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Original Article

# Associations Between Physical Activity, Blood-Based Biomarkers of Neurodegeneration, and Cognition in Healthy Older Adults: The MAPT Study

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## Abstract

Physical activity (PA) demonstrated benefits on brain health, but its relationship with blood biomarkers of neurodegeneration remains poorly investigated. We explored the cross-sectional associations of PA with blood concentrations of neurofilament light chain (NFL) and beta amyloid $(A\beta)_{42/40}$. We further examined whether the interaction between PA and these biomarkers was longitudinally related to cognition. Four-hundred and sixty-five nondemented older adults engaged in an interventional study and who had a concomitant assessment of PA levels and blood measurements of NFL (pg/mL) and $A\beta_{42/40}$ were analyzed. A composite *Z*-score combining 4 cognitive tests was used for cognitive assessment up to a 4-year follow-up. Multiple linear regressions demonstrated that people achieving 500–999 and 2000+ MET-min/week of PA had lower (ln)NFL concentrations than their inactive peers. Logistic regressions revealed that achieving at least 90 MET-min/week of PA was associated with a lower probability of having high NFL concentrations (ie, ≥91.961 pg/mL [third quartile]). PA was not associated with $(A\beta)_{42/40}$. Mixed-model linear regressions demonstrated that the reverse relationship between PA and cognitive decline tended to be more pronounced as $A\beta_{42/40}$ increased, while it was dampened with increasing levels of (ln)NFL concentrations. This study demonstrates that PA is associated with blood NFL but not with $A\beta_{42/40}$. Furthermore, it suggests that PA may attenuate the negative association between amyloid load and cognition, while having high NFL levels mitigates the favorable relationship between PA and cognition. More investigations on non demented older adults are required for further validation of the present findings.

**Keywords:** Aging, Neurofilament light chain, Physical exercise, Plasma amyloid

Brain beta amyloid (Aβ) accumulation and release of neurofilament light chain (NFL) proteins in body fluids are 2 key biomarkers of neurodegeneration (1–5) and are implicated in the early pathophysiological process of Alzheimer's disease (AD) (6–8). Aβ is a byproduct of neuron metabolism derived from the proteolytic processing of the amyloid precursor protein that plays a major role in synapse formation and function (9). It has been suggested that an imbalance between its production and clearance causes the forma-

tion of senile plaques that represent a major hallmark of AD (10). NFLs are important components of myelinated neurons that ensure the maintenance of axonal structure and size (11). Their release into the cerebrospinal fluid (CSF) and blood indicates structural damage and predicts future cognitive decline independently of Aβ (12). Increased blood levels of NFL have been reported in several neuropathological conditions including AD, frontotemporal dementia, and progressive supranuclear palsy (12). Aβ and NFL are conventionally assessed through neuroimaging and CSF measurements, but the use of these techniques remains limited by their cost as well as their time-consuming and invasive aspects, which preclude large-scale screening of the aging populations. In this respect, blood-based measurement represents a promising approach especially because recent methods, such as immunoprecipitation and mass spectrometry (IPMS), demonstrated valid relationships with CSF and imaging measures (13,14). Notably, the plasma $A\beta_{42/40}$ ratio was positively correlated with the CSF $A\beta_{42/40}$ ratio and negatively associated with brain amyloidosis measured with positron emission tomography (PET) scan (13). Such relationships are explained by the fact that a large part of Aβ produced in the brain is cleared via transportation to the blood and peripheral tissues through several pathways involving the blood–brain barrier, interstitial fluid bulk flow, and the CSF (15). Given that $A\beta_{42}$ is mainly produced in the brain while $A\beta_{40}$ is dominant in the periphery, a better $A\beta_{42}$ clearance from the brain to the periphery would lead to higher CSF and blood $A\beta_{42/40}$ ratios, indicating lower brain amyloid loads. Likewise, plasma NFL concentrations have been shown to correlate with NFL in the CSF and have been associated with several neurological conditions including AD (16,17), but the mechanisms underlying these relationships remained unknown (11).

Physical activity (PA) represents an important strategy for preventing age-related neurodegeneration as it has been associated with better brain health outcomes such as preserved cognition (18,19), lower brain amyloid deposition (20), and greater cortical thickness (21–23). Despite this evidence, the relationship between PA with blood biomarkers of neurodegeneration remains unsettled. Although higher PA levels were previously associated with lower plasma $A\beta_{42}$ concentrations and $A\beta_{42/40}$ ratios in older adults (24,25), the procedures used for amyloid quantification in these studies have been contested for their lack of precision (13) and failed to demonstrate strong correlations with PET amyloid and clinical applicability (26–30), in contrast to the IPMS technique (31). In addition, Steen Jensen et al. (32,33) reported no effect of exercise training on both Aβ species (32) and NFL (33) in the CSF of AD patients, and, to the best of our knowledge, no study has explored the relationships between blood NFL concentrations and PA levels so far. Furthermore, despite the lack of knowledge regarding the association of PA with circulating amyloid and NFL levels, and given the demonstrated favorable effect of PA on cognition (34), one could expect that the negative association between biomarkers of neurodegeneration and cognition could be moderated by PA levels. Indeed, a recent study conducted by Rabin et al. (35) indicated that PA dampens the deleterious association between brain amyloid load and cognitive decline (35). Thus, besides any potential mediation effects of amyloid and NFL levels on the relationship between PA and cognition, several mechanisms such as decreased inflammation, better insulin sensitivity, increased levels of brain-derived neurotrophic factor, reduced arterial stiffness, and preserved endothelial function (36) might compensate the negative effects of amyloid and NFL accumulation on cognition. Nonetheless, no moderation analysis study using circulating amyloid and NFL has been investigated so far.

The main objective of this study was to explore the association between PA and blood levels of NFL and $A\beta_{42/40}$ ratio in community-dwelling adults aged 70 and older. A secondary aim was to test the potential interactive association of PA and these biomarkers with the overtime evolution of cognitive function. Using IPMS-derived blood $A\beta_{42/40}$ measurement as a marker of $A\beta_{42/40}$ in the CSF and brain amyloidosis, we expected that higher amounts of PA would be associated with higher blood $A\beta_{42/40}$ ratios. Likewise, we hypothesized that, compared with sedentary people, physically active older adults would have lower blood levels of NFL. We further hypothesized that physically active people would have better cognitive outcomes compared with inactive persons, especially if they had an unfavorable biomarker status.

## Method

Data from the Multidomain Alzheimer's Preventive Trial (MAPT; clinicaltrials.gov; NCT00672685) were used for the present investigation. The MAPT study showed no significant effects, compared to the control group, of isolated omega-3 polyunsaturated fatty acid (PUFA) supplementation, multidomain intervention (cognitive training plus PA and nutritional counseling), or their combination on cognitive function over a 3-year follow-up (37). The MAPT study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the advisory Committee for the Protection of Persons participating in Biomedical Research of the Toulouse University Hospital (n° 2-07-27) and was authorized by the French Health Authority. All participants signed an informed consent before enrollment. The present study complies with all relevant ethical regulations.

### Study Population

Participants were community-dwelling older adults, aged 70 and older, without dementia but meet one of the following: spontaneous memory complaints, gait speed of 0.8 m/s or less, or difficulties in performing at least one instrumental daily-living activity. Participants with diagnosed dementia, Mini-Mental State Examination (MMSE) score less than 24, PUFA supplementation, or limitation in executing basic activities of daily living were not included.

### Plasmatic Biomarkers Dosage

#### Beta amyloid

Plasma $A\beta_{42/40}$ was determined using previously described IPMS assay methods (13). A monoclonal anti-Aβ mid-domain antibody (HJ5.1, anti-Ab13-28) conjugated to M-270 Epoxy Dynabeads (Invitrogen) was used for immunoprecipitating the $A\beta_{42}$ and $A\beta_{40}$ isoforms from 0.45 mL of plasma. Analytical internal standards were determined prior to immunoprecipitation by spiking samples with a known amount of 12C15N-$A\beta_{40}$ and 12C15N-$A\beta_{42}$. The endoprotease LysN (Pierce) was used to extract peptides from proteins. Liquid chromatography-mass spectrometry was performed as described previously (13). An orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Fisher) interface with an M-class nanoAcquity chromatography system (Waters) was used to analyze plasma as targeted parallel reaction monitoring. For Aβ species analysis, the product ions pairs and the precursor were chosen as described previously (38,39). The Skyline software package (40) was used for

analyzing derived integrated peak areas. Amounts of Aβ 40 and 42 were calculated by integrated peak area ratio to a known concentration of internal standards.

#### Neurofilament light chain

The R-PLEX human neurofilament L antibody set (Meso Scale Discovery, F217X) was used for determining plasma NFL levels from an ECL-based assay. Samples were diluted twice and assayed in duplicate. The mean value of the duplicate assay measurements was used for analyses.

### Cognitive Assessment

Cognition was assessed using a cognitive composite score (CCS) that combined the *Z*-scores of 4 cognitive tests (10 MMSE orientation items, the Category Naming Test, free and total recall of the Free and Cued Selective Reminding Test, and the Digit Symbol Substitution Test score from the Wechsler Adult Intelligence Scale—Revised) (37).

### Outcome Measures

Blood samples were collected in several time-points during the MAPT intervention. All available blood samples taken during the first-year wave were used for $A\beta_{42/40}$ ratios (continuous variable, lower is worse) and NFL (pg/mL; continuous variable, higher is worse) measurements. If the blood samples of the first-year wave were not available, the blood samples of the second-year wave were used instead. People with NFL values below the upper quartile were defined as NFL negative (NFL–); those in the upper quartile (≥91.961 pg/mL) were considered NFL positive (NFL+). Participants with plasma $A\beta_{42/40}$ of 0.107 or less were considered Aβ+, those with more than 0.107 were Aβ–. The cutoff 0.107 for plasma $A\beta_{42/40}$ was defined based on the Youden index using amyloid PET status as the reference standard. Because no consensual cutoff exists for defining plasma Aβ positivity, we also considered the lower quartile (0.1027) as an alternative cutoff, with people in the lowest quartile for plasma $A\beta_{42/40}$ (≤0.1027) as Aβ+ and those in the 3 upper quartiles as Aβ–. The biomarker values and status were the main outcome measures of the present study. Additionally, longitudinal changes in the CCS taking into account cognitive assessments performed each year from the time of the biomarker measurements until the end of the whole MAPT follow-up (5 years) were set as secondary outcome measures; cognitive tests performed before the measurement of plasma biomarkers were not used.

### PA Assessment

PA was assessed at the same time as the biomarker measurements. The short form of the Minnesota Leisure Time Activities questionnaire was used for assessing the PA level of each participant (41,42). This questionnaire asks about the frequency and the duration of the household and leisure-time PAs performed during the past 2 weeks. The questionnaire's validity for total PA volume has been demonstrated using submaximal and maximal fitness tests (43–45) with correlation coefficients of 0.57 for men (44) and 0.39 for women (45). Its reliability for total PA was also reported with correlation coefficients ranging from 0.92 to 0.69 for the 1-month and the 1-year reliability, respectively (43,46). The compendium of PAs (47) was used to estimate the intensity of each reported PA by 2 experienced raters who worked independently. The weekly PA volume was then calculated by multiplying intensity, frequency and duration of each reported PA, divided by 2, and summed together in order to obtain an overall estimation in metabolic equivalent task (MET) minutes per week (MET-min/week). Given their uncertain role on health in older adults, light PAs were not included in the calculation (48). Participants were then classified into 5 categories: 0–89 MET-min/week (roughly equivalent to <25 min/week of brisk walking), 90–499 MET-min/week (roughly equivalent to 25 min–2 h/week of brisk walking), 500–999 MET-min/week (roughly equivalent to 2–4 h/week of brisk walking), 1000–1999 MET-min/week (roughly equivalent to 4–8 h/week of brisk walking), and at least 2000 MET-min/week. The group with the lowest PA level (0–89 MET-min/week) was defined as "inactive" while the other groups were defined as "active."

### Confounders

Based on previous studies (24,25,32,33), the analyses were adjusted by age, sex, body mass index, apolipoprotein E genotype status (defined as carrying at least one *APOE-ε4* allele or not), MAPT group allocation, educational level (ordinal; no diploma, primary education, middle school, high school, or university diploma), diabetes mellitus, hypertension, hypercholesterolemia, history of stroke, and ischemic heart disease.

### Statistical Analyses

Descriptive statistics were given in mean and standard deviation for continuous variables or numbers and percentages for categorical variables. Based on the properties of the normal distribution, outliers were defined as values above or below 4 *SD* from the sample mean and were excluded from the analyses, leading to 5 and 1 exclusion for the NFL and $A\beta_{42/40}$ variables, respectively. The cross-sectional associations of plasma concentrations of the biomarkers with PA levels were assessed by performing both linear and logistic regression models. Multiple linear regressions were used to compare biomarkers levels (continuous variables) of each active group with the inactive group. In addition, logistic regressions were performed to compare the likelihood of having a bad biomarkers status (ie, being Aβ+ or NFL+) between the inactive group and each of the active groups. In both models, linear trend tests were run to assess the dose–response relationship between PA and biomarker levels using PA as a continuous variable. Lastly, longitudinal changes in the CCS according to PA and biomarker status were compared using mixed-model linear regressions (with a random effect at the participants' level and a random time slope). In this case, PA and biomarkers variables were used as continuous. The mixed model included the main terms, the 3-way interaction between biomarker, PA, and time (continuous, in years), and the two-way interactions of time with the main terms. Normality of residuals was checked using Kolmogorov–Smirnov tests and continuous outcome variables were log-transformed if needed, which led to the natural log-transformation (ln) of NFL. The software IBM SPSS statistics version 23 (Chicago, IL) was used for statistical analyses. All statistical tests were two-sided and the significance level was set at $p < .05$.

## Results

### Characteristics of the Participants

Five hundred and sixteen participants underwent blood biomarker measurements. After removing the outliers and missing data for confounders, a total of 465 participants were kept for analyses. Among them, 458 had NFL and CCS values and 433 had $A\beta_{42/40}$ values. Their baseline characteristics are presented in Table 1. Supplementary Tables 1–3 display the participant's characteristics according to NFL and $A\beta_{42/40}$ status.

**Table 1.** Characteristics of the Studied Sample at Baseline

| | Total ($n$ = 465) | |
|---|---|---|
| Age (years) | 76.8 | 4.5 |
| Female | 186 | 40.0% |
| Education | | |
| No primary education | 26 | 5.59% |
| Primary school certificate | 97 | 20.9% |
| Secondary education | 156 | 33.6% |
| High-school diploma | 66 | 14.2% |
| University diploma | 120 | 25.8% |
| BMI (kg/m$^2$) | 26.3 | 3.90 |
| Diabetes | 53 | 11.4% |
| Hypertension | 229 | 49.3% |
| Hypercholesterolemia | 160 | 34.4% |
| Stroke | 10 | 2.15% |
| Ischemic heart disease | 39 | 8.39% |
| APOE ε4 carriers | 129 | 27.7% |
| Biomarkers | | |
| NFL (pg/mL) ($n$ = 458) | 80.1 | 34.7 |
| $A\beta_{42/40}$ ratio ($n$ = 433) | 0.113 | 0.015 |
| MVPA (MET-min/week) | 1496 | 1402 |
| CCS ($n$ = 458) | 0.09 | 0.76 |

*Notes:* CCS = cognitive composite score; NFL = neurofilament light chain; Aβ = amyloid beta; BMI = body mass index; MVPA = moderate to vigorous physical activity; MET = metabolic equivalent task. Values are given in mean and standard deviation except if indicated other.

### Cross-Sectional Association Between PA and Biomarker Levels

Fully adjusted multiple linear regressions (Table 2) demonstrated that PA was significantly associated with blood lnNFL but not with $A\beta_{42/40}$. Compared to the control group, the active groups of 500–999 and 2000+ MET-min/week had lower lnNFL concentrations. Trends toward lower levels of lnNFL in the other active groups compared to the inactive one were also found.

Likewise, as given in Table 3 and Figure 1, logistic regression analyses revealed significant associations of PA with blood NFL positivity, but not with Aβ positivity. Compared to the inactive group, all of the active groups had a lower probability of being NFL+. Tests for linear trend between PA and biomarkers levels did not reach significance in both linear and logistics models.

### Interactive Associations Between PA, Biomarkers, and Cognitive Decline

Longitudinal mixed-model regressions showed a significant association between the PA × lnNFL × Time interaction and the CCS ($B$ = –0.00005, $SE$ = 0.00002; $p$ = .022). As illustrated in Figure 2A, the reverse association between PA levels and cognitive decline was dampened with increasing levels of lnNFL concentrations.

In addition, a trend toward significance was found for the interaction between PA, $A\beta_{42/40}$, and time ($B$ = –0.00090, $SE$ = 0.00046, $p$ = .051). As illustrated in Figure 2B, the reverse association between PA and cognitive decline tended to be more pronounced with decreasing plasma values of $A\beta_{42/40}$ (which is indicative of worsened amyloid profile and increased risk of cognitive decline).

## Discussion

In this study, we explored the relationship between levels of PA and blood-based biomarkers of neurodegeneration in community-dwelling adults, aged 70 years and older, free of dementia, involved in a randomized controlled trial (RCT). A higher PA level was related to lower plasma NFL concentrations, but no association was found with the $A\beta_{42/40}$ ratio. In addition, all of the active groups had lower chances of having high plasma NFL concentrations (ie, ≥91.961 pg/mL). Secondary analyses further demonstrated that the reverse association between PA and cognitive decline was attenuated with increasing levels of NFL concentrations, while it tended to be more pronounced as amyloid profile got worse (ie, decreasing levels of plasma $A\beta_{42/40}$).

### Neurofilament Light Chain

To the best of our knowledge, this study is the first that investigated the cross-sectional association between PA and plasma NFL. Our results showed that even small doses of moderate to vigorous physical activity (MVPA; ie, PA <90 MET-min/week) are associated with lower NFL values. Although stronger associations were found for people doing 500–999 MET-min/week, the fact that all PA categories were significantly associated with lower odds of being NFL+ suggests that physical inactivity (PI; defined as an insufficient amount of MVPA (49)), rather than PA level, might be a determinant of NFL. Of note, it has been demonstrated that 16 weeks of moderate to vigorous aerobic training does not reduce CSF NFL concentrations in an AD population (33). Thus, taken together, these data suggest that PA may not be able to decrease NFL levels, but rather, that PI may increase NFL production and that avoiding it would prevent such increases; a hypothesis that is further supported by the absence of dose–response relationship in both our linear and logistic models. The potential mechanisms are still to be determined but it is possible that a lack of PA exacerbates the age-related degeneration of the brain and spinal motor neurons (50), leading thus to a greater NFL production. Indeed, both brain volume reduction (51) and spinal motor neuron loss (52) have been associated with higher NFL concentrations. Furthermore, sedentary time itself (distinct from PI and defined as any waking behavior ≤1.5 MET (49)), besides PA volume, may also play a role and could explain part of the inconsistent significance across PA groups in the linear model. Indeed, it is plausible that, in active people, sedentary times may modulate NFL levels independently of, or in interaction with, PA volume as it does on other health outcomes (49,53–55). While other confounding factors such as white matter hyperintensities may also be at play (51), further studies assessing PA volume, sedentary time, and their interaction are necessary to better understand their associations with NFL concentrations.

### $A\beta_{42/40}$

Regarding the $A\beta_{42/40}$ ratio, our results do not support previous evidence of relationships between blood amyloids and PA. Stillman et al. (24) reported that higher PA levels were prospectively associated with lower plasma $A\beta_{42}$ levels, and Brown et al. (25) observed that PA level was inversely related with the $A\beta_{42/40}$ ratio. Discrepancies with our results may come from the fact that in both of these studies, blood assays were conducted using methods that did not demonstrate a strong relationship with PET-derived measures or clinical outcomes (26–28). Consistent with our results, however, is the absence of association between PA and brain amyloid (measured by PET) reported in a previous investigation that also used the MAPT study population (56). Overall, the literature about PA and Aβ remains inconclusive,

**Table 2.** Multiple Linear Regressions Showing the Cross-Sectional Associations Between PA (MET-min/week) and Biomarker Levels

| | 90–499 MET-min/week | | | | | 500–999 MET-min/week | | | | | 1000–1999 MET-min/week | | | | | 2000 MET-min/week + | | | | | Linear Trend | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | *B* | *SE* | *t* | Sig. | *n* | *B* | *SE* | *t* | Sig. | *n* | *B* | *SE* | *t* | Sig. | *n* | *B* | *SE* | *t* | Sig. | *B* | *SE* | *t* | Sig. |
| NFL (ln) | 60 | −0.145 | 0.760 | −1.91 | 0.056 | 95 | −0.221 | 0.070 | −3.15 | 0.002 | 154 | −0.126 | 0.066 | −1.90 | 0.058 | 110 | −0.164 | 0.069 | −2.37 | 0.018 | $-1.83 \times 10^{-6}$ | $1.25 \times 10^{-5}$ | −0.146 | 0.884 |
| $A\beta_{42/40}$ ratio | 57 | 0.000 | 0.003 | 0.045 | 0.965 | 92 | 0.001 | 0.003 | 0.449 | 0.654 | 141 | −0.003 | 0.003 | −1.00 | 0.318 | 106 | −0.001 | 0.003 | −0.240 | 0.810 | $6.71 \times 10^{-7}$ | $4.91 \times 10^{-7}$ | 1.368 | 0.172 |

*Notes:* NFL = neurofilament light chain; Aβ = amyloid beta; PA = physical activity; MET = metabolic equivalent task; SE = standard error; Sig. = significance. The inactive participants (PA <90 MET-min/week) were defined as the reference group.

**Table 3.** Logistic Regression Models Showing the Cross-Sectional Associations Between PA Levels (MET-min/week) and Biomarker Status

| | 90–499 MET-min/week | | | | | | 500–999 MET-min/week | | | | | | 1000–1999 MET-min/week | | | | | | 2000 MET-min/week + | | | | | | Linear Trend | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | OR | 95% CI | | $\chi^2$ | Sig. | *n* | OR | 95% CI | | $\chi^2$ | Sig. | *n* | OR | 95% CI | | $\chi^2$ | Sig. | *n* | OR | 95% CI | | $\chi^2$ | Sig. | OR | 95% CI | | $\chi^2$ | Sig. |
| NFL+ | 60 | 0.247 | 0.096 | 0.636 | 8.409 | 0.004 | 95 | 0.209 | 0.087 | 0.501 | 12.283 | 0.000 | 154 | 0.420 | 0.193 | 0.912 | 4.810 | 0.028 | 110 | 0.310 | 0.134 | 0.716 | 7.530 | 0.006 | 1.000 | 1.000 | 1.000 | 0.970 | 0.756 |
| Aβ+ (<0.107) | 57 | 0.999 | 0.390 | 2.556 | 0.000 | 0.998 | 92 | 0.977 | 0.408 | 2.338 | 0.003 | 0.958 | 141 | 1.508 | 0.667 | 3.409 | 0.974 | 0.324 | 106 | 1.225 | 0.521 | 2.877 | 0.216 | 0.642 | 1.000 | 1.000 | 1.000 | 0.231 | 0.631 |
| Aβ+ (<first quartile) | 57 | 0.917 | 0.330 | 2.550 | 0.028 | 0.868 | 92 | 0.809 | 0.311 | 2.108 | 0.188 | 0.665 | 141 | 1.276 | 0.528 | 3.081 | 0.293 | 0.588 | 106 | 1.267 | 0.506 | 3.173 | 0.255 | 0.614 | 1.000 | 1.000 | 1.000 | 0.022 | 0.881 |

*Notes:* NFL = neurofilament light chain; Aβ = amyloid beta; PA = physical activity; MET = metabolic equivalent task; OR = odd ratio; CI = confidence interval; SE = standard error; Sig. = significance. The inactive participants (PA <90 MET-min/week) were defined as the reference group.

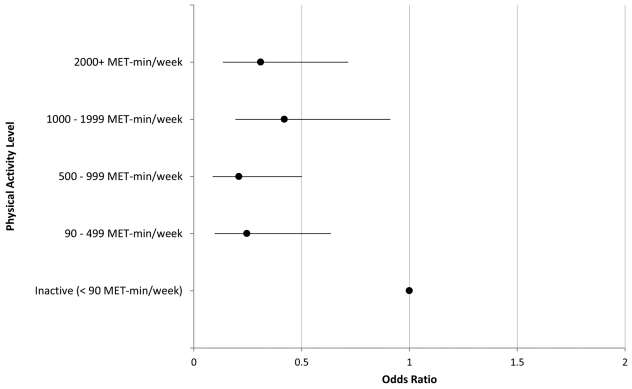


**Figure 1.** Associations between physical activity level and blood NFL status. The inactive group (<90 MET-min/week) was defined as the reference category. Error bars represent 95% confidence intervals. NFL = neurofilament light chain; MET = metabolic equivalent task.

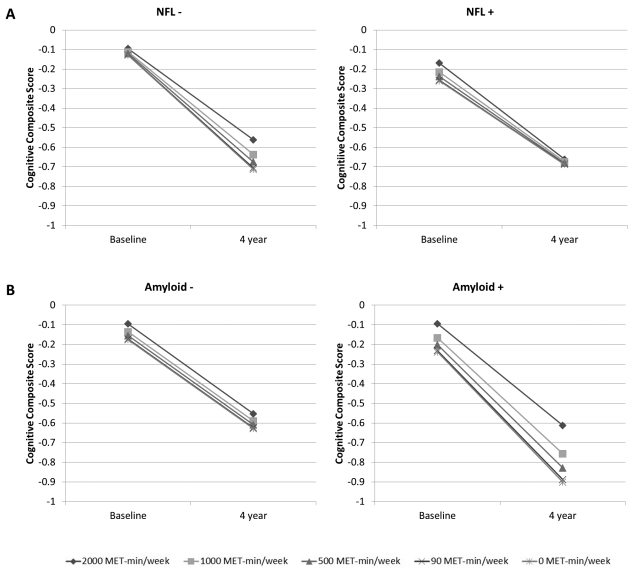


**Figure 2.** Interactive associations of a blood-based biomarker of neurodegeneration and physical activity levels with cognitive decline. For illustrative purposes, over time changes in the cognitive composite score are presented for different levels of physical activity in participants with better (NFL–/Amyloid–) or worse (NFL+/Amyloid+) blood profile in NFL (**A**) and $A\beta_{42/40}$ (**B**). NFL– and NFL+ groups were created using the first (56.9 pg/mL) and the third (93.4 pg/mL) quartiles of the distribution, respectively. Amyloid– and Amyloid+ categories were, respectively, created using the medians in the groups of individuals above and under the plasma $A\beta_{42/40}$ cutoff value of 0.107 (based on the Youden index). This corresponds to blood $A\beta_{42/40}$ ratio values of 0.119 and 0.098, respectively. The graphs indicate that the reverse association between PA and cognitive decline was attenuated with increasing levels of NFL concentrations (PA × lnNFL × Time, $p = .022$), while it tended to be more pronounced as amyloid profile got worse (PA × $A\beta_{42/40}$ × Time, $p = .051$). Aβ = beta amyloid; MET = metabolic equivalent task; NFL = neurofilament light chain; PA = physical activity.

with studies showing significant associations and others not, as reported by a recent review (20). Thus, further investigations measuring both amyloid load through PET and IPMS-derived blood $A\beta_{42/40}$ ratio are required to clarify their potential association with PA.

## Composite Cognitive Score

Although the association of PA with cognition is well established (34,57,58), its interaction with neurodegeneration biomarkers remains poorly described. To date, the study conducted by Rabin et al. (35) is the only one that tested the interactive association of PA and biomarkers of neurodegeneration with cognition (35). The authors reported that the differences in the 8-year cognitive decline between high and low brain amyloid load groups were significantly lower in active versus inactive older adults (35). In our study, the $A\beta_{42/40}$ × PA × Time interaction did not reach significance although a trend ($p = .051$) was found. In accordance with Rabin et al. (35), the favorable association between PA and cognitive decline tended to be more pronounced as blood amyloid profile load worsened. One reason for the lack of statistical significance might be the potential underlying effect of MAPT intervention on our findings, although no effect on cognition was reported (37). Moreover, discrepancies with Rabin et al. (35) might also be due to differences in the characteristics of the population, as well as in the study design. Of note, all Rabin et al.'s participants had a clinical dementia rating (CDR) score of 0, while 55.5% and 0.6% of our participants had a CDR score of 0.5 and 1, respectively. Furthermore, Rabin et al.'s follow-up was longer than ours (8 vs 4 years) which may have been more appropriate to detect an association. Another possibility is that some of the cognitive tests included in the CCS were not sensitive enough to demonstrate any association with PA and the $A\beta_{42/40}$ ratio. Consistent with this hypothesis, in their post hoc analyses, Rabin et al. found that the interaction between PA and amyloid load was significantly associated with the MMSE, the Wechsler Memory Scale—Revised logical memory delayed recall, the Free and Cued Selective Reminding Test but not with the Wechsler Adult Intelligence Scale—Revised Digit Symbol Coding Test (35). Regarding NFL, our results demonstrate that having high blood concentrations of this protein dampens the favorable association between PA and cognition, as, contrary to our hypotheses, the differences in cognitive decline between active and less active persons were reduced as NFL concentrations increased. Although the negative association between NFL and cognition is documented (59), the potential interactive effect of NFL on the benefits of PA and cognition has not been investigated yet. Thus, RCTs, assessing the effect of exercise on cognition according to different NFL status, may bring an important contribution to the field. Taken together, our results suggest that PA may favorably modulate the association between blood amyloid profile and cognitive decline while, on the other hand, high NFL concentrations may attenuate the positive association between PA and cognition.

## Strengths and Limitations

Our study has 3 main strengths. First, this is the first study that tested the associations of PA and plasma Aβ using the more accurate method for Aβ measurement that combines immunoprecipitation with chromatography-mass spectrometry. Second, this study is the first that investigated the association between PA and blood NFL concentrations, which adds further understanding of the possible effect of PA on neuronal alteration in older adults. Lastly, our study enriches the poor literature about the interactive association between biomarkers of neurodegeneration and PA with cognition. The main limitation of our study is the use of self-reported PA; investigations with objective measurements of PA such as accelerometers are needed (60). Another limitation is the fact that we used data from an

RCT aiming to prevent cognitive decline and in which half of the population received the multidomain intervention (composed of nutritional counseling and cognitive training plus PA), which may have had an impact on the investigated associations although no effect of the intervention was found (37). An additional weakness of this study lies in the uneven sample sizes of the PA groups, as well as the use of quartiles to define the biomarker categories, which also led to uneven groupings. Furthermore, the PA levels of the participants may have changed throughout the MAPT follow-up, which also could have had an impact on the outcome measured in this study.

## Conclusions

This study demonstrates that PA level is associated with NFL production but is not associated with blood $A\beta_{42/40}$, in a sample of 465 people aged 70 years and older involved in an RCT. Our results further indicate that neuronal deterioration modulates the favorable association between PA and cognition and also suggest that PA may moderate the amyloid-related cognitive decline. Future investigations should couple both powerful blood-based biomarkers and neuroimaging assessment to bring further understanding of the physiopathology behind cerebral aging and its prevention through PA. Another important question to address is whether and how sedentary behavior, as well as PA patterns, besides their volume itself, are related to these biomarkers.

## Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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## Conflict of Interest

None declared.

## Author Contributions

J.R. and P.S.B. conceptualized and designed this study. J.R. analyzed data, performed statistical analyses, and wrote the manuscript. P.S.B. analyzed data and revised the manuscript critically for intellectual content. Y.R. and B.V. revised the manuscript for intellectual content. G.A., A.D.N., J.E.M., Y.L., and R.J.B. played a major role in data acquisition and revised the manuscript for intellectual content. All authors have read and approved the final manuscript before publication.

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