Cardiorespiratory fitness attenuates adverse influence of poor sleep on CSF biomarkers in an at-risk cohort

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ABSTRACT

BACKGROUND: Previous studies have found a bidirectional relationship between physical activity (PA) and sleep, such that increased PA improves sleep quality and better sleep boosts levels of PA. Both exposures have also been favorably associated with Alzheimer's disease (AD) pathophysiology, including reduced amyloid-beta (Aβ) and tau burden. However, few studies have examined sleep and PA in the same analysis. Specifically, no studies have examined whether increased PA attenuates the adverse effects of poor sleep on AD biomarkers. Therefore, the objective of this study was to i) examine the relationship between sleep and cerebrospinal fluid (CSF) biomarkers among healthy late-middle-aged adults at risk for the disease and ii) determine whether PA may modify this association.

METHODS: This study included seventy-four adults from the Wisconsin Registry for Alzheimer's Prevention. Sleep was evaluated using the validated Medical Outcomes Study Sleep Scale. We specifically focused on the Sleep Problems Index I (SPI) score, which incorporates domains of sleep disturbance, somnolence, sleep adequacy, and shortness of breath. Higher SPI scores indicate greater sleep problems. Participants also underwent a graded exercise test to assess aerobic fitness—an index of habitual PA—using peak oxygen consumption (VO_2 peak) as the measure of fitness. CSF was collected via lumbar puncture, from which Aβ42, total-tau (t-tau) and phosphorylated-tau (p-tau) were immunoassayed. Regression analyses were used to examine the association between SPI scores and CSF biomarkers, as well as the interaction between SPI and aerobic fitness on these same biomarkers, adjusting for age at fitness assessment, sex, and apolipoprotein-ε4 status.

RESULTS: Higher SPI scores were associated with higher levels of t-tau (p=.046) and p-tau (p=.017), as well as higher t-tau/Aβ42 (p=.015) and p-tau/Aβ42 (p=.009) ratios. Importantly, analyses also revealed significant SPI*VO_2 peak interactions for t-tau (p=.034) and p-tau

(p=.046). Specifically, the relationship between poorer sleep and higher levels of t-tau and p-tau was significant among less fit individuals, but not among high-fit individuals.

CONCLUSION: In a late-middle-aged at-risk cohort, aerobic fitness attenuated the association between poor sleep and tau levels. These findings suggest physical activity may play an important role in the prevention of AD by protecting against biomarker alterations even within the context of impaired sleep.

INTRODUCTION

Alzheimer's disease (AD) is quickly developing into one of the most pressing public health concerns in the United States, currently affecting more than 5 million Americans and projected to affect nearly 16 million by the year 2050.[1] As such, research into possible preventative measures to delay onset of the disease is becoming increasingly urgent. Of particular interest are easily interventions targeted to modifiable lifestyle factors during the preclinical phase of AD, in which pathophysiological brain changes, such as amyloid-beta (Aβ) plaques, neurofibrillary tangles, and neurodegeneration may occur without any outward cognitive symptoms.[2] Two such interventions, improved sleep and increased physical activity (PA), have previously been associated with these preclinical brain alterations, among other AD outcomes, and may have the potential to arrest progression of the disease.

Self-reported sleep quality has been associated with age-related alterations in cognition, such that those reporting worse sleep quality also exhibited greater cognitive changes.[3] Poorer sleep has also been associated with greater rates of cortical atrophy in frontal, temporal, and parietal brain regions among late-middle-aged adults,[4] as well as reduced gray matter volume.[5] With regard to the hallmark pathologies of AD, measures of sleep quality—including sleep efficiency, adequacy, latency, duration, and somnolence—have been associated with reduced amyloid burden, as measured by positron emission tomography[5-7] and cerebrospinal fluid (CSF) markers.[8] A recent study by Sprecher and colleagues also found sleep quality to be associated with tau pathology.[9] These studies suggest sleep may play an important role in AD pathological processes.

In addition to sleep, PA has emerged as a viable option for protecting against changes related to AD. Studies examining the relationship between PA and these AD pathologies indicate increased PA is associated with lower Aβ and tau burden.[10,11] PA may also have the capacity to attenuate the deleterious effect of age[12] and the apolipoprotein E (*APOE*) ε4

allele[13,14] on Aβ deposition. For this particular study, we chose to index PA using cardiorespiratory fitness (CRF), a measure of habitual PA. Increased[14] CRF has been associated with favorable AD outcomes, including improved cognition, increased gray matter volumes, and reduced white matter hyperintensities,[15-17] as well as a lower risk of dementia[18] and dementia mortality.[19] Few studies have examined CRF and its association with Aβ and tau markers, though a recent study by our group found CRF may lessen the adverse effect of genetic risk factors on Aβ and tau burden.[14]

These two exposures have been shown to have a complex relationship, such that better sleep boosts levels of PA and increased PA improves sleep quality.[20] Furthermore, sleep-disordered breathing[21] and sleep apnea[22] have both been associated with decreased CRF, while sleep deprivation has been shown to lower CRF in the short-term.[23,24] Higher levels of CRF have been associated with better sleep efficiency,[25] increased sleep duration, and improved overall sleep quality.[26] To our knowledge, few studies have examined both sleep and either PA or CRF in the same analysis[27-29] and only one has examined their possible interaction with respect to CSF biomarkers. As such, our objective with this study was to i) examine the association between sleep and CSF biomarkers in a cohort of healthy, late-middle-aged adults with risk factors for AD, and ii) determine whether CRF modifies this association.

METHODS

Participants

This study included 74 participants from the Wisconsin Registry for Alzheimer's Prevention (WRAP). WRAP is a longitudinal registry of over 1500 cognitively healthy, late-middle-aged adults between the ages of 40 and 65 at study entry.[30] Participants for the present study were selected based on completion of a graded exercise test (GXT), select self-report sleep questionnaires, and lumbar puncture for collection of CSF. The sample was enriched with persons with risk factors for AD, specifically individuals with a parental family history (79.7%)

and/or carrying ≥ 1 apolipoprotein E ε4 (APOE4) allele (40.5%). **Table 1** displays the participants' relevant background characteristics. All study procedures were approved by the University of Wisconsin Institutional Review Board and each subject provided informed consent prior to participation.

Sleep Assessment

To assess sleep, participants completed the Medical Outcomes Study (MOS) Sleep Scale,[31] a validated questionnaire that assesses 8 domains of sleep over the previous 4 weeks. The first question asks participants how long it takes them to fall asleep, ranging from 1 (*0-15 minutes*) to 5 (*more than 60 minutes*). The second question asks the average number of hours slept each night and is free-response style. The last 10 questions assess other qualities of sleep and are rated on a 6-point scale ranging from 1 (*all the time*) to 6 (*none of the time*). Responses were then converted to a 0-100 point scale, such that higher values denote more of the characteristic being measured. Scores were then summed to give totals for 8 domains of sleep: sleep disturbance, somnolence, sleep adequacy, snoring, awakening short of breath or with a headache, sleep quantity, and 2 global indices of sleep problems.[32] We specifically focused on the Sleep Problems Index I (SPI), which incorporates the domains of sleep disturbance, somnolence, sleep adequacy, and shortness of breath into a single score. **Table 2** shows the full questionnaire, and indicates which questions contributed to the SPI score.

Graded Exercise Testing

GXT was performed using a modified Balke protocol.[33] Comfortable brisk walking speeds were determined prior to testing as a safety precaution and to ensure a valid test. For participants who were capable of walking at 3.5 miles per hour comfortably, this speed was used throughout the test. For participants who found this walking speed uncomfortable, a slower speed was chosen. The grade of the treadmill was increased by 2.5% every two minutes until the participant reached volitional exhaustion. Oxygen uptake (VO_2), carbon dioxide production,

minute ventilation, HR, and work rate were measured continuously using a metabolic cart and two-way non-rebreathing valve (TrueOne® 2400, Parvomedics, Sandy, UT). The system was calibrated 4 hours prior to each test using standard gases with known concentrations and with a calibrated three-liter syringe. Peak oxygen consumption (VO_2 peak, mL/kg/min) during exercise was used as the index of CRF.

CSF Assessment

Lumbar puncture for collection of CSF was performed the morning after a 12-hour fast, with a Sprotte 24- or 25-gauge spinal needle at L3/4 or L4/5 using gentle extraction into polypropylene syringes. Each sample consisted of 22 mL of CSF, which was then combined, mixed, and centrifuged at 2000g for 10 minutes. Supernatants were frozen in 0.5 mL aliquots in polypropylene tubes and stored at -80°C. The samples were immunoassayed for Aβ42, t-tau, and p-tau (phosphorylated at threonine 181) using INNOTEST enzyme-linked immunosorbent assays (Fujirebio, Gent, Belgium) by board-certified laboratory technicians who were blind to clinical data and used protocols accredited by the Swedish Board for Accreditation and Conformity Assessment as previously described.[34] We additionally computed t-tau/Aβ42 and p-tau/Aβ42 ratios using the INNOTEST assay values. The average time between GXT and CSF collection was 1.37 $\pm$ 1.06 years.

Statistical Analyses

To examine whether sleep problems, as indexed by SPI score, were associated with CSF biomarkers of AD, we fitted a series of linear regression models—one for each CSF biomarker—that were adjusted for age, sex, and *APOE* ε4 status.

In order to further investigate whether CRF has the capacity to modify the relationship between sleep problems and CSF biomarkers, we additionally refitted the models to incorporate a SPI*VO_2 peak interaction term, while still adjusting for age, sex, and APOE4 status. Where

significant, this interaction term would indicate that the association between SPI and CSF biomarkers depends on level of aerobic fitness, suggesting that CRF may have the potential to ameliorate the deleterious effect of poor sleep on CSF biomarkers. All analyses were conducted using IBM SPSS, version 24.0. Only findings with $p \leq 0.05$ (two-tailed) were considered significant.

RESULTS

Participant Characteristics

Table 1 details the relevant background characteristics of the participants. The sample had an average age of 64.38 ± 5.48 years at the time of GXT completion and was majority female (68.9%). 79.7% had a parental family history of AD and 40.5% were *APOE* ε4-positive. Overall, the sample was well-educated, with an average of 16.26 ± 2.11 years of education. The average body mass index (BMI) was 28.31 ± 5.42 kg/m^2, which is in the overweight category.

Association between Sleep Problems and CSF Biomarkers

Greater sleep problems (i.e. higher SPI scores) was associated with higher levels of t-tau (p=.046) and p-tau (p=.017), as well as higher t-tau/Aβ42 (p=.015) and p-tau/Aβ42 (p=.009) ratios. However, SPI scores were not associated with CSF Aβ42 levels. These findings are reported in **Table 3**.

Cardiorespiratory Fitness and Sleep-Related Alterations in CSF Biomarker Levels

There were significant SPI*VO_2 peak interactions for t-tau (p=.034) and p-tau (p=.046), as reported in **Table 4.** To display this graphically we followed standard procedure for generating plots for interactions between two continuous variables, which entails solving the regression equation at specific “anchor points” for each of the continuous variables. In our case, we solved the equation at ±1 standard deviation away from the mean for both SPI score and VO_2 peak, representing Good vs. Poor Sleep and Low vs. High VO_2 peak, respectively. These graphs,

shown in **Figure 1A-B**, revealed that greater fitness (i.e., higher VO_2 peak) was associated with less adverse effect of poor sleep on CSF t-tau and p-tau. Specific regression estimates (i.e., simple main effects) for the influence of poor sleep on levels of CSF biomarkers in those with Low vs. High VO_2 peak are as follows: **for t-tau,** β (SE)=105.74 (35.68), p=.004 in the Low VO_2 peak group vs. β (SE)=-17.56 (44.17), p=.692 in the High VO_2 peak group; **for p-tau,** β (SE)=14.30 (4.48), p=.002 in the Low VO_2 peak group vs. β (SE)=-.23 (5.55), p=.968 in the High VO_2 peak group.

DISCUSSION

To our knowledge, this is the first study to examine both sleep and CRF in the same analysis with respect to CSF biomarkers, particularly in a cohort of late-middle-aged individuals at risk for AD. We found increased sleep problems were associated with higher levels of t-tau and p-tau, as well as higher ratios of t-tau/Aβ42 and p-tau/Aβ42. However, we also found CRF may have the potential to mitigate this adverse association between poor sleep and CSF biomarkers, such that individuals with higher fitness may be protected from the impact of sleep problems of CSF biomarkers.

Our findings of an association between sleep disturbance and greater AD pathology are consistent with previous reports. In a recent study from our group, Sprecher and colleagues[9] found that self-report of inadequate sleep, somnolence, and sleep problems were associated with higher CSF t-tau/Aβ42 and p-tau/Aβ42. Analyses also revealed a correlation between lower sleep adequacy and higher t-tau. Disrupted breathing during sleep has also been associated with greater tau burden. Osorio and colleagues[35] reported that individuals with sleep-disordered breathing exhibited higher levels of CSF t-tau and p-tau compared to controls. Similarly, in a study by Liguori and colleagues,[36] patients with obstructive sleep apnea (OSA) displayed higher ratios of t-tau/Aβ42, compared to patients without OSA. Taken together, these findings indicate

a link between poor sleep quality and AD pathology, suggesting interventions to improve sleep quality may be a viable pathway to protect against AD pathological changes.

With regard to the interaction between sleep and CRF, our results are relatively novel. To our knowledge, few studies have examined sleep and PA in the same analysis and most have focused on cognition. Wilckens and colleagues[28] assessed PA and sleep in relation to executive function. They found sleep efficiency, but not sleep duration, served as a mediator for the positive association between PA and performance on measures of executive of control. This suggests that improved sleep efficiency may serve as a mechanism by which PA improves cognition. Another study[27] investigated the interaction between objectively-measured sleep and PA in adult women. Analogous to our findings, they found that poor sleep efficiency was associated with worse cognitive performance, but only among those who engaged in low levels of PA. Finally, a preliminary study by Brown and colleagues[29] found that, among APOE4 carriers, greater self-reported PA was associated with reduced Aβ42 deposition only among those reporting good sleep quality. It should be noted that these studies examined various measures of PA, rather than CRF. Even so, taken together with our findings, these studies underscore the intricate relationship between PA and sleep hygiene.

This study is not without limitations. First, its cross-sectional design limits the ability to establish causality. Future studies with a prospective design will be vital in determining whether sleep quality causes the observed differences in biomarker levels, or vice versa, and what role CRF plays in this relationship. Another potential limitation is the use of self-report measures to assess sleep quality. There is considerable variability in individual interpretations of sleep quality[37] and self-report also limits our ability to identify true sleep disorders, such as sleep apnea or sleep-disordered breathing. Studies utilizing polysomnography or actigraph-measured sleep would be beneficial in providing objective measures of sleep quality. However, it should also be noted that self-reported sleep measures add an important dimension to the analysis, as

objective measures may not fully capture sleep quality.[38] Finally, our sample was relatively homogeneous with regard to race and education, being mostly well-educated non-Hispanic whites. This limits the generalizability of our findings to the larger population.

In conclusion, this study reveals novel findings regarding the relationship between sleep, CRF, and CSF biomarkers among late-middle-aged adults at risk for AD. Sleep problems were associated with greater AD pathology. However, this relationship was attenuated among high fit individuals. Given the novelty of our study, further studies of a similar design are needed to validate our findings. Overall, these results suggest improving sleep quality and increasing PA may be practical targets to protect against AD pathophysiological changes and slow progression to the disease in at-risk individuals.

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Table 1. Participant Characteristics (N=74)

Characteristic	Value*
Age, years	64.38 (5.48)
Female, %	68.9
Education, years	16.26 (2.11)
Family history positive, %	79.7
APOE ε4 positive, %	40.5
VO_2 peak, mL/kg/min	24.86 (6.04)
MMSE	29.27 (1.08)
Body mass index, kg/m^2	28.31 (5.42)
Total cholesterol, mg/dL	208.69 (39.63)
HDL cholesterol, mg/dL	66.80 (20.39)
Systolic blood pressure, mmHg	128.64 (17.65)
Diastolic blood pressure, mmHg	75.32 (9.38)
Hypertension, %	24.3
Diabetes, %	2.7
Smoker (ever), %	50.0

*Values indicate mean and standard deviation, unless otherwise indicated.

APOE4=ε4 allele of apolipoprotein E gene; VO_2 peak=peak volume of oxygen consumed during graded exercise test; MMSE=Mini-Mental State Examination; HDL=high-density lipoprotein

Table 2. Medical Outcomes Study Sleep Scale

	Sleep Problems Index I
During the past 4 weeks…	
1. How long did it usually take for you to fall asleep?[a]	
2. On the average, how many hours did you sleep each night?[b]	
How often did you…[c]	
3. Feel that your sleep was not quiet (moving restlessly, feeling tense, speaking, and so forth, while sleeping)?	
4. Get enough sleep to feel rested upon waking in the morning?	**o**
5. Awaken short of breath or with a headache?	**o†**
6. Feel drowsy or sleepy during the day?	
7. Have trouble falling asleep?	**o†**
8. Awaken during your sleep time and have trouble falling asleep again?	**o†**
9. Have trouble staying awake during the day?	**o†**
10. Snore during your sleep?	
11. Take naps (5 min or longer) during the day?	
12. Get the amount of sleep you needed?	**o**

Responses were converted to a 0-100 scale and summed and averaged to produce the total SPI score.

o indicates item included in SPI score

† indicates item score reversed before computing SPI score

[a] Responses were on 15-minute increments from 1 (0-15 minutes) to 5 (more than 60 minutes).

[b] Responses were free entry.

[c] Responses were on a 6-point scale from 1 (all of the time) to 6 (none of the time).

SPI=Sleep Problems Index I

Table 3. Association between sleep problems and CSF biomarkers

CSF Biomarker	SPI Score	
	β (SE)	p
Aβ42	-1.13 (28.65)	.969
t-tau	**30.74 (15.13)**	**.046**
p-tau	**4.62 (1.89)**	**.017**
t-tau/Aβ42	**.11 (.04)**	**.015**
p-tau/Aβ42	**.02 (.01)**	**.009**

Models were adjusted for age at GXT, sex, and *APOE* ε4 status.

CSF=cerebrospinal fluid; SPI=Sleep Problems Index I; β=regression estimate; SE=standard error; Aβ42=amyloid-beta 42; t-tau=total tau; p-tau=phosphorylated tau; GXT=graded exercise test; *APOE* ε4= the ε4 allele of the apolipoprotein E gene

Table 4. CRF attenuates the adverse effect of poor sleep on CSF biomarkers

CSF Biomarker	SPI x VO_2 peak[§]		SPI (Low VO2 peak)[†]		SPI (High VO2 peak)[‡]	
	β (SE)	p	β (SE)	p	β (SE)	p
Aβ42	-7.49 (4.82)	.125	-	-	-	-
t-tau	-5.46 (2.52)	**.034**	105.74 (35.68)	**.004**	-17.56 (44.17)	.692
p-tau	-.64 (.32)	**.046**	14.30 (4.48)	**.002**	-.23 (5.55)	.968
t-tau/Aβ42	-.01 (.01)	.477	-	-	-	-
p-tau/Aβ42	-.01 (.01)	.575	-	-	-	-

[§] The regression estimates and associated p values are for the SPI x VO_2 peak interactive term in each CSF biomarker's model. This term assesses whether VO_2 peak modifies the effect of sleep on the examined CSF biomarkers.

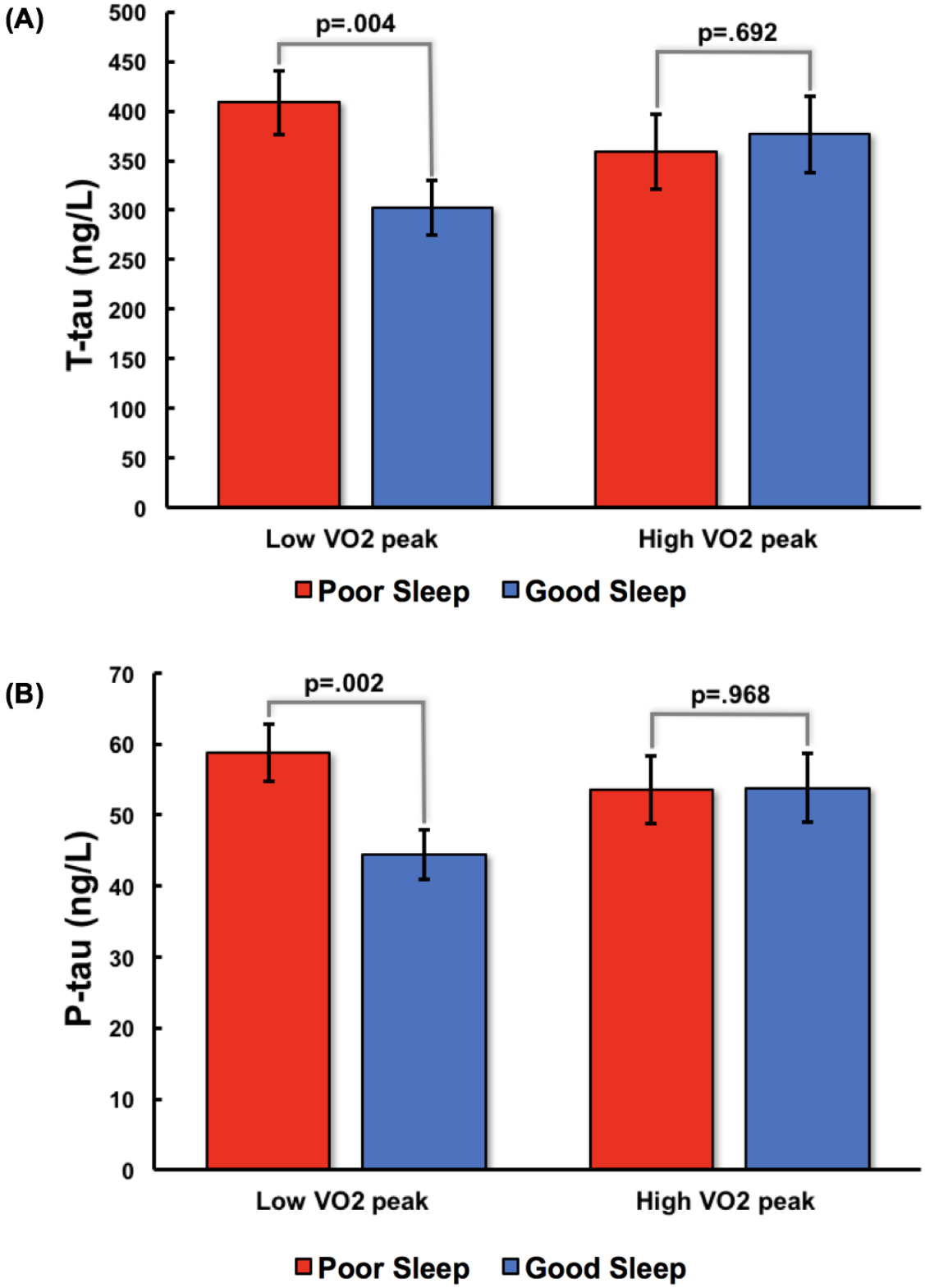
[†] The regression estimates and associated p values are for the simple main effect for the influence of sleep problems on each CSF biomarker *within the Low VO_2 peak group.*

[‡] The regression estimates and associated p values are for the simple main effect for the influence of sleep problems on each CSF biomarker *within the High VO_2peak group.*

Variables included in the model were age at GXT, sex, *APOE* ε4 status, SPI score, VO_2 peak, and a SPI x VO_2 peak interaction, with the SPI x VO_2 peak interaction term being the effect of primary interest.

CRF=cardiorespiratory fitness; CSF=cerebrospinal fluid; SPI=Sleep Problems Index I; VO_2 peak=peak volume of oxygen consumed during graded exercise test; β=regression estimate; SE=standard error; Aβ42=amyloid-beta 42; t-tau=total tau; p-tau=phosphorylated tau; GXT=graded exercise test; *APOE* ε4=the ε4 allele of the apolipoprotein E gene

Figure 1. CRF attenuates adverse effect of poor sleep on CSF biomarker levels



Figures display adjusted means and standard errors from analyses that modeled t-tau (A) and p-tau (B) as a function of age, sex, *APOE* ε4 status, Sleep Problems Index I, VO_2 peak, and a Sleep Problems Index I*VO_2 peak interaction. The Sleep Problems Index I*VO_2 peak interaction term was the effect of primary interest in all models.

Although Sleep Problems Index I and VO_2 peak were included in the analyses as continuous variables, for the purposes of graphing the study findings, we chose two anchor points (i.e., ±1 standard deviation away from the mean) to represent Good vs. Poor Sleep and Low vs. High VO_2 peak.

CRF=cardiorespiratory fitness; CSF=cerebrospinal fluid; t-tau=total tau; VO_2 peak=peak volume of oxygen consumed during graded exercise test; p-tau=phosphorylated tau; *APOE* ε4=the ε4 allele of the apolipoprotein E gene