Effect of 1 Night of Total Sleep Deprivation on Cerebrospinal Fluid β-Amyloid 42 in Healthy Middle-Aged Men
A Randomized Clinical Trial

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IMPORTANCE Increasing evidence suggests a relationship between poor sleep and the risk of developing Alzheimer disease. A previous study found an effect of sleep on β-amyloid (Aβ), which is a key protein in Alzheimer disease pathology.

OBJECTIVE To determine the effect of 1 night of total sleep deprivation on cerebrospinal fluid Aβ42 protein levels in healthy middle-aged men.

DESIGN, SETTING, AND PARTICIPANTS The Alzheimer, Wakefulness, and Amyloid Kinetics (AWAKE) study at the Radboud Alzheimer Center, a randomized clinical trial that took place between June 1, 2012, and October 1, 2012. Participants were cognitively normal middle-aged men (40-60 years of age) with normal sleep (n = 26) recruited from the local population.

INTERVENTIONS Participants were randomized to 1 night with unrestricted sleep (n = 13) or 1 night of total sleep deprivation (24 hours of wakefulness) (n = 13).

MAIN OUTCOMES AND MEASURES Sleep was monitored using continuous polysomnographic recording from 3 PM until 10 AM. Cerebrospinal fluid samples were collected using an intrathecal catheter at defined times to compare cerebral Aβ42 concentrations between evening and morning.

RESULTS A night of unrestricted sleep led to a 6% decrease in Aβ42 levels of 25.3 pg/mL (95% CI [0.94, 49.6], P = .04), whereas sleep deprivation counteracted this decrease. When accounting for the individual trajectories of Aβ42 over time, a difference of 75.8 pg/mL of Aβ42 was shown between the unrestricted sleep and sleep deprivation group (95% CI [3.4, 148.4], P = .04). The individual trajectories of evening and morning Aβ42 concentrations differed between the unrestricted sleep and sleep deprivation groups (P = .04) in contrast to stable Aβ40, tau, and total protein levels.

CONCLUSIONS AND RELEVANCE Sleep deprivation, or prolonged wakefulness, interferes with a physiological morning decrease in Aβ42. We hypothesize that chronic sleep deprivation increases cerebral Aβ42 levels, which elevates the risk of Alzheimer disease.

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The amyloid cascade hypothesis of Alzheimer disease (AD) argues that this disease is initiated by deposition of the β-amyloid (Aβ) protein, of which the Aβ42 iso-type is known to be the greatest contributor. Causes for this Aβ deposition remain unknown in sporadic AD but are thought to reflect an imbalance between production and clearance of Aβ.

Epidemiological studies have identified many potential risk factors for AD and increasing evidence suggests that poor sleep is among these factors. Mechanistic studies suggest that neural activity leads to increased production and secretion of Aβ such that wakefulness (increased activity) augments Aβ production. In turn, sleep (reduced activity) is associated with increased clearance of Aβ and lower Aβ production. Indeed, Aβ levels in the cerebrospinal fluid (CSF) in both humans and rodents showed a marked decrease during sleep, compared with wakefulness. Moreover, rodent studies revealed increased cerebral Aβ levels and subsequent deposition of Aβ after extended wakefulness. These findings suggest that poor sleep through chronic partial sleep deprivation may interfere with a physiological sleep-related decrease in cerebral Aβ, leading to sustained higher Aβ levels and, possibly, Aβ accumulation.

We assessed the effect of 1 night of total sleep deprivation (24 hours of wakefulness) on CSF Aβ42 levels in healthy men. We hypothesized that sleep deprivation would lead to higher CSF Aβ42 levels compared with a night with unrestricted sleep. Because of its strongest contribution to Aβ plaque formation, CSF Aβ42 was chosen as the main outcome parameter. We included CSF Aβ40, phosphorylated tau (P-tau), and total tau (T-tau) as secondary outcome parameters because they are typically affected in a later stage in the development of AD and are thought to be biomarkers that may be initiated independently from Aβ. The total protein level was chosen as a control parameter.

### Methods

#### Participants

We recruited 26 healthy men who were medication free, were cognitively normal with a Mini-Mental State Examination score greater than 28, and had normal sleep quality defined as a Pittsburgh Sleep Quality Index of 5 or more (Table). Participants were randomized to a sleep deprivation group (n = 13) or a group with unrestricted sleep (n = 13).

#### Table. Overview of Baseline Characteristics, CSF, and Sleep Data

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unrestricted Sleep (n = 13)</th>
<th>Sleep Deprivation (n = 13)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline characteristic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>49.4 (5.5)</td>
<td>50.4 (4.9)</td>
<td>.63</td>
</tr>
<tr>
<td>BMI</td>
<td>25.7 (3.4)</td>
<td>25.6 (1.8)</td>
<td>.95</td>
</tr>
<tr>
<td>MMSE score</td>
<td>29.5 (0.8)</td>
<td>29.8 (0.6)</td>
<td>.27</td>
</tr>
<tr>
<td>PSQI</td>
<td>2.4 (1.2)</td>
<td>2.9 (1.3)</td>
<td>.27</td>
</tr>
<tr>
<td><strong>Polysomnographic variable</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WASO, min</td>
<td>92.2 (49.4)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Sleep efficiency, %</td>
<td>77.3 (12.1)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Sleep, min</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>382 (59)</td>
<td>19 (32)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>NREM</td>
<td>355 (98)</td>
<td>9.3 (17)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>REM</td>
<td>72.4 (28.9)</td>
<td>4.5 (14.4)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>CSF value</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aβ42, pg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evening</td>
<td>426 (181)</td>
<td>474 (126)</td>
<td>.16</td>
</tr>
<tr>
<td>Morning</td>
<td>401 (165)</td>
<td>477 (148)</td>
<td>.04</td>
</tr>
<tr>
<td>Aβ40, pg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evening</td>
<td>6153 (1863)</td>
<td>6907 (2251)</td>
<td>.15</td>
</tr>
<tr>
<td>Morning</td>
<td>7041 (2719)</td>
<td>7769 (2993)</td>
<td>.27</td>
</tr>
<tr>
<td>P-tau, pg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evening</td>
<td>36.9 (13.3)</td>
<td>34.4 (14.5)</td>
<td>.47</td>
</tr>
<tr>
<td>Morning</td>
<td>42.5 (14.3)</td>
<td>36.9 (16.8)</td>
<td>.12</td>
</tr>
<tr>
<td>T-tau, pg/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evening</td>
<td>61.5 (15.7)</td>
<td>72.9 (28.0)</td>
<td>.02</td>
</tr>
<tr>
<td>Morning</td>
<td>67.7 (20.4)</td>
<td>70.8 (33.5)</td>
<td>.61</td>
</tr>
<tr>
<td>Total protein, mg/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evening</td>
<td>313 (94)</td>
<td>313 (96)</td>
<td>.20</td>
</tr>
<tr>
<td>Morning</td>
<td>341 (95)</td>
<td>336 (96)</td>
<td>.42</td>
</tr>
</tbody>
</table>

Abbreviations: Aβ, β-amyloid; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CSF, cerebrospinal fluid; MMSE, Mini-Mental State Examination; NA, not applicable; NREM, nonrapid eye movement; PSQI, Pittsburgh Sleep Quality Index; P-tau, phosphorylated tau; REM, rapid eye movement; T-tau, total tau; WASO, wake after sleep time onset.

No group differences were observed on baseline.

A score of 5 or lower is considered as normal sleep.

Variables show successful sleep deprivation, contrasted by sufficient sleep in the unrestricted sleep group.

Levels are presented as the average of the 3 morning or evening samples for the unrestricted sleep group and sleep deprivation group.

P value represents difference between evening CSF levels between the unrestricted sleep and sleep deprivation groups.

P value represents difference between the morning CSF levels between the unrestricted sleep and sleep deprivation groups.
They were blinded for group allocation until arrival at the study center. Figure 1 shows the flow diagram for this study. At baseline, groups were similar in their CSF profiles except for a minor difference in T-tau, which was, however, within the normal range in both groups (Table). Concentrations of CSF Aβ42 for all participants were well above a previously determined cutoff level of 192 pg/mL11 (Figure 2), suggesting that these participants were devoid of cerebral amyloid deposition.

This study was approved by the institutional review board at Radboud University Medical Center and was performed according to good clinical practice rules. Written informed consent was obtained from all participants.

Study Design
During the entire study period, blood pressure, temperature, pulse, and the catheter insertion point were monitored. Intravenous saline at 100 mL/h was administered to guarantee hydration and reduce the risk of postspinal headache.12
The unrestricted sleep group was instructed to go to sleep at 10 PM and 8, 9, and 10 AM. Continuous polysomnographic recording was performed from 3 PM until 10 AM using a titanium ambulant electroencephalogram recording system (Embla Systems). Sleep monitoring and scoring were performed according to the American Academy of Sleep Medicine guidelines.13

CSF Collection and Analysis
An indwelling catheter was placed at the L3/L4 interspace under local anesthesia and aseptic conditions by an experienced anesthesiologist. Samples of CSF were taken at 5, 8, 9, and 10 PM and 8, 9, and 10 AM. The sleep deprivation group had extra sample collections at 12, 2, 4, and 6 AM; these were not collected in the unrestricted sleep group to avoid sleep disruption. Sampling during the night has shown to be a major confounder on the sleep quality in our previous serial sampling study.14 Each CSF sample was collected in duplicate (each 3 mL) to decrease intranidividual variation. All samples were clear on visual inspection. Within 30 minutes after collection, samples were centrifuged at 2000g for 5 minutes and stored in aliquots at −80°C in 2-mL polypropylene tubes.

Cerebrospinal fluid, Aβ42, P-tau, and T-tau were determined using the xMAP-based Innobia assay (Innogenetics) and CSF Aβ40 was measured using an enzyme-linked immunosorbent assay (The Genetics Company). The total protein level was determined by a turbidimetric assay using benzethonium chloride. All CSF samples were analyzed in duplicate and all samples from the same participant were measured in the same plate to avoid interplate variation.

Statistical Analysis
All analyses were performed using SPSS version 20.0 (IBM). Statistical significance was set at P < .05.

The primary analysis for both the primary (Aβ42) and secondary (Aβ40, T-tau, and P-tau) outcomes was a paired t test comparing the average of the 3 evening samples with the average of the 3 morning samples.

As secondary analyses, we investigated the relationship between sleep duration and Aβ42 levels using a Pearson correlation test between the parameters total sleep time and the difference in Aβ42 level between the first (5 PM) and last (10 AM) samples.

Finally, we compared the individual trajectories of all CSF markers in both groups using random-intercept mixed-modeling analysis. Statistical analyses were performed with raw data and were repeated after log transformation of these raw data because of the known large intraindividual variability in these CSF biomarkers.14 The sample size for this study was based on the primary analysis, with 80% power to detect a 10% reduction (17% was previously observed in rodents7) in CSF Aβ42 with 13 in each group, based on CSF Aβ42 data as observed in a previous study.14
Results

Sleep Patterns

The unrestricted sleep group slept an average of 6.4 hours with a normal sleep architecture. The sleep deprivation procedure was successful in the sleep deprivation group (Table).

Effect of Sleep and Sleep Deprivation on CSF Total Protein, Aβ42, Aβ40, P-tau, and T-tau Levels

Figure 3A shows the CSF Aβ42 values for both groups. Baseline data of the 3 evening samples in the unrestricted sleep and sleep deprivation group did not differ (95% CI [−78.7, 173.5 pg/mL], P = .45). In the unrestricted sleep group, the lowest Aβ42 values were observed at 10 AM. Despite the expected relatively large interindividual variation in Aβ42 levels, the average of the 3 morning samples was 6% lower than the average of the 3 evening samples for unrestricted sleep (25.3 pg/mL, 95% CI [0.94, 49.6], P = .04), but not for sleep deprivation. Neither sleep nor sleep deprivation affected Aβ40, T-tau, or P-tau (Table).

TotalsleepdurationwascorrelatedwiththemaximumreductioninAβ42 (r = −0.50, P = .04) (Figure 4). This largest reduction in Aβ42 (~38.4 pg/mL, 95% CI [3.9, 149.9], P = .04) was observed between the 2 times, 5 PM and 10 AM (Figure 3A). This effect was not found for Aβ40, P-tau, and T-tau.

The individual trajectories of the serial CSF Aβ42 concentrations as assessed by mixed modeling differed between individuals who were allowed unrestricted sleep or were sleep deprived (75.8 pg/mL, 95% CI [3.4, 148.4], P = .04) (Figure 3A). After log transformation of the data, this effect remained (P = .01). Again, this effect was not observed for Aβ40, P-tau, and T-tau. In both groups, we observed increases over time of the individual trajectories of CSF Aβ40, P-tau, and T-tau (Figure 5). The CSF total protein levels remained stable in both the unrestricted sleep and sleep deprivation groups during the entire period. Figure 3B illustrates that the morning decrease observed for CSF Aβ42 was not found for CSF total protein levels.

Discussion

We showed that CSF Aβ42 levels decrease during a night of sleep in healthy human individuals. The magnitude of this decrease correlated with the total amount of sleep. Moreover, 1 night of total sleep deprivation abolished this decrease in Aβ42. These sleep-related effects were not observed for CSF Aβ40, P-tau, T-tau, and total protein levels.
Unrestricted overnight sleep has previously been associated with a decrease in CSF Aβ levels. Rodents have a 25% decrease in interstitial fluid levels of Aβ during sleep (human Aβ1-x in transgenic mice and murine Aβx-40 in wild-type mice). In human CSF, an amplitude-mesor ratio of 8.3% in Aβ42 was observed over a 24-hour period, with the lowest values observed in the morning. We confirmed this morning effect, and these combined data suggest that a morning decrease in CSF Aβ42 levels is a normal physiological phenomenon related to sleep. Interestingly, this morning decrease was most prominent at 10 AM. In the previous human study, it was also found that the lowest levels were at 10 AM and that Aβ42 was inversely correlated with sleep after a 6-hour delay. The authors attributed this effect to the previously reported delay in clearance of Aβ from interstitial fluid to the CSF. Although previous studies suggested that this sleep-related decrease also applied to Aβ40, we were unable to confirm this. It is not unlikely that the metabolism of the different isoforms of Aβ is not the same in response to physiological changes. This assumption is based on various levels of evidence that the metabolism of Aβ42 is different from that of Aβ40: (1) it is well established that Aβ42 aggregates faster than Aβ40; (2) Aβ40 and Aβ42 may each be produced at different intracellular locations and by different mechanisms; and (3) Aβ42 and Aβ40 may be cleared from the brain by different mechanisms.

Aside from showing an effect of sleep on CSF Aβ42, we demonstrated that sleep deprivation abolishes this morning decrease in CSF Aβ42. Sleep deprivation has been applied in a transgenic rodent model, where a short period of sleep deprivation, compared with periods of normal sleep, resulted in a 16.8% increase in hippocampal interstitial fluid levels of human Aβ1-x. Our data show that a comparable effect is present in healthy middle-aged men.

We hypothesize that this effect of sleep deprivation (ie, extended wakefulness) on Aβ42 is mainly explained by the relationship between neuronal activity, synaptic strength, and Aβ42 production. In animal models, wakefulness and brain activation increase synaptic strength and Aβ levels, while periods of sleep or reduced brain activation decrease syn-
aptic strength and Aβ levels. In humans, brain activation has also indirectly been linked to Aβ production. Brain areas that display the highest levels of activity during wakefulness, the so-called default mode network, are also the regions most prone to Aβ aggregation. In patients with traumatic brain injury, higher levels of consciousness (compared with coma) led to higher interstitial fluid levels of Aβ. A decrease in Aβ clearance during sleep deprivation may exaggerate the increase in CSF Aβ. A recent rodent study observed a 60% increase in the interstitial space volume during periods of sleep, relative to the awake state using real-time iontophoresis. As a result of higher CSF flow through the dilated interstitial space, there was twice as much clearance of radiolabeled Aβ during sleep compared with the awake state.

Previous studies of CSF serial sampling suggested that both high sample frequency and a large sampling volume could lead to linear increases in CSF Aβ levels. One hypothesis for this linear increase is that Aβ is not homogeneously distributed in the extracellular fluid and that serial sampling affects the flow to the subarachnoid space to increase lumbar CSF Aβ to concentrations that may reflect brain vesicular levels. A very important point of discussion is that the 4 extra CSF samples taken from the sleep deprivation group during the night could have augmented any sampling-related increases over time. In contrast to these considerations, Figure 5 clearly illustrates that there were no effects of sample frequency on CSF Aβ40, P-tau, and T-tau in both groups, which was also confirmed in the mixed-modeling analyses. The tau protein has previously been proven to be a relatively stable protein in CSF, with a much longer in vivo turnover rate than Aβ (11-day half-life). A response of CSF T-tau to physiological changes such as decreased neuronal activity can only be detected after several days and will likely not be present in our experimental design. Therefore, major changes in CSF T-tau would only reflect changes in CSF composition due to serial sampling. The absence of differences between the 2 groups in T-tau supports the fact that the 4 extra samples in the sleep deprivation group did not affect the composition of CSF. In addition, CSF total protein levels remained stable in both groups, even during the periods of extra sampling in the sleep deprivation group, further supporting our conclusion that the difference in sampling frequency in the 2 groups is likely not the cause of the observed differences in Aβ42 protein (Figure 3B).

A previous study has demonstrated that an increase over time for either Aβ or tau protein levels is not observed when the sampling frequency is once every 2 hours. This is in line with our sampling frequency during the night in sleep-deprived participants. Taken together, and also considering that the decrease in Aβ42 in the unrestricted sleep group is correlated with the total amount of sleep, we argue that there is no evidence to suggest the extra samples in the deprivation group affected the concentration of Aβ42.

Conclusions

We have shown that 1 night of total sleep deprivation increases CSF Aβ42 levels relative to normal sleep in a group of healthy middle-aged men. One night of total sleep deprivation has previously been shown to have similar effects on behavioral alertness, reductions in working memory performance, and cognitive performance that are similar to those observed in cases with chronic sleep restriction to 6 hours or less per night for 14 days. This suggests total sleep deprivation mimics physiological processes as seen in chronic partial sleep deprivation. Therefore, we hypothesize chronic partial sleep deprivation, as seen in disrupted sleep, might contribute to gradual Aβ42 accumulation, which may initiate Aβ oligomer formation and Aβ deposition. This hypothesis is supported by several studies that suggest disrupted sleep is an increased risk factor for cognitive decline and AD. Specifically, sleep quality, more than sleep duration per se, may play an important role in Aβ deposition. Because our study already showed an effect in a limited number of participants, it is important to further investigate this effect in a larger sample and extend observations over a longer period. It would be especially interesting to study the effects of chronic sleep deprivation on amyloid accumulation, using CSF Aβ42 or amyloid imaging to further unravel the role of sleep deprivation in the development of AD.

Conflict of Interest Disclosures: None reported.
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Additional Contributions: Marijke Beenes, Radboud University Medical Center, performed the cerebrospinal fluid analysis of all participants. She received financial compensation.
REFERENCES


