The psycho-metabolic consequences of sleep loss in people

LIEVE T. VAN EGMOND
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Abstract

Night work is vital for maintaining our 24/7 society; however, in the long run, it may have adverse health consequences like obesity and Alzheimer’s disease. By performing one of the most extensive experimental in-laboratory studies to date, I sought to investigate how sleep deprivation impacts important features like how a person responds to others and how well a person can sustain attention and wakefulness during simulated night work. To this end, in Paper I, I used eye tracking to show that young adults were less visually attentive to faces after sleep deprivation, irrespective of the displayed emotion. Additionally, participants rated faces as less trustworthy and attractive after the nocturnal vigil. In conclusion, the observed effects suggest that night work may impact emotional regulation. Whether the change in face processing increases the odds of negative affect and social withdrawal remains unclear.

Using the same cohort in Paper II, I found that women and people with obesity struggled more with overnight wakefulness (measured by questionnaires, vigilance, and electroencephalography) than men and people with normal weight, respectively. Strikingly, these groups also exhibited increased blood levels of brain health biomarkers following total sleep loss. These results indicate that a person’s biological sex and weight status may moderate to which extent night work adversely affects brain health and occupational performance.

Sleep deprivation drives the development of obesity. However, whether similar mechanisms accounting for this weight-promoting effect of sleep loss apply to people who already have obesity is not well researched. Additionally, most experimental studies focused on the effects of acute sleep loss on the energy balance in men. With these gaps in mind, using the above-described cohort, Paper III focused on three prominent endocrine regulators of energy balance, namely leptin, known to promote satiety, and the hunger-promoting hormones ghrelin and adiponectin. Overall, I observed that lower blood leptin concentrations followed one night of total sleep deprivation while those of ghrelin and adiponectin increased. These results indicate that a person’s biological sex and weight status may moderate to which extent night work adversely affects brain health and occupational performance.

While acute sleep loss may predispose humans to gain weight, what we eat can influence our sleep. At age 70, 970 participants from the Uppsala Longitudinal Study of Adult Men (ULSAM) filled out a seven-day food diary and questionnaires surveying for possible sleep problems. Thus, in Paper IV, I investigated whether healthy dietary habits were associated with lower odds of suffering from subjective sleep disturbances. Contrary to my hypothesis, neither the Mediterranean diet nor the Healthy Diet Indicator (based on WHO recommendations) was associated with sleep outcomes. Thus, more controlled interventional studies are needed to systematically evaluate how dietary habits may influence sleep in older men.

Keywords: Sleep, nutrition, metabolism, sleep deprivation, sex differences, weight differences, obesity, cognition, mediterranean diet, brain health, social evaluation, night work, occupational performance, emotion, vigilance, energy balance, diet, elderly

Lieve T. van Egmond, Functional Pharmacology and neuroscience, 593, Uppsala University, SE-75124 Uppsala, Sweden. Department of Pharmaceutical Biosciences, Box 591, Uppsala University, SE-75124 Uppsala, Sweden.

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No man knows what he is capable of, until he has tried everything.

-Willem F. Hermans, Beyond sleep
List of Papers

This thesis is based on the following papers, referred to in the text by their Roman numerals.


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Additional Papers


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# Abbreviations

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<th>Description</th>
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<tr>
<td>AOI</td>
<td>Area of interest</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<td>CI</td>
<td>Confidence interval</td>
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<td>CNS</td>
<td>Central nervous system</td>
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<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>EE</td>
<td>Energy expenditure</td>
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<td>EEG</td>
<td>Electroencephalography</td>
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<td>EI</td>
<td>Energy intake</td>
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<tr>
<td>HDI</td>
<td>Healthy Diet Indicator</td>
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<tr>
<td>GLM</td>
<td>Generalized linear model</td>
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<tr>
<td>GLMM</td>
<td>Generalized linear mixed models</td>
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<tr>
<td>KSS</td>
<td>Karolinska sleepiness scale</td>
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<tr>
<td>LSD</td>
<td>Least significant difference</td>
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<tr>
<td>MD</td>
<td>Mediterranean diet</td>
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<tr>
<td>MVLR</td>
<td>Multivariate logistic regression model</td>
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<tr>
<td>NFL</td>
<td>Neurofilament light chain</td>
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<td>NREM</td>
<td>Non-rapid eye movement</td>
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<td>ODI</td>
<td>Oxygen desaturation index</td>
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<tr>
<td>OSA</td>
<td>Obstructive sleep apnea</td>
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<td>PA</td>
<td>Physical activity</td>
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<td>PSG</td>
<td>Polysomnography</td>
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<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
</tr>
<tr>
<td>PVT</td>
<td>Psychomotor vigilance test</td>
</tr>
<tr>
<td>REM</td>
<td>Rapid eye movement</td>
</tr>
<tr>
<td>SCN</td>
<td>Suprachiasmatic nuclei</td>
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<tr>
<td>SDB</td>
<td>Sleep-disordered breathing</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SFA</td>
<td>Saturated fatty acid</td>
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<td>SWS</td>
<td>Slow-wave sleep</td>
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<tr>
<td>TFD</td>
<td>Total fixation duration</td>
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<tr>
<td>TFP</td>
<td>Total fixation points</td>
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<tr>
<td>ULSAM</td>
<td>Uppsala Longitudinal Study of Adult men</td>
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<tr>
<td>VAS</td>
<td>Visual analogue scale</td>
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<tr>
<td>WMT</td>
<td>Wake maintenance test</td>
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Introduction

Sleep

Sleep is one of the main pillars for preserving physical and mental health across the lifespan. However, we have only started to unravel sleep’s many functions, and a clear consensus regarding the core definition of sleep has not yet been reached\(^1\). Carskadon and Dement define sleep as “a reversible behavioral state of perceptual disengagement from, and unresponsiveness to the environment”\(^2\). Despite the seemingly inactive bodily state, many processes are ongoing during sleep to facilitate rest, recovery, and restoration, therewith enabling health and daytime functioning.

![Figure 1](hypnogram of a participant from the MetSleep study with lab-typical sleep. Abbreviations: N1, NREM1; N2, NREM2; N3, NREM3; R, REM; W, wake.)

Sleep architecture

Most adults need between seven and nine hours of sleep per night\(^3\). During this time, the body goes through different sleep stages that cycle in blocks of ~90 minutes\(^4\). Figure 1 shows a typical in-lab sleep night of around eight hours. The first three sleep stages involve non-rapid eye movement (NREM) sleep. Stage 1 (also called NREM 1) is the transitional phase from wakefulness to sleep. Stage 2 (NREM2) is the most prominent sleep stage and is characterized by light sleep with sleep spindles and k-complexes. During this stage, the body relaxes, and the heart and breathing rates slow down. Stage NREM3, also known as deep or slow-wave sleep (SWS), can be identified by its slow and high-voltage brain activity. It is important for f.e. glucose homeostasis, brain cleansing, and immunity\(^5,6\). Rapid eye movement (REM) sleep is the fourth sleep stage, well-known for its intermittent rapid eye movements. During this stage, the skeletal muscles are paralyzed, and the brain shows rapid, low-voltage cortical brain activity. REM sleep is important for f.e. emotional memory consolidation and brain plasticity\(^7,8\). Whereas the first night half is
predominated by NREM sleep, the second half mainly consists of REM sleep. The gold standard for identifying these different sleep stages is electroencephalography (EEG), which measures the electrical activity in the brain using non-invasive electrodes.

The sleep-wake cycle

Two processes drive the sleep-wake cycle. The first is sleep propensity, also known as process S or the homeostatic sleep drive. Sleep propensity increases with extended wakefulness when the waste products in the brain build up and create sleep pressure, and it decreases again during sleep. The second process is the circadian drive for arousal, also known as process C. The circadian rhythm fluctuates around 24 hours and is driven by the suprachiasmatic nuclei (SCN). These central circadian pacemakers regulate the sleep-wake cycle and metabolic oscillations via different outputs, including cortisol, core body temperature, and melatonin. During the day, the environmental light signals to the SCN to block the release of sleep-promoting melatonin in the pineal gland. When the night falls and environmental light decreases, melatonin production increases, thereby promoting sleep. Light is one of the strongest external cues, also called Zeitgebers, that can help synchronize the internal circadian rhythm with the external environment. Other Zeitgebers include exercise, meal timing, and social activity. Processes S and C also influence each other’s functioning: hampering one process will also lead to impaired functioning of the other. For example, one study found that high sleep pressure due to partial sleep deprivation decreased the effectiveness of light to phase shift the circadian rhythm. Therefore, both processes should be considered to ensure good sleep-wake behavior.

Sex* differences in sleep

It is well known that sleep differs between the sexes. A study using over 11 million nights from almost 70,000 non-shift working adults from all over the world found that men sleep on average half an hour less than women per night across the lifespan. These differences were most prominent during early and middle adulthood. Additionally, women experienced more sleep problems during the night, including night-time awakenings. A separate study also revealed that women have a longer sleep latency than men, meaning they require more time to fall asleep. Using PSG, researchers showed in 2,685 adults that women had more than twice as much SWS than men, where men had more

*Please note that throughout this thesis, sex refers to the biological sex of a person, male or female, and the biological and physiological attributes that accompany this (reproductive organs, chromosomes, hormones). Gender differences in sleep are outside the scope of this doctoral thesis.
NREM1 and NREM2\textsuperscript{14}. Women also have a 41\% higher risk of developing insomnia than men\textsuperscript{15}. Sex hormones could explain some of the differences in women’s sleep health, f.e. when looking at the menstrual cycle. Women experience more sleep spindles during NREM in the luteal phase, when progesterone and estradiol are high, compared to the follicular phase at the beginning of the cycle, with low progesterone and estradiol levels\textsuperscript{16}. Sleep quality and upper airway resistance, which could lead to sleep-disordered breathing (SDB), also vary during the menstrual cycle\textsuperscript{17}. Changes in a woman’s hormone balance, as happens during menarche, pregnancy, and menopause, are also key moments when sleep and the prevalence of sleep problems can alter substantially\textsuperscript{18}. Unfortunately, most sleep studies are conducted in men or male animals, so the translatability of findings to the female counterpart is uncertain.

The influence of weight on sleep
As will be discussed in more detail later, sleep loss can have a causal effect on markers of weight gain, possibly leading to excess weight\textsuperscript{19}. However, it is essential to understand how sleep is altered in people that are overweight or obese. Being overweight is defined as having a body mass index (BMI) between 25 - <30 kg/m\textsuperscript{2}, whereas obesity is defined as a BMI $\geq$ 30 kg/m\textsuperscript{2} and/or a waist circumference $>$102 cm for men and $>$88 cm for women\textsuperscript{20}. Weight gain increases the risk of developing insomnia, sleep maintenance problems, and daytime sleepiness\textsuperscript{21}. Having obesity also increases the risk of developing sleep disorders, including SDB, which worsens sleep quality\textsuperscript{22}. Over 70\% of people with obstructive sleep apnea (OSA), a form of SDB, suffer from obesity\textsuperscript{23}. In turn, OSA is associated with metabolic syndrome, higher cardiovascular morbidity, and mortality\textsuperscript{24}. Therefore, understanding the relationship between excessive weight and sleep is crucial to be able to implement sleep as a weight loss program tool. Nevertheless, the importance of good sleep in obesity treatment is often neglected\textsuperscript{25}. This neglect is unfounded, as one study showed that already one hour of sleep restriction for five nights during a weight-loss intervention in people with overweight and obesity led to a smaller proportion of fat mass loss, despite losing similar amounts of weight\textsuperscript{26}. Furthermore, longitudinal studies revealed that people with higher degrees of sleep disturbances at baseline also had a higher risk of failure to lose weight at six months of a weight-loss intervention\textsuperscript{27}.

Sleep in the elderly
Sleep naturally changes with healthy aging, including advanced sleep timing, shorter sleep duration, more night-time awakenings, more daytime naps, and less slow-wave sleep\textsuperscript{28}. Around half of the people over 65 years of age report
difficulties with falling or staying asleep. These sleep alterations could be due to age-related structural and functional changes in the SCN and pineal gland, leading to difficulties regulating sleep and wake and a decreased nocturnal melatonin release. However, aging is often accompanied by other changes, such as medical conditions and lifestyle alterations, which could cause and worsen sleep problems in the aging population.

Consequences of sleep loss
Notwithstanding the importance of good sleep, many people suffer from sleep problems like insomnia. An insomnia diagnosis is given when a person experiences difficulty initiating sleep, maintaining sleep, or premature awakenings, or has chronically nonrestorative or poor sleep. These symptoms must be present for at least three nights per week over at least three consecutive months, despite adequate sleep opportunities and circumstances, and must cause some form of daytime impairment. Annually, 35-50% of the general adult population experiences insomnia symptoms for at least one month, and 12-20% qualify for a clinical insomnia disorder.

Acute sleep problems can lead to daytime fatigue, attention problems, mood disturbances, and physical discomfort. In addition, chronic sleep disturbances are a risk factor for metabolic syndrome, mental disorders, hypertension, Alzheimer’s (see also section “Sleep loss and neurodegeneration”), cardiovascular disease, and overall increased mortality.

Effects of sleep loss on mood and facial evaluation
Sleep loss impacts our mood and emotions, e.g., by feeling more unstable, stressed, irritable, and anxious. This increased negative affect also reflects in the behavior of the sleep-deprived. For example, sleep loss causes social withdrawal and loneliness and makes people less motivated to help others. Vice versa, one study found that sleep-deprived individuals are perceived as less healthy and less attractive. Additionally, well-slept raters were less inclined to interact with the sleep-deprived individual. This could further exacerbate loneliness and social isolation.

An increase in negative mood is also measured when focusing on the brains of people following sleep loss. Exposure to negative stimuli after sleep deprivation increases the activity of the amygdala, a brain region important in emotion regulation. Other studies also indicate an increased reactivity to pleasure-evoking stimuli, further strengthening the theory of sleep-loss-induced emotional dysregulation.
The amygdala is also essential in emotion recognition, and uses facial cues to assess possible danger. For example, people with lesions in the amygdala cannot focus on the eye-region of faces, which is one of the strongest social cues for emotion detection. This impairment weakened the ability to perceive and identify fearful faces. In addition, the amygdala is not only important for recognizing others’ fearful emotions but also increases activity when processing other facial expressions.

Sleep deprivation decreases the accuracy and increases the time necessary to identify salient facial expressions. Additionally, patients with insomnia misinterpret angry faces as fearful. However, if faces with strong expressions are also processed and evaluated differently following sleep loss is unclear.

Shift work

In 2021, 18% of the workforce in the European Union performed shift work, which is work conducted outside the regular daytime (between 07:00 and 18:00h). Shift work is necessary for maintaining our 24/7 society, due to the around-the-clock need for critical services, law enforcement, transport, and manufacturing. However, shift workers often suffer from sleep problems and fatigue. Their circadian rhythm is not aligned with their work-induced sleep-wake rhythm: their inner rhythm promotes wakefulness when daytime sleep is planned. This often results in shorter sleep duration, with a spill over effect on the days off. The circadian misalignment also resembles lower melatonin levels in night shift workers during both day and night. Shift work has also been associated with negative behaviors, including smoking, alcohol misuse, and a low-quality diet. These behaviors, together with circadian misalignment, could create highly adverse health outcomes. For example, chronic shift work has been associated with a higher risk of developing obesity, type II diabetes, cancer, and cardiovascular disease.

Night-time performance

Night work not only impacts post-work performance and well-being but also affects performance during the night. Shift-induced short and misaligned sleep causes fatigue and sleepiness during the night shift. For example, a study on train drivers found that people working in railway transportation had a six to fourteen times higher risk for severe sleepiness on the night shift than those during the day shift. In addition, air traffic controllers on the night shift reported increased fatigue and confusion and decreased vigor and general activ-
Increased sleepiness and fatigue can have serious consequences. For example, a study investigating over 6,000 single-vehicle traffic accidents demonstrates a prominent accident peak between 00:00 and 07:00h, with the top between 01:00 and 04:00h, possibly due to sleepiness behind the wheel. Furthermore, a study modeling several industries' accident and incident risks found that the risk of injuries and accidents during the nightshift was ~28% higher than the morning shift. Even without accidents, night work impacts cognitive performance during the shift. For example, a night of simulated night shift work caused substantial impairments in cognitive performance, including inhibitory control, learning, and vigilance. Lastly, a study implementing a mimic firefighting scenario found that total sleep deprivation increased attention lapses, accuracy, and reaction time compared to sleep.

Brain health

The glymphatic system and aging

Sleep is vital to regulate glymphatic clearance. During sleep stage NREM3, the glymphatic system exchanges cerebrospinal fluid with interstitial fluid. In this way, neurotoxic waste that is built up throughout the day, like β-amyloid and tau, is excreted. Glymphatic activity declines with aging, leading to an accumulation of β-amyloid plaques and tau tangles, contributing to the development of cognitive decline and neurodegenerative diseases, including Alzheimer’s disease. In the early stages of neurodegenerative diseases, the loss of wake-promoting neurons disturbs the sleep-wake homeostasis, leading to more sleep disturbances, neurotoxic waste accumulation, and further deterioration of the illness.

Sleep loss and neurodegeneration

Increases in neurotoxic biomarkers might already appear after one night of sleep deprivation, as seen in healthy young men. These findings are confirmed in studies investigating short or more chronically disturbed sleep. For example, a study in >1,100 cognitively unimpaired adults found that short sleep duration (<7h) was associated with higher cerebral spinal fluid (CSF) and plasma tau levels than people with longer sleep duration. Additionally, having more sleep disturbances was associated with a decrease in CSF β-amyloid. Of note, subsample longitudinal analyses in 332 participants further confirmed that CSF β-amyloid-42 declined over a one-and-a-half-year period in the group with more sleep problems.

Shift work has been identified as another factor that could worsen cognitive decline and the development of neurodegenerative diseases. For example,
nurses working nights for at least six years had a 50% higher risk of dementia than those working nights for less than one year. A separate study using data from the Swedish Twin Registry found that ever performing nightshift work was associated with a 12% increased risk of dementia. Additionally, a study of retired nurses vs. teachers found that those who worked night shifts at least once per week for more than one year had a higher risk of cognitive decline and dementia than controls that did not perform shift work.

Sex and weight differences in neurodegeneration

Twice as many women have Alzheimer’s disease as men. Even after controlling for possible age differences (women, on average, live longer than men), women still have a 15% higher risk of dementia and a 56% higher odds of developing Alzheimer’s disease than men. Furthermore, from the moment of diagnosis, cognitive deterioration in women also occurs more rapidly. As these sex differences might be driven by hormonal or lifestyle changes earlier in life, small changes in brain health and cognitive performance might already be present at an earlier age. This further stresses the need to understand sex differences in risk factors, neuropathological development, and symptomatology.

People with obesity experience impairments in almost all cognitive domains compared to normal-weight people. However, little is known about how excessive weight impacts cognitive decline and the development of neurodegenerative diseases. Indications of an association come from epidemiological findings. A longitudinal study in older English adults found that people with obesity at baseline had a 34% higher risk of dementia, independent of f.e. sex and age. Additionally, people having both obesity and central obesity (defined as a BMI ≥30 kg/m² and a waist circumference >102 cm for men or >88 cm for women) had a 28% higher dementia risk than normal-weight people without central obesity. A meta-analysis found that obesity when being below 65 years of age was associated with a 41% risk of dementia. However, obesity from 65 years or older resulted in a 17% lower risk of dementia. Forecasts of dementia prevalence also showed that when considering obesity in the forecast models, dementia prevalence would be 19% higher in the United States and China compared to only considering demographic changes. These findings show that more attention needs to be paid to possible weight differences in cognitive performance and cognitive decline to better understand pathology risk and disease development in these groups.
Energy homeostasis and food intake

The regulation of food intake

A healthy and stable body weight is essential for good health. Therefore, the body needs to maintain energy homeostasis, meaning that the amount of energy intake is similar to the amount of energy that is expended. This energy balance is regulated by hormones, of which the most well-known are leptin and ghrelin. Leptin is a hormone secreted by the subcutaneous adipose tissue, and once in the brain it decreases hunger and appetite. Its plasma concentration reflects the state of peripheral fat energy stores. Where leptin is a more long-term regulator, ghrelin is a fast-acting hormone. The stomach releases ghrelin during fasting to promote appetite signaling in the hypothalamus and is suppressed after food intake, indicating a role in meal initiation.

Rodent studies suggest that leptin is an upstream regulator of ghrelin, as it blocks ghrelin’s orexigenic effects in the hypothalamus. However, human studies found contradicting results. For example, one study found a negative correlation between plasma ghrelin and leptin levels in people with obesity. This correlation was not confirmed in a study including children and adolescents with obesity. Additionally, a study including 120 healthy men and women found that ghrelin was negatively associated with leptin and found higher ghrelin levels in women than men. However, a three-day fast decreased leptin without increasing 24h ghrelin levels, suggesting independent pathways for leptin and ghrelin in regulating energy homeostasis.

Adiponectin is another adipokine secreted by the adipose tissue. This hormone increases insulin sensitivity and holds anti-apoptotic and anti-inflammatory properties. For example, it increases glucose uptake and fat storage in the adipose tissue, increases insulin sensitivity in the liver, and inhibits vascular inflammation. Adiponectin secretion is also inversely correlated with fat mass and is reduced in people with obesity. Of note, adiponectin also has typical properties of a hunger hormone: serum and CSF adiponectin levels increase after fasting in mice and decrease after feeding. It also increases food intake by activating orexigenic pathways in the hypothalamus.

Sleep loss and energy homeostasis

Sleep loss shifts the energy balance to the positive side, mainly due to, e.g., increased food intake, and possibly yet conflicting results exist due to reduced energy expenditure. If persisting over more extended periods, the sleep loss-induced positive energy balance could promote weight gain, including an increased risk of metabolic comorbidities. Moreover, a study using
the UK Biobank with over 80,000 people found that short sleep duration increases the risk on f.e. obesity, cardiovascular disease, and diabetes\textsuperscript{110}.

On the acute level, one night of total sleep loss in young men led to an increase in hunger ratings, portion size, and higher consumption of snacks\textsuperscript{105}. The shift towards increased food intake could be partly due to disturbances in the endocrine hormones regulating food intake. For example, acute sleep loss in healthy young men resulted in higher ghrelin levels the following morning\textsuperscript{105,106}. A separate study found that leptin levels increased after one night of total sleep loss\textsuperscript{111}. In contrast, some studies indicate an increase in ghrelin levels but not in leptin following one night of sleep deprivation\textsuperscript{112}. Similarly, a study involving 88h of sustained wakefulness found a reduction in the diurnal amplitude of leptin\textsuperscript{113}. When investigating patients with chronic insomnia, ghrelin levels across the night were significantly lower than in matched healthy controls, but no differences were found in leptin levels\textsuperscript{114}. The discrepancy in these findings could be due to the duration and chronicity of the sleep loss condition and need further investigation.

A study involving healthy young men found sleep-loss-induced tissue-specific DNA methylation changes in adipose tissue that indicate an increased fat storage in the adipose tissue\textsuperscript{115}. This could be caused by increases in adiponectin, which stimulates food intake and lipid storage in the adipose tissue\textsuperscript{97}. One study found differences in 24h adiponectin levels in men after short sleep (four hours), which was mainly caused by a decrease in night-time adiponectin, but could not confirm these findings in women\textsuperscript{116}. In contrast, a study in healthy young men found no difference in plasma adiponectin levels following sleep restriction\textsuperscript{117}. To date, no studies have focused on possible changes in adiponectin levels following total sleep deprivation.

The interplay of sleep loss, sex, and energy homeostasis

Sex could be a potential modulator of the association between sleep loss and energy homeostasis\textsuperscript{118}. A study investigating the effects of five days of short sleep found that women consumed more food and gained weight compared to total sleep, whereas men did not show these differences\textsuperscript{119}. However, a separate study did not find sex differences in energy intake (EI) following sleep restriction\textsuperscript{120}. When looking at the hormones regulating EI, a study implementing five nights of sleep restriction found a rise in leptin in all participants following the sleep restriction intervention, but with a more pronounced increase in women than men\textsuperscript{121}. Total ghrelin levels increased in men but not women when exposed to four hours of sleep compared to normal sleep\textsuperscript{116}. However, no differences were found for leptin between the conditions or the sexes\textsuperscript{116}. Twenty-four-hour adiponectin levels seemed lower in men but not
women after short sleep\textsuperscript{116}. A separate study found that adiponectin levels changed in women but not men following five nights of short sleep (four hours)\textsuperscript{122}. Whereas adiponectin decreased in Caucasian women, it increased in African American women\textsuperscript{122}. With these scarce and contradicting results in mind, more studies are needed to disentangle possible sex differences in the consequences of sleep loss on energy homeostasis.

The interplay of sleep loss, weight, and energy homeostasis

As described before, sleep loss can cause changes in energy homeostasis, which increases food intake, possibly decreases energy expenditure (EE), and can lead to weight gain. However, few studies have investigated if people's weight status alters these unwanted consequences of sleep loss. One study implementing a four-hour sleep schedule for five nights found that although all participants had higher leptin levels than after normal sleep, participants with a higher BMI had significantly greater increases in leptin levels than participants with a lower BMI\textsuperscript{121}. A separate within-subject study in adolescents found that total ghrelin concentrations in saliva were blunted after total sleep loss compared to sleep, with more pronounced differences in adolescents with overweight and obesity\textsuperscript{123}. Lastly, a study investigating the effects of partial sleep deprivation on the effectiveness of a calorie-restricted diet in people with overweight found that sleep restriction resulted in 55\% less weight loss by fat and in an increase in 24h blood ghrelin concentrations compared to total sleep\textsuperscript{124}. However, more studies are needed to disentangle how weight status differences can impact sleep loss's effect on energy homeostasis. On a positive note, one hour of sleep extension in people with overweight resulted in a significant EI decrease of 270 kcals compared to controls\textsuperscript{125}.

Food and diet as a possible sleep aid

Short-term sleep loss can lead to behavioral choices and endocrine changes that favor weight gain\textsuperscript{103–109}. Vice versa, the food we eat can also alter our sleep, possibly in a cyclical way\textsuperscript{126}. For example, a high intake of carbohydrates moderately decreases the duration of SWS but increases the time and proportion of REM sleep compared to a low carbohydrate intake\textsuperscript{127}. Additionally, adhering to a diet with a high glycaemic index was found to be a risk factor for insomnia in postmenopausal women\textsuperscript{128}. There are also indications that foods high in melatonin can improve sleep quality\textsuperscript{129}. Additionally, epidemiological findings have shown that diets rich in plant-derived foods and seafood but low in processed and sugar-rich foods are associated with better sleep quality\textsuperscript{130}.

One diet that has received a lot of attention for its possible health benefits is the Mediterranean diet (MD), which consists of primarily plant-based foods,
like vegetables, fruits, grains, as well as olive oil, fish, and shell food. Meat and dairy products, as well as alcohol, are only included in moderation. One study found that people with a moderate to high adherence to the MD were more likely to reach six to seven hours of sleep and report fewer insomnia symptoms than participants with a low MD adherence\textsuperscript{131}. In turn, a separate study of over 1,600 older adults found an association between sleep quality but not sleep duration and MD adherence\textsuperscript{132}. Where the previously mentioned results did not reveal any sex differences\textsuperscript{131,132}, other studies found that women but not men adhering to MD suffered fewer insomnia symptoms\textsuperscript{133}. A study on women also showed that higher adherence to the MD was associated with better sleep quality, efficiency, and fewer sleep disturbances\textsuperscript{134}. Altogether, more studies are needed before the MD can be considered a non-pharmacological sleep aid.

**Study rationale**

In 2021, 18% of the workforce in the European Union worked outside the usual nine-to-five schedule, e.g., having evening or night shifts\textsuperscript{50}. Although these non-traditional work times are necessary for our 24/7 society, they disrupt the circadian rhythm and increase the risk of developing f.e., obesity, diabetes, and cancer\textsuperscript{58,135}. As sleep is one of the main pillars for sustaining physical and mental health, it is essential to unravel how overnight wakefulness affects our well-being and health.

In the last 20 years, numerous studies have suggested that a lack of sleep compromises cognitive performance, adversely affects emotional regulation, and harms brain health. For example, following sleep loss, the brain reacts stronger to positive and negative emotional stimuli\textsuperscript{39,41,136}. It also becomes harder to identify facial expressions, which is important to assess the other person's emotional state and ease social interaction\textsuperscript{46,47}. Overnight wakefulness also impairs vigilance and inhibitory control\textsuperscript{66}, possibly explaining why performance errors and work accidents peak at night\textsuperscript{64,65}. When chronic, night shift work could severely affect brain health. For example, nurses working nights for at least six years had a 50% higher risk of dementia than those working nights for less than one year\textsuperscript{77}.

Additionally, studies including healthy young men showed that a night of sleep loss increased blood markers that are indicative of neurodegeneration\textsuperscript{74,75}. Of note, women and people with obesity are at a greater risk of developing Alzheimer's disease\textsuperscript{81,86}. Despite evidence suggesting that having a good night's sleep improves several aspects of central nervous system function and health, several significant knowledge gaps remain. For example, it is unknown whether faces with strong negative or positive expressions are more
attention-captivating when the viewer is sleep-deprived and if this also causes a difference in subjective evaluation. Furthermore, it is unclear whether sex and weight status impact the acute effects of sleep loss on night-time cognitive performance and brain health markers.

Besides the central nervous system (CNS) effects of sleep loss, an overwhelming body of evidence has demonstrated that sleep loss increases the risk of adverse weight gain and obesity, even in the short term. For example, following acute sleep loss, the brain’s reward response to food increases, and people eat more than they need, possibly leading to weight gain\textsuperscript{104,137–139}. The sleep loss-induced increase in food intake could be due to a decrease in satiety-promoting leptin and an increase in hunger-driving ghrelin hormone levels\textsuperscript{140}. However, outcomes on sex differences in the sleep-loss-induced endocrine response to food intake have been contradictory\textsuperscript{119,141}. Additionally, little is known about whether appetite regulatory hormones are differently affected by sleep deprivation in people that already suffer from obesity compared to normal-weight.

While experimental studies suggest that acute sleep loss leads to behavioral and endocrine changes in favor of poor dietary choices, it is not well-researched whether the quality of the diet a person chooses may impact sleep. Some studies have found positive associations between, e.g., adherence to the MD and sleep variables. However, the present body of literature regarding the association between dietary preferences and sleep is relatively thin and has produced somewhat contradictory findings\textsuperscript{131–133}.
Figure 2, overview of the study aims.
Study aims

This thesis aimed to investigate the psycho-metabolic consequences of sleep loss in people, focusing on social appearance and exploration, overnight performance, brain health, the regulation of energy metabolism, and dietary patterns (Figure 2). The specific aims were:

1) Do images of angry, happy, scared, and neutral faces differ in their social appearance if the observer is sleep-deprived? (Paper I)

2) Does a sleep-deprived person visually explore others' faces differently? (Paper I)

3) Do the effects of overnight wakefulness on subjective and objective alertness differ between biological sexes and weight groups? (Paper II)

4) Does the brain health response to sleep loss vary by biological sex and weight status? (Paper II)

5) Does acute sleep loss affect hormonal pathways involved in food intake and the energy balance differently when focusing on men vs. women and participants with normal weight vs. obesity? (Paper III)

6) Is there an association between subjective sleep disturbances and adherence to common dietary patterns? (Paper IV)
Methods

This thesis work consists of an experimental part (Paper I – III) and an epidemiological part (Paper IV). A comprehensive description of all methods can be found in the corresponding manuscripts (see List of Papers). In my experimental study, the consequences of one night of total sleep loss in young adults with normal weight and obesity were investigated. I focused on visual attention and evaluation following sleep loss (Paper I), as well as sex and weight differences in the ability of the brain to cope with overnight wakefulness (Paper II), and the endocrine regulation of metabolic homeostasis (Paper III). Lastly, I used epidemiological methods to investigate the association between sleep problems and adherence to healthy dietary patterns in older adults (Paper IV).

Experimental work (Paper I-III)

Participants

Possible participants were recruited via an online recruitment website and local billboards. A total of 508 people filled out an online survey to evaluate their health status and lifestyle behavior, including sleep habits. Potential participants were excluded when indicating poor sleep quality, extreme chronotypes (as assessed by the morningness-eveningness questionnaire\textsuperscript{142}), a sleep duration <7 hours, having physical or mental illnesses, regular medication usage, drugs or nicotine use, more than five standard units of alcohol or caffeine per day, uncorrected vision problems, and time-zone travel within three months of the study period or between study sessions. Women also needed hormonal contraceptives due to possible confounding by the menstrual cycle on sleep, emotion processing, and cognition\textsuperscript{17,143,144}. Of the people filling in the online survey, 81% met at least one of the exclusion criteria. The remaining 96 candidates visited the laboratory for an onsite screening to further assess health, well-being and discuss possible study questions. Following, 49 people were excluded, leaving 47 participants available for inclusion in the study (mean ± standard deviation (SD), age: 25 ± 3 years; 21 women). Twenty-six participants were identified as having a normal weight, as defined by a waist circumference <94 cm for men and <80 cm for women\textsuperscript{20}. Obesity (n=21) was defined as having a waist circumference >102 cm for men and >88 cm for women\textsuperscript{20}.
Seven single eye tracker recordings were unavailable for the analyses in Paper I due to unforeseen hardware and software issues. Additionally, sleep scoring revealed one participant that slept 02:45h in the sleep condition. Hence, this participant’s sleep condition data were also excluded from the analysis. The final analytical sample consisted of 45 participants, of which 20 were women.

For Paper II, no further exclusions needed to be made, leaving a sample of 47 participants. However, for the blood analyses of Paper II and Paper III, blood was available for 44 participants (of which 24 men and 20 women; 25 subjects with normal weight and 19 with obesity). For Paper II, 40 participants were included in the blood analysis due to missing covariate data exclusions (25 men; 25 subjects with normal weight).

The study was performed according to the Declaration of Helsinki. The ethical board of Uppsala approved all experimental procedures before the onset of the study (DNR2017/560). Subjects provided written informed consent before the study sessions and were compensated for their participation. All experiments were performed between March 2018 and November 2020.

General study setup
The full study setup can be found in Figure 3. This within-subject crossover study comprised two conditions that were separated by at least one week: one night with total sleep loss versus one night with an eight-hour sleep opportunity. The order of conditions was randomized and counterbalanced across the participants. Female participants did not have any of their sessions in their menstrual phase. An adaptation night was scheduled within seven days of the first experimental session to accommodate participants to the laboratory environment and equipment.

During the experimental sessions, a standardized dinner was served upon arrival at 19:00h. Polysomnography (PSG) measures were recorded during the night in both sessions using SOMNO HD (10-20 system; SOMNOmedics GmbH, Randersacker, Germany). In the sleep condition, sleep was allowed between 23:00 and 07:00h. Sleep stage scoring was performed according to the criteria from the American Academy of Sleep Medicine. The pulse oximeter from SOMNO HD was used to estimate the Oxygen Desaturation Index (ODI) per hour during the sleep night. This measure is suggested to be as valuable as the more classic apnea-hypopnea index to diagnose and grade obstructive sleep apnea. The ODI was defined as decreases in blood oxygen saturation that were >3% higher than the baseline. In the wake condition,
Figure 3, Complete study setup of the experimental investigation into the psychometabolic consequences of sleep loss. The top box shows the general study setup. The middle box further explains the setup of the sleep deprivation condition. Lastly, the eye-tracking paradigm is explained in the bottom window. Abbreviations: BD, blood drawing; EEG, electroencephalography; ET, eye tracking; KSS, Karolinska Sleepiness Score questionnaire; PVT, psychomotor vigilance task.

Subjects performed a battery of tests every two hours from 23:00h and spent the rest of the time with a researcher conducting sedentary leisure activities (e.g., watching movies, reading, studying) under normal light conditions.
(~500 lux). No food nor beverages (except water) were allowed. The test battery consisted of the Karolinska Sleepiness Scale (KSS; *Paper II*, for subjective sleepiness;\(^{148}\)), a psychomotor vigilance test (PVT; *Paper II*, testing reaction time;\(^{149}\)), and a 2-min eyes-closed wake maintenance test (WMT; *Paper II*, testing the ability to stay awake). In the morning following both conditions, blood was drawn around 07:30h to detect blood markers of brain health (*Paper II*) and metabolism state (*Paper III*). The eye-tracking task for facial exploration and evaluation assessment (*Paper I*) was performed around 08:00h.

**Facial exploration and evaluation (***Paper I***)

For *Paper I*, an eye-tracking task was conducted where high-resolution and standardized images of the faces of adult male and female Caucasian actors (18-40 years) were shown. These were derived from the Chicago Face Database\(^ {150}\). Three male and three female actors were randomly selected from four facial expression categories: happy, fearful, angry, and neutral. This resulted in a total of 24 pictures from 24 individuals. The images were identical for both experimental conditions but presented in a differently randomized order in each version to avoid order effects.

A Tobii Pro Spectrum eye tracker was used sampling at 600Hz with binocular tracking, which showed the stimuli on a 23.8”, 1920x1082 pixels screen (Tobii© Technology AB, Stockholm, Sweden). Participants were located within 60 to 70cm of the eye tracker screen and used a headrest to minimize movement. Participants freely explored each stimulus for five sec, after which a message appeared to rate the actor's healthiness, attractiveness, and trustworthiness on a 100mm visual analog scale (VAS; 0= not at all; 100= very much) without a time limit. A fixation cross was shown between each stimulus for three seconds to redirect the participant’s gaze to the center of the screen. Before the onset of the experimental paradigm, a test round was performed to ensure all instructions were understood. Following, the paradigm was performed alone.

The Tobii Pro Lab software (Tobii© Technology AB, Stockholm, Sweden) was used to create areas of interest (AOIs) for the upper and lower part of the face in a standardized manner (Figure 4) and to calculate the total fixation duration (TFD) and total fixation points (TFP). The TFD was defined as the time during which participants’ eyes rested on the AOI using the classifications from the Tobii I-VT fixation filter\(^ {151}\). The TFP was defined as the total amount of times that the participant fixated in each AOI.
The first 300 msec of each facial stimulus recording were discarded. The variables of interest were the TFD and TFP of the whole face as well as the upper and lower parts of the face. Single probes were rejected if the eye tracker could not detect the eyes.

**Test battery during overnight wakefulness (Paper II)**

*Paper II* focused on the effects of overnight wakefulness on performance during the night. To this aim, a test battery was conducted every two hours, i.e., at 23:00, 01:00, 03:00, 05:00, and 07:00h. First, the 9-point KSS\textsuperscript{148} was given to assess the subjective sleepiness of the participant. Then the PVT\textsuperscript{149} was conducted to measure sustained attention during a 3-min computer task. Here, the participants needed to respond to a red dot presented in the center of a black screen, with changing intervals. The reaction time and attentional lapses were the variables of interest. At the end of each test battery, participants performed a WMT, resting their heads on a chinrest while closing their eyes for two min during which brain activity was measured with EEG. They were instructed not to fall asleep. Sleep scoring was used to identify possible microsleep and sleep episodes.

**Blood sampling and analyses (Paper II & III)**

Fastened blood was drawn via venipuncture at 07:30h following both experimental nights. Blood was collected into PSTII tubes (BD Sweden, Stockholm)
and centrifuged at 1300rcf for 10 min at 4°C. The supernatant was stored at -80°C until analysis.

For Paper II, various brain health biomarkers were measured after sleep and sleep loss. Phosphorylated tau levels, pT181 and pT231, were analyzed using in-house single molecule arrays (Simoa) assays. The amyloid-beta peptides Aβ40 and Aβ42, glial fibrillary acidic protein (GFAP), and neurofilament light chain (NfL) were measured with the Quanterix Neurology 4-Plex E Advantage Kit (Quanterix, Billerica, Massachusetts). All biomarkers were measured on the ND-X Analyser (Quanterix, Billerica, Massachusetts).

In Paper III, the Human Leptin Quantikine ELISA Kit (DLP00; Biotechne) was used to measure serum leptin concentrations. Serum adiponectin levels were determined with the Human Total Adiponectin/Acrp30 Quantikine ELISA Kit (DRP300; Biotechne). The total Human Ghrelin ELISA kit (EZGRT-89K; Millipore, Billerica, MA) was used for quantifying total ghrelin plasma levels.

Statistical analysis
All analyses were performed using IBM SPSS Statistics 26 and 28 (SPSS Inc. Chicago, IL, USA). Visual inspection and the Shapiro-Wilk test assessed the normal distribution of the variables. A p<0.05 was considered significant. In Paper I, generalized linear mixed models (GLMM) were used to investigate the main effects of SLEEP (within-subjects; sleep vs. sleep deprivation) and EMOTION (happy vs. neutral vs. angry vs. fearful) or face evaluation (healthiness vs. attractiveness vs. trustworthiness). Additionally, possible interactions between these factors were modeled. Pairwise t-tests with least significant difference (LSD) adjustment for multiple comparisons were used for post-hoc testing.

Skewed data in Paper II were log-transformed to approach normality. First, independent t-tests were used to assess baseline differences (i.e., 23:00h) between sex and weight groups. Potential baseline differences of the variables between the sleep and sleep deprivation condition were examined with paired t-tests. The PVT reaction time was adjusted for baseline differences by expressing the mean reaction time at 01:00, 03:00, 05:00, and 07:00h as a percentage of the baseline. Absolute differences to the baseline were used for PVT lapses and micro sleep and sleep episodes in the WMT, as most participants had zero events at baseline. Due to large inter-individual differences in brain health biomarkers, these were baseline-adjusted using the participants’ sleep condition values. GLMMs were used to examine if sleepiness, vigilance, and the ability to stay awake vary by sex and weight status during the sleep deprivation night, including TIME (within-subjects; 01:00 vs. 03:00 vs. 05:00
vs. 07:00h), SEX (between-subjects; fixed; male vs. female), and WEIGHT (between-subjects; fixed; normal-weight vs. obese). Generalized linear models (GLMs) were used for the CNS health biomarkers, including SEX (between-subjects; fixed; male vs. female) and WEIGHT (between-subjects; fixed; normal-weight vs. obese). All GLM and GLMM analyses included time in bed in the night before the experimental wake condition as a covariate. Pairwise t-tests with LSD adjustments for multiple comparisons were conducted for post-hoc testing. Possible correlations were tested with Spearman’s rho tests. Unless otherwise stated, all data is reported as estimated marginal means [95% confidence interval (CI)].

The data for the analyses in Paper III were not normally distributed (Kolmogorov-Smirnov test; P<0.05). Thus, the Wilcoxon signed-rank test was used to test possible differences between sleep and sleep deprivation in the whole group and for the sex- and weight-stratified analyses. Individual sleep-wake differences between the sex and weight groups were compared using Mann-Whitney U tests. Effect sizes were calculated by dividing the Z statistic from the Wilcoxon signed-rank test by the square root of the number of observations. Effect sizes 0.10 – <0.30 were considered a small effect, 0.30 - <0.50 a moderate effect, and ≥0.50 a large effect154.

Epidemiological work (Paper IV)

Population and study design

In this epidemiological study, data from the Uppsala Longitudinal Study of Adult men (ULSAM) was used to assess if older men with sleep initiation or maintenance problems adhere differently to healthy dietary patterns, i.e., the MD or Healthy Diet Indicator (HDI), than those without sleep issues. The primary aim of ULSAM was to identify cardiovascular risk factors in men from Uppsala, Sweden. All men were born between 1920 and 1924 and investigations started at age 50. In 1990, 1,211 men participated in the 70y follow-up. Sleep variables and dietary assessments were available for 1,093 participants. After excluding participants with missing covariate data, 970 participants were available for final analysis. All participants in ULSAM provided written informed consent before the study onset, and the ethical review board in Uppsala approved the study (251/90 and 97/329).

Sleep measures

Sleep initiation and maintenance problems were assessed via paper questionnaires. “Do you have difficulties falling asleep at night?” measured sleep initi-
tiation problems. Sleep maintenance problems were evaluated with the question: “Do you often wake up in the early hours, unable to get back to sleep?” Participants could answer both questions with “yes”, “no”, or “I don’t know”. The latter option was treated as not having that sleep problem.

Assessment of dietary adherence

Participants recorded their dietary intake in a pre-coded menu-book for seven consecutive days, from which their daily energy (EI) and nutrient intake were calculated. Over- and under-reporters of dietary intake were identified using the Goldberg 2 cut-off for adequate reports of energy intake as modified by Black\textsuperscript{155}, which considers physical activity (PA; as assessed via questionnaires), basal metabolic rate (using the age-adjusted Schofields formula), and reported EI. After applying this cut-off, 54\% of the included men were classified as adequate energy reporters (n=519).

As described in\textsuperscript{156} and summarized in Table 1, MD adherence scores were calculated by determining the different components of the MD from the pre-coded menu-book, leading to a score between 0 (not at all adherent) to 8 points (very adherent). The components included: fat quality, vegetables, fruits, cereals, fish, meat, dairy, and alcohol intake. Population medians for each dietary characteristic were used as a cut-off, and several adjustments were made to better adjust the MD characteristics to the Swedish food products and habits.

The HDI score was based on the dietary guidelines from the World Health Organisation. It included saturated fatty acids (SFAs), polyunsaturated fatty acids (PUFAs), proteins, total carbohydrates, sucrose, fiber, fruits and vegetables, cholesterol, and fish. Modifications were made to the pre-set cut-off values to align with the Swedish national guidelines. The total HDI score ranged from -1 to 8 points, with 8 being highly adherent. All components are summarized in Table 1. See\textsuperscript{157} for a more detailed description.

Health and lifestyle factors

Several health and lifestyle factors were assessed during the age-70 follow-up. Body measurements, including weight, height, waist circumference, and blood pressure, were taken during a clinic visit via conventional methods. The BMI (kg/m\textsuperscript{2}) was calculated by dividing the weight (kg) by the square height (meters). Hypertension prevalence was diagnosed if the participant had a supine diastolic blood pressure \( \geq 95 \) mmHg and/or anti-hypertensive drug treatment. An oral glucose tolerance test was administered to screen for diabetes prevalence. Plasma glucose and insulin were measured in blood before and 30, 60, 90, and 120 min after ingestion of a high concentrated glucose solution.
Table 1. Scoring of the subcomponents of the MD and HDI.

<table>
<thead>
<tr>
<th>MD Score</th>
<th>Components</th>
<th>HDI Score</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 8</td>
<td>Total</td>
<td>-1 - 8</td>
<td>Total</td>
</tr>
<tr>
<td>1</td>
<td>High PUFAs/SFAs*</td>
<td>1</td>
<td>0-12% energy from SFAs</td>
</tr>
<tr>
<td>1</td>
<td>High intake of vegetables and legumes*</td>
<td>1</td>
<td>5-10% energy from PUFAs</td>
</tr>
<tr>
<td>1</td>
<td>High intake of fruit and berries*</td>
<td>1</td>
<td>10-20% energy from protein</td>
</tr>
<tr>
<td>1</td>
<td>High intake of cereals, incl. potato*</td>
<td>1</td>
<td>50-70% energy from total carbohydrates</td>
</tr>
<tr>
<td>1</td>
<td>High intake of fish*</td>
<td>-1</td>
<td>&gt;10% energy from sucrose</td>
</tr>
<tr>
<td>1</td>
<td>Low intake of meat and meat products*</td>
<td>1</td>
<td>≥3 g/MJ fiber</td>
</tr>
<tr>
<td>1</td>
<td>Low intake of milk and dairy products*</td>
<td>1</td>
<td>&gt; 400 g/day fruit and vegetables</td>
</tr>
<tr>
<td>1</td>
<td>Moderate alcohol intake#</td>
<td>1</td>
<td>0-300 mg/day cholesterol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>≥ 35 g/day fish</td>
</tr>
</tbody>
</table>

Abbreviations: HDI, Healthy Diet Indicator; MD, Mediterranean Diet; SFAs, saturated fatty acids; PUFAs, polyunsaturated fatty acids. *high and low intake defined as > and < population median, respectively; # Defined as residual adjusted intake of 10–50 g/d and no biochemical signs of alcohol abuse (i.e., aspartate aminotransferase:alanine aminotransferase ratio < 2).

A diabetes diagnosis was given if the 120min and one or more of the 30-90min glucose levels were ≥11.1 mmol/L. Current smoking status was assessed verbally during the clinic visit. A standardized self-administered questionnaire was used to assess leisure time PA levels. Regular leisure time PA was defined as performing an active sport or heavy gardening ≥three hours per week and/or conducting hard physical training or engaging in a competitive sport. Educational status was divided into university education vs. upper secondary school (sixth form) or below. Participants were also asked about the possible occurrence of the following disease-states: heart infarction, angina pectoris, cancer, and joint problems. Answer options were “yes”, “no”, or “I don’t know”, of which the latter was treated as “no”.

Statistical analyses

Statistical analyses were performed using SPSS version 24 (SPSS Inc. Chicago, IL, USA). The following possible confounders were included in the analyses as binary variables, unless otherwise stated: exact age (continuous), BMI (continuous), waist circumference (continuous), smoking status, hypertension prevalence, diabetes prevalence, leisure-time PA, educational level, heart infarction prevalence, angina pectoris prevalence, cancer prevalence, and having joint problems. Alcohol intake data (as % of daily EI; continuous) was extracted from the food diaries. As the season of assessment could also affect sleep behavior, the season of the clinic visit was also added as a potential confounder (ordinal).
First, bivariate tests were performed between independent (i.e., MD or HDI diet scores and possible confounders) and dependent variables (sleep initiation or sleep maintenance problems) using unpaired t-tests and $\chi^2$ tests. To avoid overloading the model, as suggested by 159, only the variables with a $p<0.2$ from the bivariate tests were then entered as potential confounders into the multivariate logistic regression model (MVLR; enter method). Statistical significance was reached when the 95% CI did not straddle zero.
Results

Paper I

First, an eye tracker was used to investigate alterations in the gaze patterns of facial expressions (happy, fearful, angry, and neutral) following sleep and total sleep loss, using the TFD and TFP. Using a GLMM, it was found that the TFD was 8% lower after sleep loss than after sleep (mean [95%CI]; 72.7% [71.3%, 74.0%] vs. 81.0% [80.0%, 81.9%]; p<0.001 for SLEEP). The TFD did not differ by EMOTION (p=0.497), and no significant interaction between SLEEP and EMOTION was found (p=0.235).

As the eyes are the most potent nonverbal social cue for conveying emotions\(^{44}\), additional analyses were conducted for the different face regions. GLMM analysis showed that the TFD for the upper part of the face was 8.2% lower following sleep loss (sleep loss vs. sleep: 49.3% [47.8%, 50.8%] vs. 57.5% [56.2%, 58.7%]; p<0.001). Additionally, the TFD on the upper part of the face differed by the shown emotion irrespective of the experimental condition (happy vs. fearful vs. angry vs. neutral faces; 51.8% [49.8%, 53.8%] vs. 51.4% [49.5%, 53.3%] vs. 55.3% [53.3%, 57.3%] vs. 55.0% [53.1%, 56.9%], p=0.005). However, the SLEEP*EMOTION interaction did not reach significance (p=0.770).

When focusing on the lower part of the face, no difference was found between the experimental conditions (sleep loss vs. sleep; 22.5% [21.3%, 23.8%] vs. 23.2% [22.3%, 24.2%]; p=0.381). Though, the displayed emotion did seem to impact the TFD (happy vs. fearful vs. angry vs. neutral faces: 24.2% [22.7%, 25.8%] vs. 24.5% [22.9%, 26.0%] vs. 20.4% [19.0%, 20.9%] vs. 22.4% [20.8%, 23.9%], p<0.001). The SLEEP*EMOTION interaction was not significant (p=0.065), but given the trend to significance, exploratory comparisons were performed. This indicated that the TFD on the lower part of angry faces was 4.6% lower following sleep loss (p=0.015). However, no such trends were present for the other emotional expressions (p≥0.171).

Similar results were found when analyzing the TFP on the whole face and when focusing on either the upper or lower part of the face. Additionally, the individual faces within each emotion category did not affect the TFD and TFP outcomes following sleep and sleep loss.
After exploring each face, participants were asked to rate the face regarding attractiveness, healthiness, and trustworthiness on a 100mm VAS scale. Following sleep loss, faces were evaluated as less attractive (mean difference from sleep [95%CI]; −3.6 mm [−5.6 mm, −1.7 mm]) and less trustworthy (−2.6 mm [−4.4 mm, −0.8 mm]; both p<0.004). No difference was found in perceived healthiness following sleep loss (−1.2 mm [−3.0 mm, 0.6 mm]; p=0.180). Additionally, the attractiveness, trustworthiness, and healthiness also differed by the facial expression (all p<0.001 for EMOTION). Exploratory post-hoc comparisons indicated that angry faces were less trustworthy (−4.9 mm, p= 0.005) and healthy (−4.2 mm, p=0.020) following sleep loss. Fearful and neutral faces were seen as less attractive (−3.9 mm and −5.5 mm, respectively; p≤0.030). However, as the interaction between SLEEP and EMOTION was not significant (p≥0.187), these exploratory analyses should be interpreted with caution. No differences were found in the effects of sleep on the ratings when considering the individual faces of each emotion category.

Paper II

This study assessed how overnight occupational performance parameters (subjective sleepiness, vigilance, and sustained wakefulness) differ between men and women with normal weight and obesity during a night of sleep loss. To this aim, a test battery with the KSS, PVT, and WMT was executed at 23:00, 01:00, 03:00, 05:00, and 07:00h. Blood biomarkers for brain health were also measured after the sleep and sleep deprivation conditions.

As measured by the KSS (scale range 1-9; 9 being extremely sleepy), subjective sleepiness was 2.6 points higher at the end of the sleep loss condition (07:00 vs. 23:00h; p<0.001). At each time point in the night, female scores were, on average, 1.3 points higher than in males (mean [95%CI]; female vs. male; 7.4 [7.0, 7.8] vs. 6.1 [5.8, 6.4]; p<0.001), although no interaction between SEX and TIME was found (p=0.963). People with normal weight and people with obesity were similarly affected in their sleepiness throughout the night (p=0.685 for WEIGHT), and the WEIGHT*TIME interaction did not reach significance (p=0.841).

The PVT assessed the reaction time to respond to an unpredictable stimulus and the number of lapses during this test. Over the whole night, the reaction time became around six percent longer (07:00 vs. 23:00h; p= 0.016), but no significant sex difference was found for the increase in reaction time (difference between 07:00 and 23:00h; men vs. women; +3.7% [1.9%, 5.6%] vs. +4.1% [1.6%, 6.5%]; p=0.838). Similar null findings were noticed for the weight groups (difference between 07:00 and 23:00h; normal weight vs. obesity; +4.0% [2.1%, 5.9%] vs. +3.8% [1.5%, 6.2%]; p=0.896). Additionally, no
interactions of the sex and weight groups with TIME were found for reaction
time during overnight wakefulness (p ≥ 0.381). The number of attentional
lapses did not change by TIME, SEX, or WEIGHT (all p ≥ 0.303), nor were
there any interactions of SEX or WEIGHT with TIME (all p ≥ 0.802).

The WMT was a 2-min quiet wake test, where participants were instructed to
stay awake with their eyes closed. Using EEG, the duration of microsleep and
sleep could be recorded. Over the whole night, participants had +7.7sec mi-
crosleeps and +45.5sec sleep duration compared to the 23:00h baseline
(p=0.01 and p<0.001, respectively). Additionally, females experienced, on av-
average, longer microsleeps than males during the WMT tests (+8.1sec [1.3sec,
5.6sec] vs. +2.9sec [1.2sec, 4.6sec]; p=0.001), but no difference in sleep du-
ration existed between the groups (females vs. males; +23.5sec [13.3sec,
33.8sec vs. +23.5sec [16.6sec, 30.4sec]; p=0.994). Participants with normal
weight and those with obesity did not significantly differ in their microsleep
duration (+5.5sec [3.7sec, 7.3sec] vs. +5.5sec [3.1sec, 7.8sec]; p=0.996).
However, participants with obesity slept 1.8 times longer during the WMT
compared to the participants with normal weight (normal weight vs. obese;+
16.6sec [9.3sec, 23.8sec] vs. +30.4 sec [20.9sec, 40.0sec]; p=0.015). No in-
teractions were found between the time points during the night and SEX or
WEIGHT (all p ≥ 0.166).

Analyses of the brain health markers revealed that pTau-181 blood levels fol-
lowing the sleep deprivation night were around 25% higher than after sleep
(p=0.003). None of the other brain health markers (pTau231, NfL, Aβ40,
Aβ42, and GFAP) were significantly altered by the sleep loss condition
(p ≥ 0.054). Followingly, the individual ratios for the blood biomarkers were
calculated for the subgroup analyses by dividing the individual’s sleep loss
condition by their sleep condition blood values. Women’s NfL blood levels
were more increased following sleep loss than men’s (ratios sleep loss/sleep
female vs. male; 1.16 [1.04, 1.28] vs. 0.92 [0.83, 1.01]; p=0.002). Addition-
ally, participants with obesity had higher increases in pTau181 after the sleep
loss condition than participants with normal weight (1.58 [1.34, 1.83] vs. 1.09
[0.91, 1.27]; p=0.001).

Exploratory correlational analyses did not show an association between the
total duration of microsleep episodes and the NfL ratio in women (Spearman’s
rho=−0.469; p=0.124). Women’s higher NfL ratio was also not explained by
the cumulative KSS score (Spearman’s rho=−0.208; p=0.408). When focusing
on the participants with obesity, no significant correlations were found be-
tween the total sleep duration during the WMT and the pTau181 ratio (Spear-
man’s rho=−0.408; p=0.212). As participants with obesity also had a higher
ODI than normal weight participants (mean ± SD; 4.8 ± 5.2 vs. 1.4 ± 1.5;
p=0.02; independent t-test), possible correlations of the ODI score and
pTau181 ratios were explored. However, no correlation was detected (Spearman’s rho=0.278; p=0.117).

As expected, participants also felt sleepier at 07:15h after the sleep loss condition than after the sleep condition, as measured by the KSS (7.5 [7.0, 8.0] vs. 3.3 [2.8, 3.9]; p<0.001; paired t-test). The reaction time (as measured by the PVT) was also longer following sleep loss than sleep (340 msec [325 msec, 354 msec] vs. 312 msec [300 msec, 323 msec]) and the number of lapses was higher (3.1 [1.5, 4.7] vs. 1.6 [0.5, 2.6]; p≤0.014 for both; paired t-tests).

Paper III

The third paper focused on the endocrine regulation of energy homeostasis following total sleep deprivation. Satiety-promoting leptin concentrations decreased to 17.3 ± 2.6 after the night of sleep loss, a ~7% decrease from sleep (18.6 ± 2.8 ng/ml; p=0.037; r=0.22). This sleep loss-induced reduction in leptin was confirmed in women (sleep loss vs. sleep; 25.8 ± 4.3 vs. 28.1 ± 4.7 ng/ml; p=0.030; r=0.34) but not in men (sleep loss vs. sleep; 10.1 ± 2.4 vs. 10.6 ± 2.3 ng/ml; p=0.458; r=0.11). When focusing on weight differences, serum leptin seemed lower in participants with normal weight (sleep loss vs. sleep; 6.2 ± 1.0 vs. 7.1 ± 1.2 ng/ml; r=0.26) and in participants with obesity (31.8 ± 3.9 vs. 33.6 ± 4.3 ng/ml; r=0.22) following sleep loss, however, these differences were not significant (p≥0.069). Additionally, individual leptin levels were assessed between the sex and weight groups. No significant differences in leptin were found between men and women in their individual wake-sleep differences (−0.5 ± 0.7 vs. −2.3 ± 0.9 ng/ml; p=0.138). Similar null findings were detected when comparing the individual wake-sleep differences between participants with normal weight and those with obesity (−0.9 ± 0.4 vs. −1.9 ± 1.2 ng/ml; p=0.337).

Hunger-promoting ghrelin levels were around 13% higher after the sleep loss condition than after sleep (839.4 ± 77.5 vs. 741.4 ± 63.2 pg/ml; p=0.003; r=0.32). These sleep-loss-induced increases in ghrelin were also present when only investigating men (sleep loss vs. sleep; 703.6 ± 56.6 vs. 616.2 ± 56.1 pg/ml; p=0.024; r=0.34) and women (988.8 ± 145.3 vs. 879.1 ± 111.2 pg/ml; p=0.049; r=0.31). Similar patterns were found for the subset with normal weight (913.0 ± 130.4 vs. 833.5 ± 106.0 pg/ml; p=0.095; r=0.25) and the subset with obesity (750.4 ± 65.8 vs. 629.9 ± 47.1 pg/ml; p=0.007; r=0.44). However, when comparing the individual wake-sleep differences, no significant differences were found between the sexes (males vs. females; 87.4 ± 51.9 vs. 109.7 ± 52.5 pg/ml; p=0.762), nor between the weight groups (79.5 ± 58.5 vs. 120.4 ± 39.9 pg/ml; p=0.471).
Lastly, adiponectin, which has food-intake-promoting properties, increased by ~10% following sleep loss (compared to sleep; 7.5 ± 0.6 vs. 6.8 ± 0.6 μg/ml; p=0.003; r=0.31). Stratification analyses showed that these patterns were also present in the female subgroup (9.4 ± 1.0 vs. 8.4 ± 0.9 μg/ml; p=0.025; r=0.35) and the participants with normal weight (8.1 ± 0.8 vs. 7.4 ± 0.7 μg/ml; p=0.040; r=0.29). No significant effects of sleep deprivation on adiponectin were found in men (men: 5.9 ± 0.5 vs. 5.6 ± 0.6 μg/ml; p=0.056) nor in participants with obesity (6.6 ± 0.8 vs. 6.1 ± 0.8 μg/ml; p=0.053). Lastly, men and women did not significantly differ in their increases in adiponectin (0.3 ± 0.2 vs. 1.0 ± 0.5 μg/ml; p=0.164). Additionally, the rise in adiponectin levels was not significantly different in participants with normal weight than in those with obesity (0.8 ± 0.3 vs. 0.5 ± 0.3 μg/ml; p=0.951).

Paper IV

Using data from the ULSAM cohort at age 70, this research examined if adhering to the MD or HDI was associated with less sleep initiation and sleep maintenance issues. First, no association was found between having sleep initiation problems and the level of adherence to the MD (score ranging from 0 to 8; 8 being very adherent) in the full cohort (mean ± SEM; having sleep initiation problems vs. not having: 3.73 ± 0.14 vs. 3.89 ± 0.05 points; p=0.32; unpaired t-test). Similar results were found in the subgroup of adequate reporters (3.78 ± 0.21 vs 3.92 ± 0.07 points; p=0.51; unpaired t-test). When investigating the separate components of the MD, men with a low intake of milk and dairy products had 36% lower odds of having sleep initiation problems than men with a high intake of milk and dairy products after adjusting for potential confounders. However, this finding could not be confirmed in the sub-cohort of adequate reporters of EI. The other dietary components of the MD did not show an association with sleep initiation problems (all p ≥0.2 on the bivariate tests in both the full cohort and sub-cohort of adequate reporters).

Sleep maintenance issues were also not associated with the level of MD adherence in the entire cohort (having sleep maintenance issues vs. not having: 3.81 ± 0.11 vs. 3.89 ± 0.06 points; p=0.57; unpaired t-test) nor in the sub-cohort of adequate reporters (3.94 ± 0.14 vs. 3.90 ± 0.08 points, p=0.83; unpaired t-test). The components “High intake of cereals” and “moderate alcohol intake” were identified to be possibly associated with sleep maintenance issues in the whole cohort (p=0.017 and p=0.09, respectively; χ² test) as well as in the subgroup of adequate reporters (p=0.04 and p=0.126, respectively; χ² test). However, when entered in the MVLR with other potential confounders with a bivariate test p<0.2, the two MD categories did not show an association with sleep maintenance issues in the entire cohort (OR [95%CI]; 1.20 [0.85,
1.68] and 0.77 [0.53, 1.11], respectively) nor in the subcohort of adequate reporters (1.63 [0.99, 2.68] and 0.72 [0.41, 1.24], respectively).

Bivariate testing also showed that sleep initiation issues were not associated with the total HDI scores (ranging between -1 and 8; 8 being very adherent) in the full cohort (having sleep initiation issues vs. not having: 3.56 ± 0.20 vs. 3.52 ± 0.06 points; p=0.84) as well as in the adequate reporters (having sleep initiation issues vs. not having: 3.53 ± 0.24 vs. 3.37 ± 0.08 points; p=0.53). None of the HDI components were eligible for inclusion into the MVLR model in the whole cohort (all p≥0.2 on bivariate tests). However, when focusing on the subgroup of adequate reporters, bivariate tests showed a possible association with sleep initiation issues for the components “0-12% energy from SFAs” (p=0.08; χ² test) as well as “50-70% energy from total carbohydrates” (p=0.10; χ² test). MVLR analyses adjusted for selected possible confounders, including either of these components, showed no association between the HDI component and sleep initiation issues (OR [95%CI]; 1.59 [0.81, 3.12] and 1.41 [0.71, 2.80], respectively).

Lastly, no associations were found between the total HDI scores and sleep maintenance issues in the entire cohort (having sleep maintenance issues vs. not having: 3.54 ± 0.13 vs. 3.52 ± 0.06 points; p=0.88; unpaired t-test). These findings were confirmed in the subgroup of adequate reporters (having sleep maintenance issues vs. not having: 3.45 ± 0.18 vs. 3.38 ± 0.08 points; p=0.73; unpaired t-test). In the full cohort, the HDI components “5-10% energy from PUFAs”, “10-20% energy from protein”, “50-70% energy from total carbohydrates”, and “≥3 g/MJ fiber” were indicated to possibly be associated with sleep maintenance issues (all p<0.2 on bivariate tests). None of these variables reached significance in the covariate-adjusted MVLR models (OR [95%CI]; 0.72 [0.51, 1.03], 0.82 [0.58, 1.16], 1.28 [0.88, 1.87], and 1.12 [0.78, 1.61], respectively). Only considering the adequate reporters, only “50-70% energy from total carbohydrates” reached significance on the bivariate test with sleep maintenance issues (p<0.2) but was not significantly associated with sleep maintenance issues when adjusting for possible confounders (OR [95%CI]; 1.38 [0.84, 2.24]; MVLR).
Discussion

In the overarching experimental study, 47 young men and women participated in one night of sleep and one night of total sleep deprivation. During the sleep deprivation night, a battery of tests assessed the overnight performance. Additionally, blood samples were drawn in the morning following both conditions, and a facial exploration task was performed using eye-tracking. This wide array of tests helped shine a light on areas possibly affected by sleep loss: social appearance and exploration, overnight performance, and metabolism.

For Paper I, the main aims were to investigate if sleep-deprived people visually explore other people's faces differently and if angry, happy, scared, and neutral faces differ in their social evaluation if the observer is sleep-deprived. This study showed that participants spent less time fixating on faces after sleep loss. This could be due to a sleep loss-induced decrease in attention\(^\text{160}\) and an increase in sleepiness\(^\text{161}\). Additionally, sleep deprivation impairs the drive to engage in any behavior that does not contribute to initiating sleep\(^\text{161}\), which could also explain why the brain is less driven to explore the different facial expressions, irrespective of whether an emotional or neutral face was shown.

When focusing on the separate parts of the face, sleep loss decreased the time spent focusing on the upper parts of the face, including the eyes. This reduction was not found for the lower parts of the face except in angry faces. As the eyes and the mouth are the most potent cues for identifying facial expressions\(^\text{44}\), the decreased fixation time on the eyes (and mouth of angry faces) could be the basis of why sleep-deprived people struggle with assessing emotionally expressive faces\(^\text{46,47}\).

Sleep deprivation also altered the evaluation of the different facial expressions, as angry faces were perceived as less trustworthy. Furthermore, healthy, neutral, and fearful faces were rated as less attractive. However, faces showing happy expressions did not receive a different evaluation following sleep loss. This finding is in line with results from a study investigating chronic sleep restriction. They found that people with less positive and more negative facial features were evaluated as more dangerous when the reviewer was sleep-deprived, but not after sleep\(^\text{162}\). The impairment in emotional identification and a more negative judgement could highly impact the functioning of people who
often suffer from compromised sleep and rely heavily on a quick assessment of other people’s emotional state, like police and security officers. Additionally, the sleep-loss-induced negativity bias could increase social withdrawal, in the long run leading to loneliness and depression. 

**Paper II** aimed to unravel the effects of overnight wakefulness on subjective and objective alertness and if this differs between men and women as well as normal-weight and obese people. Additionally, possible sex and weight differences in the brain health marker response to sleep loss were explored. The analyses demonstrated that women felt sleepier than men during overnight wakefulness and struggled more to stay awake, as indicated by a longer total time spent in microsleep. Moreover, women but not men experienced higher levels of NfL in the morning following sleep deprivation than after sleep. This marker is indicative of neuro-axonal damage. Comparative analyses between the weight groups revealed that participants with obesity had a longer time spent unintentionally asleep during the sleep deprivation night than participants with normal weight. Furthermore, the sleep-loss-induced increase in pTau181 levels, a predictor for brain atrophy, was higher in participants with obesity than in normal-weight participants.

A recent systematic review on the relation between chronic shift work and cognitive performance suggests that circadian rhythm desynchronization, lack of sleep, and fatigue resulting from night work may negatively impact workers’ cognitive efficiency. Similar findings come from a meta-analysis, where acute sleep loss (between 24 and 48h) substantially decreased cognitive performance over multiple domains, including attentional lapses. A separate study of sixty male control room shift workers found that those working the night shift made more omission and commission errors and experienced more subjective sleepiness during the shift than those working the day shifts. Additionally, those working nights also had a lower subjective sleep quality than the daytime workers.

Though some studies include both sexes, few studies focus on possible sex differences in night-time cognitive performance. One study, including 18 women and 16 men, found that women had a more substantial impairment in cognitive performance in the early morning hours of overnight wakefulness compared to men. Yet, both groups felt equally sleepy throughout the night. In contrast, the women in the present study experienced higher levels of sleepiness than men, but there were no sex differences in cognitive performance. As the women in this study were all using hormonal contraceptives, opposite to the previous study where half of the included females were free-cycling, this could possibly elucidate the discrepancy in findings. Namely, attentional failure during a 30h wakefulness protocol varied significantly over the different phases of the menstrual cycle.
Women are at a higher risk of developing Alzheimer’s than men and can already show changes in brain health markers up to 10 years before diagnosis. Hence, the present study aimed to unravel possible sex differences in brain health markers following one night of sleep loss. Women but not men showed an increase in NfL, possibly indicating a higher stress load on the female brain due to forced wakefulness. However, no correlations existed between the subjective sleepiness or microsleep duration and NfL levels.

Night shift workers have a 1.2 times higher risk of becoming overweight or obese than people that work during the day. Additionally, people with obesity experience a higher degree of cognitive impairments and have a higher risk of dementia. However, the present study is among the first to investigate how people with obesity are affected in their overnight cognitive performance compared to their normal-weight counterparts. Although no weight differences were detected regarding sleepiness and vigilance, participants with obesity spent more time unintentionally asleep during a sustained wakefulness task. This indicates that non-demanding wakefulness periods during the night could be more challenging for this group. The more pronounced increase in pTau-181 in obese participants following the sleep-deprivation night did not correlate with the time spent unintentionally asleep during the night. Still, these findings could pinpoint a higher sleep pressure in participants with obesity. It is known that obesity is associated with a higher risk of developing SDB, and could lead to fragmented non-restorative sleep. Although not measured in the night preceding the sleep-deprivation condition, participants with obesity did have higher ODI scores during their sleep condition. The higher ODI and therewith possibly less-restorative sleep could indicate a higher sleep pressure before the wake condition's onset. Of note, the ODI scores and pTau-181 levels were not significantly correlated in this subgroup. Low-grade inflammation, as often occurs in obesity, could be a different explanation for the increase in sleep pressure. However, the higher sleep pressure was not reflected in the subjective sleepiness levels of the participants with obesity.

Lastly, the found rises in NfL and pTau-181 following overnight wakefulness could be due to compromised glymphatic activity and increased physiological stress on the brain. However, future studies should investigate if chronic sleep problems or night shift work lead to more pronounced alterations in brain health markers and cognitive performance in women and people with obesity.

Sleep-loss effects on the hormonal regulation of the energy balance and possible sex and weight differences herein were the main focus of Paper III. Here, it was found that key hormones for appetite regulation were altered following a night of sleep loss. Where the satiety-promoting leptin decreased after the
sleep deprivation night, blood levels of the hunger-promoting ghrelin and adiponectin increased. Adiponectin has both protective properties, including increasing insulin sensitivity and anti-inflammation\textsuperscript{96,97}, but also promotes lipid storage in adipocytes and increases food intake, as shown in rodent studies\textsuperscript{99,174}. It is unknown if the herein observed hormonal shift towards promoting EI would also lead to an increased food intake behavior and, after more chronic sleep loss, could result in weight gain\textsuperscript{107,108}.

When stratifying the analyses by sex, women experienced a more prominent decrease in leptin and a slight increase in adiponectin following sleep deprivation. However, as the interaction between sex and the sleep condition was not significant, these results should be interpreted as hypothesis-generating only. A previous study showed that a four-day short sleep schedule increased total ghrelin levels in men but not in women following the short sleep period\textsuperscript{116}. In turn, glucagon-like peptide 1, a satiety-promoting hormone, was decreased in women but not men\textsuperscript{116}. These results might indicate that there might be different mechanisms for shifting the energy balance towards a higher energy intake in men and women, but this needs to be confirmed in future studies.

The link between chronic sleep loss, weight gain, and obesity is well established. In this study, there were some indications that participants with obesity had a more substantial increase in ghrelin levels following sleep loss. Still, no interactions between weight and sleep condition could confirm this.

In summary, one night of sleep loss altered the hormonal regulation of the energy balance favoring energy intake, thereby possibly leading to weight gain. More studies are needed before any conclusions can be drawn regarding the indicated sex and weight differences in these hormone alterations.

As discussed before, acute sleep loss can lead to behavioral and endocrine changes in favor of poor dietary choices. However, it is not well-researched whether the quality of a person's diet may impact sleep. Therefore, \textit{Paper IV} investigated possible associations between subjective sleep disturbances and the adherence to healthy dietary patterns using the ULSAM dataset including 970 older men. This study concluded that adhering to the MD or HDI was not associated with less sleep initiation and sleep maintenance problems in 70-year-old men. However, adhering to a low intake of milk and dairy products, an MD compound, decreased the odds of having sleep initiation problems.

These findings add to several studies investigating MD adherence and sleep quality. Similar to these null findings in older men, one study in 5,886 older adults found that women, but not men, had fewer sleep problems when better adhering to the MD\textsuperscript{133}. In contrast, some studies with both men and women
found a positive association between MD adherence and sleep quality, sleep duration, or both\textsuperscript{131,132}. Following the dietary recommendations of the World Health Organisation, HDI adherence was also not associated with better sleep in the present cohort. This was surprising, as some of the recommendations, such as a high fiber intake and a low saturated fat and sugar intake, are associated with more restorative sleep with fewer arousals in middle-aged adults\textsuperscript{175}.

One of the components of the MD, a low intake of milk and dairy products, was linked to a decrease in sleep initiation issues. Particularly hot milk has the reputation of improving sleep, but these effects could not be substantiated by scientific evidence\textsuperscript{176,177}. The association found in the ULSAM cohort could be explained by beta-casein proteins, which are naturally present in cow milk and could cause milk intolerance. These proteins can give gastrointestinal problems, which comes with pain and discomfort\textsuperscript{178} and lead to sleep initiation problems\textsuperscript{179}. However, it needs to be kept in mind that the association between low milk and dairy intake and a decrease in sleep initiation problems could not be confirmed in the subgroup of adequate reporters, further stressing the importance of adequate assessment of food reports.
If I waited for perfection,  
I would never write a word.

-Margaret Atwood
Reflections and future perspective

*Paper I* to *III* and the overall experimental study, as well as the epidemiological investigation of *Paper IV* hold various strengths, limitations, and lessons and wishes for the future, which will be discussed in this chapter.

The strength of the overall experimental study lies in the highly-controlled conditions, which helped to differentiate the effects of sleep loss from possible interferences or moderators (like caffeine/food during the night and ambient light exposure) on the different outcomes of interest. Including both men and women in the study makes the findings more publicly relevant, and possible sex differences could be assessed. Furthermore, as all women were on monophasic hormonal contraceptives, potential confounding from the menstrual cycle could be reduced. Natural hormonal fluctuations during the menstrual cycle are known to impact sleep\textsuperscript{16,17} but also influence f.e. facial exploration and evaluation\textsuperscript{143,180,181}, as well as the energy balance\textsuperscript{182}. However, hormonal contraceptive use lies between 30-45\% among women in the fertile age range in the Nordic countries\textsuperscript{183}. Therefore, the findings in *Paper I* to *III* cannot be extrapolated to free-cycling women and call for further investigation in these groups to better understand how these women are affected by sleep loss throughout the cycle and how to mitigate this\textsuperscript{184}.

Only young, healthy adults without nightshift work experience were included in the experimental study and were exposed to a single night of sleep deprivation. The naivety to night shift work is a strength as it prevents possible built-up impairments due to night work ahead of the experimental study that could mask or amplify study outcomes. However, future studies are needed to investigate how the different study outcomes are affected by chronic sleep deprivation and/or night shift work in both laboratory and real-life environments. Additionally, participants were fasting and mainly sedentary during the sleep deprivation night. Night workers are known and recommended to eat, drink (e.g. caffeinated beverages), and take naps if possible during the shift\textsuperscript{185,186}. These factors can impact performance and alertness during the night shift\textsuperscript{185,186}. Thus, studies in real-life environments with long-term shift workers are warranted to investigate the translatability of the findings to daily practice.
Participants with obesity (and those with normal weight) all reported good sleep quality and duration and did not have any medical diagnosis nor were receiving (pharmaceutical) treatment at the onset of the study. However, although these participants were seemingly healthy, the ODI during sleep was slightly higher in participants with obesity than in those with normal weight, possibly pointing towards sleep apnea in this group. Of note, many people with OSA remain undiagnosed due to a lack of visible symptoms (e.g., daytime sleepiness, decreased vigilance)\(^{187}\).

In the current experimental study, blood was only sampled at one timepoint in the morning after both the sleep and the sleep deprivation condition. Therefore, possible changes or circadian fluctuations of the blood markers during the sleep deprivation night or later in the day could not be assessed. Additionally, it was not tested if there were any behavioral changes following the alterations in the hormones regulating the energy balance due to sleep loss. Thus, whether these hormone changes lead to increased food intake and weight gain remains unknown.

The epidemiological study of *Paper IV* only included 70-year-old men, which limits the translatability of the findings to women and other age groups. Because of the cross-sectional design, inverse causation for the association between low milk and dairy intake and less sleep initiation problems cannot be ruled out. Thus, follow-up studies to assess dietary adherence and sleep parameters over a longer period of time or dietary intervention studies with follow-up could further disentangle the diet-sleep association and unravel causal relationships. In ULSAM, no objective sleep assessments were performed, such as polysomnography or sleep wearables. Additionally, no standardized questionnaires were used to map the severity and frequency of sleep problems. However, the analyses conducted in *Paper IV* were controlled for a wide range of potential confounders, including smoking, hypertension, and diabetes. Moreover, food diaries and nutritional epidemiological studies are prone to misreporting, primarily by over and underreporting food intake\(^{188}\). To overcome this issue, the Goldberg criterion\(^ {155}\) was applied in this study. By these means, around half of the cohort was identified to have misreported their energy intake. Additionally, the association between low dairy intake and less sleep initiation problems was significant in the whole cohort but not in the subgroup of adequate reporters. This highlights the importance of assessing adequate reporting before any possible conclusions can be drawn.

Reflecting on the experimental study of *Paper I to III*, there are several things I would like to have planned and set up differently. Although this study is one of the more extensive studies investigating sleep loss in a within-subject experimental setting to date, an increased number of participants would increase the power to give more certainty about the detected null findings and group
differences. Unfortunately, time and logistical boundaries, as well as the pandemic that delayed the study for more than six months, made this impossible. A Fitbit or other sleep wearable would also be helpful to objectively monitor participants' adherence to the study instructions before and in between the experimental sessions and to better estimate their sleep-wake behavior and activity patterns. It would also be exciting to replicate this study in normal-weight and obese men and women suffering from sleep problems or those with night shift work experience. This could give a better view of how long-term circadian desynchronization and short sleep affect social appearance and exploration, brain health, metabolism, and diet. As discussed, including women in all phases of their menstrual cycle is necessary, and better methods are needed to confirm these phases during experimental studies. Alternatively, increasing the number of blood samplings before, during the sleep deprivation night, during the day post-session, and after recovery sleep would be valuable. This way, circadian variation, sleep-loss-induced alterations, and recovery of hormone levels and brain health markers could be mapped. Lastly, changing the static facial images in Paper I to a virtual reality world, where participants could encounter people with different expressions would also allow the investigation of visual exploration and behavior in a more real-life setting. Additionally, this would enable measuring bodily responses to the stimuli (e.g., backing off vs. approaching, skin conductance due to stress).

Unfortunately, in science, we are tightly bound by the logistical and financial resources available, making me aware that many of the ideas that started brewing from conducting and evaluating these studies might never come to light. Hence, this can only enable me to call for agencies and institutions to increase their funding of experimental studies and longitudinal investigations with thorough sleep assessments (e.g., standardized questionnaires and objective measures). This would help young and experienced researchers to disentangle sleep in various cohorts and test implementation of scientific findings. In this way, we could optimize and personalize healthcare to help people sleep and thus function better.


Den experimentella delen av studien inkluderade unga friska vuxna män och kvinnor. Deltagarna hade antingen en hälsoam vikt eller var kraftigt överviktiga (fetma). En natt fick deltagarna sova över i sömnlabbet, andra natten höll de sig vakna. Olika tester genomfördes under natten och morgonen och därefter samlades blodprover in.


vilket tyder på en möjlig högre stressnivå i kvinnors hjärna. Överviktiga personer hade också högre biomarkörvärden än personer med en hälsosam vikt.

Del III undersökte inverkan av sömnbrist på hormoner som är involverade i reglering av matintag. Vår forskning fann att nivåerna av de aptitstimulerande hormonerna ghrelin och adiponektin var förhöjda efter sömnbrist medan det aptitdämpande hormonet leptin faktiskt minskade. Detta kan leda till ökat matintag och eventuell viktökning på längre sikt. Dessa hormonella förändringar var störst hos kvinnor (jämfört med män) och överviktiga personer (jämfört med friskviktiga).

Sömnbrist påverkar inte bara reglering av matintaget, våra kostvanor har också inverkan på sömnen. Därför studerade vi under del IV sambandet mellan att upprätthålla en hälsosam kost och sömnproblem hos äldre män, som oftare lider av sömnproblem. Vi hittade inget samband mellan att följa en hälsosam kost, såsom medelhavsdieten, och färre sömnproblem i denna grupp. Mer kontrollerade studier behövs för att avgöra om matvanor kan påverka sömnen hos äldre män.
Samenvatting (Summary in Dutch)


Dit onderzoek omvat een experimenteel (deel I, II & III) en epidemiologisch gedeelte (deel IV). De focus lag op de gevolgen van slaapgebrek: de invloed op het beoordelingsvermogen van anderen, en de alertheid en breingezondheid gedurende de nacht. Ook keken we naar de invloed op onze energiebalans en het verband tussen slaapproblemen en gezonde voedingspatronen.

Het experimentele gedeelte van het onderzoek omvatte ogenschijnlijk gezonde jongvolwassen mannen en vrouwen. De deelnemers hadden ofwel een gezond gewicht of zwaar overgewicht (obesitas). Één van de nachten sliepen de deelnemers op het slaaplaboratorium, de andere nacht bleven ze wakker. Gedurende de nacht en in de ochtend werden diverse testen uitgevoerd en werd er bloed afgenomen.

Uit deel I van het onderzoek blijkt dat slaaptekort ervoor zorgt dat je minder goed kijkt naar andermans gezichten en deze ook negatiever beoordeeld. Na de slaaploze nacht vonden deelnemers mensen met boos gelaat minder betrouwbaar en minder gezond. Mensen met een angstig of neutraal gezicht werden gezien als minder aantrekkelijk. Slaaploze deelnemers konden zich bovendien ook minder lang focussen op de verschillende gezichten. Gezichtsexpressies zijn cruciaal om de emotionele toestand van anderen te begrijpen en hierop te kunnen reageren. Slaapgebrek kan er dus toe leiden dat de emoties van anderen verkeerd of te laat worden geïnterpreteerd.
In deel II onderzochten we hoe je presteert als je gedurende nacht wakker blijft. Ook keken we of hier sekse- en gewichtsverschillen in bestaan. Vrouwen voelden zich vermoeider dan mannen na de slapeloze nacht, maar we vonden geen sekse verschillen in reactievermogen. Toen de deelnemers tijdens een korte test geacht werden om zonder stimulans wakker te blijven, zagen we dat vrouwen sneller wegdoezelden dan mannen. Ook sliepen de deelnemers met obesitas onbedoeld meer dan zij met een gezond gewicht. In de ochtend werden biomarkers gemeten die een idee geven over de gezondheid van het brein. In vergelijking met mannen hadden vrouwen hogere biomarkerwaardes door het slaaptekort, wijzende op een mogelijk hoger stressniveau in het brein van vrouwen. Mensen met obesitas hadden ook hogere biomarkerwaardes dan mensen met een gezond gewicht.

In deel III is gekeken naar de invloed van slaapgebrek op hormonen die betrokken zijn bij het reguleren van de voedselinname. Uit ons onderzoek bleek dat de niveaus van de eetlustopwekkende hormonen ghreline en adiponectine verhoogd waren na slaapgebrek. Het eetlustremmende hormoon leptine was juist verminderd. Dit zou kunnen leiden tot een verhoogde voedselinname en mogelijk ook gewichtstoename op de langere termijn. Deze hormonale veranderingen waren het grootst bij vrouwen (in vergelijking met mannen) en mensen met obesitas (in vergelijking met mensen met een gezond gewicht).

Slaaptekort beïnvloedt niet alleen de regulering van voedselinname, maar wat we eten beïnvloedt ook onze slaap. Daarom bestudeerden we in deel IV de relatie tussen het aanhouden van een gezond voedingspatroon en slaapproblematiek in oudere mannen, die vaak last hebben van slaapproblemen. Wij vonden geen verband tussen het volgen van een gezond voedingspatroon, zoals het Mediterraans dieet, en minder slaapproblemen in deze groep. Meer gecontroleerde onderzoeken zijn nodig om vast te stellen of eetpatronen de slaap bij oudere mannen kunnen beïnvloeden.
Find a group of people who challenge and inspire you, spend a lot of time with them, and it will change your life forever.
- Amy Poehler
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