or reduced amyloid buildup.

## 5. Conclusions

Due to the relatively limited progress of pharmacological and antibody therapies for cognitive and behavioral deficits, alternative interventions must be rigorously investigated and studied. The findings of the current study imply that a non-pharmacological intervention based on PE and/or with the simultaneous performance of cognitive training may halt the progression of MCI towards AD. More specifically, data from our study showed that individuals with MCI who underwent the PE intervention had significant improvements in crucial AD pathological hallmarks. In parallel with an improvement of general cognitive function, memory, and executive functions implied by a plethora of previous studies, including of our scientific team, these improvements may beneficially affect the patients' real-world function (RWF) and quality of life (QOL). However, these aspects are to be verified in future studies.

The present study is accompanied by several challenges for future research. Firstly, the implementation of this cognitive training was not verified to be more beneficial than PE alone in terms of the studied biomarkers. It is hypothesized that the improvement of amyloid and tau pathology found in patients following the Mixed intervention program could be linked with the suppression of inflammation processes, most probably attributed to TNF-α. On the other side, the positive effects found in the group following PE without cognitive training can allegedly be attributed to the attenuation of amyloid pathology. It should be noted that, while the design of the study included elements that could be related to moderation and mediation, the statistical analyses conducted were intended to assess the direct effects of the interventions on biomarker outcomes rather than to test a model of moderation or mediation. A backbone study with more extended periods of exercise with MCI or AD patients is expected to give more evidence about the long-term protective effects of PE against other dementias. Finally, further investigation in a more significant cohort of human patients should be attempted.

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## Declaration of competing interest

The authors declare no competing interests.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nsa.2024.104085.

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