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The Pulse of Sleep: Sleep and Cardiometabolic Health

By

Vyoma D Shah

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Psychology

in the

Graduate Division

of the

University of California, Berkeley

Committee in Charge:

Professor Matthew P. Walker, Chair
Professor William Jagust
Professor Lance Kriegsfeld
Professor Aric Prather

Fall 2023

The Pulse of Sleep: Sleep and Cardiometabolic Health

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by

Vyoma D Shah

Abstract

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Doctor of Philosophy in Psychology

University of California, Berkeley

Professor Matthew Walker, Chair

Two of the top killers of the human race are cardiovascular disease and type 2 diabetes. These diseases, which have shared etiology, collectively form the domain of cardiometabolic health. Multiple functions of cardiometabolic health are perturbed by impaired sleep, and conversely, restored by increased sleep quantity and/or improved sleep quality. That is, impairments in sleep are closely related to impairments in cardiometabolic health. This leads to the proposition that specific features of sleep act as biomarkers for specific features of cardiometabolic health. This thesis aims to determine the impact of sleep on three major domains of cardiometabolic health: 1) atherosclerosis (arterial plaque accumulation), 2) dyslipidemia (unhealthy blood lipids), and 3) hyperglycemia (high blood sugar). Accordingly, three key findings emerge that comprise the three chapters of this report. 1) The quality of human sleep, specifically the degree of fragmentation, raises inflammatory-related white blood cells, thereby conferring an increased risk for atherosclerosis. This was true of sleep fragmentation assessed across a week or a single night, which predicted increasingly higher CAC scores through a mediating association with increased neutrophils. 2) Unique features of an individual's sleep (duration, efficiency, timing, regularity) predict aspects of triglyceride metabolism under fasting (homeostatic) and post-food intake (allostatic) conditions. First, later sleep timing predicts higher fasting triglycerides, emphasizing a unique relation with triglyceride homeostasis (not allostasis). Second, this homeostatic link is mediated by increased systemic inflammation (GlycA levels). Third, and conversely, declining sleep efficiency negatively impacts post-food lipid clearance, reflecting allostatic regulation. Fourth, these effects are replicated in a second independent cohort. 3) The coupling of NREM sleep spindles and slow oscillations the

night prior is associated with improved next-day peripheral glucose control. Further, this sleep-associated glucose pathway may influence glycemic status through altered insulin sensitivity, rather than through altered pancreatic beta cell function. Moreover, these associations are replicated in an independent dataset of over 1900 adults. Of therapeutic significance, the coupling between slow oscillations and spindles was the most significant sleep predictor of next-day fasting glucose, even more so than traditional sleep markers, relevant to the possibility of an EEG index of hyperglycemia. Collectively, beyond these scientific insights, these results support a sleep–cardiometabolic homeostasis framework, such that unique features of sleep act as biomarkers for unique aspects of cardiometabolic health. Importantly, these findings help reinform public health sleep-related guidelines to reduce the mortality and economic burden of cardiometabolic disease globally.

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General Introduction

Two of the top causes of human mortality are cardiovascular disease and type 2 diabetes [1]. Every year, cardiovascular disease (ischemic heart disease and stroke) is responsible for over 1 in 4 deaths globally, claiming over 14 million lives [1–3]. Concurrently, diabetes is an increasingly concerning worldwide epidemic. The number of deaths resulting from diabetes is steadily increasing, up 70% over the last two decades. Every year, diabetes costs the global economy over 6.7 million lives [4] and $966 billion [5]. Importantly, cardiovascular disease and diabetes mellitus are not completely independent. Rather, they are often co-morbid, with a subset of shared underlying risk factors and core pathological mechanisms [6,7], and can influence and exacerbate each other [8]. Indeed, hyperglycemia causes 1 in 5 cardiovascular deaths [9]. Taken together, these diseases form the broader, multifaceted disease category of cardiometabolic disease - a leading cause of death, and grave public health concern. A deeper, nuanced understanding of the different aspects of cardiometabolic health is crucial to developing effective therapeutic interventions and minimizing preventable deaths.

Today, there is a plethora of causal and associational studies implicating poor sleep as a contributing factor to ill cardiometabolic health [10]. Ill cardiometabolic health is multifaceted, with numerous intermingling components. Of these components, three cornerstones are: 1) atherosclerosis - the build-up of plaque in the walls of arteries, 2) lipemia - abnormal levels of lipids in the bloodstream, a contributing factor to atherosclerosis, and 3) irregular glucose metabolism - principal to maintaining blood sugar homeostasis in the bloodstream, and a causal factor for cardiovascular disease. Sleep has been associated with each of these three pillars of ill cardiometabolic health. However, given the multifactorial nature of sleep, as well as the high dimensionality of cardiometabolic health, multiple fundamental questions remain unanswered.

First, poor sleep quality leads to atherosclerosis. Yet, the potential pathways explaining this association remain a mystery. Previous studies have linked poor sleep to impaired immune function [11], and inflammation to increased risk of cardiovascular events [12,13]. Therefore, one candidate pathway through which fragmented sleep causally triggers cardiovascular disease is via the up-regulation of inflammatory-associated white blood cells, which incite atherosclerosis. Yet, this proposition remains untested in humans.

Second, varied macro aspects of sleep such as short sleep duration and obstructive sleep apnea are linked to impaired lipid profiles [14,15], and resultantly, myocardial infarction, heart failure, stroke, and atherosclerosis A common etiology that is a pathway to these cardiovascular diseases, is that of elevated triglycerides. However, the underlying mechanisms, and the specific features of an individual's objective sleep, that impair triglyceride metabolism remain to be understood. Current studies examining the associations between sleep and triglyceride regulation are cross-sectional and do

not control for multiple major risk factors already known to influence lipid profiles, such as meal content, meal timing, and genetic contributions. Furthermore, despite the understanding that different mechanisms regulate fasting versus post-meal lipid profiles [16], it remains untested which specific features of sleep govern the homeostatic regulation of lipid metabolism in the fasted state, versus the allostatic regulation of lipid metabolism in the postprandial state.

Third, a lack of sleep impairs glucose control and increases the risk of diabetes in humans [17], but why? Despite the broad clinical, therapeutic, and public health implications, the underlying sleep-related central brain mechanism explaining this impaired glucose regulation remains unknown. Specifically, examining the role of macro sleep features in regulating metabolism, and how electrical brain oscillations during sleep are associated with metabolic glucose homeostasis in humans is currently untested.

Despite these overarching links between sleep and cardiometabolic health, at least three key unanswered questions have yet to be addressed, focused on 1) atherosclerosis (arterial plaque accumulation), 2) dyslipidemia (unhealthy blood lipids), and 3) hyperglycemia (high blood sugar). Building on these data, this thesis sought to address these three fundamental issues, formulated in three independent hypotheses, each assessed in three independent studies:

- Hypothesis 1 (Study 1): The impact of fragmented sleep on atherosclerotic pathology is governed, in part, through the novel mediating influence of increased inflammation (as measured by neutrophil and monocyte levels). Furthermore, this sleep-related disease pathway is robust when multiple alternate cofactors (disease mechanisms) known to impact cardiovascular health are being controlled for (Chapter 1).

- Hypothesis 2 (Study 2): Sleeping at a later time is associated with the homeostatic dysregulation of fasted triglyceride levels. Lower sleep efficiency predicts the allostatic dysregulation of post-meal triglyceride levels. Further, these sleep-related associations hold true for VLDL cholesterol (high in triglyceride composition), and remain robust after accounting for risk factors known to influence lipid metabolism (Chapter 2).

- Hypothesis 3 (Study 3): A function of synchronized (i.e., temporally coupled) NREM slow oscillation—sleep spindle events in humans is the brain-body regulation of optimal glucose homeostasis. More specifically, both the extent and quality of coupled NREM slow oscillations—spindle events in humans predict optimal next-day regulation of peripheral blood glucose levels (Chapter 3).

Chapter 1. The heart of the matter: Broken sleep predicts hardened blood vessels

Introduction

Sleep disruption is associated with atherosclerosis. Why is this? One potential pathway through which fragmented sleep causally triggers cardiovascular disease is via the upregulation of inflammatory-associated white blood cells, which incite atherosclerosis [18,19]. However, the proposition that sleep fragmentation in humans is associated with atherosclerosis through the mediating influence of increased neutrophil and monocyte counts remains unexplored [20–22]. Moreover, that such a pathway is evident even when accounting for common contributing factors leading to atherosclerosis in humans, such as age, sex, ethnicity, BMI, smoking status, blood pressure, use of antihypertensive medication, sleep apnea, and insomnia, is untested.

Here, we address these unresolved questions. Specifically, we tested the hypothesis that the impact of fragmented sleep on atherosclerotic pathology is governed, in part, through the novel mediating influence of increased neutrophil and monocyte levels, and furthermore, that this sleep-related disease pathway remains robust when controlling for multiple alternate cofactors (disease mechanisms).

To do so, we examined the association between sleep fragmentation (measured using two independent sources of objective data: polysomnography (PSG) and multiple nights of wrist-based actigraphy), white blood cell count, and in vivo measures of subclinical atherosclerosis in a diverse sample of the population (n=3305).

The characteristics of the cohort, stratified by atherosclerosis severity category, are shown in **Table 1**, with the sleep parameters of the cohort, presented in **S1 Table**. The unadjusted bivariate correlation described in the next paragraphs are shown in **S1 Fig**.

Results

Actigraphy

Focusing first on *direct* associations (prior to testing the mediation hypothesis and the inclusion of cofactors), actigraphy-measured sleep fragmentation positively and significantly predicted CAC score ($r=0.18$, $p<0.001$; **Fig 1A** and **S1 Fig**). Second, this same objective measure of sleep fragmentation positively predicted higher neutrophil count ($r=0.08$, $p<0.01$), and was not significantly correlated with monocyte count

(r=0.04, p=0.17). Third, both neutrophil and monocyte counts were positively associated with the CAC score (r=0.12, p<0.001 and r=0.14, p<0.001, respectively).

Having established each individual direct association, we next tested the hypothesis that the relationship between sleep fragmentation and atherosclerosis pathology (CAC score) was not direct, but instead, statistically influenced through the *indirect* mediating impact of fragmented sleep on raised neutrophil count, which in turn, predicted CAC score. Supporting this proposed pathway, the impact of sleep fragmentation on CAC scores was significantly mediated through the indirect pathway of raised level of neutrophils (n=1110, β=0.71, 95% CIs=0.18-1.65).

Thus, sleep fragmentation was associated with atherosclerosis risk, yet this relationship was, in part, indirectly contributed to through the influence of fragmented sleep quality on increased neutrophil count. Consistent with the lack of a significant pairwise association between sleep fragmentation and monocytes, there was no indirect effect with monocyte count (β=0.35, 95% CIs=-0.11-1.14), potentially suggesting a greater mediating role of neutrophil activity.

Numerous factors to date have been demonstrated to increase atherosclerotic risk, including age, sex, ethnicity and BMI [23–25] as well as sleep-related features, including the presence of sleep apnea [26] and insomnia [27]. Importantly, the above mediation effect remained significant when controlling for the factors of age, sex, ethnicity, BMI, smoking status, blood pressure, use of antihypertensive medication, as well as sleep apnea and insomnia diagnoses (β=0.44, 95% CIs=0.02-1.27; **Fig 1A and Supplementary Methods**). The mediation also remained significant when excluding participants with a CAC score of zero (n=746, β=1.05, 95% CIs=0.27-2.52), and in this more select cohort, again showed a significant mediation after controlling for all the above-mentioned covariates (β=0.85, 95% CIs=0.11-2.36). Related, the mediation similarly remained significant when excluding 104 participants diagnosed with sleep apnea (β=0.64, 95% CIs=0.11-1.71, controlled for all above covariates).

While in the same direction, the mediation effect was not statistically significant when adjusting for sleep apnea using the apnea-hypopnea index (AHI) estimated from the PSG night (β=0.35, 95% CIs=-0.02-1.18). This indicates that sleep apnea cannot be excluded as a contributing factor in the mediation. However, post-hoc analysis using AHI instead of sleep fragmentation as the exposure variable did, however, demonstrate that there was no indirect effect of AHI on CAC via an increase in monocyte/neutrophil counts (neutrophil: β=0.12, 95% CIs=-0.02-0.44; monocytes: β=-0.05, 95% CIs=-0.27-0.04). That is, sleep fragmentation, beyond AHI, appears to have a specific relationship with inflammatory-related increases in atherosclerosis.

Polysomnography

Having quantified the association between atherosclerosis and home-based sleep, measured using wrist-actigraphy, we further tested these same relationships using PSG-recorded sleep. Congruent with the actigraphy findings, the severity of PSG-measured fragmentation (arousals during NREM sleep), directly and positively predicted CAC score severity (r=0.14, p<.001). Once again, this association was indirect. Specifically, the impact of the PSG-measured arousal index in NREM fragmentation on CAC scores was mediated through raised levels of neutrophils (n=1046, β=0.42, 95% CIs=0.13-0.94) and raised monocytes (n=1046, β=0.32, 95% CIs=0.07-0.75). This effect was specific to NREM sleep, with no such significant associations with the arousal index measured during REM sleep (r=0.003, p=.92).

The PSG-based mediation effect with neutrophils remained significant after controlling for age, sex, ethnicity, smoking status, and blood pressure (β=0.19, 95% CIs=0.01-0.61; **Fig 1B**). However, unlike the actigraphy-based measures, the effect did not remain significant after adjusting for BMI, sleep apnea, insomnia, and use of antihypertensive medication. One interpretation is that one week of wrist-based actigraphy sleep measurement, relative to a single night of PSG sleep recording, is more capable of detecting the sleep-dependent link between neutrophils and atherosclerosis when considering relevant co-factors.

The indirect mediation effect with monocytes did remain significant after controlling for age and ethnicity (β=0.16, 95% CIs=0.01-0.49), but not after adjusting for sex, suggesting that the atherosclerotic impact of sleep fragmentation on monocytes (but not neutrophils) is partially regulated by sex.

It is noteworthy that the sleep-atherosclerosis measured using PSG was significant for both neutrophil and monocyte counts, while actigraphy-measured sleep only showed a significant mediation effect with neutrophils (β=0.36, 95% CIs=-0.11-1.14). This may suggest greater sensitivity of PSG measures in quantifying this atherosclerosis disease pathway with multiple inflammatory-related factors, while still appreciating the above PSG results concerning co-morbidities.

Subjective sleep

Having tested the association between atherosclerosis and *objective* measures of sleep, we tested for an equivalent relationship using *subjective* reports of sleep fragmentation. Self-reported sleep fragmentation was not associated with neutrophil count (r=0.009, p=0.77), monocyte count (r=-0.056, p=0.08), or CAC score (r=-0.042, p=0.11), and provided no indirect mediation effect of the association between white

blood cells and atherosclerosis (neutrophils: β=0.73, 95% CIs=-4.5-6.3, monocytes: β=-4.3, 95% CIs=-10.7-0.11). In addition, there was no direct or indirect effect of habitual daytime sleepiness (measured by the Epworth sleepiness scale, [28]) on CAC score. These findings suggest that, unlike objective assessments, self-reported sleep quality and daytime sleepiness do not offer statistically sensitive measures in the predictive mediation pathway between sleep, inflammation, and atherosclerosis.

Exploratory analyses

Finally, we tested whether other objective sleep parameters, beyond sleep fragmentation, were similarly associated with atherosclerosis via an elevation in neutrophil and/or monocyte counts. Specifically, we looked at both actigraphy and PSG measures of sleep quantity and quality.

Consistent with the above findings, PSG-defined WASO was indirectly associated with increased CAC through an increase in monocyte count (β=0.05, 95% CIs=0.01-0.13). Similarly, higher sleep efficiency (averaged across 7 days of actigraphy) negatively predicted a lower CAC score via a reduction in neutrophil count (β=-1.12, 95% CIs=-2.83--0.13). However, neither of these relationships remained significant after controlling for the above-mentioned cofactors. Thus, fragmented sleep, more than other sleep features, appears to be a particularly sensitive predictor of white-blood-cell mediated atherosclerosis.

Discussion

Together, these findings affirm a pathway in which the quality of human sleep, specifically the degree of fragmentation, raises inflammatory-related white blood cells, thereby conferring an increased risk for atherosclerosis. This was true of sleep fragmentation assessed across a week or a single night, which predicted increasingly higher CAC score through a mediating association with increased neutrophils.

To the best of our knowledge, these data are the first to report such an association with sleep fragmentation and subclinical atherosclerosis in humans. Our findings confirm recent seminal work in mice demonstrating that experimentally induced sleep fragmentation, associated with increases in blood levels of monocytes and neutrophils, results in larger atherosclerotic lesions [18,19]. Furthermore, these rodent data provide added mechanistic insight, such that sleep fragmentation reduced hypocretin levels in the hypothalamus, signaling bone marrow-related increases in the production of monocytes and neutrophils.

Advancing this research, we establish a sleep fragmentation—white blood cell—atherosclerosis association in a population-based sample of human adults, and demonstrate these effects remained robust when accounting for multiple other common atherosclerosis risk factors present in humans: age, sex, ethnicity, BMI, smoking status, blood pressure, use of antihypertensive medication, as well as sleep apnea and insomnia diagnoses. Finally, we show that this indirect pathway can be quantified with objective sleep metrics, either using one week of wristwatch actigraphy, or a single night of PSG recording.

Importantly, however, we demonstrate that this same disease sensitivity is not observed when using self-reported *subjective* sleep fragmentation or other metrics of sleep quantity and/or quality. This may be pertinent for clinicians and researchers in determining which sleep measures should be focused on in this context.

Though our statistical models remained significant after adjusting for age (in addition to other cofactors), this does not challenge the well-established and independent links between i) aging and increases in monocytes and neutrophils [29,30], ii) increases in atherosclerosis risk [31], and iii) decreases in sleep quantity and quality [32]. Rather, our findings simply indicate that the mediation relationship between sleep fragmentation, white blood cells and atherosclerosis persists when chronological age is considered.

Decreasing sleep duration and fragmented sleep are independently associated with an increased risk of atherosclerosis [33,34]. However, the pathways through which the impact of sleep impairment operates have remained largely unknown. Building on rodent models [18], our findings suggest that one candidate pathway through which sleep fragmentation can raise atherosclerotic risk in humans may be through raised levels of inflammatory-associated neutrophil and monocyte counts. This proposal is consistent with findings that insufficient sleep (acute and prolonged) triggers low-grade inflammation [11], decreases and increases in discrete immune factors, and enhances upstream signaling mechanisms of inflammation, including those regulated by monocytes [11]. Moreover, both monocytes and neutrophils have a recognized role in atherosclerosis, including the modulation of proatherogenic reactive oxygen species and neutrophil extracellular traps that encourage monocyte accumulation to the plaque site [12,13,35–38].

What it is about sleep fragmentation that triggers this inflammatory blood cell pathway continues to be defined, though it is known that sleep fragmentation inhibits hypocretin production in the hypothalamus, thereby promoting neutrophil production in the bone marrow [18]. Additionally, sleep fragmentation results in hypercortisolemia [39,40], which can prevent the inhibition of granulocyte macrophage colony-stimulating factor (GCSF) that otherwise limits neutrophil levels [41], and may therefore further increase neutrophil production [42,43].

In the broader context of public health, these data suggest that improved sleep continuity (i.e., lowering of sleep fragmentation) may offer a novel preventative strategy for lowering inflammatory status, and thus lowering relative atherosclerosis risk. More broadly, these findings could help inform public health guidelines that focus on societal sleep health, one benefit of which may be lowering atherosclerotic burden.

Limitations

A first limitation is that our analyses were constrained by the use of cross-sectional data, which precludes definitive assessment of the directionality of associations. For example, it could be that cardiovascular disease (or associated treatments) may also drive sleep fragmentation in addition to, or rather than, the other way around. Although post-hoc sensitivity analyses (Supplementary Results) indicated that the incorporation of measures of cardiovascular disease did not substantively alter the significance of mediation effects in our cohort, this possibility remains. Prospective longitudinal controlled studies will be needed to directly address the issue of reverse causality.

Second, it is important to note that while the indirect mediation pathways were statistically significant, the effect sizes of the pairwise associations were overall small. This suggests that raised inflammation (our a priori study focus) is likely one of several potential pathways through which insufficient sleep contributes to atherosclerosis. Other potential pathways include altered autonomic nervous system activity, increased oxidative stress, impaired glucose metabolism, and endothelial dysfunction [40,44–46]. While we were unable to explore each of these pathways, post-hoc analyses revealed that the mediation pathway was also significant when using heart rate variability (HRV) during sleep as the exposure variable in the mediation pathway—a well-established marker of the autonomic nervous system, instead of sleep fragmentation (see Supplementary Results). Sleep disruption is also associated with raised levels of apolipoprotein(Apo) B — a strong predictor of cardiovascular disease [47]; though see [48]. Still, our findings indicate that one potential atherosclerotic pathway in humans involves the influence of fragmented sleep on raised inflammatory-associated neutrophil and monocyte count.

The observed associations were not significant when adjusting for the AHI measured by PSG. Sleep apnea is well known to cause sleep fragmentation, and thus, these results are consistent with sleep apnea as a factor contributing to sleep fragmentation. While our post-hoc analyses suggest that AHI per se (independently of sleep fragmentation) is not significantly associated with the inflammatory-related increases in atherosclerosis, it is likely that apnea-induced cortical and autonomic arousals play a mechanistic role in this indirect association between sleep, leukocytes, and atherosclerosis.

Our reported mediation effect was stronger for neutrophils, relative to monocytes, and is consistent with a recent report demonstrating significant indirect associations between overnight heart rate, neutrophil counts and obstructive sleep apnea [46]. However, another limitation is that the current study was not powered or designed to differentiate these individual cell contributions. We propose three speculative, non-mutually exclusive, explanations for this stronger neutrophil relationship that may warrant future investigation. First, sleep disruption is linked to a larger relative increase in neutrophils as compared to monocytes [49]. Neutrophils may therefore be the more sleep-sensitive, and thus important, disease-related immune cell factor of this particular pathway. Second, neutrophils are more numerous than monocytes, making up 60 to 70 percent of the total white blood cell count. As such, a perturbation of white blood cells (for example, by sleep disruption) may lead to their influence being more pronounced. Third, the measure of neutrophil count is encoded as a continuous variable, whereas monocyte count is encoded as a quasi-categorical variable (see **Supplementary Figure 2**), which may reduce monocyte sensitivity.

While the current study accommodated for common comorbidities and co-factors (e.g., insomnia and sleep apnea diagnoses, obesity, sex, age, ethnicity, smoking status, blood pressure, and hypertensive medication), it must be recognized that this does not exclude the contribution of all possible comorbidity influences that may nevertheless be influential, and remain unaccounted for. We also cannot rule out the possibility that our findings may be influenced by selection bias. The original cohort consisted of individuals free of known cardiovascular disease. This may have led to an underrepresentation of individuals with early-onset cardiovascular disease. A small proportion (~2%) of individuals were excluded from the MESA sleep exam due to regular CPAP, oral appliance or oxygen use, thus, potentially reducing the representation of those with clinically significant sleep apnea. However, the participation rate in the MESA sleep study subset was high (~44%), and health profiles were generally similar between the participants who did enroll in the sleep study versus those who did not [24].

Taken together, our findings are consistent with the emerging idea of a pivotal role of neutrophils in atherogenesis [50], and establish that this association is in part mediated by sleep quality.

Methods

Ethics Statement

Institutional Review Board approval was obtained at each study site and written informed consent was obtained from all participants.

Procedure

The data were derived from the Multi-Ethnic Study of Atherosclerosis (MESA) Exam 5, [51], using information from its Exam 5 clinic exam and the MESA Sleep Ancillary Study, which included one night of home polysomnography (PSG), seven consecutive days of wrist actigraphy (Actiwatch Spectrum, Philips Respironics, Murrysville, PA), and a sleep questionnaire. All participants in the main MESA were invited to participate in the additional Sleep study at Exam 5, with the exception of those regularly using CPAP or an oral device for sleep apnea. The demographic characteristics of this subset of individuals in the sleep study relative to those of the overall full study cohort have been described elsewhere (see Table S1 in [24]).

The Multi-Ethnic Study of Atherosclerosis (MESA) is a multi-center prospective study of more than 6,000 ethnically diverse men and women aged 45-84 from six communities in the United States. MESA was designed to investigate the prevalence and progression of subclinical cardiovascular disease (CVD) as well as to identify CVD risk factors predicting the development of clinically overt CVD in an ethnically diverse population [51].

There have been four follow-up exams to date since the initial exam, in the years 2003-2004 (Exam 2), 2004-2005 (Exam 3), 2005-2007 (Exam 4), and 2010-2012 (Exam 5). The MESA 5 Core exam occurred from April 2010 to February 2012, 10 years after the initial exam. Similar to the prior follow-up exams, Exam 5 collected interval medical history, anthropometrics, blood pressure readings, fasting venipuncture, spot urine collection, nutrition and physical activity surveys, smoking history, ankle/arm index, retinal photography, and ECG. In addition, cardiac MRI was repeated in participants who underwent cardiac MRI at exam 1, and cognitive function testing was newly performed in all MESA Exam 5 participants. Randomly selected participants were invited to participate in the MESA ancillary study (70% of the MESA cohort), which performed cardiac CT imaging for measurement of CAC and carotid ultrasound for IMT measurement.

All MESA participants were also invited to participate in the MESA Sleep ancillary study at MESA Exam 5 (2010-2013). Sleep exams were scheduled to occur after the MESA 5 core exam. The purpose of MESA Sleep was to obtain quantitative measures of sleep and sleep-disordered breathing (SDB) to better characterize specific sleep traits and sleep disorders and their CVD risk associations across ethnic groups, as well as to determine the association of sleep indices with incident CVD. The sleep protocol included one night of home polysomnography (PSG), seven consecutive days of wrist actigraphy (Actiwatch Spectrum, Philips Respironics, Murrysville, PA), and a sleep questionnaire.

In-home polysomnography (PSG) was performed using the Compumedics Somte System (Compumedics LTd., Abbostville, Australia. The recording montage consisted of cortical electroencephalograms (C4-M1, Oz-Cz, and Fz-Cz leads), bilateral EOG, chin EMG, thoracic and abdominal respiratory inductance plethysmography (by auto-calibrating inductance bands); airflow (by nasal-oral thermocouple and pressure recording from a nasal cannula); ECG; leg movements, and finger pulse oximetry. EEG, EOG, EMG and ECG were all sampled at 256 Hz. Nocturnal recordings were transmitted to the centralized reading center at Brigham and Women's Hospital and data were scored by trained technicians using current guidelines [59,60].

Actigraphy was performed using the Actiwatch Spectrum wrist actigraph (Philips Respironics, Murrysville, PA) worn on the participant's non-dominant wrist. Output was sent to the Sleep Reading Center at Brigham and Women's Hospital where records were scored with the use of the corresponding sleep diary. Specifically, actigraphy data were aggregated in 30-second epochs and automatically scored as sleep or wake by a validated algorithm implemented in the Actiware-Sleep v.5.59 analysis software (Mini Mitter Co., Inc.), after manually editing the sleep period using sleep diary data and event and light markers. Two scorers scored MESA actigraphy studies. Intra-scorer reliability for average sleep duration, sleep efficiency, and WASO were 0.91, 0.97, and 0.91, respectively. Sleep fragmentation was defined as the sum percent mobile epochs and percent immobile bouts less than 1-minute duration to the number of immobile bouts, for the given interval. This is also known as the restlessness index or movement and fragmentation index.

White blood cells (WBC) were assessed in blood samples collected at Exam 5 at a central laboratory. Blood assays included total WBC count, and leukocyte subsets (basophils, eosinophils, neutrophils, lymphocytes, and monocytes) were determined as complete blood count with differential analysis.

Mediation models were adjusted for factors known to affect cardiovascular risk, specifically age, sex, race/ethnicity, body mass index (BMI; in kg/m2), smoking status, use of antihypertensive medication, blood pressure as well as medical diagnosis of sleep apnea and insomnia. Information on doctor-diagnosed sleep apnea/insomnia was obtained from the sleep questionnaire survey: "Have you been told by a doctor that you have any of the following:? a) Sleep Apnea (or obstructive sleep apnea, OSA) b) Insomnia". Those who answered 'yes' were defined as having doctor-diagnosed sleep apnea and/or insomnia, respectively. Smoking was defined as never, former (no smoking within the past 30 days), or current. Resting blood pressure was measured three times in the seated position and the average of the second and the third served as systolic and diastolic blood pressure.

Briefly, the subset of participants who enrolled in the sleep study were more likely to be younger, of non-White ethnicity, non-smoker, and normotensive as compared to the MESA participants who did not enroll. Self-report doctor-diagnosed sleep apnea and

other health characteristics (e.g. diabetes, obesity, myocardial infarction, asthma) were equivalent in both groups. White blood cell counts were assayed from blood from a morning blood draw the Exam 5 visit. Coronary Artery Calcification (CAC) imaging from Exam 5 provided an in vivo assessment of atherosclerosis, resulting in a standard Agatston score [52]. In-depth details of the study design, sleep evaluations, blood evaluations and CAC imaging can be found elsewhere [24,51–53].

Three validated markers of fragmented sleep were used as a priori predictor variables: 1) fragmentation index, which reflects the proportion of total sleep epochs characterized by movement, calculated separately for each night and then averaged across the seven nights of actigraphy, 2) the number of arousals per hour of NREM sleep (the arousal index, a measure that correlates with autonomic markers of arousal [54]), estimated during the PSG night [55], and 3) the participant self-reported sleep fragmentation (*"Overall, was your typical night's sleep during the past 4 weeks"*: 0 = very sound to 4 = very restless). Second, we conducted exploratory analyses with other sleep parameters, such as actigraphy- and PSG-defined measures of sleep quality and quantity (e.g. overall duration, wake after sleep onset (WASO), and, for the PSG night, percent time in each sleep stage and arousal index in REM sleep).

After removing participants with absent values on either the main predictor variables (i.e. objective and subjective measures of sleep fragmentation) and/or the main outcome variable (CAC score, or atherosclerosis Agatston score), the final sample size was 1630 participants (752 males, mean ± SD age=68.5 ± 9.2 yrs, Body Mass Index (BMI)=28.9 ± 5.5 kg/m^2) of diverse ethnicities (602 White, 451 Black, 393 Hispanic and 184 Asian). This sample represents 34.6% of all the participants included in MESA 5 core exam (n=4716), and 72.1% of all participants that also took part in the MESA 5 sleep exam (n=2261, of which 2060 participants had successful PSG data, 2156 had actigraphy data, and 2240 completed sleep questionnaires). For mediation analyses, the sample size was further reduced by removing participants with absent values on the mediator variable (e.g. the neutrophil count, final sample size, n=1110).

The hypothesis was tested using a formal mediation analysis with sleep fragmentation as the independent variable, monocyte and neutrophil counts as the mediator variables, and CAC score as the dependent variable. Specifically, the goal was to statistically determine whether monocyte and neutrophil counts could be deemed mediators of the effect of sleep fragmentation on CAC score. The relevant outcome of a formal mediation analysis is the indirect effect, which quantifies the difference between the effect of the independent variable on the dependent variable when the mediator is accounted for versus when it is not. Since both the mediators and dependent variables were continuous (**Supplementary Figure 1**), ordinary least squares regression was used to model direct and indirect associations. Mediation analysis was performed using the *mediation_analysis* function of the Pingouin statistical package for Python [56], modeled on the mediation R package [57]. As

recommended for mediation analysis reporting [58], all effects were considered significant only if the 95% bias-corrected bootstrap confidence interval (CIs; of the indirect effect) was entirely above or below zero. CIs were derived from 10,000 bootstrap samples. Consistent with current guidelines, we do not report the ratio of the indirect effect over the total effect as a measure of effect size, as this ratio can be any real number and is not bounded by 0 and 1 [58].

Figures & Tables

Table 1. Participants characteristics by atherosclerosis severity (CAC category).

	Very low (CAC=0)	Low (CAC=1-100)	High (CAC=101-400)	Very high (CAC>401)	*p-value*
Count	367	333	210	200	-
Age	64.4 ± 8.0	68.3 ± 9.2	71.6 ± 8.8	73.5 ± 8.0	***<.001***
BMI	29.7 ± 5.7	29.2 ± 5.5	30.0 ± 4.9	29.4 ± 5.4	*0.397*
Male sex	29.7%	50.8%	51.9%	64.0%	***<.001***
Race, White	31.9%	35.4%	45.2%	52.0%	***<.001***
Race, African American	36.2%	29.4%	22.9%	18.5%	***<.001***
Race, Hispanic	31.3%	34.2%	31.4%	28.5%	*0.580*
Race, Chinese	0.5%	0.9%	0.5%	1.0%	*0.873*
Smoking, Never	43.6%	44.4%	35.2%	30.0%	***0.002***
Smoking, Former	48.2%	48.3%	55.2%	62.5%	***0.004***
Smoking, Current	8.2%	6.6%	9.5%	7.5%	*0.656*
Any hypertension medication	43.9%	56.8%	66.2%	69.5%	***<.001***
SBP	122.0 ± 21	122.4 ± 19	126.1 ± 24	125.4 ± 20	***0.045***

DBP	68.4 ± 9.7	68.6 ± 9.4	68.3 ± 11.2	67.7 ± 10.1	*0.769*
WBC count	5.8 ± 1.7	5.8 ± 1.5	6.4 ± 2.0	6.6 ± 4.1	***<.001***
Neutrophils count	3.4 ± 1.4	3.4 ± 1.2	3.8 ± 1.5	3.9 ± 1.6	***<.001***
Monocytes count	0.4 ± 0.2	0.4 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	***<.001***
Sleep apnea	7.9%	6.9%	7.1%	5.0%	*0.703*
Insomnia	5.2%	3.9%	3.8%	3.5%	*0.745*

Data are shown as mean ± SD for continuous variables, and as percentages for categorical variables. P-values were calculated using one-way ANOVA for continuous variables and chi-square test of independence for categorical variables. BMI=body mass index (kg/m^2); SBP=seated systolic blood pressure (mmHg); DBP=seated diastolic blood pressure; WBC=white blood cells. Only the characteristics of the participant included in the complete-case mediation analysis (n=1110) are reported.

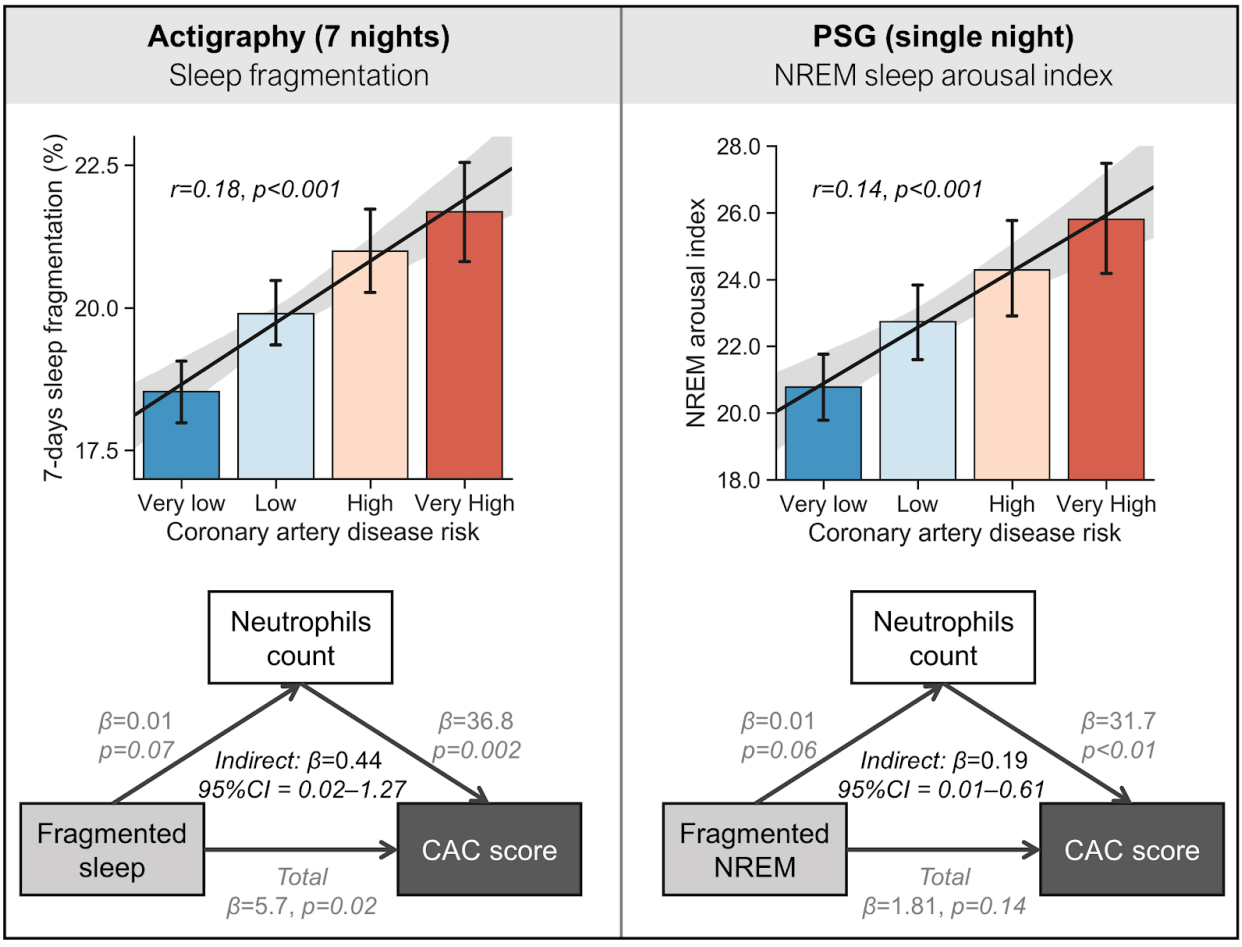


Fig 1. Results. (A) Actigraphy-measured sleep fragmentation is positively associated with coronary artery disease risk (Very low = 0 Agatston units, Low = 1-100, High = 101-400, Very high > 401). Mediation analysis demonstrated a significant association between actigraphy measured sleep fragmentation and increased absolute neutrophils count, which consequently predicts higher CAC scores. Thus, the link between fragmented sleep and atherosclerosis risk is, in part, governed by the impact of fragmented sleep on elevated neutrophils. (B) PSG measured sleep fragmentation (arousal index in NREM sleep) and the positive association with coronary artery disease risk. Here again, mediation analysis revealed that the association between PSG measured sleep fragmentation in NREM and increased absolute neutrophils count, which in turn, predicts higher CAC scores. Cofactors controlled for in the mediation models included age, sex, ethnicity, BMI, smoking status, blood pressure, use of antihypertensive medication, as well as sleep apnea and insomnia diagnosis, described in the main text.

Supplementary Materials

Supplementary Methods

The Multi-Ethnic Study of Atherosclerosis (MESA) is a multi-center prospective study of more than 6,000 ethnically diverse men and women aged 45-84 from six communities in the United States. MESA was designed to investigate the prevalence and progression of subclinical cardiovascular disease (CVD) as well as to identify CVD risk factors predicting the development of clinically overt CVD in an ethnically diverse population [51].

There have been four follow-up exams to date since the initial exam, in the years 2003-2004 (Exam 2), 2004-2005 (Exam 3), 2005-2007 (Exam 4), and 2010-2012 (Exam 5). The MESA 5 Core exam occurred from April 2010 to February 2012, 10 years after the initial exam. Similar to the prior follow-up exams, Exam 5 collected interval medical history, anthropometrics, blood pressure readings, fasting venipuncture, spot urine collection, nutrition and physical activity surveys, smoking history, ankle/arm index, retinal photography, and ECG. In addition, cardiac MRI was repeated in participants who underwent cardiac MRI at exam 1, and cognitive function testing was newly performed in all MESA Exam 5 participants. Randomly selected participants were invited to participate in the MESA ancillary study (70% of the MESA cohort), which performed cardiac CT imaging for measurement of CAC and carotid ultrasound for IMT measurement.

All MESA participants were also invited to participate in the MESA Sleep ancillary study at MESA Exam 5 (2010-2013). Sleep exams were scheduled to occur after the MESA 5 core exam. The purpose of MESA Sleep was to obtain quantitative measures of sleep and sleep-disordered breathing (SDB) to better characterize specific sleep traits and sleep disorders and their CVD risk associations across ethnic groups, as well as to

determine the association of sleep indices with incident CVD. The sleep protocol included one night of home polysomnography (PSG), seven consecutive days of wrist actigraphy (Actiwatch Spectrum, Philips Respironics, Murrysville, PA), and a sleep questionnaire.

In-home polysomnography (PSG) was performed using the Compumedics Somte System (Compumedics LTd., Abbostville, Australia. The recording montage consisted of cortical electroencephalograms (C4-M1, Oz-Cz, and Fz-Cz leads), bilateral EOG, chin EMG, thoracic and abdominal respiratory inductance plethysmography (by auto-calibrating inductance bands); airflow (by nasal-oral thermocouple and pressure recording from a nasal cannula); ECG; leg movements, and finger pulse oximetry. EEG, EOG, EMG and ECG were all sampled at 256 Hz. Nocturnal recordings were transmitted to the centralized reading center at Brigham and Women's Hospital and data were scored by trained technicians using current guidelines [59,60].

Actigraphy was performed using the Actiwatch Spectrum wrist actigraph (Philips Respironics, Murrysville, PA) worn on the participant's non-dominant wrist. Output was sent to the Sleep Reading Center at Brigham and Women's Hospital where records were scored with use of the corresponding sleep diary. Specifically, actigraphy data were aggregated in 30-second epochs and automatically scored as sleep or wake by a validated algorithm implemented in the Actiware-Sleep v.5.59 analysis software (Mini Mitter Co., Inc.), after manually editing the sleep period using sleep diary data and event and light markers. Two scorers scored MESA actigraphy studies. Intra-scorer reliability for average sleep duration, sleep efficiency, and WASO were 0.91, 0.97, and 0.91, respectively. Sleep fragmentation was defined as the sum percent mobile epochs and percent immobile bouts less than 1-minute duration to the number of immobile bouts, for the given interval. This is also known as the restlessness index or movement and fragmentation index.

White blood cells (WBC) were assessed in blood samples collected at Exam 5 at a central laboratory. Blood assays included total WBC count, and leukocyte subsets (basophils, eosinophils, neutrophils, lymphocytes, and monocytes) were determined as complete blood count with differential analysis.

Mediation models were adjusted for factors known to affect cardiovascular risk, specifically age, sex, race/ethnicity, body mass index (BMI; in kg/m^2), smoking status, use of antihypertensive medication, blood pressure as well as medical diagnosis of sleep apnea and insomnia. Information on doctor-diagnosed sleep apnea/insomnia was obtained from the sleep questionnaire survey: *"Have you been told by a doctor that you have any of the following:? a) Sleep Apnea (or obstructive sleep apnea, OSA) b) Insomnia"*. Those who answered 'yes' were defined as having doctor-diagnosed sleep apnea and/or insomnia, respectively. Smoking was defined as never, former (no smoking within the past 30 days), or current. Resting blood pressure was measured

three times in the seated position and the average of the second and the third served as systolic and diastolic blood pressure.

Supplementary Results

Importantly, our analyses were limited by the use of cross-sectional data, and while a causal mediation framework was used, this precludes a definitive assessment of the directionality of associations. The rationale for interpreting our findings in the specific direction of sleep leading to atherosclerosis is twofold. First, the present article was principally motivated by directionally specific findings in rodents. In those models, the authors used the causal manipulation of fragmented sleep in otherwise healthy animals without pre-existing atherosclerosis, which resulted in raised inflammatory blood cell markers that, in turn, led to the development of atherosclerotic plaques [18]. Second, our analyses focused on CAC, and not cardiovascular disease (CVD), as the outcome variable. While CAC is one of the most well-established predictors of future cardiovascular disease, a high CAC score per se is not deterministic of present-state CVD and/or associated treatments. In the current study, we therefore wanted to test the experimental hypothesis by looking at CAC relationships in the early inception stages of CVD (i.e., subclinical atherosclerosis) in an effort to minimize the issue of reverse causality to a degree.

To empirically address this point in a more direct manner, we conducted mediation analyses that specifically excluded participants with a history of cardiovascular events, whilst adjusting for the same covariates that were included in our full cohort analysis. The main mediation between actigraphy-defined sleep fragmentation, neutrophil counts and atherosclerosis remained significant when excluding participants with history of congestive heart failure (β=0.44, 95% CIs=0.03-1.34), peripheral vascular disease (β=0.41, 95% CIs=0.002-1.26), stroke (β=0.39, 95% CIs=0.03-1.23; and with trend significance when removing participants with history of myocardial infarction: β=0.36, 95% CIs=-0.004-1.08). Furthermore, two-sided Welch's t-test comparing the actigraphy and PSG sleep fragmentation of participants with or without history of cardiovascular events yielded no significant differences for any of the outcomes considered (all p's $>$ 0.3). Altogether, this set of post-hoc analyses tentatively suggests that a history of cardiovascular events is a less parsimonious factor driving sleep fragmentation in this specific cohort. Parenthetically, data has also indicated that reductions in sleep fragmentation (by means of CPAP treatment in apnea patients) are associated with decreased atherosclerosis (Drager et al. 2007), suggesting at least a partial mechanistic and directionally specific role of sleep fragmentation in atherosclerosis risk.

We also conducted post-hoc analyses to determine whether additional markers of autonomic arousal during sleep could be associated with increased subclinical

atherosclerosis via higher counts of neutrophil/monocyte. We specifically focused on well-established measures of heart rate variability (HRV). After adjusting for the same covariates that were included in our main mediation analysis, we indeed found that worse HRV outcomes (reflecting lower parasympathetic tone), both in the time and frequency domains, were significantly associated with an increase in neutrophil counts, which in turn predicted a higher CAC score.

First, we found that a lower log-transformed absolute spectral power of all normal-to-normal (NN) intervals between 0.003 and 0.04 Hz (i.e. very low frequency power, or VLF), calculated across the entire night of polysomnography-recorded sleep, predicted a higher CAC score, via an increase in neutrophil counts (n=932, β=-4.56, 95% CIs=-12.6--0.70). Similarly, a lower percentage of differences between adjacent NN intervals greater than 10 and 20 ms (pNN10 and pNN20, respectively), calculated across the entire night of polysomnography-recorded sleep, was significantly associated with higher CAC, via a raise in neutrophil counts (n=932, pNN10: β=-0.24, 95% CIs=-0.59--0.05; pNN20: β=-0.16, 95% CIs=-0.41--0.03).

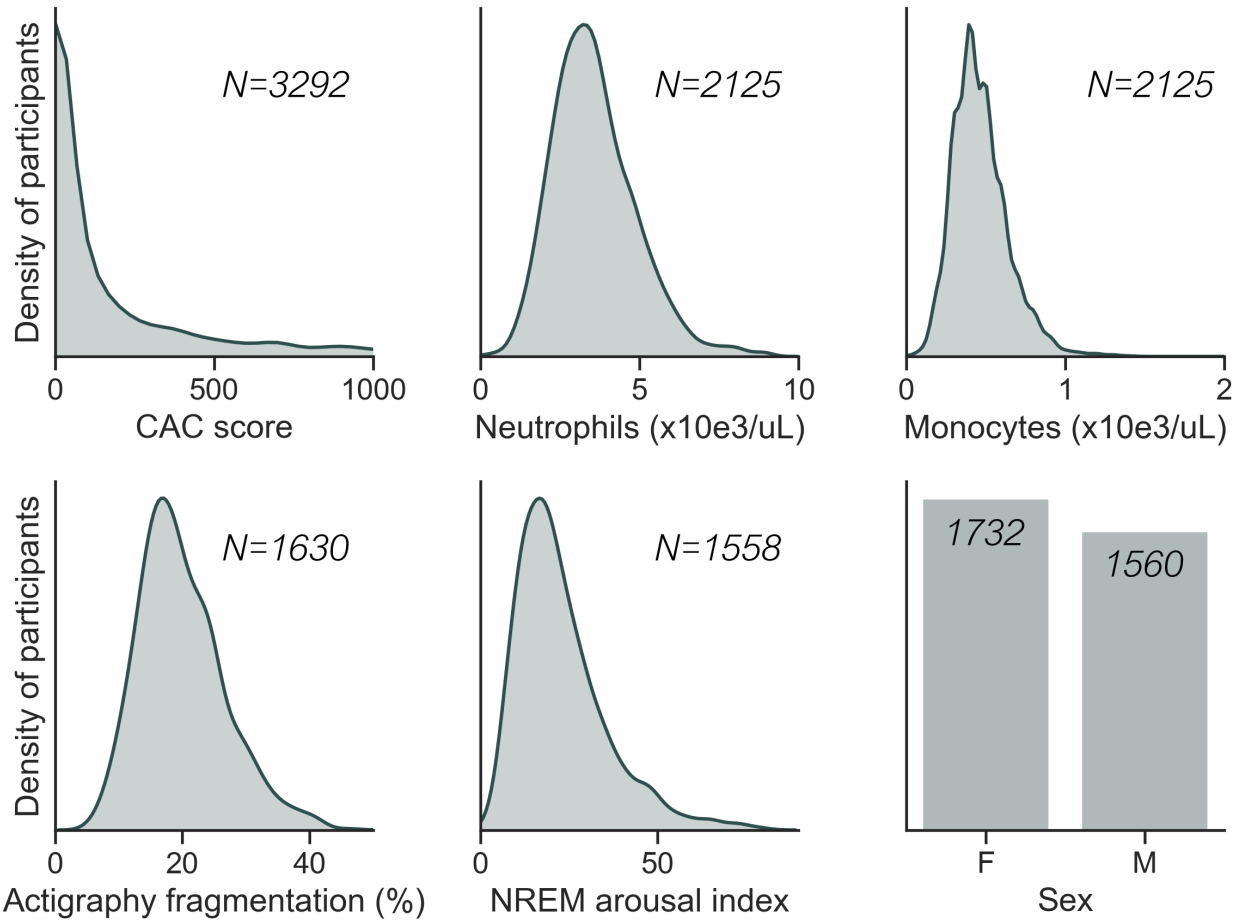
Decreased VLF power has been associated with higher levels of inflammation and higher cardiac mortality [61]. The pNNx family is a well-known marker of parasympathetic activity, with higher values being associated with lower cardiovascular disease risk [62]. These additional post-hoc analyses therefore help support the proposal that one potential pathway through which sleep fragmentation may raise inflammatory-related white blood cells and thus atherosclerosis risk is autonomic dysfunction. Such data lead to the testable hypothesis that the measurement of HRV during sleep e.g., by using wearable photoplethysmography sensors, is sensitive enough to detect this inflammatory-related increased risk for atherosclerosis.

Finally, to determine whether our main mediation pathways between sleep fragmentation, neutrophils/monocytes, and atherosclerosis were sex-specific, we conducted post-hoc analysis specifically for males and females. While not adequately powered, we did not find any significant mediation effect between sleep fragmentation, neutrophils/monocytes, and atherosclerosis for either sex when adjusting for all the covariates (excluding sex). Interestingly, unadjusted models did reveal a significant mediation in females only between the average actigraphy fragmentation, neutrophil count, and CAC score (n=595, β=0.81, 95% CIs=0.13-2.15), suggesting that the neutrophil-related association between sleep fragmentation and CAC might be somewhat stronger in females than in males.

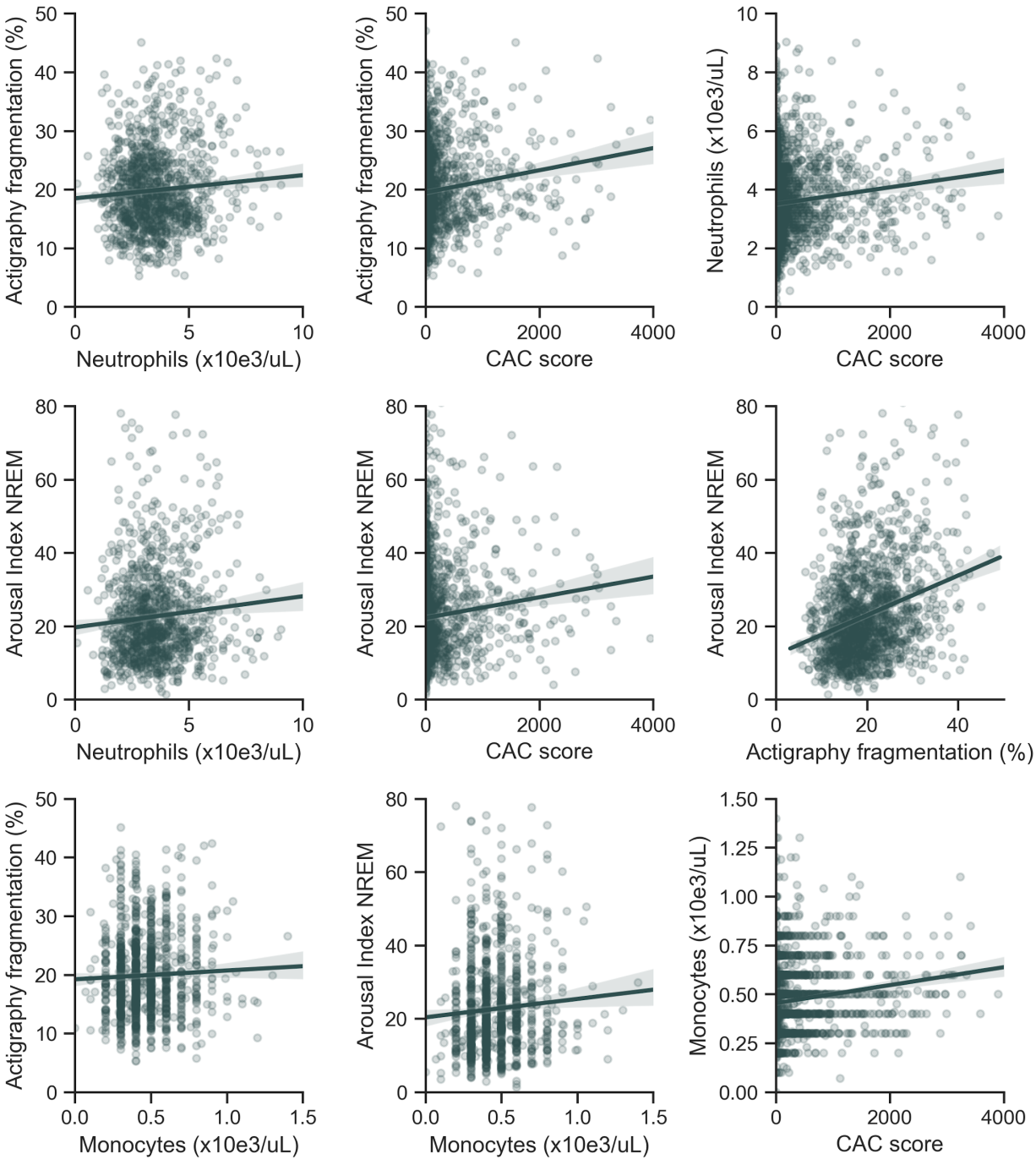
	Very low (CAC = 0)	Low (CAC = 1 - 100)	High (CAC = 101 - 400)	Very high (CAC > 401)	*p*
Actigraphy					
Fragmentation (%)	18.5 ± 6.2	19.9 ± 6.4	21.0 ± 6.8	21.7 ± 7.4	***<.001***
Efficiency (%)	90.1 ± 3.4	89.8 ± 3.3	89.4 ± 4.0	89.7 ± 3.8	***0.047***
Total Sleep Time (min)	393.2 ± 72.1	387.9 ± 78.3	378.0 ± 91.2	402.5 ± 86.2	***0.002***
WASO (min)	36.4 ± 15.8	37.2 ± 15.6	37.9 ± 17.6	39.3 ± 17.6	*0.106*
Polysomnography					
Total Sleep Time (min)	371.5 ± 80.2	367.3 ± 81.0	340.4 ± 80.2	360.8 ± 77.7	***<.001***
Sleep Efficiency (%)	78.0 ± 13.2	76.8 ± 12.6	72.5 ± 14.5	74.7 ± 13.2	***<.001***
N1 sleep (%)	12.5 ± 8.4	14.1 ± 8.1	15.1 ± 9.6	17.5 ± 10.4	***<.001***
N2 sleep (%)	57.3 ± 10.0	56.9 ± 9.8	57.6 ± 10.9	58.4 ± 10.5	*0.289*
N3 sleep (%)	11.3 ± 9.2	10.0 ± 8.8	10.2 ± 9.7	7.6 ± 7.7	***<.001***
REM sleep (%)	18.9 ± 6.6	19.0 ± 6.6	17.0 ± 6.9	16.5 ± 6.5	***<.001***
WASO (min	82.5 ± 62.5	92.1 ± 60.6	109.1 ± 74.9	104.0 ± 66.1	***<.001***
AI (all)	20.2 ± 11.2	21.9 ± 11.6	23.3 ± 12.6	24.5 ± 12.5	***<.001***
AI (REM)	17.0 ± 11.2	18.0 ± 11.8	17.9 ± 12.7	17.4 ± 11.7	*0.580*
AI (NREM)	20.8 ± 12.0	22.7 ± 12.3	24.3 ± 13.4	25.8 ± 13.5	***<.001***
AHI	20.0 ± 17.4	24.5 ± 20.3	25.3 ± 19.0	26.8 ± 19.4	***<.001***

REM latency	105.7 ± 72.9	103.8 ± 74.7	119.1 ± 82.8	110.4 ± 76.2	***0.039***

S1 Table. Participants sleep parameters by atherosclerosis severity (CAC category). Data are shown as mean ± SD. P-values were calculated using one-way ANOVA for continuous variables and chi-square test of independence for categorical variables. WASO = wake after sleep onset (min); AI = arousal index (number of events per hour); AHI = All Apneas + Hypopneas With >=3% Desat Or Arousal - Index (AHI). Statistical significance, p-value < 0.05.



Supplementary Figure 1. Distribution (kernel density estimation) plots of the main variables.



Supplementary Figure 2. Bivariate regression plots.

Chapter 2. Counting Z's and calories: Unique features of sleep predict different profiles of dyslipidemia

Introduction

More humans die of heart disease than any other single disease cause [1]. Currently, cardiovascular disease (ischemic heart disease and stroke) is responsible for more than 1 out of every 4 deaths in the world, claiming over 14 million lives annually [1–3]. In the United States alone, the annual associated cost of cardiovascular disease is estimated to be $320 billion [63].

What accounts for heart disease? To date, numerous factors, including impaired lipid profile, hypertension, diabetes mellitus, physical inactivity, unhealthy diet, smoking, and abdominal obesity, have been recognized as associations with, many of them causally, heart disease [64]. One common central pathway through which such factors contribute to heart disease is lipid dysregulation, including high triglyceride concentration, as well as the imbalance of high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL). Specifically, high levels of VLDL and LDL, are causally associated with increased build-up of plaque in the arteries. The composition of VLDL in particular is majorly triglycerides. Therefore, an increase in VLDL levels can signal excessive levels of triglycerides being transported from the liver to tissues around the body, which in turn is related to both increased inflammation and atherosclerosis.

Triglycerides are a type of fat (lipid) in the blood. When an individual eats, any calories not needed are converted to triglycerides. Therefore, assessing triglyceride levels offers critical insight into an individual's lipid metabolism. Triglyceride levels themselves can be measured in a fasted state and/or postprandially (after a meal). Fasted triglyceride levels and postprandial triglyceride levels each represent a different aspect of lipid regulation, and when impaired, dyslipidemia [16,65]. One metric of the homeostatic regulatory state of an individual's lipid metabolism is their fasted triglyceride levels. Moreover, triglyceride levels when fasted have long been predictors of cardiovascular disease, including nonfatal myocardial infarction, nonfatal ischemic stroke, coronary revascularization, and cardiovascular death. However, the responsivity of the body regarding raised levels of triglyceride in response to standardized food challenges provides a distinctly different metric, one that evaluates the dynamic allostatic ability of an individual's metabolic system to manage lipid levels. Indeed, this measure of allostatic management measures is an even stronger predictor of cardiovascular events (nonfatal myocardial infarction, nonfatal ischemic stroke, coronary revascularization, or cardiovascular death), than fasted triglycerides.

Beyond classical factors linked to cardiovascular disease, such as diabetes mellitus, physical inactivity, unhealthy diet, smoking, and abdominal obesity [66,67], a more recent disease-related role has been identified for sleep. Experimental studies in humans and animals have begun to suggest that poor sleep predicts cardiovascular disease, including emerging links with lipid metabolism. For example, in humans, short sleep duration and the condition of obstructive sleep apnea, are both associated with increased dyslipidemia and impaired lipid metabolism. Specifically, short sleep duration is associated with high triglycerides and low HDL [14,15]. Similarly, the presence of obstructive sleep apnea is associated with increased total triglycerides, low HDL, high LDL, and high total cholesterol [14,15]. However, experimental studies to date have examined sleep across just one night of sleep and averaged cross-sectionally. While having many merits, such studies understandably fail to capture the real-world fact that sleep differs markedly across individuals, and even within an individual, there are marked differences in sleep duration, timing, efficiency, and consistency from night to the next. Micro-longitudinal studies employing multiple nights of sleep data for each individual provide one solution to addressing both of these current limitations, allowing for the ability to test distinctly different hypotheses from those evaluated to date.

Another key barrier to a deeper characterization of the associations between sleep and lipid metabolism in humans has been the logistical difficulty of controlling for multiple known risk factors for dyslipidemia in small cohorts. These include disambiguating the effects of genetic influences on cardiovascular risk, carefully quantifying and tracking, day-to-day, what an individual eats, and when they eat it across the 24-hour circadian clock face, all of which have known impacts on lipid management [68–70].

Another methodical challenge to more accurately understand the relationship between sleep and triglyceride metabolism is that both sleep and triglyceride regulation themselves are multifaceted, and can be characterized in numerous ways. Broadly, four macro pillars of sleep, that each represent a unique asleep of sleep, are: sleep duration, sleep timing, sleep consistency, and sleep efficiency. Currently, the specificity with which each of these fundamental sleep factors is associated with triglyceride metabolism, either fasted or postprandially, remains unknown. Regarding triglyceride metabolism, different mechanisms underlie the regulation, and clinical implications, of fasted versus postprandial triglycerides. Raised levels of fasted triglycerides represent impaired homeostatic regulation of triglyceride levels, whereas raised levels of postprandial triglycerides represent an allostatic impairment in metabolizing post-meal triglyceride levels from the bloodstream. The association of sleep with the homeostatic versus allostatic regulation of triglycerides remains unknown.

Addressing the above gaps in the literature, this study untangles the complex relationship between each of the fundamental pillars of sleep (sleep duration, sleep timing, sleep consistency, and sleep efficiency) with triglyceride metabolism, in a highly controlled context. First, the dataset includes non-twins, as well as a significant

percentage of identical and fraternal twins, allowing to control for the influence of genetic makeup on lipid metabolism. Second, this is the first such large-scale study in a cohort that includes 10-14 nights of sleep data per individual, carefully defined standardized meal composition, meal context (e.g. exercise, sleep, meal ordering, time of day), with fasted and postprandial measurements at multiple timepoints. Finally, the sleep-related measures derived themselves are more robust and accurate than those employed in traditional studies, as they utilize multiple nights of data.

Specifically, and based on previous work, this study tests three key predictions. First, sleeping at a later time is associated with the homeostatic dysregulation of fasted triglyceride levels. Second, lower sleep efficiency predicts the allostatic dysregulation of post-meal triglyceride levels. Third, a shorter sleep duration is associated with higher triglyceride levels in both the fasted and postprandial states. Further, all these sleep-related associations hold true for VLDL cholesterol (high in triglyceride composition), and remain robust after accounting for risk factors known to influence lipid metabolism.

Results

Sleep and Homeostatic Lipid Regulation

Analyses first tested the prediction that the apriori feature of later sleep timing, and a shorter sleep duration, are associated with elevated (worse) total triglyceride levels under the fasted state, more reflective of the current lipid homeostatic condition of the body. Supporting the hypothesis, later sleep timing significantly predicted higher fasting total triglyceride levels (composed of a glycerol backbone esterified with three fatty acids) in Cohort 1. That is, participants with a later average sleep timing (indexed by sleep midpoint) displayed significantly elevated total triglycerides levels in the fasted state, most reflective of current homeostatic status, absent a food provocation ($n = 959$, $p < 0.001$, $r = 0.11$; **Figure 3A**). Also consistent with the experimental hypothesis, neither sleep efficiency nor sleep consistency were associated with fasting triglycerides.

However, contrary to the hypothesis, and prior single night sleep measure cross-sectional data [71], sleep duration did not predict fasting total triglyceride levels. Expounding in the Discussion, one possible explanation is that the current data reflect the first longitudinal study with repeated sleep measurements to quantity an individual's sleep signature robustly, or at least, in a way that single, one-time sleep assessments are less sensitive to. This, plus the fact that the current study controls for the other sleep-related cofactors in the same a priori analysis (i.e., controlled for sleep timing, sleep efficiency, and sleep consistency.)

Taken together, these results suggest a statistically independent contribution of sleep midpoint to fasting triglyceride levels and do so separately from previously recognized risk factors known to influence fasting triglyceride levels.

These first analyses establish the longitudinal within-person relationships between late sleep timing and fasted triglyceride levels. However, multiple other factors have been identified that influence lipid metabolism, including age, sex, race, body mass index (BMI), education, smoking, physical activity, and even the sleep features identified here (sleep duration, sleep efficiency, sleep timing, and sleep consistency). A next series of analyses therefore sought to factor in all these covariates and test whether or not the original associations were robust and remained significant, performed using a multilevel regression model fitted to adjust for all these known co-risk factors, including the sleep factors themselves.

With all factors included in the analysis model (age, sex, race, body mass index (BMI), education, smoking, physical activity, family ID as a random effect, sleep duration, sleep efficiency, and sleep consistency), the relationship between sleep timing and fasting triglycerides remained significant ($\beta = 0.05$, $p = 0.045$).

Having determined that the timing of each individual's sleep phase (midpoint index) is associated with their own respective basal lipid status (fasted) in the first cohort, the next set of analyses sought to determine whether or not this association replicates in the second independent cohort (PREDICT2), using the same methodological approach. Here in Cohort 2, a later timing of an individual's sleep was again associated with higher fasted basal levels of triglycerides, representing their current homeostatic lipid state. As with Cohort 1, this association remained robust after adjusting for age, sex, race, BMI, education, smoking, physical activity, and the three remaining sleep factors (sleep duration, sleep efficiency, and sleep consistency), also noted in Cohort 1 ($\beta = 0.04$, $p= 0.011$). Thus, across two independent cohorts, later sleep timing statistically and significantly predicted progressively elevated levels of fasting triglyceride levels. Importantly, this association (fitting the body's homeostatic lipid status) was independent of how much that individual sleeps, the efficiency of their sleep, as well as the night-to-night consistency of their sleep. That is, sleep *timing* is unique in this regard.

Sleep and Allostatic Lipid Regulation

The former analysis established an association between sleep that is timed later into the night and fasted triglycerides, more reflective of the stable basal, or homeostatic, state of circulating lipids. The next series of analyses explored an orthogonal experimental hypothesis. Specifically, unique sleep features predicted one's metabolic ability to dynamically manage a standardized food bolus provocation, representing an

allostatic triglyceride challenge or provocation i.e., the ability to appropriately regulate the shift out of lipid homeostasis caused by food intake, resulting in an allostatic load to be managed (assessed using postprandial circulating blood measures of triglycerides levels unfolding across several hours). Analyses focused on the a priori (see introduction) target of sleep quality.

Here, analyses tested the hypothesis that worse sleep efficiency predicted significantly elevated post-meal provocation levels of total triglycerides. Importantly, given that fasting and postprandial triglyceride levels are often correlated, all postprandial values (T=6 hours) were adjusted for each individual's baseline triglyceride values in the fasted state, thus resulting in a person-normalized relative change in lipid allostasis. In accordance with the experimental hypothesis, decreased sleep efficiency predicted greater elevated (baseline-adjusted) postprandial triglyceride levels in Cohort 1 (PREDICT1; $r = -0.065$, $p = 0.059$, $n = 842$; **Figure 3B**). That is, lower sleep efficiency appears to be a biomarker for impaired allostatic regulation of postprandial triglyceride levels.

Importantly, and similar to the previous association between sleep timing and fasted total triglycerides, this sleep efficiency--postprandial total triglycerides association remained robust after accounting for the aforementioned risk factors (age, sex, race, BMI, education, smoking, physical activity, sleep timing, sleep duration, and sleep consistency) ($\beta = -1.47$, $p = 0.049$). Thus, the efficiency with which an individual maintains sleep is reliably associated with the body's ability to manage triglyceride levels following a food provocation, above and beyond the influence of sleep timing, sleep duration, sleep consistency, age, sex, race, BMI, education, smoking, and physical activity. However, and contrary to the experimental hypothesis, the duration (rather than efficiency) of an individual sleep was not similarly predictive of postprandial triglyceride levels. Therefore, when assessed in a longitudinal within subjects, the qualitative measure of sleep efficiency, rather than the quantity of sleep, was correlated to the individual's capacity to manage an allostatic load caused by a standardized food challenge.

Collectively, these results suggest a double dissociation in which the sleep timing of one's sleep on the 24-hour clockface most accurately predicts the metabolic state of lipid homeostasis (fasted), while the qualitative metric of sleep efficiency is, in contrast, related to how the body manages triglyceride levels after a meal that is more reflective of allostatic lipid regulation.

Lipid Homeostasis vs. Allostasis: Double Dissociation

The hydrolysis of multiple types of triglyceride-rich lipoproteins can each result in an atherogenic profile [72,73]. Specifically, increased levels of triglyceride-rich lipoproteins are

a strong predictor of future atherosclerotic cardiovascular disease [73]. Of these lipoproteins, very low-density lipoprotein (VLDL) in specific, is a cholesterol-related metabolite that is high in triglyceride composition, and therefore a key candidate for reflecting the same specificity of sleep features with the homeostatic versus allostatic regulation of triglyceride metabolism. Therefore, having established a double dissociation for specific features and triglyceride regulation, the next set of analyses tested the prediction that VLDL cholesterol would specifically reflect this same double dissociation of sleep timing versus sleep efficiency.

Concordant with the earlier results, later sleep timing predicted worse VLDL cholesterol ($r = 0.10$, $n = 906$, $p = 0.002$; **Figure 3C**) in Cohort 1. Importantly, this association remained robust when accounting for risk factors known to influence fasted VLDL outcomes - specifically, age, sex, race, BMI, education, smoking, physical activity, sleep duration, sleep efficiency, and sleep consistency ($\beta = 0.02$, $p = 0.017$). Therefore, more than the efficiency of an individual's sleep, or the amount of sleep they obtain, or also the regularity of their sleep, instead, it is the timing of an individual's sleep on the 24-hour clockface that is most associated with the *fasted* lipid state, specifically basal, unfed levels of circulating VLDL cholesterol. However, these data do not address how the four main a priori sleep factors (timing, quantity, efficiency, regularity) are linked to lipid particle profiles in response to the allostatic challenge of a standardized meal, which the next series of analyses address.

Specifically, having assessed fasted lipid particle levels, more indicative of an individual's homeostatic lipid status, the next set series of analyses sought to test the hypothesized sleep association between postprandial triglyceride level following the standardized food meal provocate, evaluating an individual's allostatic response-ability. Congruent with the experimental hypothesis, it was the worsening quality of an individual's sleep, as measured by sleep efficiency that predicted a higher rise in Cohort 1 ($r = -0.07$, $n = 828$, $p = 0.04$; **Figure 3D**). Of note, this association remained statistically significant ($\beta = -0.20$, $p = 0.034$) after accounting for the aforementioned risk factors (age, sex, race, BMI, education, smoking, physical activity, and even sleep factors themselves (sleep timing and sleep duration) known to influence postprandial VLDL outcomes. Therefore, a lower quality (efficiency) of sleep that an individual has been suffering predicts higher postprandial VLDL levels in response to a standard food challenge, reflecting a less capable ability to manage an allostatic load. Moreover, sleep efficiency expresses this significant association beyond the influence of traditional cardiac risk factors, as well as other sleep factors previously linked with dyslipidemia. Taken together, these results further support a double dissociation framework such that later sleep *timing* impairs metabolic lipid (especially triglyceride, fasted) homeostasis, whereas lower sleep *efficiency* blunts meal-reactive allostasis.

LDL is a principal carrier of cholesterol in the bloodstream, and therefore atherogenic, it has traditionally been a key target in lipid-lowering therapies [74,75]. HDL, also a carrier of

cholesterol, helps remove cholesterol from the bloodstream and is associated with a reduced risk of cardiovascular disease [76]. Recently, increasing epidemiological evidence suggests that triglyceride-rich lipoproteins such as VLDL are strong, independent predictors of atherosclerotic cardiovascular disease [73]. Reflecting that VLDL is majorly a carrier of triglycerides, in Cohort 1, the levels of total triglycerides and VLDL cholesterol were highly correlated in both the fasted state ($r = 0.76$, $p < 0.001$; Supplementary Figure 2A) and postprandial state ($r = 0.66$, $p < 0.001$; Supplementary Figure 2B). Importantly, in the current cohort, and in contrast to VLDL, measures of LDL were not significantly associated with any of the four sleep measures, either in the fasted or postprandial state. This suggests a specificity of the interrelationship between sleep and homeostatic, as well as allostatic, lipid metabolism. Specifically, the sleep associations appear to be specific to fat-related lipid pathways (reflected in the relationships with VLDL), rather than cholesterol-related lipid pathways (reflected in the relationships with LDL), something we return to in the Discussion. Notably, increased sleep efficiency (but not sleep duration, timing, or consistency) was associated with higher HDL cholesterol in the fasted state ($\beta = 0.57$, $p = 0.028$), whereas increased sleep consistency (but not sleep duration, timing, or efficiency) predicted improved (higher) HDL cholesterol in the postprandial state ($\beta = 0.02$, $p = 0.034$). This suggests that different mechanisms of sleep regulate reducing atherosclerotic burden (as measured by VLDL cholesterol) versus increasing atheroprotective reserve (as measured by HDL cholesterol).

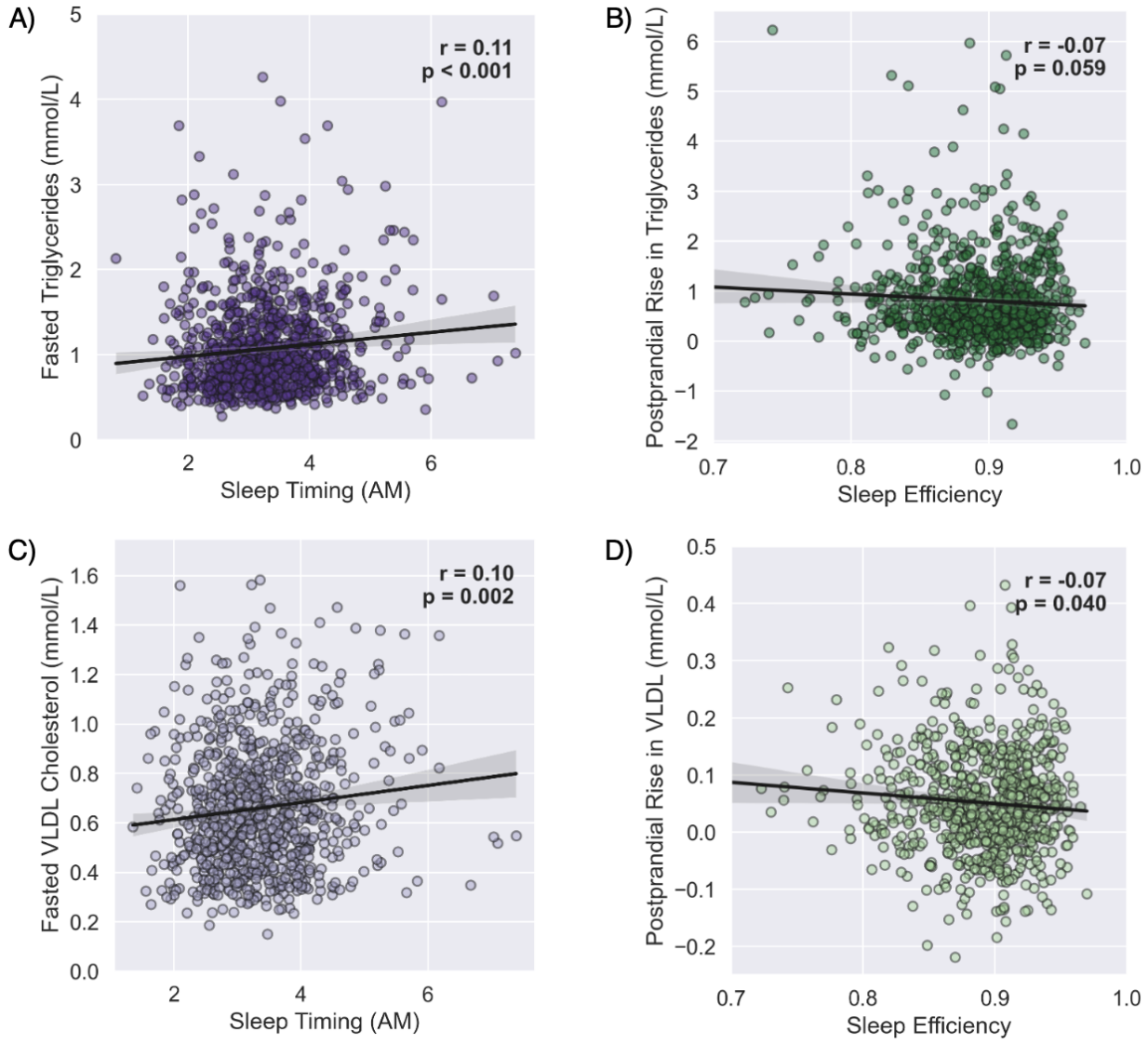


Figure 3: A double dissociation in Cohort 1. A) A later sleep timing was significantly associated with higher total triglycerides in the fasted state. **B)** Independently, higher sleep efficiency predicted an increased baseline-adjusted rise in postprandial total triglycerides. **C)** A later sleep timing was significantly associated with higher VLDL cholesterol in the fasted state. **D)** Independently, higher sleep efficiency predicted an increased baseline-adjusted rise in postprandial VLDL cholesterol. Translucent bars represent 95% bootstrapped confidence intervals.

Inflammation Mediation

Recent studies have shown that shorter sleep duration, later sleep timing, and irregular sleep are all predictors of increased inflammation [77]. However, it remains unknown if sleep timing-dependent inflammation further contributes to triglyceride-related hyperlipidemia. The final series of analyses sought to test the hypothesis that the

above-reported association between sleep timing and fasted triglyceride levels is mediated by increased inflammation (using the composite systemic inflammation biomarker, glycoprotein acetylation; GlycA), offering insight into a potential mechanistic pathway.

First, and in Cohort 1, later sleep timing predicted increased inflammatory levels of GlycA ($r = 0.13$, $p < 0.001$; Supplementary Figure 3). This relationship remained significant when other sleep features (duration, consistency, and efficiency), as well as the aforementioned covariates (age, sex, race, BMI, education, smoking, and physical activity), were controlled for ($\beta = 0.01$, $p = 0.007$). Second, increased inflammation status was significantly associated with higher (worse) fasting triglyceride levels - an association that also remained robust after controlling for the above covariates (age, sex, race, BMI, education, smoking, physical activity, sleep duration, sleep consistency, and sleep efficiency ($\beta = 2.98$, $p < 0.001$). Finally, and consistent with the experimental interaction-path hypothesis the relationship between an individual's later sleep timing and higher next-day fasted triglycerides was not simply direct, but also indirectly mediated through higher inflammation, which in turn, significantly predicted a higher fasting triglycerides state within the body (n = 816, $\beta = 0.03$, 95% CIs = -0.01-0.06, p = 0.008; **Figure 4A**).

To assess the replicability of this mediation, the same analysis was conducted in Cohort 2. Here again, the relationship between later sleep timing and higher fasted triglyceride status was indirectly mediated through higher inflammation (n = 816, $\beta = 0.03$, 95% CIs = -0.01-0.06, p = 0.008; **Figure 4B**), which was significantly associated with higher fasting triglycerides state within the body. The mediation in Cohort 2 similarly remained significant after controlling for the aforementioned risk factors (age, sex, race, body mass index (BMI), education, smoking, physical activity, family ID as a random effect, sleep duration, sleep efficiency, and sleep consistency; $\beta = 0.02$, 95% CIs = 0.00-0.04, p = 0.026; **Figure 4B**).

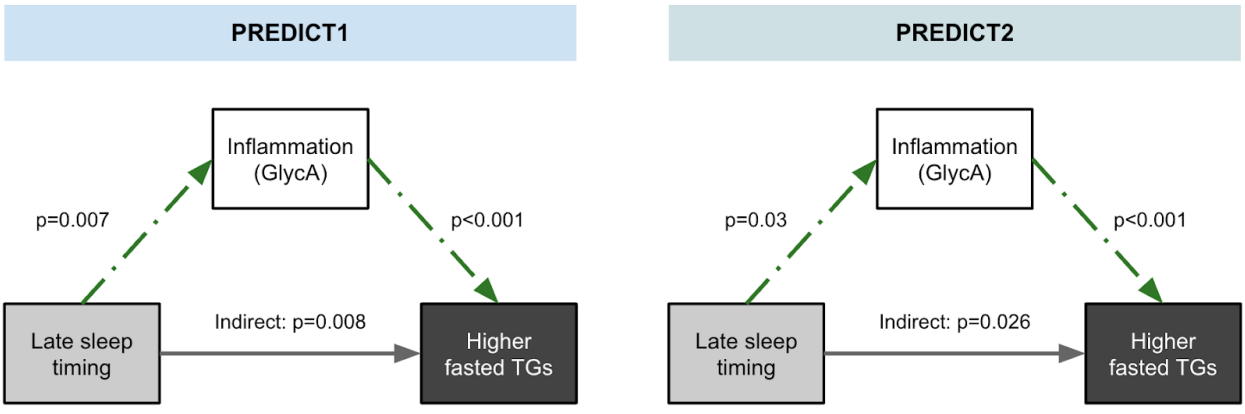


Figure 4: Later sleep timing predicts higher fasted triglycerides (TGs) via an indirect, mediating effect of inflammation, as measured by glycoprotein acetylation (GlycA). A) Fully adjusted regression

model for Cohort 1 (the PREDICT1 dataset.) **B)** Fully adjusted regression model for Cohort 2 (the PREDICT2 dataset.)

Discussion

Together, these data support a novel sleeping-brain-triglycerides metabolism framework, suggesting that selective and specific sleep features are related to unique and different homeostatic and allostatic markers of dyslipidemia, and were replicated in two independent cohorts. Specifically, the later an individual's timing of sleep, the greater the fasted total triglyceride levels, the more indicative of basal, homeostatic lipid state. In contrast, the lower the quality of an individual's sleep, notably the efficiency of sleep, the worse the allostatic capability to clear lipids from the bloodstream following a food challenge, resulting in higher levels of postprandial triglycerides. Finally, aspects of the relationship between sleep and hyperlipidemia were, in part, mediated by sleep-associated increases in systemic inflammation.

Sleep and Homeostatic Lipid Regulation

Later timing of sleep was associated with significantly greater fasted total triglyceride levels, yet there was no such sleep-timing relationship with the allostatic lipid postprandial response following a standardized food provocation. Later timing of sleep has been linked to a variety of factors, most notably a late chronotype ('night owls', or 'evening types'). Although causal mechanisms between being a late chronotype and ill health are not well understood, being a late chronotype is associated with higher BMI, worse metabolism, and unhealthier eating habits [78,79]. Specifically, late chronotypes are likely to have increased difficulty falling asleep on time, circadian misalignment, more likely to skip breakfast, have increased late-evening food consumption, and poorer quality diet (higher consumption of processed and ultra-processed foods) [78–80]. Regarding food intake, later chronotypes tend to distribute a higher proportion of macronutrient content later in the day which in turn, is related to worse metabolic health [81] even after controlling for total calories and macronutrient composition; [81]. Additionally, late chronotypes are more insulin resistant, such that their bodies both 1) require more insulin than early chronotypes to lower glucose levels, and 2) their bodies favor carbohydrates as an energy source, over fats [82]. Taken together, these findings suggest that later chronotypes may have chronic circadian misalignment, which in turn is associated with impaired homeostatic lipid metabolism [83]. One parsimonious explanation of the current findings linking later sleep timing and hyperlipidemia is that later schedules promote frequently eating more, and more unhealthy foods, at a later phase of the 24-hour circadian clock, which is a time linked to worse metabolic

regulation of both glucose and lipids. If causal manipulations support this thesis it would suggest that therapeutic interventions aimed at shifting bedtimes earlier would meaningfully augment superior management of lipids across individuals with such later sleep timing.

Interestingly, and rejecting the hypothesis prediction, the total amount of sleep an individual obtained (rather than how that sleep was timed), did not predict fasted levels of triglyceride. Previous findings have described a relationship between sleep quantity and worse lipid metabolism [84,85], or self-reported sleep quality. Of possible explanatory relevance, these studies were all cross-sectional. However, within individuals, from one night to the next, sleep varies non-trivially [86] including the quantity, efficiency, physiology EEG quality, consistency, and timing. To the best of our knowledge, the current study is the first to utilize a micro-longitudinal design, allowing the characterization of individual-specific features of sleep across numerous nights, rather than a single-night snapshot (with both approaches being meaningful). This dissonance in results highlights the importance of a micro-longitudinal study design with multiple nights of sleep data to accurately represent an individual's sleep. Additionally, this is the first study to control for the influence of other fundamental sleep factors (sleep timing, sleep efficiency, sleep consistency) on triglyceride regulation. One explanation of these differences is that associations observed in prior studies linking sleep duration to triglyceride metabolism did not include other related sleep factors, namely sleep timing (for fasted triglycerides) and sleep efficiency (for postprandial triglycerides) into the statistical models, and if included, sleep duration would no longer remain significant. Importantly, these results suggest that in the fasted state, the influence of sleep timing is beyond, and statistically independent of, that of sleep duration. Conversely, in the postprandial state, the influence of sleep efficiency is greater than that of sleep duration. One potential interpretation of this is that sleeping later primarily impairs triglyceride homeostasis through a pathway of increasing insulin resistance (and thereby dysregulating triglyceride clearance [87], whereas fragmented sleep causally increases oxidative stress and sympathetic overactivity [88], which in turn delays post-meal triglyceride clearance. This suggests that different features of sleep, at least partially, independently regulate different aspects of triglyceride metabolism through different mechanistic pathways.

Third, and returning to sleep timing and the homeostatic fasted lipid status, the later that an individual's sleep occurred into the night, the higher their daytime VLDL cholesterol. Given that VLDL is primarily a carrier of triglycerides, in which higher levels are associated with worse cardiovascular outcomes, the signature of increased circulating VLDL levels reflects a lowered ability of the body to clear and thus manage triglyceride lipid metabolism. Regarding sleep, one mechanistic pathway that could explain the result of the current study concerns insulin. Specifically, the recognized impact of later sleep in reducing the body's sensitivity to insulin (i.e., insulin resistance, which in turn, results in known overproduction of VLDL, and dysregulation of VLDL

synthesis and clearance [87]. If demonstrated, such a link would impress the relevance of clinically inquiring or measuring) the timing of sleep in patients with diabetic dyslipidemia. Should the sleep be of a later timed profile, therapeutically targeting early sleep schedules may aid in clinically significant improvements in atherogenic lipid and lipoprotein abnormalities, both of which are metabolically interrelated and emerge from VLDL overproduction [87]. That is, for optimal homeostatic lipid status, the timed decision to terminate the waking period and initiate earlier sleep is less about demarkating the end of the prior day, but instead, represents a metabolic influence that begins the next day.

Sleep and Allostatic Lipid Regulation

The association of later sleep timing and increased total triglyceride levels was exclusive to the fasted state, reflecting the (unfed) basal homeostatic lipid state. Yet when an individual was then metabolically challenged with a standardized meal probe (providing an allostatic lipid load that the body must dynamically manage), sleep again was significantly linked with postprandial triglyceride levels. However, the feature of sleep associated with this allostatic lipid regulation was not timing. Instead, the quality of their sleep (reflected in the efficiency of sleep) was linked to the allostatic, postprandial capability of an individual to clear triglycerides from the bloodstream associated with food intake. Prior animal models have established that factors causing lower sleep efficiency will prolong the clearance of triglycerides from the bloodstream, postprandially, suggesting a sleep-efficiency—metabolic-inefficiency pathway [88]. Moreover, this pathway is mechanistically explained, in part, by the impact of worse sleep efficiency on increased oxidative stress, which accelerates the progression of dyslipidemia [88]. Given that elevated postprandial triglycerides are a stronger predictor of future cardiovascular disease than fasted triglycerides, our results suggest focusing on sleep interventions that improve sleep efficiency, and continued emphasis on CPAP treatments in sleep apnea patients, to improve allostatic lipid metabolism, and reduce preventable cardiovascular mortality. Previous studies assessing associations between subjective reports of sleep quality and postprandial triglycerides in humans have not found robust associations [89]. Our results suggest that multiple nights, and the objective assessment of sleep efficiency, are more sensitive prognostic tools for predicting postprandial triglyceride values, than a single night, or subjective sleep of quality data.

Lipid Homeostasis vs. Allostasis: Double Dissociation

Importantly, the absence of a significant association of postprandial triglyceride levels with sleep timing highlights that different features of sleep specifically govern the mechanism of homeostatic regulation of triglycerides in the fasted state, versus the

allostatic regulation of triglycerides in the postprandial state. Specifically, there exists a double dissociation, such that when an individual sleeps is associated with the homeostatic process of downstream regulation of triglyceride metabolism, whereas sleep efficiency is specifically associated with the allostatic process of regulating postprandial triglyceride metabolism. That is; different features of sleep govern different regulatory mechanisms of triglyceride metabolism, such that sleeping later impairs the usage of triglycerides to be mobilized for energy, whereas low sleep efficiency influences the transport and storage of triglycerides. Notably, the association between sleep timing--homeostatic lipid metabolism, and sleep efficiency--allostatic lipid metabolism, was specific to measures of triglyceride-rich lipoprotein (VLDL cholesterol) and not LDL or HDL cholesterol. This suggests that the pathway by which sleep helps regulate lipid metabolism is: 1) specific to triglycerides (not cholesterol or proteins), and 2) by way of reducing atherosclerotic burden, rather than increasing atheroprotective reserve. Of clinical relevance, this double dissociation helps reinform public health sleep-related guidelines to mitigate dyslipidemia and reduce the mortality and economic burden of cardiovascular disease. Specifically, these findings support steering the emphasis of public health guidelines away from increasing sleep duration, to interventions focused on earlier sleep timing to lower fasted triglyceride outcomes, and increasing sleep efficiency to improve the postprandial clearance of triglycerides from the bloodstream.

Critically, and of clinical relevance, these associations remained significant when controlling for key risk factors that themselves influence lipid metabolism - age, sex, race, BMI, education, smoking, physical activity, genetic influence (family ID), and even other sleep measures themselves, such as sleep duration, and sleep consistency. That is, the association between the late timing of sleep and impaired homeostatic regulation of increased fasted triglycerides is independent of other co-factors known to influence fasted triglyceride levels. Moreover, the influence of sleep efficiency on allostatic postprandial triglyceride regulation is statistically independent of risk factors already known to influence lipid metabolism. These findings establish the measure of sleep timing as a biomarker of the homeostatic regulation of triglycerides, and independently, sleep efficiency as a biomarker of the allostatic regulation of triglycerides. Notably, these associations were validated in an independent, larger replication dataset, suggesting that the effects are less likely to be driven by single cohort-specific idiosyncrasies. Rather, these replications offer increased support and robustness to the framework of sleep timing and sleep efficiency as biomarkers of metabolic homeostatic versus allostatic regulation of triglyceride levels.

Inflammation Mediation

Impaired sleep is robustly associated with inflammation [11,90,91]. Independent of sleep, increased inflammation has been associated with impaired triglyceride levels [92].

However, that sleep dysregulation-related hyperlipidemia is in part, explained through the intermediary influence on inflammation has yet to be investigated or reported. This previously unexamined link, which we show exists in the current study, is important. Specifically, it would suggest that there is at least one potential new therapeutic target—reducing sleep-impairment-related inflammation—that could potentially mitigate some of the harm that an individual's dysregulated sleep is causing to their dislipidemia state. Pharmacological methods for blunting or suppressing the inflammatory response are well known, and many are largely safe and may represent an easier first intervention target than the (still required and important) goal of optimizing sleep (using methods such as cognitive behavioral therapy for insomnia.

Importantly, the current study needs to be understood within the context of numerous limitations. First, this prospective longitudinal study is simply associational, and thus cannot determine causality. The causal mechanisms by which sleep timing impacts the homeostatic regulation of triglycerides, and by which sleep efficiency regulates the allostatic metabolism of triglycerides, need further exploration. Yet, this study does utilize a micro-longitudinal design in a highly controlled setting, and allows critical insight into the association of specific sleep features with unique aspects of lipid metabolism. These findings motivate the design of longitudinal studies capable of testing bidirectional causality. For instance, interventions of sleep timings and sleep fragmentation to alter the homeostatic versus allostatic metabolism of triglycerides; and conversely, altering meal nutritional content and timing to potentially alter sleep factors, would help test the double dissociation framework suggested by these results. Additionally, the sleep assessments from this study are accelerometer-based. Future studies using polysomnography would help gain a deeper understanding of the neural underpinnings of triglyceride regulation, at the level of brainwave activity across the duration of sleep. Additionally, this study lacks insight into triglyceride metabolism across the day. Future studies assessing the relationship between these same sleep features and meals later in the day would provide valuable insight into the allostatic regulation of postprandial triglycerides measured across the day.

Methods

Study population and experimental design

Cohort 1: PREDICT1 study

The hypotheses were first tested in cohort 1, which was the Personalized Responses to Dietary Composition Trial 1 (or “PREDICT 1”). The PREDICT studies are registered clinical trials designed to quantify and predict individual variations in postprandial responses to standardized meals in a real-world setting, while also testing the impact of lifestyle factors such as sleep and physical activity. The PREDICT1 study is a

single-arm, single-blind micro-longitudinal intervention study, the methodology of which (described below) affords the ability to characterize glucose, insulin, lipid, and added postprandial responses to foods based on individual-specific characteristics, including molecular biomarkers, nutritional composition of the food and lifestyle factors. The official start and end dates for the study were 5 June 2018 and 8 May 2019, respectively. The first participant was enrolled on 4 August 2018 and the last clinical visit was completed on 24 April 2019, with the primary cohort based at King's College London in the UK and a second cohort (that underwent the same profiling as in the UK) assessed at Massachusetts General Hospital in Boston, MA, USA. In the UK subset, participants (target enrollment, 1,000 participants) were recruited from the TwinsUK cohort, a prospective cohort study, and online advertising. In the USA, participants (target enrollment, 100 participants) were recruited through online advertising and research participant databases. Of the 1041 total participants, a total of 969 individuals had robust quality sleep actigraphy data and fasting and postprandial lipid profile measurements. Within this dataset, 60% of the participants were fraternal and/or identical twins. The written informed consent and ethical committee approvals covered all analyses reported in the current study in addition to the key primary outcomes described in [93].

The trial was registered on ClinicalTrials.gov (registration number: NCT03479866, first posted on March 27, 2018) as part of the registration for the PREDICT program of research, which also includes two other study protocol cohorts. The trial was run in accordance with the Declaration of Helsinki and Good Clinical Practice. The study was approved in the UK by the Research Ethics Committee and Integrated Application System (IRAS 236407) and in the US by the Institutional Review Board of Partners Healthcare (Protocol # 2018P002078). Participants did not receive financial compensation for taking part in the study.

Study participants were healthy individuals aged 18–65 years who were able to provide written informed consent. Exclusion criteria included ongoing inflammatory disease; cancer in the last three years (excluding skin cancer); long-term gastrointestinal disorders including irritable bowel disease or Celiac disease (gluten allergy), but not including irritable bowel syndrome; taking immunosuppressants or antibiotics as daily medication within the last three months; capillary glucose level of >12 mmol l–1 (or 216 mg dl–1), or type 1 diabetes mellitus, or taking medication for type 2 diabetes mellitus; currently experiencing acute clinically diagnosed depression; heart attack (myocardial infarction) or stroke in the last 6 months; pregnant; and vegan or experiencing an eating disorder or unwilling to consume foods that are part of the study [93].

Replication cohort: PREDICT2 study

The PREDICT2 study, with 928 participants, is a single-arm mechanistic micro-longitudinal intervention study that aimed to further understand lipid responses to dietary intake as well as their modulation by meal sequence and time of day. The trial was registered on ClinicalTrials.gov (NCT03983733, first posted in June 2019), with data collected between June 2019 and March 2021. The study design is roughly similar to the PREDICT1 study. The full description of the study is available on ClinicalTrials.gov. For this particular study, only the fasting lipid profile measurements were available.

The experimental protocol of the PREDICT1 and PREDICT2 studies is very similar (**Figure 1**), and included a baseline day with comprehensive metabolic profiling, followed by a 14-day home phase during which participants wore a wrist-watch actigraphy to measure their sleep/wake patterns. Demographics and descriptive statistics of the two cohorts are reported in Table 1. Of note, participants in the replication cohort were significantly younger, with higher BMI, education, and levels of physical activity.

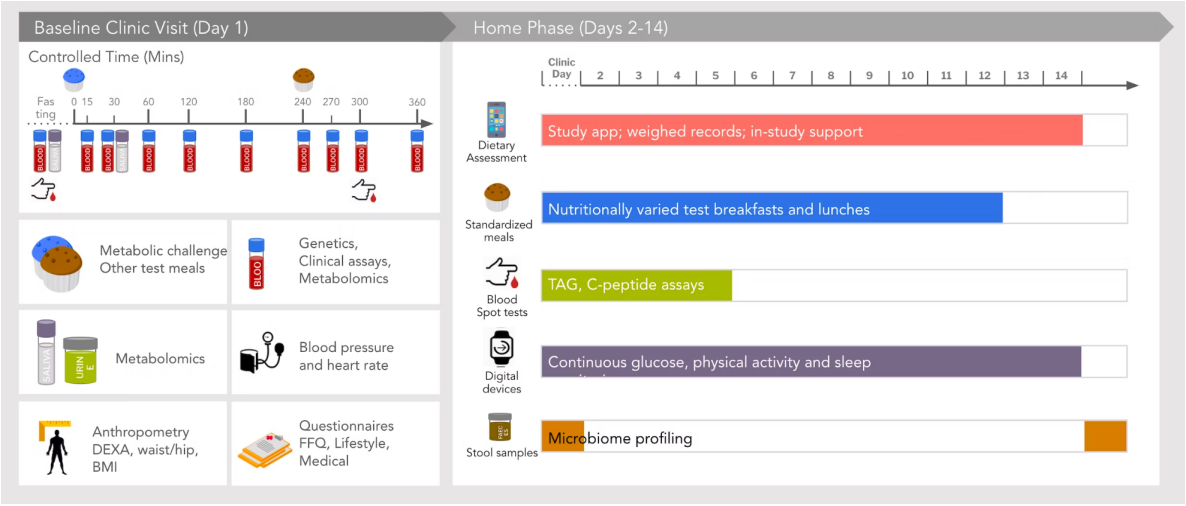


Figure 1: Study design.

Table 1: Participants' characteristics

Metric	PREDICT1	PREDICT2	p
No. of participants	n=1041	n=928	
Twin status	460 MZ / 170 DZ	0 MZ / 0 DZ	
Age	45.44 ± 12.15	43.75 ± 11.62	**<0.001**
Sex	27% male	27% male	-
BMI	25.68 ± 5.09	26.57 ± 5.97	**<0.001**
Waist-hip ratio	0.84 ± 0.08	0.85 ± 0.09	**0.089**
Race White	81%	82%	-
Triglycerides (fasted)	1.06 ± 0.54	1.07 ± 0.63	0.669
ApoB (fasted)	0.89 ± 0.22	0.97 ± 0.22	**<0.001**
Non-HDL-c (fasted)	3.56 ± 0.91	3.98 ± 0.94	**<0.001**
GlycA (fasted)	0.86 ± 0.11	0.82 ± 0.13	**<0.001**
Sleep duration	7.72 ± 0.77	7.74 ± 0.88	0.641
Sleep midpoint	3.31 ± 0.85	3.20 ± 1.11	**0.016**
Sleep efficiency	0.89 ± 0.04	0.89 ± 0.04	0.072
Sleep consistency	0.01 ± 0.47	-0.01 ± 0.53	0.240
PSQI score	6.02 ± 2.86	6.03 ± 2.99	0.949

Sleep measurements

Raw accelerometer processing

In addition to the self-report Pittsburgh Sleep Quality Index (PSQI) [94], sleep/wake patterns were measured using a triaxial accelerometer (AX3, Axivity, Newcastle Upon Tyne, UK). The accelerometer was fitted by clinical practitioners at the baseline clinic visit on the non-dominant wrist and worn for the duration of the study (except during water-based activities, including showers and swimming), after which they were removed on the last day and mailed back to the study staff. The accelerometer was programmed to measure acceleration at 50 Hz with a dynamic range of ±8 g (where g refers to the standard acceleration of gravity, i.e., approximately 9.81 m/s^2). Non-wear

periods were defined as windows of at least 1 hour with less than 13 mg for at least 2 out of 3 axes, or where 2 out of 3 axes measured less than 50 mg [95].

Raw accelerometer data was analyzed using the `GGIR` R package version 1.10-7 [96]. Sleep/wake detection was then quantified using the validated algorithm described in [97], which uses the variance in the accelerometer z-axis angle together with a set of heuristic rules to determine sleep periods. This algorithm does not require a sleep diary and has been validated against gold-standard polysomnography in both healthy individuals and patients with sleep disorders, with a mean concordance statistic of 0.86 and 0.83, respectively [97]. For each night, the following sleep metrics were calculated: sleep onset, sleep midpoint, sleep offset, sleep duration (defined as the elapsed time from sleep onset through sleep offset, or sleep period time [SPT]), wake after sleep onset (WASO), total sleep time (TST; = SPT - WASO), sleep efficiency (SE; = TST / SPT). Sleep efficiency was calculated using the SPT and not the more common total time in bed as the denominator because the absence of sleep diary data precludes the accurate estimation of bedtime prior to sleep. For the same reason, the algorithm is unable to characterize sleep onset latency (the time between going to bed and falling asleep). The GGIR algorithm is not able to detect naps and therefore only nighttime sleep parameters were included in subsequent analyses.

A set of thresholds was then applied to remove invalid nights or participants, consistent with typical practices [98]. First, any nights with a TST outside the range of 2.5 to 12 hours were excluded. Second, nights with more than 30% of epochs classified as invalid by the GGIR algorithm were excluded. Third, nights with a sleep onset outside of 7 pm to 6 am or a sleep offset before 2 AM were excluded, as well as nights with a sleep offset occurring exactly at noon (indicating cropping by the GGIR algorithm). Finally, participants with less than 4 days of valid sleep data were removed.

Derivation of traits sleep factors

For each participant, a set of four unique sleep factors were derived from the above longitudinally measured sleep metrics and used as the main predictors of interest in all statistical models below. Importantly, these four sleep parameters all cover a unique aspect of sleep, as indicated by their relatively weak relationships (**Figure 2**). These are:

1. The average sleep duration, expressed in hours, defined as the individual's typical sleep duration across the micro-longitudinal 10-14 day study.
2. The average sleep efficiency, is defined as the percentage of the sleep period time that was spent sleeping.

3. The average midpoint of sleep, expressed as a deviation from midnight in hours and defined as (waketime – bedtime) / 2. Sleep midpoint is a standard metric to estimate chronotype [99].

4. The average sleep consistency, expressed in standardized units, which measures whether a given participant is consistent or not in the amount and timing of their sleep. Sleep consistency was created by taking the first component of a principal component analysis (PCA) on the standard deviation of sleep midpoint and the standard deviation of sleep duration.

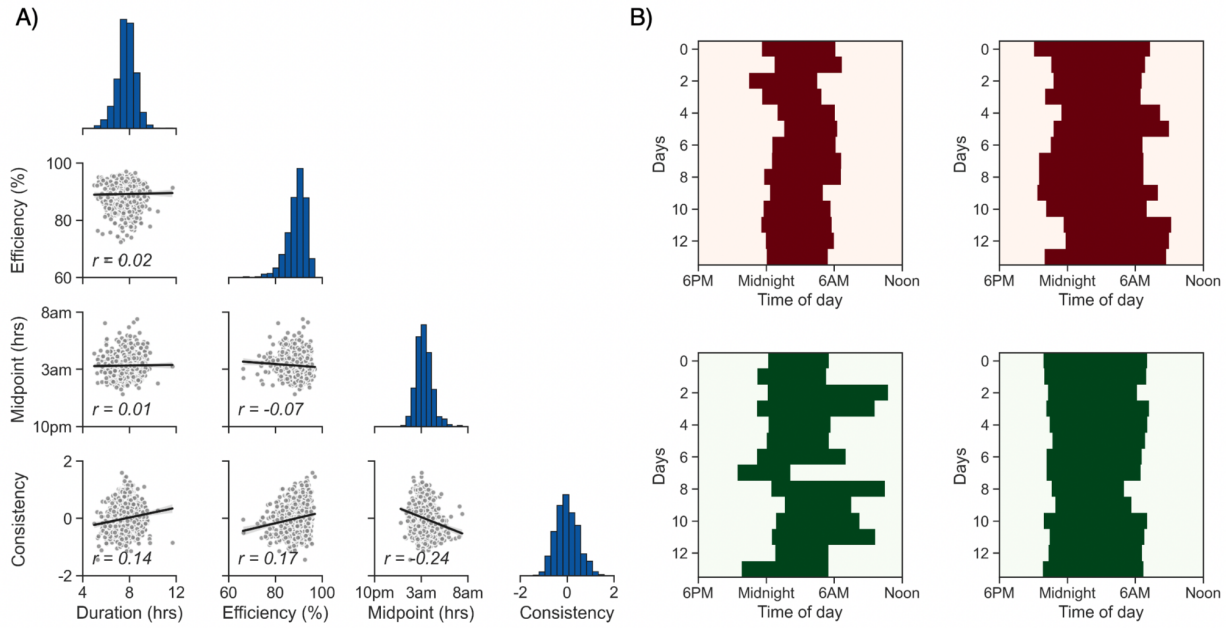


Figure 2: Estimation of sleep traits in Cohort 1 (the PREDICT1 dataset). A) For each participant, four sleep factors were derived from the longitudinal wrist-worn actigraphy recordings: sleep duration, efficiency, midpoint, and consistency (see Methods). These four sleep parameters all cover a unique aspect of sleep, as indicated by the relatively weak linear relationships. B) Actograms showing representative participants with short sleep duration (upper left), long sleep duration (upper right), inconsistent sleep (lower left), and stable sleep (lower right). Dark and light blue periods indicate sleep and wake, respectively.

Lipid profile measurements

Participants ate standardized nutrient-controlled meals twice across a 4-hour period: the first measure reflecting the food-intake administration (time 0 hour, or the T=0 hour time point), the second consumed 4 hours later (i.e., T=4 hours). As in prior studies of lipid assessment [16,100], lipid status was assessed twice, the first measurement taken at

T=0 hours, but importantly, before the first meal, reflecting the baseline lipid state. The second was taken 6 hours later (T=6 hours, i.e. 6 hours after the first meal, and 2 hours after the second meal). Standard nuclear magnetic resonance (NMR) spectroscopy was used on blood samples (plasma) to perform a full lipid panel. NMR was applied on the blood samples at both T=0 and T=6hrs. Triglyceride and VLDL cholesterol levels were measured at T=0 (fasting) and T=6hrs following a first standardized metabolic challenge meal at T=0 and then a second meal at T=4hrs. For each of the postprandial triglyceride measures, the difference (rise) between postprandial and fasting levels was calculated. The 6-hour time point was selected having been established as a meaningfully accurate physiological-response index to the allostatic load challenge that a standardized meal induces [100]. In such protocols, triglyceride levels peak 3-5 hours after a meal in metabolically healthy individuals [16,101], and return to baseline approximately 6 hours after a meal. This profile is considered an adaptive response to the allostatic food overload, one that returns the system back into lipid homeostasis. However, metabolically unhealthy individuals do not respond in the same dynamic way, resulting in persistently higher relative levels of triglycerides, indicative of impaired lipid management and allostatic overload [16,102,103]. Of clinical relevance, postprandial triglyceride levels are a stronger biomarker of cardiovascular illness than fasting triglyceride levels [16,65].

Statistical Analysis

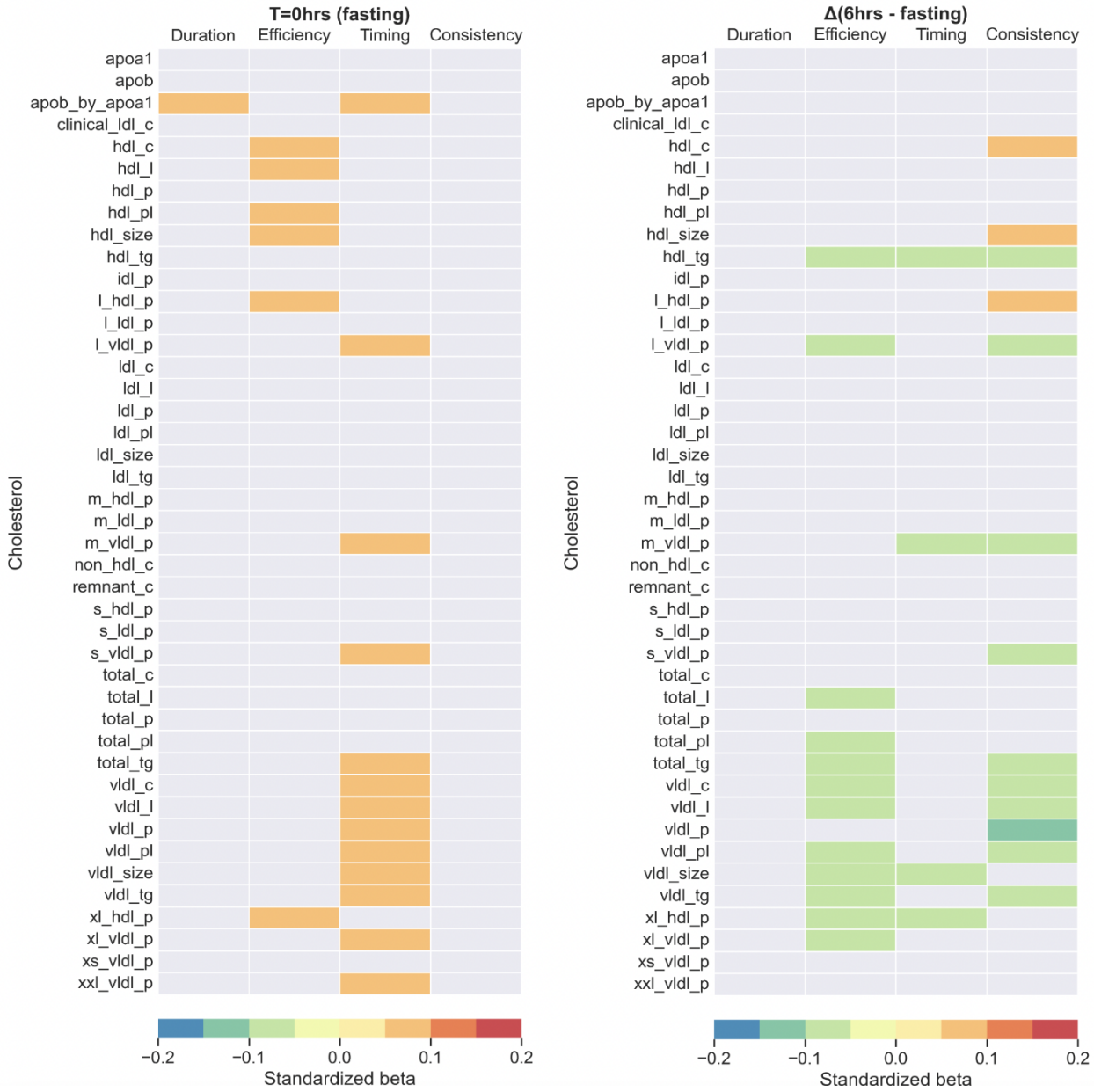
Standard linear regression models were used to measure the statistical association between sleep and lipid outcomes. Unless specified otherwise, all models were adjusted for age, sex, body mass index (BMI), race, education, smoking, and physical activity. Since Cohort 1, PREDICT1, included twins from the same family, a multilevel regression was used with family identifier as a random effect. All regression analyses were conducted in R.

For all regression models, the variance inflation factor (VIF) was used to check for multicollinearity. Diagnostic plots were used to assess the validity of the fitted models. For each model, these included scatterplots of standardized residuals by fitted values and observed versus fitted values. Normal quantile plots (Q–Q plots) were used to check the assumption of normality of the residuals and random effects.

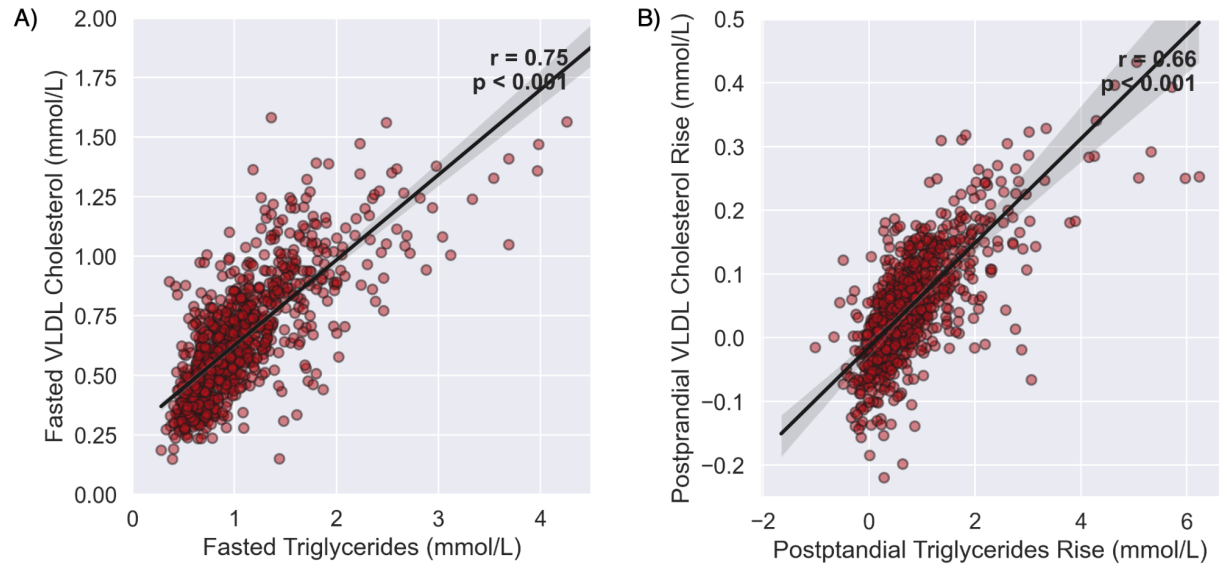
Mediation analyses were conducted with the `mediation` R package [57] and default parameters.

Supplementary Materials

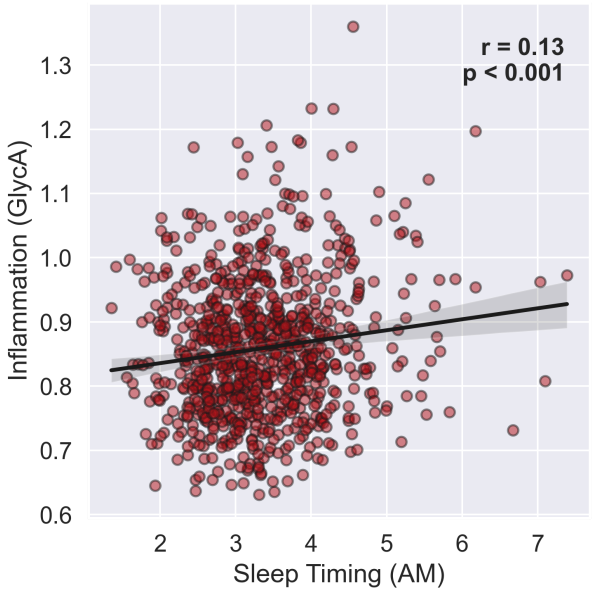
Supplementary Figures



Supplementary Figure 1: Sleep and cholesterol metabolite associations from NMR data. A later sleep timing was significantly associated with worse VLDL outcomes in the fasted state. However, higher sleep efficiency predicted a lower rise in postprandial VLDL outcomes. Grayed cells are not significant. Beta and p-values calculated from a fully adjusted multilevel model.



Supplementary Figure 2: Triglyceride levels are positively correlated with VLDL cholesterol levels. **A)** Total triglyceride levels in the fasted state are significantly associated with total VLDL cholesterol levels in the fasted state. **B)** The baseline-adjusted postprandial rise in total triglyceride levels is significantly associated with the baseline-adjusted rise in VLDL cholesterol levels.



Supplementary Figure 3: Sleep timing is positively correlated with inflammation in the fasted state.

Chapter 3. Sweet slumber: Coordinated human sleeping brainwaves map peripheral body glucose homeostasis

Introduction

Diabetes—a condition of marked glucose dysregulation—is a major cause of death globally. The World Health Organization estimates that over 420 million people are suffering from the condition, which carries a direct societal cost of $760 billion each year [104]. These preventable mortality and financial costs are projected to increase markedly over the next decade [104,105].

Experimental studies in humans and animals have demonstrated that one causal factor impairing blood glucose equilibrium is insufficient sleep [10,17]. Both acute and chronic partial sleep restriction, including that of non-rapid eye movement (NREM) slow-wave sleep, impair glucose tolerance and insulin sensitivity [106–109]. Conversely, sleep extension improves glucose metabolism [110].

But why? Currently, the mechanism(s) through which sleep optimally governs next-day glucose homeostasis in humans remains unknown. A recent seminal study in rodents has offered one candidate pathway [111]. Specifically, hippocampal sharp-wave ripples — which are temporally coupled with NREM slow oscillations and sleep spindles [112–114] — were associated with the top-down regulation of peripheral blood glucose through activation of the hypothalamus (which itself provides autonomic control of peripheral circulating hormones, including insulin) [111,115].

Collectively, these findings lead to the hypothesis that one function of synchronized (i.e., temporally coupled) NREM slow oscillation—sleep spindle events in humans is the brain-body regulation of optimal glucose homeostasis. More specifically, that both the extent and quality of coupled NREM slow oscillations—spindle events in humans would predict optimal next-day regulation of peripheral blood glucose levels.

Results

In short (see STAR Methods and Table S1), a total of 647 humans with overnight polysomnography data and next morning glucose and insulin measurements were analyzed. Together with electrophysiological analysis of sleep oscillations and circulating morning measures of glucose, insulin resistance and pancreatic beta cell function were further quantified using the validated homeostasis assessment models (HOMA-IR and HOMA-B respectively; see STAR Methods and Figure S1 for details). Using these evaluations, we tested the prediction that coupled NREM slow

oscillations—spindles the night prior are associated with improved next-day peripheral blood glucose levels. To examine the robustness of these findings, we then validated the associations between NREM slow oscillation—spindle coupling and peripheral blood glucose levels in an independent, larger replication cohort of 1996 humans with the same sleep and glucose indices.

Focusing first on the cohort of 647 participants, and as expected, NREM slow oscillations (SO, <1 Hz) were functionally coupled with sleep spindles (mean: 87.6%, SD: 3.35, Table S1), such that the phase of the slow oscillation modulated the amplitude of the spindle-related frequency band (12-16 Hz), hereafter referred to as slow oscillation—spindle coupling (for conciseness). The strongest coupling between slow oscillation and spindle-related activity occurred ~0.4 sec after the negative peak of the slow oscillation (Figure 1A, Table S1). For most individuals, the maximum coupling occurred near the up-phase of the slow oscillation (-12.15° ± 28.32, Figure 1B, Table S1).

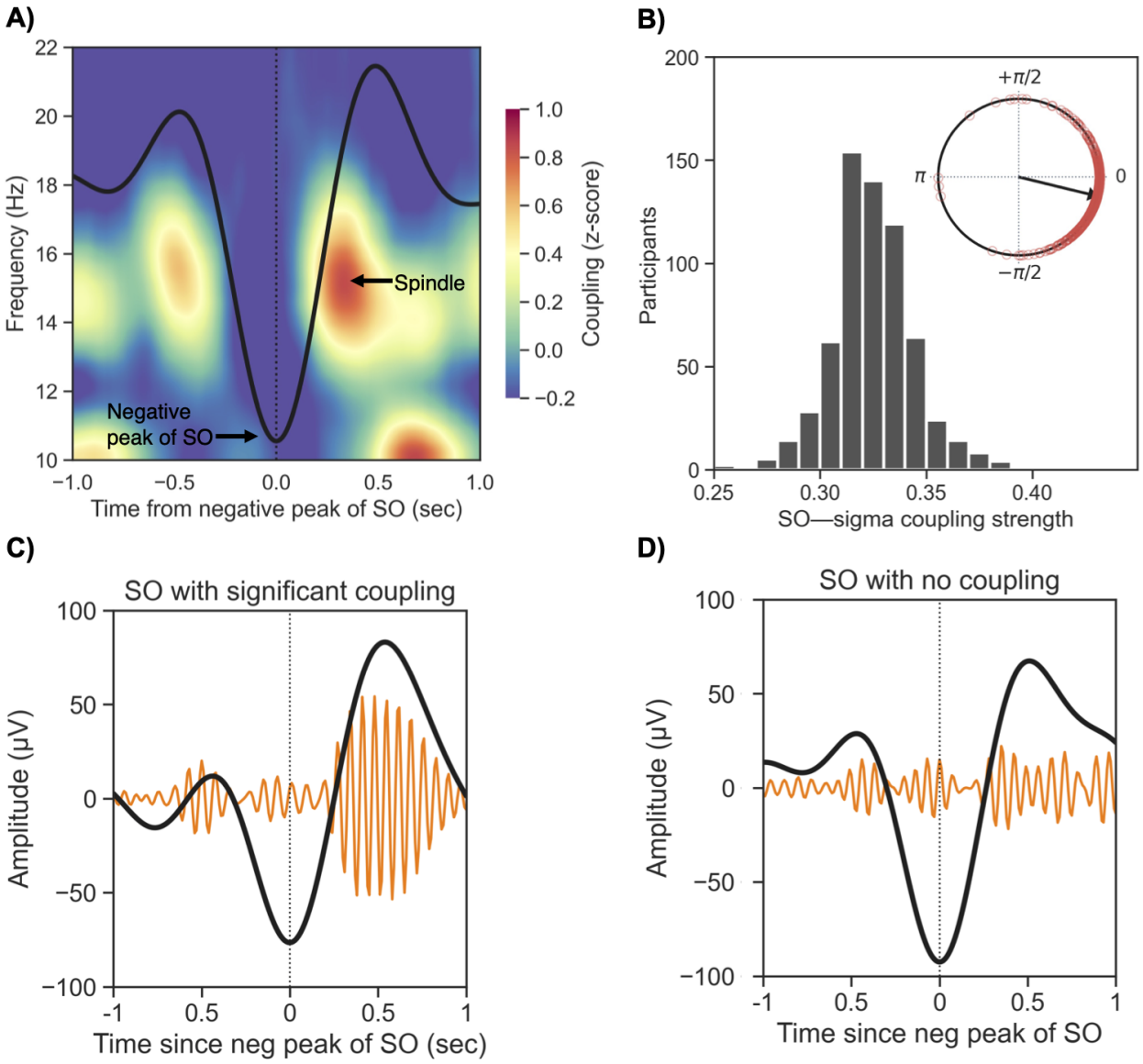


Figure 1: Slow oscillations are functionally coupled with sleep spindles.

A) In human NREM sleep, slow oscillations (SO, <1 Hz) are functionally coupled with sleep spindles, such that the phase of the slow oscillation modulates the amplitude of the spindle-related frequency band (12-16 Hz). This plot shows the average peak-locked slow oscillation calculated across all the participants (black thick line) and the associated time-frequency representation of the coupling strength [116]. Warmer color indicates higher phase-amplitude coupling. The strongest coupling between slow oscillation and spindle-related activity occurs ~0.4 sec after the negative peak of the slow oscillation. **B)** Histogram of the average slow oscillations—spindle coupling strength across all participants. The coupling strength is calculated using the ndPAC method [117]. The circular plot shows the histogram of the preferred phase of the coupling. For most individuals, the maximum coupling occurs near the up-phase of the slow oscillation (0°). **C)** Example of a coupled slow oscillation (SO). The thick black line shows the SO-filtered signal (0.3-1.5 Hz), whereas the orange lines show the associated spindle-filtered (12-16 Hz) signal, scaled by a factor of 4 for illustrative purposes. **D)** Example of an uncoupled slow oscillation (SO)

from the same individual as in **C)**. No statistical SO—spindle coupling was detected for this SO (see **STAR Methods**).

Next, we tested the prediction that the degree of such coupling of NREM sleep oscillations was associated with glycemic control the following day. Supporting the hypothesis, greater slow oscillation—spindle coupling at night predicted lower next-day fasting blood glucose levels (partial correlation adjusted for age, r=-0.20, n=631, p<0.001; Figure 2A). Beyond the simple quantity of synchronized slow oscillation—spindle events, the strength of the temporal synchrony (meaning, the precision of the timing of the coupling) between slow oscillations and spindle activity was similarly associated with lower subsequent fasting blood glucose levels (partial r=-0.17, n=631, p<0.001; Figure 2B).

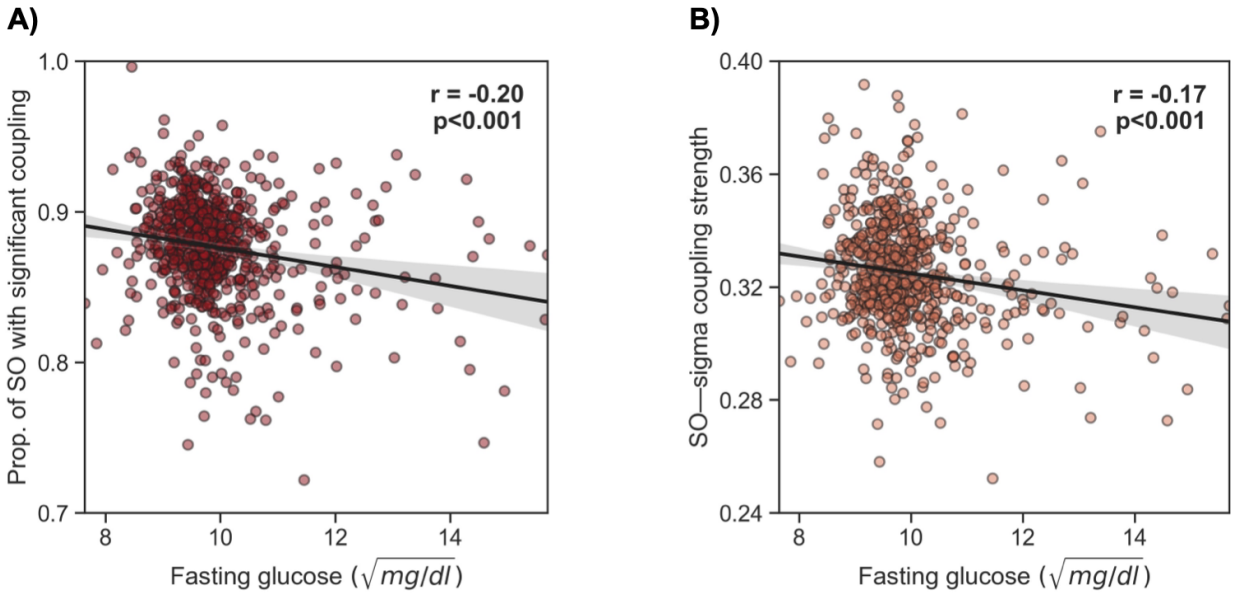


Figure 2: Slow oscillation—spindle coupling predicts lower next-day fasting glucose in the CFS dataset.

A) Partial correlation adjusted for age between the extent of slow oscillation—spindle coupling (i.e. the proportion of slow oscillations that are significantly coupled, see **STAR Methods**) and next-day fasting blood glucose levels. **B)** Partial correlation adjusted for age between slow oscillation—spindle coupling strength and next-day fasting blood glucose levels. Translucent bars represent 95% bootstrapped confidence intervals. Fasting glucose levels were normalized using a square-root transformation (see **STAR Methods**). Of note, both coupling measures remained significantly correlated with fasting glucose levels when removing fasting glucose values above 12 (= 144 mg/dL; r=-0.20, p<0.001 and r=-0.15, p<0.001, respectively).

To date, multiple other factors have been identified that influence glycemic control beyond sleep. Prima facie examples include age, gender, race, body mass index (BMI), hypertension, and even certain sleep features, such as apnea-hypopnea index (AHI), the quantity of sleep, and specific sleep stages [118,119].

To ensure that the relationship between slow oscillation—spindle coupling and blood glucose levels was robust, multilevel regression models were fitted to adjust for these known co-risk factors. With all factors included in the analysis model (age, gender, race, BMI, hypertension, AHI, sleep duration, sleep efficiency, and family as a random effect), the relationships between higher slow oscillation—spindle coupling and lower next-day fasting blood glucose levels remained significant (p=0.001 and p=0.020 respectively; Tables S2 and S3). This suggests a statistically independent contribution of coordinated sleep oscillations to the mapping of next-day blood glucose control beyond these other classic factors known to govern glycemic state.

An additional sensitivity analysis was conducted adjusting for diabetes status as an additional covariate, to check if this association is different in normoglycemic versus diabetic individuals. The association between coupling quantity and lower fasting blood glucose levels remained at trending significance when including diabetes status as an additional covariate in the regression analysis (β = -1.79, p = 0.063). However, the association between coupling strength and fasting glucose did not remain significant (β = -2.59, p = 0.133).

Two other important risk factors for metabolic health are smoking status and education level [120,121]. These two factors were not included in the main regression model because of a high missingness of data, which resulted in the exclusion of ~30% of participants from the analysis. However, the association between coupling quantity and lower fasting blood glucose levels remained significant when including smoking status and education level as additional covariates in the regression analysis (β=-4.22, p=0.002). This was not true of the coupling strength (β=-2.65, p=0.29). Based on in vivo cellular recordings in animal models, the proportion of slow oscillation—spindle coupling may be a better metric of hippocampal sharp-wave ripple density, which is causally associated with peripheral blood glucose levels via a hypothalamic signaling pathway [111], than the strength of that brainwave coupling [114]. Taken together, such selectivity may suggest that the proportion of coupled slow oscillation—spindle events represents the most sensitive sleep biomarker of glucose homeostasis.

Beyond the predictive relationships with fasted blood glucose levels, similar associations were observed with two-hour postprandial glucose values following an oral glucose tolerance test (OGTT). To assess whether overnight sleep was associated with next-day OGTT glucose levels at all, we re-ran the models reported for fasting blood glucose and slow oscillation—spindle coupling, similarly adjusted for known risk factors including age, gender, race, BMI, hypertension status, AHI, sleep duration, and efficiency. Here again, both the proportion (β = -5.36, p = 0.037) and strength (β =

-9.31, p = 0.044) of slow oscillation—spindle coupling were significantly associated with lower (superior) next-day OGTT values. Therefore, slow oscillation—spindle coupling demonstrated interrelationships both with the fasted condition of the glucose state, and further, the body's dynamic reaction to a metabolic glucose challenge, requiring a regulatory glycemic response.

The next series of analyses sought to test the replicability and robustness of the slow oscillation—spindle coupling reflecting a marker of glucose homeostasis in a larger, independent cohort. For this purpose, we examined the Multi-Ethnic Study of Atherosclerosis (MESA [51]; see STAR Methods, Figure 3A and Table S4) of over 1900 participants who had fasting glucose measurements and overnight polysomnography data.

Consistent with the results in the first dataset, slow oscillation—spindle coupling during NREM sleep once again predicted superior fasting peripheral blood glucose (partial correlation adjusted for age, r=-0.103, n=1968, p<0.001; Figure 3B). Moreover, the strength of the temporal synchrony between slow oscillation—spindle coupling was similarly associated with lower fasting blood glucose levels (partial r=-0.130, n=1968, p<0.001 respectively; Figure 3C).

As in the first cohort, both the proportion and strength of slow oscillation—spindle coupling remained significantly associated with fasting blood glucose after adjusting for assessed risk factors (age, gender, race, BMI, hypertension, AHI, and the quantity and quality of sleep; p=0.034 and p=0.011, respectively, Tables S5 and S6).

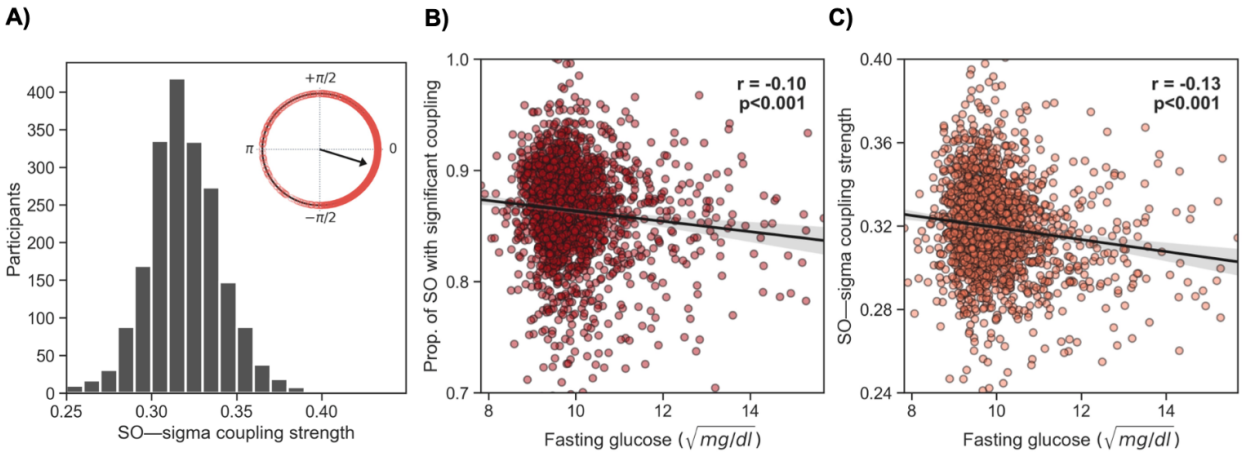


Figure 3. SO-spindle coupling during sleep is a prominent marker of glucose homeostasis, in an independent (MESA) dataset

A) Histogram of the average slow oscillations—spindle coupling strength across all participants in the MESA dataset. The coupling strength is calculated using the ndPAC method [117]. The circular plot shows the histogram of the preferred phase of the coupling. For most individuals, the maximum coupling occurs near the up-phase of the slow oscillation (0°). **B)** Partial correlation adjusted for age between the extent of slow oscillation—spindle coupling (i.e. the proportion of slow oscillations that are significantly

coupled, see **STAR Methods**) and next-day fasting blood glucose levels, in the MESA dataset. **C)** Partial correlation adjusted for age between slow oscillation—spindle coupling strength and next-day fasting blood glucose levels. Translucent bars represent 95% bootstrapped confidence intervals, in the MESA dataset. Fasting glucose levels were normalized using a square-root transformation (see **STAR Methods**).

Taken together, these results replicate the association between slow oscillation—spindle coupling and fasting blood glucose levels from the first dataset, and in a larger cohort, support the association of slow oscillation—spindle coupling as a central brain marker of peripheral body glycemic status.

Glucose homeostasis is governed by several independent mechanisms, key among them being the function ability of pancreatic beta cells, which initially sense increases in glucose and lead to the release of insulin, and separately, the sensitivity of cells in the body to the signal of insulin (the impairment of which results in insulin resistance). Having established the association between coupled NREM sleep oscillations and peripheral body glucose state, we next sought to determine whether this sleep biomarker was mapping one or both of these glucose homeostasis pathways within the first main cohort. This was accomplished using the added measures of HOMA-IR, offering a representation of insulin resistance/sensitivity, and HOMA-B, an index of insulin secretory function [122–124].

Lower slow oscillation—spindle coupling predicted higher (i.e., worse) next-day insulin resistance the following day, quantified using the validated metric of HOMA-IR ($r=-0.213$, $n=634$, $p<0.001$; Figure 4A). However, suggesting a mechanistic dissociation, no such sleep associations were identified with next-day pancreatic beta cell secretory function, evaluated with the metric of HOMA-B ($r=-0.072$, $n=626$, $p=0.074$).

Furthermore, both the proportion and strength of slow oscillation—spindle coupling remained significantly associated with HOMA-IR after adjusting for all aforementioned risk factors ($p=0.005$ and $p=0.016$, respectively, Tables S7 and S8).

Beyond the simple number (quantity) of synchronized slow oscillation—spindle events, the quality of coupling (indexed by the strength of temporal synchrony between slow oscillations and spindle activity) was similarly associated with improved next-day blood glucose homeostasis, as assessed by fasted glucose levels ($r=-0.170$, $n=634$, $p<0.001$; Figure 2B), as well as next-day insulin sensitivity as measured by HOMA-IR ($r=-0.197$, $n=634$, $p<0.001$; Figure 4B). Once again, there was no such association with the pancreatic beta cell function measure of HOMA-B ($r=-0.066$, $n=626$, $p=0.097$).

Such results further support the proposal that the association between NREM sleep oscillations and next-day glucose homeostasis is best understood through altered

insulin sensitivity within the body, rather than changes in pancreatic beta cell function and corresponding insulin release.

One candidate pathway explaining the association between slow oscillation—spindle coupling and next-day glucose homeostasis is an alteration in heart rate variability (HRV) during sleep, an indirect measure of autonomic parasympathetic activity. Accordingly, we conducted a mediation analysis, which revealed that HRV (see **STAR Methods**), significantly mediated the association between both proportion and strength of slow oscillation—spindle coupling and next-day fasting glucose levels in the MESA dataset (indirect effect: $p = 0.0014$ and $p = 0.0008$ respectively; **Figures S2A** and **S2B**). Specifically, the greater the proportion of coupled slow oscillations during sleep, the higher the HRV (indicative of greater parasympathetic dominance) during sleep ($p = 0.001$; adjusted for all aforementioned cofactors; see also 1) that, in turn, was linked to superior (i.e. lower) next-day fasting blood glucose levels ($p < 0.001$). In the CFS dataset, a similar effect was observed for HRV during sleep mediating the association between the proportion of slow oscillation—spindle coupling and insulin resistance, with trending significance (indirect effect: $p = 0.076$; **Figure S4A**), such that a higher proportion of coupled slow oscillation—spindle events was associated with higher HRV ($p = 0.037$), through the statistical mediation pathway, further predictive of lower insulin resistance ($p = 0.022$). However, HRV in the CFS cohort did not significantly mediate the association between the proportion (indirect effect: $p = 0.135$; **Figure S3A**) or strength (indirect effect: $p = 0.66$; **Figure S3B**) of slow oscillation—spindle coupling and next-day fasting glucose levels.

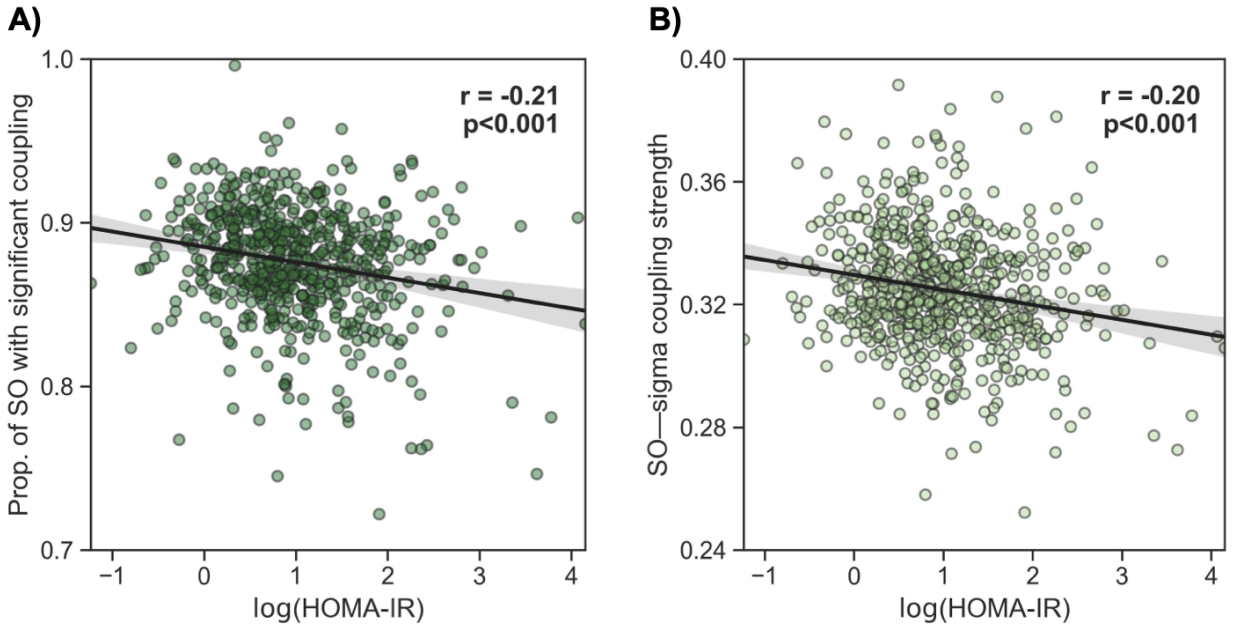


Figure 4: Insulin resistance is significantly correlated with the coupling between slow oscillations and spindle-related activity in the CFS dataset.

A) Partial correlation adjusted for age between the extent of slow oscillation—spindle coupling (i.e. the proportion of slow oscillations that are significantly coupled, see **STAR Methods**) and next-day HOMA-IR. **B)** Partial correlation adjusted for age between slow oscillation—spindle coupling strength and next-day HOMA-IR. Translucent bars represent 95% bootstrapped confidence intervals.

Since impaired glucose function has been associated with broad, macro-level sleep features, such as sleep apnea severity, sleep duration, and certain stages of sleep [118,119], we next examined the predictive sensitivity of our a priori micro-sleep measures of slow oscillation—spindle oscillation coupling, and how it ranked relative to all other sleep metrics. Notably, after adjusting for known risk factors for glucose homeostasis (specifically, age, gender, BMI, hypertension, and family as a random effect), slow oscillation—spindle coupling was the single strongest sleep predictor of next-day fasting glucose levels and insulin resistance relative to all other traditional sleep metrics (Figure 5). This included the amount of time (number of minutes and percentage) in each sleep stage (N1, N2, N3, and REM), sleep duration and sleep efficiency, wake after sleep onset (WASO), the arousal index, sleep apnea severity as measured with the AHI, individual morphological features of either slow oscillations or spindles (density, frequency, amplitude), and spectral band power in REM or NREM sleep (slow delta, fast delta, total delta, theta, alpha, sigma, beta; see Figure 5 and Methods). Together, these findings indicate that slow oscillation—spindle coupling is a predominant sleep marker associated with next-day glucose homeostasis.

For a purely ecological context setting, we examined the effect-size association between superior to inferior slow oscillation—spindle coupling and next-day glucose homeostasis balance. Going from the 1st percentile of the proportion of coupled slow oscillations (77%) to the 99th percentile (94%) represented a decrease of 13.2 mg/dL in fasting blood glucose levels (holding all other covariates constant). Similarly, going from the 1st percentile to the 99th percentile value of the coupling strength represented a decrease of 9.9 mg/dL in fasting glucose levels. For reference, the current CDC guidelines indicate that a difference of ~15 mg/dL in fasting glucose levels reflects the difference between an individual in a normoglycemic zone to being prediabetic (e.g. from 95 to 110 mg/dL), or from a prediabetic state to being diabetic (e.g. from 115 to 130 mg/dL).

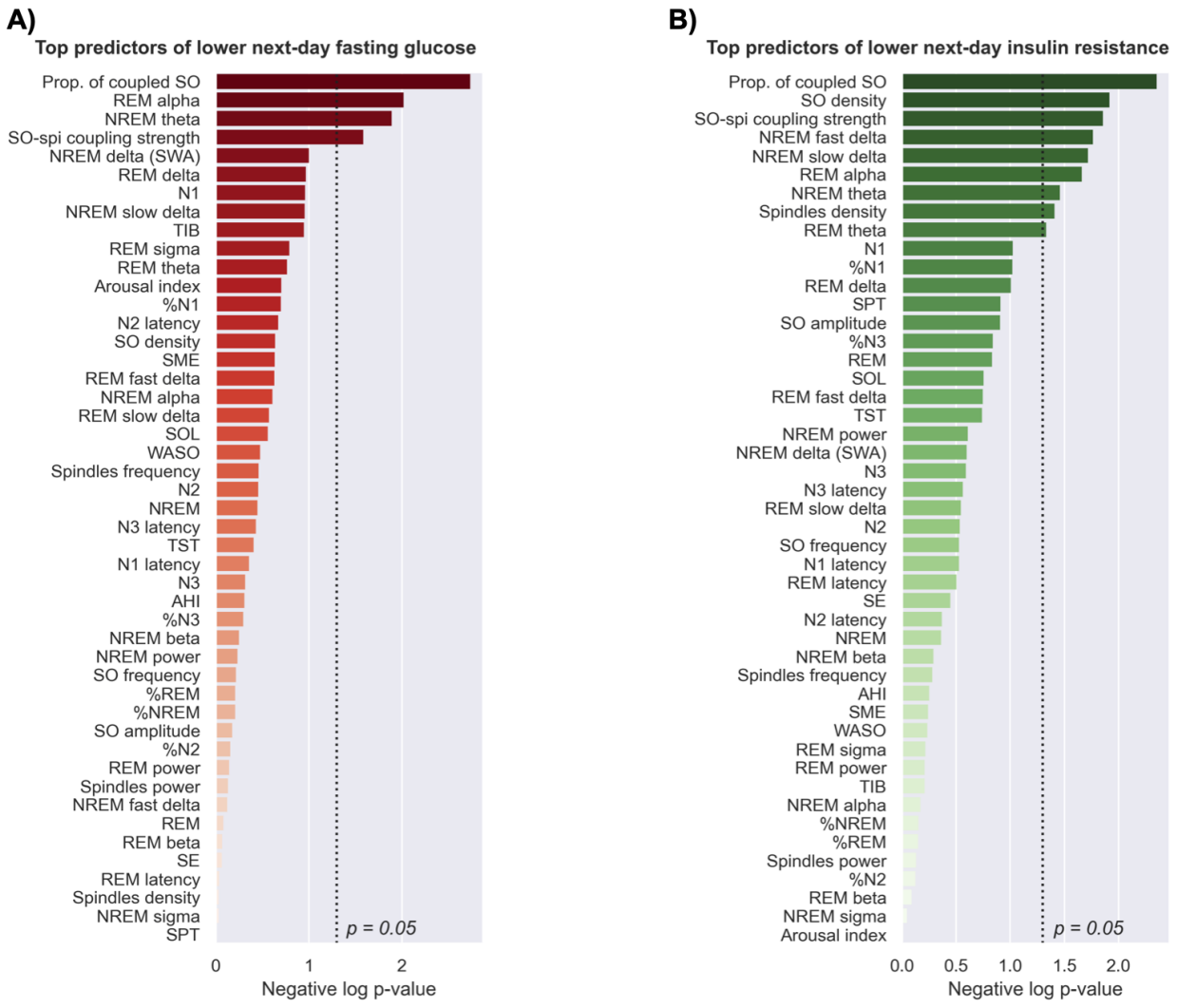


Figure 5: Slow oscillation—spindle coupling is the top sleep predictor of next-day glucose homeostasis.

A) Top sleep predictors of lower next-day fasting glucose, ranked in descending order of significance (negative log10 p-value). **B)** Top sleep predictors of lower next-day insulin resistance (HOMA-IR), ranked in descending order. The proportion of SO with significant coupling was the best sleep predictor of both fasting glucose and insulin resistance. Unadjusted two-tailed P-values were obtained by fitting, for each sleep predictor separately, a multilevel regression model adjusted for age, gender, BMI, race/ethnicity, hypertension, and family ID. A total of 47 sleep parameters were included in the rank analysis. NREM refers to N2 + N3 sleep (N1 excluded). A full description of these parameters is provided in **Tables S9** and **S10**.

Discussion

Taken together, these findings support a NREM sleep-oscillation-body framework of glucose homeostasis in humans; one that describes a mapped association between prior slow oscillation—spindle coupling and next-day glucose homeostasis.

Prior observations in rodents have demonstrated that hippocampal ripples during sleep decrease peripheral blood glucose levels, in part through a hypothalamic signaling pathway [111]. Considering that coupled slow oscillation—spindle activity coincides subcortically with hippocampal sharp wave ripples [112,114], our results indicate the presence of a similar sleeping-brain—glycemic association observable in humans. Furthermore, Tingley et. al. reported that isolated ripples did not show an association with peripheral glucose levels, whereas bursts of hippocampal ripples did. There is also evidence, in humans, that the nesting of ripples in spindle troughs during slow oscillation—spindle coupling, is stronger associated with ripple bursts, as compared to isolated ripples [112–114,125]. These findings indicate the possibility that the slow oscillation—spindle coupling-glucose homeostasis association is driven by collective burst trains of hippocampal sharp wave ripples, as opposed to isolated ripples. Such oscillation trains may therefore underlie part of the mechanistic pathway accounting for the associations reported here in humans.

The above-noted NREM sleep-oscillation-body framework of glucose homeostasis can be considered across at least two different time scales, though they may not be mutually exclusive. The first, as we describe here in humans, involves a temporally longer, feed-forward association such that NREM slow oscillation—spindle coupling predicts superior next-day glucose homeostasis. The second, previously observed in rodents [111], involves a short-term feedback loop between hippocampal sharp-wave ripple activity and concurrent changes in circulating glucose during sleep. Both processes, either independently, or interactively (e.g., moment-to-moment changes in glucose across the night cumulatively determine next-morning glucose status), may aid in generalized glycemic homeostasis. These pathways may further offer disease insights into the brain(sleep)-body(glucose) mechanisms that help explain the well-characterized sleep between short and disrupted sleep, hyperglycemia, and type 2 diabetes [17].

Moreover, and critical from a clinical and public health perspective, we further establish that these associations remained significant when controlling for prototypical factors that themselves are known to impact blood glucose, including age, gender, race, BMI, hypertension, and even sleep measures, such as the apnea-hypopnea index (AHI), and the quantity and quality of sleep. That is, an association between slow oscillation—spindle coupling and glucose homeostasis that is independent of other

co-factors influencing glycemic control—both in measures of fasted blood glucose assessment and following the standard metabolic challenge of an oral glucose tolerance test (OGTT).

Importantly, the association between slow oscillation—spindle coupling and peripheral glucose homeostasis was also validated in an independent, larger replication dataset, suggesting that the effects are less likely to be driven by single cohort-specific idiosyncrasies. Rather, the replication of the association between slow oscillation—spindle coupling and fasting blood glucose levels offers added support to the framework of slow oscillation—spindle coupling metric in a predictive or supervisory role of glucose homeostasis [111], above and beyond other traditional sleep metrics.

Adding to these insights, slow oscillation—spindle coupling predicted next-day improved (enhanced) insulin sensitivity, but not pancreatic beta call function. Our findings thus indicate a potential dissociation between two key glycemic control mechanisms: 1) the sensitivity of pancreatic beta cells to the glucose status of the body, which can release insulin in the presence of sensed high glycemic load, and 2) the sensitivity of cells within the body to that consequential signal of insulin released by the pancreas, resulting in the cellular uptake of glucose from the blood [118]. The results reported here suggest that the link identified between slow oscillation—spindle coupling and glucose homeostasis is not one associated with a dual-action regulation of glycemic control. Rather, relationships were observed only for the measure of HOMA-IR (indexing cellular insulin sensitivity), and not HOMA-B, reflecting pancreatic beta cell sensing. Therefore, the association with blood glucose stasis appears to be most parsimoniously explained by a link between NREM sleep oscillations and a select alteration in insulin sensitivity [126,127], rather than regulating pancreatic beta cell function or insulin synthesis/secretion [124,128].

Alterations in fasting glucose levels and impairments in OGTT, as we identify in the current study, each reflect different aspects of insulin resistance. The former has been linked to hepatic insulin resistance, while the latter is primarily associated with impaired muscle insulin resistance [129]. It is important to note, however, that the OGTT findings were only assessed in normoglycemic individuals. Future examinations in hyperglycemic cohorts are needed to explore whether this sleep-associated allostatic response is different in diabetes.

One proposed mechanism explaining the recognized link between deficient sleep and impaired blood glucose control is an alteration of autonomic sympathovagal balance resulting in a biased state of sympathetic activity over parasympathetic activity [130], which may chronically lead to insulin resistance and metabolic dysfunction [131]. Addressing this question, we conducted a mediation analysis to test whether coupled NREM oscillations and superior glycemic status were mediated through an association with increased parasympathetic autonomic activity during sleep. HRV, a measure of

autonomic parasympathetic activity, significantly mediated the association between both, the proportion of slow oscillation—spindle coupling and next-day fasting glucose levels, in the MESA dataset, but not in the CFS dataset. Parasympathetic activity may be only one partial pathway linking slow oscillation—spindle coupling with next-day glucose homeostasis. It further indicates that other such pathways may exist that account for the additional variance in mediation that is not explained by parasympathetic activity in this sleep-glycemic relationship.

To date, associations between sleep loss, blood glucose status, and diabetes risk have productively focused on traditional sleep statistics, including sleep duration, sleep efficiency, amount of each sleep stage (particularly the loss of deep NREM sleep [108,109]), and markers of sleep disorders (e.g. AHI) [17,132]. However, exactly what it is within sleep that accurately maps glycemic control in humans has remained unknown. Addressing this issue, we demonstrate that slow oscillation—spindle coupling is not only a sensitive glycemic index, but of all sleep features, including sleep stages, and all other sleep electrical oscillation spectra, such coupling offers the highest predictive sensitivity of next-day glucose homeostasis. Indeed, this predictive relationship with glucose status exceeded that of all other sleep measures assessed, including total sleep amount, sleep efficiency, NREM slow wave sleep, as well as sleep apnea severity (AHI score). Our findings in no way challenge these now robust links between those aforementioned sleep measures and diabetes risk and/or blood glucose status [17,133]. Rather, our results establish the measure of slow oscillation—spindle coupling as an additional, independent contributing feature of sleep, one that offers insights into potential disease pathways associated with diabetes considering recent rodent data causally linking slow oscillation—spindle coupling with momentary glucose regulation.

In conclusion, our findings suggest a sleeping-brain—body framework of insulin-associated glucose homeostasis in humans, and re-emphasize the importance of sleep in the clinical management of hyperglycemia.

Limitations

Our study must be appreciated within the context of important limitations. First, though our findings describe a temporal association between sleep the night before and peripheral glucose homeostasis, the results do not establish causality. The mechanism(s) by which slow oscillation—spindle coupling impacts next-day glucose homeostasis in humans needs further exploration. Given that our data are non-invasive and only measure next-day glucose, we are unable to gain causal and temporal insight into the association between hippocampal sharp-wave ripple activity and slow oscillation—spindle coupling and glucose homeostasis. Future studies in intracranial patients, along with continuous glucose monitoring, would help provide further

mechanistic insight. However, multiple studies have shown that hippocampal sharp-wave ripples are temporally coupled with NREM slow oscillations and sleep spindles [112–114], making slow oscillation—spindle coupling a promising non-invasive marker of hippocampal sharp-wave ripple bursts. Our findings motivate the design of studies capable of testing bi-directional causality e.g., manipulating slow oscillation—spindle coupling in humans [134] to alter glucose regulation or vice versa. Second, the effect sizes observed in this study are, as expected, in the small-to-moderate range, and similar to those recently reported in rodents [111]. Expected, considering that an individual's blood glucose level is determined by multiple factors including genetics, food intake/diet, and gut microbiome [135–137]. Sleep—an indirect lifestyle factor, is therefore anticipated to account for a modest, yet still clinically meaningful, proportion of between-person variability in glucose levels [138], as noted above regarding the difference between those in the upper and lower quartiles of slow oscillation—spindle coupling activity. Future studies that provide longitudinal repeated assessment will help examine how potential individual differences in baseline general health could contribute to differences in slow oscillation-spindle coupling and metabolic deficiencies. Finally, measures of glucose in the main dataset (CFS) were assessed in the morning for closest proximity to sleep, affording a test of the sleep-dependent hypothesis. Nevertheless, these measures do not provide insight into glucose regulation across the entire day, although it should be noted that there is a significant correlation between blood glucose levels measured across the day [139]. Still, temporal knowledge of glycemic status across the day can have important benefits to understanding metabolic dysfunction, requiring continuous glucose monitoring across the 24-hour period as an ideal next experimental step [140,141].

Methods

Experimental model and subject details

Two independent cohorts were used to test the hypothesis. The first (main cohort) was the Cleveland Family Study data set (CFS; [142,143]), and the second (replication cohort) was the Multi-Ethnic Study of Atherosclerosis (MESA; [51]) data set. Both the CFS and the MESA datasets followed the guidelines of the National Sleep Research Resource (NSRR), and Institutional Review Board (IRB) approval was obtained at each study site.

The former Cleveland Family Study (CFS) data set is a longitudinal family-based epidemiological study of sleep apnea with over 2400 participants. Families were selected based on the presence of a proband diagnosed with Obstructive Sleep Apnea (OSA; [144]). Neighboring families without a diagnosis of OSA were used as controls. A subset of 728 participants was selected for a study that involved collecting sleep, cardiovascular and metabolic measures, between July 2001 and June 2005 (visit 5).

Prepubertal children were excluded from subsequent analyses by using 15 years old as the cut-off age (n=73, 655 participants remaining). The protocol was approved by the institutional review boards of the local hospitals from where the participants were recruited. All participants provided informed written consent.

The latter Multi-Ethnic Study of Atherosclerosis (MESA [51];) data set is a multi-center, longitudinal investigation of factors associated with the development of cardiovascular disease. There have been five follow-up visits to date, approximately once every two years. All participants provided written informed consent and all MESA activities were approved by the institutional review boards of the participating institutions. All subsequent analyses are based on the MESA Exam 5, which was collected from 2010-2013.

Method details

Measurement of glycemic levels, insulin sensitivity and health covariates

CFS

Fasting glucose and insulin values were derived from all individuals (n=728) using blood samples collected via venipuncture at 7 AM on the morning after PSG (Sulit et. al., 2006). In non-diabetic participants (n=596), this was followed by the administration of an oral glucose tolerance test (OGTT). During the oral glucose tolerance test, participants orally consumed 75 grams of anhydrous glucose, and glucose levels were measured 2 hours later via venipuncture. OGTT values were measured as 2-hour post glucose serum load (in mg/dl). Impaired glucose tolerance criteria were defined by self-reported use of diabetes medication, as fasting glucose ≥ 110 mg/dl, or as 2 hours post glucose serum load ≥ 140 mg/dl. A square root transformation was used to reduce skewness in fasting and postprandial glucose levels and thus minimize the influence of outliers, consistent with prior assessment measures [137].

Insulin resistance and pancreatic beta cell function were quantified using the standardized homeostasis assessment model (HOMA-IR and HOMA-B respectively) scores. HOMA-IR was calculated as fasting serum insulin multiplied by fasting plasma glucose (in mg/dL), divided by 405, as described previously [122,123,145]. HOMA-B was calculated as fasting serum insulin multiplied by 360, divided by fasting plasma glucose (in mg/dL) minus 63 [122,124]. HOMA-IR and HOMA-B values were then log transformed to reduce skewness, consistent with standard practices [146,147]. High scores indicate low insulin sensitivity or high insulin resistance.

Before coming in for their PSG session, all participants completed the Cleveland Health and Sleep Questionnaire, which is a standardized and validated questionnaire

assessing sleep habits and symptoms, medical history, health habits, and medication use, including diabetic and antihypertensive medications. BMI was measured as the ratio of weight to the square of height (kg/m^2). Weight was measured to the nearest 0.1 kg using a calibrated scale. Height was measured to the nearest centimeter using a wall-mounted stadiometer.

MESA

Fasting glucose was measured during the MESA Exam 5 clinic visit. Participants fasted for 12 hours and avoided smoking and heavy physical activity for 2 hours before the examination. Fasting blood samples were drawn between 7:30 and 10:30 AM. Fasting blood glucose (serum) was measured by the glucose oxidase method on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Rochester, New York) [148]. As in CFS, fasting glucose values outside the range of 60 to 250 mg/dl were masked (n=8). Then, a square root transformation was used to further reduce skewness in fasting glucose levels and minimize the influence of outliers, consistent with prior assessment measures [137]. Age, gender, race/ethnicity, smoking status, education, and income were collected at MESA Exam 5 via self-report questionnaires.

EEG analysis

Sleep recording and sleep staging

CFS: Fourteen-channel overnight PSG recordings were collected using Compumedics E Series System, at a dedicated clinical research facility. Details about the montage and sampling rate can be found here. Sleep scoring was performed by trained research technologists, using R&K rules [149]. For subsequent analyses, NREM stages 3 and 4 were collated into a single stage (N3) to conform with the most recent guidelines [59].

MESA: Sleep studies were scheduled to occur after the MESA Exam 5 clinic visit. The average gap between the MESA sleep study and MESA Exam 5 clinic visit was 341 days, with a standard deviation of 200 days. At-home full overnight PSG recordings were collected in 2237 participants from the parent cohort (age range = 54-95 years) using the Compumedics Somte System (Compumedics Ltd., Abbotsford, Australia). The recording montage consisted of three cortical EEG (central C4-M1, occipital Oz-Cz, and frontal Fz-Cz leads), bilateral EOG, chin EMG, as well as several other sensors to measure heart rate, respiration, and leg movements.

Quantification and statistical analysis

Spectral analyses

EEG power in specific bands were calculated separately for NREM sleep (excluding N1) and REM sleep, using a Welch periodogram with a 4-sec hamming window. Spectral bands were defined as: slow delta (0.5-1.25 Hz), fast delta (1.25-4 Hz), total delta (i.e. slow wave activity [SWA], 0.5-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), sigma (12-16 Hz), beta (16-30 Hz). EEG powers were expressed as a proportion of the total power summed across all bands. The correlation analyses also included the total summed power in NREM and REM (expressed in microVolts-squared).

Slow oscillations event-locked phase-amplitude coupling

All EEG analyses for the CFS dataset were conducted on the C3-M2 channel, after downsampling to 100 Hz and inverting the polarity (to fix a known issue, see here). All EEG analyses for the MESA dataset were conducted on the C4-M1 channel. PSG data were sampled at 256 Hz and a hardware low-pass filter with a cutoff frequency of 100 Hz was applied during recording. Nocturnal recordings were transmitted to the centralized reading center at Brigham and Women's Hospital and data were scored by trained technicians using current guidelines.

Slow oscillations (SO) detection was performed on NREM sleep (excluding N1 sleep) using the YASA Python library [150]. The algorithm uses amplitude and duration thresholds [151,152] to detect SO on the bandpass-filtered signal (0.3-1.5 Hz), coupled with an outlier removal step to remove invalid events. Based on previous findings showing that the standard amplitude threshold of 75 µV is not adequate for older adults [153], a more liberal amplitude threshold of 60 µV for peak-to-peak amplitude and 32 µV for the negative peak amplitude was chosen. For each PSG night, the average SO density (= number of SO per min of NREM), frequency (Hz), and amplitude (µV) were calculated.

To calculate event-locked cross-frequency coupling [112], first, each detected SO was cut to 1 sec before and after the negative peak of the SO event. For each event-locked 2-sec window, Hilbert transforms were used to extract the instantaneous phase of the SO band (0.3-1.5 Hz) and the instantaneous amplitude of the sigma band (12-16 Hz), which is highly correlated to spindle amplitude and density [154], and has been previously used to measure SO-spindle coupling [155]. To avoid filter edge artifacts, the instantaneous phase and amplitude time-series were calculated on the entire signal before running the SO detection. The strength of the coupling between the SO phase and the sigma amplitude was calculated, for each SO, using the normalized direct PAC (ndPAC) method [117]. The ndPAC is conceptually similar to the traditional mean vector

length method [156], with two exceptions. First, the amplitude signal is z-scored to help eliminate distortions in the PAC estimate due to direct current components in data. Second, ndPAC includes a statistical thresholding to reject false estimates arising from distortions of non-coupled oscillation powers. As such, and unlike other PAC methods, the ndPAC does not require a permutation-based surrogate normalization. The ndPAC coupling value ranges from 0 (no coupling) to 1 (perfect coupling). Formally, the ndPAC is defined as:

$$ndPAC = \frac{1}{N}\left|\sum_{n=1}^{N} a(n)e^{i\phi(n)}\right|$$

Where $a(n)$ is the normalized (mean removed and variance made unity) amplitude signal and $\phi(n)$ is the phase from high- and low-bandpass filtered signals with data length N, respectively. The closed-form statistical threshold is given by:

$$x_{th} = 2 \times N \times \left[erf^{-1}(1 - p)\right]^2$$

With p the confidence level, and erf^{-1} the inverse error function [117]. Every value of coupling exceeding the threshold x_{th} is considered reliable, at the given confidence level. Otherwise, coupling is considered unreliable and values are set to zero. The proportion of SO that are coupled with the spindle-related sigma band therefore represents a simple metric of the coupling *quantity*. Noteworthy, another approach that has been used to estimate the quantity of SO events that are coupled is to apply an automatic spindle detection on the signal and then find spindles that occur within a certain range of the negative peak of the SO. However, the ndPAC approach has the advantage of being data-driven and as such does not rely on arbitrary thresholds for the spindle detection and events co-occurrence.

A single summary value of coupling strength per participant was obtained by averaging all the valid ndPAC values, that is, all the SO-spindle coupling values that were not rejected by the statistical thresholding. In addition, the proportion of SO that had a valid (= significant) SO-spindle coupling was calculated for each participant. A value of 1 therefore indicates that all the detected SO have a significant phase-amplitude coupling with the sigma band, whereas a value of zero indicates that none of the detected SO show a functional coupling with the sigma band. Lastly, the preferred phase (in radians) of the SO at the maximum sigma amplitude within each 2-sec window was extracted as a measure of coupling directionality. To this end, the amplitude values were first binned according to 18 phase slices (360 deg / 18 bins = 20 degrees each). The preferred phase was then defined as the phase bin for which the distribution of amplitude is maximum.

An outlier removal step was applied which consisted of masking the coupling values with an absolute z-score above 4 for either the coupling strength or the coupling quantity (n=8 in CFS, n=9 in MESA).

For illustrative purposes, a time-frequency representation of the SO-spindle coupling was calculated using the event-related phase-amplitude coupling (ERPAC) method [116]. ERPAC is based on a circular-linear correlation that evaluates, across all detected SO for a given night/individual, the instantaneous amplitude at each specific frequency with the sine and cosine of the instantaneous phase. As with a traditional Pearson correlation, values can range between -1 to 1, with higher positive values indicating a strong coupling at that specific event-locked time between the amplitude and phase time series. All coupling analyses were performed in Python using the Tensorpac package [157].

Heart rate variability

Heart rate variability (HRV) across the night was calculated from the ECG channel using non-overlapping windows of 5 minutes. The ECG was first high-pass-filtered at 0.5 Hz using a 5th-order Butterworth filter and the R-peaks were detected and corrected for each 5-min window using the default parameters in the neurokit2 Python toolbox [158]. Windows with less than 175 NN intervals were excluded. Based on the experimental hypotheses, the analyses were focused on the root mean square of successive differences between normal heartbeats (RMSSD) — a widely-used HRV metric that reflects vagally-mediated short-term variability in heart rate [61]. Of note, although HRV metrics are widely used as a marker of parasympathetic activity, heart rate variability is also impacted by endocrine and reproductive factors, including but not limited to growth hormone [159], luteinizing hormone [160], and thyroid hormones [161]. Formally, given a time-series of beat-to-beat interval RR of length N, the RMSSD is defined as:

$$RMSSD = \sqrt{\frac{\sum_{i=1}^{N-1} (RR_i - RR_{i+1})^2}{N-1}}$$

The median RMSSD across all 5 min epochs was calculated to get a single RMSSD value per participant. The resulting values were then log-transformed to reduce skewness, consistent with standard practices [61].

Statistical analyses

A strict inner merge was used to combine the health data (demographics and glucose) with the SO-spindle coupling variables. In other words, only participants with

non-missing glucose and coupling data were included in subsequent analyses (n=647 participants). A more liberal left merge was then used to combine the EEG spectral power data with the main dataframe.

Correlations between dependent variables were calculated using the Pearson correlation coefficient. Partial correlations were performed in Python using the Pingouin package [56]. All regression analyses were performed using the `lmer` R function [162]. Models were adjusted for age, gender, race/ethnicity [163], BMI, hypertension status, apnea-hypopnea index (AHI), sleep period time (SPT), and sleep efficiency (SE, calculated as total sleep time divided by sleep period time [24]). Since the CFS study includes participants from the same family, multilevel models were used with family ID as a random effect. P-values for the regression models were obtained from two-tailed Wald tests. Marginal effects were calculated using the `ggeffect` R function [164].

The preprocessing and analysis steps were identical between the CFS main cohort and the MESA replication cohort. One notable exception is that MESA does not include participants from the same families and therefore a standard (non-multilevel) linear regression was used to test associations between predictors of interest and glucose outcomes. A total of 1996 unique MESA participants were remaining after combining the health data and EEG coupling data. There was no participant under the age of 15 in MESA.

Assessment of the ranking of the sleep predictors was performed by extracting, independently for each sleep predictor, the p-value of that predictor in a multilevel regression model adjusted for age, gender, race/ethnicity, BMI, and hypertension status. AHI, SPT and SE were not included as covariates in the model since all three were included, as predictors, in the ranking analysis. The unadjusted p-values from all sleep predictors were then log-transformed with base 10 and negated for illustrative purposes.

Key resources table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Cleveland Family Study data	Zhang et al. 2018 [142,143] Redline et al. 1995 [142,143]	https://sleepdata.org/datasets/cfs
Multi Ethnic Study of Atherosclerosis data	Zhang et al. 2018 [24,143]; Chen et al. 2015 [67]	https://biolincc.nhlbi.nih.gov/studies/mesa/
Software and algorithms		
YASA	Vallat and Walker 2021 [150]	https://github.com/raphaelvallat/yasa
Tensorpac	Combrisson et al. 2020 [157]	https://github.com/EtienneCmb/tensorpac
Pingouin	Vallat 2018 [56]	https://pingouin-stats.org/
Code for all data preprocessing and analysis	This paper	https://github.com/raphaelvallat/vallat2023_coupling_glucose

Supplementary Materials

Supplementary Tables

Table S1. Demographics of the Cleveland Family Study (CFS; visit 5). Related to STAR Methods.

Variables	Statistics
No. unique participants	647
No. unique families	144
Age (yrs)	44.73 ± 17.39 (range = 15-89)
Sex	359 F / 288 M
BMI (kg/m^2)	33.47 ± 9.17 (range = 17-85)
Race/ethnicity	350 African / 280 Caucasian / 17 Other
Smoking status	324 No / 169 Yes / 154 Missing
Hypertension status	428 No / 211 Yes
Diabetes status	530 No / 115 Yes
Fasting glucose (mg/dl)	100.56 ± 25.65 (range = 66-246)
log(HOMA-IR)	0.99 ± 0.77 (range = -1-4)
log(HOMA-B)	4.81 ± 0.63 (range = 3-8)
Apnea-hypopnea index (AHI)	13.78 ± 20.17 (range = 0-125)
% of participants with AHI >30	13.6%
Sleep efficiency (SE, %)	81.18 ± 12.74 (range = 27-99)
Sleep period time (SPT, min)	459.13 ± 82.01 (range = 52-660)
Total sleep time (TST, min)	369.83 ± 74.26 (range = 40-576)
N1 (% of TST)	5.32 ± 4.72 (range = 0-63)
N2 (% of TST)	58.55 ± 12.41 (range = 21-100)
N3 (% of TST)	17.71 ± 10.99 (range = 0-57)
REM (% of TST)	18.41 ± 7.61 (range = 0-44)
SO density (per min of NREM)	2.50 ± 1.98 (range = 0-11)
Spindles density (per min of NREM)	3.28 ± 1.37 (range = 0-7)
Proportion of SO with significant coupling	87.60 ± 3.35 (range = 72-100)
SO—spindle coupling strength	0.32 ± 0.02 (range = 0.25-0.39)
SO—spindle preferred phase (º)	-12.15 ± 28.32

SO = slow oscillations.

Table S2. Slow oscillation—spindle coupling quantity significantly predicts next-day fasting blood glucose in the CFS dataset. Related to Figure 2.

Predictors	β	Standardized β	95% CI	p
Proportion of NREM SO with significant coupling	-3.88	-0.12	-6.16 – -1.60	**0.001**
Age	0.01	0.15	0.00 – 0.01	**<0.001**
Male	0.41	0.19	0.26 – 0.57	**<0.001**
Race/ethnicity [Black]	0.15	0.14	-0.04 – 0.34	0.128
Race/ethnicity [Other]	0.18	0.16	-0.34 – 0.69	0.502
BMI	0.03	0.28	0.02 – 0.04	**<0.001**
Hypertension	0.44	0.19	0.25 – 0.62	**<0.001**
Apnea-hypopnea index (AHI)	-0.00	-0.06	-0.01 – 0.00	0.158
Sleep efficiency	-0.01	-0.07	-0.01 – 0.00	0.105
Sleep period time	-0.01	-0.02	-0.07 – 0.05	0.686

The number of participants with complete data included in the multilevel regression analysis was 623. Family ID was set as a random effect (n=144 unique groups). The dependent variable, fasting blood glucose, was transformed using a square root transformation to reduce skewness. The reference category for race/ethnicity was White. Age, sex, BMI, and hypertension were all significant predictors of fasting blood glucose levels. Being a male, older, having a higher BMI, and having hypertension were all associated with higher levels of next-day fasting blood glucose. Race, sleep duration, sleep efficiency, and AHI were not significant predictors of next-day fasting blood glucose.

Table S3. Slow oscillation—spindle coupling strength significantly predicts next-day fasting blood glucose in the CFS dataset. Related to Figure 2.

Predictors	β	Standardized β	95% CI	p
SO—spindle coupling strength	-4.90	-0.08	-9.04 – -0.77	**0.020**
Age	0.01	0.16	0.00 – 0.02	**<0.001**
Male	0.41	0.18	0.25 – 0.56	**<0.001**
Race/ethnicity [Black]	0.16	0.15	-0.03 – 0.35	0.102
Race/ethnicity [Other]	0.18	0.16	-0.34 – 0.69	0.502
BMI	0.03	0.29	0.03 – 0.04	**<0.001**
Hypertension	0.44	0.19	0.25 – 0.62	**<0.001**
Apnea-hypopnea index (AHI)	-0.00	-0.05	-0.01 – 0.00	0.164
Sleep efficiency	-0.00	-0.05	-0.01 – 0.00	0.198
Sleep period time	-0.01	-0.01	-0.07 – 0.05	0.746

The number of participants with complete data included in the multilevel regression analysis was 623. Family ID was set as a random effect (n=144 unique groups). The dependent variable, fasting blood glucose, was transformed using a square root transformation to reduce skewness. The reference category for race/ethnicity was White. Age, sex, BMI, and hypertension were all significant predictors of fasting glucose levels. Being a male, older, having a higher BMI, and having hypertension were all associated with higher levels of next-day fasting blood glucose. Race, sleep duration, sleep efficiency, and AHI were not significant predictors of next-day fasting blood glucose.

Table S4. Demographics of the MESA sleep study. Related to STAR Methods.

Variables	Statistics
No. unique participants	1996
Age (yrs)	68.43 ± 9.17 (range = 54-93)
Sex	359 F / 288 M
BMI (kg/m^2)	28.64 ± 5.49 (range = 17-56)
Race/ethnicity	729 Caucasian / 550 African / 478 Hispanic / 239 Asian
Smoking status	930 Never / 912 Former / 142 Current
Gap between PSG and glucose Measures (days)	341 ± 200 (range = 0-1024)
Hypertension status	1130 Yes / 866 No
Diabetes status	1197 Normal / 409 Impaired / 356 Treated / 34 Untreated
Fasting glucose (mg/dl)	100.46 ± 21.81 (range = 62-249)
log(HOMA-IR)	0.99 ± 0.77 (range = -1-4)
log(HOMA-B)	4.81 ± 0.63 (range = 3-8)
Apnea-hypopnea index (AHI)	19.73 ± 18.54 (range = 0-111)
% of participants with AHI >30	21.8%
Sleep efficiency (SE, %)	78.53 ± 13.44 (range = 10-99)
Sleep period time (SPT, min)	462.20 ± 91.48 (range = 94-1084)
Total sleep time (TST, min)	359.89 ± 82.15 (range = 32-601)
N1 (% of TST)	14.18 ± 9.16 (range = 0-79)
N2 (% of TST)	58.00 ± 11.05 (range = 19-100)
N3 (% of TST)	9.98 ± 9.05 (range = 0-51)
REM (% of TST)	17.84 ± 6.92 (range = 0-59)
SO density (per min of NREM)	1.48 ± 1.18 (range = 0-13)
Spindles density (per min of NREM)	2.68 ± 1.43 (range = 0-12)
Proportion of SO with significant coupling	86.34 ± 4.44 (range = 67-100)
SO—spindle coupling strength	0.32 ± 0.02 (range = 0.23-0.41)
SO—spindle preferred phase (°)	-12.15 ± 28.32

SO = slow oscillations.

Table S5. Slow oscillation—spindle coupling quantity significantly predicts next-day fasting blood glucose in the MESA dataset. Related to Figure 3.

Predictors	β	Standardized β	95% CI	p
Proportion of NREM SO with significant coupling	-1.04	-0.05	-2.00 – -0.08	**0.034**
Age	0.00	0.02	-0.00 – 0.01	0.390
Male	0.22	0.11	0.13 – 0.30	**<0.001**
Race/ethnicity [Black]	0.02	0.02	-0.09 – 0.13	0.688
Race/ethnicity [Asian]	0.37	0.38	0.23 – 0.51	**<0.001**
Race/ethnicity [Hispanic]	0.36	0.37	0.25 – 0.47	**<0.001**
BMI	0.04	0.20	0.03 – 0.04	**<0.001**
Hypertension	0.23	0.12	0.14 – 0.32	**<0.001**
Apnea-hypopnea index (AHI)	0.00	0.03	-0.00 – 0.00	0.201
Sleep efficiency	0.00	0.01	-0.00 – 0.00	0.698
Sleep period time	-0.00	-0.02	-0.00 – 0.00	0.380

The number of participants with complete data included in the multilevel regression analysis was 1966. The dependent variable, fasting blood glucose, was transformed using a square root transformation to reduce skewness. The reference category for race/ethnicity was White. Sex, race BMI, and hypertension were all significant predictors of fasting blood glucose levels. Being male, being Asian, being Hispanic, having a higher BMI, and having hypertension were all associated with higher levels of next-day fasting blood glucose. Age, sleep duration, sleep efficiency, and AHI were not significant predictors of fasting blood glucose.

Table S6. Slow oscillation—spindle coupling strength significantly predicts next-day fasting blood glucose in the MESA dataset. Related to Figure 3.

Predictors	β	Standardized β	95% CI	p
Proportion of NREM SO with significant coupling	-2.57	-0.06	-4.56 – -0.59	**0.011**
Age	0.00	0.02	-0.00 – 0.01	0.464
Male	0.22	0.11	0.13 – 0.30	**<0.001**
Race/ethnicity [Black]	0.02	0.02	-0.09 – 0.13	0.710
Race/ethnicity [Asian]	0.37	0.38	0.23 – 0.51	**<0.001**
Race/ethnicity [Hispanic]	0.36	0.37	0.25 – 0.47	**<0.001**
BMI	0.03	0.19	0.03 – 0.04	**<0.001**
Hypertension	0.23	0.12	0.14 – 0.32	**<0.001**
Apnea-hypopnea index (AHI)	0.00	0.03	-0.00 – 0.00	0.210
Sleep efficiency	0.00	0.01	-0.00 – 0.00	0.691
Sleep period time	-0.00	-0.02	-0.00 – 0.00	0.387

The number of participants with complete data included in the multilevel regression analysis was 1966. The dependent variable, fasting blood glucose, was transformed using a square root transformation to reduce skewness. The reference category for race/ethnicity was White. Sex, race BMI, and hypertension were all significant predictors of fasting blood glucose levels. Being male, being Asian, being Hispanic, having a higher BMI, and having hypertension were all associated with higher levels of next-day fasting blood glucose. Age, sleep duration, sleep efficiency, and AHI were not significant predictors of fasting blood glucose.

Table S7. Slow oscillation—spindle coupling quantity significantly predicts next-day HOMA-IR in the CFS dataset. Related to Figure 4.

Predictors	β	Standardized β	95% CI	p
Proportion of NREM SO with significant coupling	-2.20	-0.10	-3.73 – -0.68	**0.005**
Age	-0.0	0.02	-0.00 – 0.00	0.660
Male	0.17	0.11	0.07 – 0.27	**0.001**
Race/ethnicity [Black]	0.14	0.19	0.02 – 0.27	**0.025**
Race/ethnicity [Other]	0.13	0.17	-0.21 – 0.47	0.458
BMI	0.04	0.44	0.03 – 0.04	**<0.001**
Hypertension	0.23	0.14	0.11 – 0.35	**<0.001**
Apnea-hypopnea index (AHI)	0.00	0.01	-0.00 – 0.00	0.826
Sleep efficiency	-0.00	-0.01	-0.01 – 0.00	0.797
Sleep period time	-0.03	-0.05	-0.07 – -0.01	**0.141**

The number of participants with complete data included in the multilevel regression analysis was 626. Family ID was set as a random effect (n=144 unique groups). The dependent variable, HOMA-IR, was log-transformed to reduce skewness. The reference category for race/ethnicity was White.

Table S8. Slow oscillation—spindle coupling strength significantly predicts next-day HOMA-IR in the CFS dataset. Related to Figure 4.

Predictors	β	Standardized β	95% CI	p
SO—spindle coupling strength	-3.39	-0.08	-6.14 – -0.64	**0.016**
Age	0.00	0.02	-0.00 – 0.00	0.580
Male	0.17	0.11	0.06 – 0.27	**0.002**
Race/ethnicity [Black]	0.15	0.19	0.03 – 0.27	**0.018**
Race/ethnicity [Other]	0.14	0.18	-0.20 – 0.48	0.428The
BMI	0.04	0.44	0.03 – 0.04	**<0.001**
Hypertension	0.23	0.14	0.11 – 0.35	**<0.001**
Apnea-hypopnea index (AHI)	0.00	0.01	-0.00 – 0.00	0.829
Sleep efficiency	0.00	0.00	-0.00 – 0.00	0.973
Sleep period time	-0.03	-0.05	-0.07 – -0.01	**0.170**

The number of participants with complete data included in the multilevel regression analysis was 626. Family ID was set as a random effect (n=144 unique groups). The dependent variable, HOMA-IR, was log-transformed to reduce skewness. The reference category for race/ethnicity was White.

Table S9. Multilevel regression between sleep features and next-day fasting blood glucose in the CFS dataset. Related to Figure 5.

Sleep predictors	Std. beta	n	p
Proportion of coupled SO	-0.109	639	**0.002**
REM alpha	0.097	626	**0.01**
NREM theta	-0.088	647	**0.013**
SO–spindle coupling strength	-0.079	639	**0.026**
REM delta	-0.059	626	0.107
NREM delta (SWA)	0.058	647	0.1
N1	-0.057	647	0.11
REM sigma	0.056	626	0.161
NREM slow delta	0.056	647	0.11
TIB	-0.055	647	0.112
SO density	0.055	647	0.23
Arousal index	-0.049	640	0.197
REM theta	0.049	626	0.171
%N1	-0.047	647	0.2
SME	-0.047	647	0.231
REM fast delta	-0.046	626	0.233
N2 latency	-0.044	647	0.214
NREM alpha	-0.041	647	0.245
REM slow delta	-0.039	626	0.266
SOL	-0.039	647	0.274
WASO	0.037	647	0.333
NREM	-0.034	647	0.354
Spindles frequency	-0.033	647	0.346
N2	-0.033	647	0.349
N3 latency	0.032	617	0.37
TST	-0.032	647	0.392
N3	0.029	647	0.483
N1 latency	-0.028	640	0.438
%N3	0.027	647	0.502
AHI	-0.027	647	0.491
SO frequency	-0.021	647	0.601

NREM beta	-0.02	647	0.56
SO amplitude	-0.02	647	0.664
NREM power	0.019	647	0.58
%REM	0.017	647	0.614
%NREM	-0.017	647	0.616
%N2	-0.015	647	0.696
Spindles power	0.013	647	0.736
REM power	-0.013	626	0.718
NREM fast delta	-0.011	647	0.75
REM	-0.008	647	0.823
REM beta	-0.007	626	0.851
SE	-0.007	647	0.86
REM latency	0.004	626	0.918
Spindles density	-0.004	647	0.921
NREM sigma	-0.003	647	0.927
SPT	0.001	647	0.988

All regressions were adjusted for age, sex, BMI, race/ethnicity, hypertension and family ID. Regressions are sorted in descending order of significance. NREM refers to N2 + N3 sleep (N1 excluded). Sleep features that significantly predict *higher* levels of fasting glucose = worse outcome) are highlighted in red. A total of 47 sleep parameters were included in the correlation analysis. Two-sided p-values were not corrected for multiple comparisons. The spectral frequency bands are: slow delta (0.5-1.25 Hz), fast delta (1.25-4 Hz), delta (0.5-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), sigma (12-16 Hz), beta (16-30 Hz), and total power (in microvolts-squared, 0.5-30 Hz). AHI = Apnea–hypopnea index, SE = sleep efficiency, SO = slow oscillations, SME = sleep maintenance efficiency, SOL = sleep onset latency, SPT = sleep period time, TST = total sleep time, WASO = wake after sleep onset.

Table S10. Multilevel regression between sleep features and next-day insulin resistance (HOMA-IR) in the CFS dataset. Related to Figure 5.

Sleep predictors	Std. beta	n	p
SO density	0.11	647	**0.012**
Proportion of coupled SO	-0.096	639	**0.004**
SO–spindle coupling strength	-0.084	639	**0.014**
REM alpha	0.083	626	**0.022**
NREM fast delta	-0.081	647	**0.017**
NREM slow delta	0.079	647	**0.019**
Spindles density	-0.072	647	**0.039**
NREM theta	-0.072	647	**0.035**
REM theta	0.068	626	**0.047**
SO amplitude	0.067	647	0.124
REM delta	-0.059	626	0.099
%N1	-0.059	647	0.095
N1	-0.058	647	0.095
%N3	0.056	647	0.145
SPT	-0.052	647	0.123
REM	-0.05	647	0.147
REM fast delta	-0.05	626	0.178
TST	-0.049	647	0.182
SOL	0.047	647	0.175
N3	0.045	647	0.256
SO frequency	-0.041	647	0.297
NREM delta (SWA)	0.039	647	0.252
NREM power	0.039	647	0.246
N3 latency	0.039	617	0.274
REM slow delta	-0.037	626	0.285
N1 latency	0.036	640	0.297
N2	-0.036	647	0.292
REM latency	0.035	626	0.314
SE	-0.033	647	0.356
NREM	-0.028	647	0.436
N2 latency	0.027	647	0.427

NREM beta	-0.022	647	0.511
AHI	0.022	647	0.561
Spindles frequency	-0.022	647	0.526
SME	0.021	647	0.571
WASO	-0.02	647	0.583
REM sigma	0.02	626	0.605
REM power	0.017	626	0.615
TIB	-0.017	647	0.616
NREM alpha	-0.014	647	0.677
%NREM	0.013	647	0.703
%REM	-0.013	647	0.706
Spindles power	0.012	647	0.738
%N2	-0.011	647	0.752
REM beta	-0.008	626	0.812
NREM sigma	0.004	647	0.9
Arousal index	-0.001	640	0.978

All regressions were adjusted for age, sex, BMI, race/ethnicity, hypertension and family ID. Regressions are sorted in descending order of significance. NREM refers to N2 + N3 sleep (N1 excluded). Sleep features that significantly predict *higher* HOMA-IR values (= worse outcome) are highlighted in red. A total of 47 sleep parameters were included in the correlation analysis. Two-sided p-values were not corrected for multiple comparisons. The spectral frequency bands are: slow delta (0.5-1.25 Hz), fast delta (1.25-4 Hz), delta (0.5-4 Hz), theta (4-8 Hz), alpha (8-12 Hz), sigma (12-16 Hz), beta (16-30 Hz), and total power (in microvolts-squared, 0.5-30 Hz). AHI = Apnea–hypopnea index, SE = sleep efficiency, SO = slow oscillations, SME = sleep maintenance efficiency, SOL = sleep onset latency, SPT = sleep period time, TST = total sleep time, WASO = wake after sleep onset.

Supplementary Figures

Figure S1. Assessment of insulin resistance (IR) and pancreatic beta cell function (B) using the standardized homeostasis assessment model (HOMA). A) HOMA-IR is positively correlated with fasting glucose. B) HOMA-B is negatively correlated with fasting glucose. Related to STAR Methods.

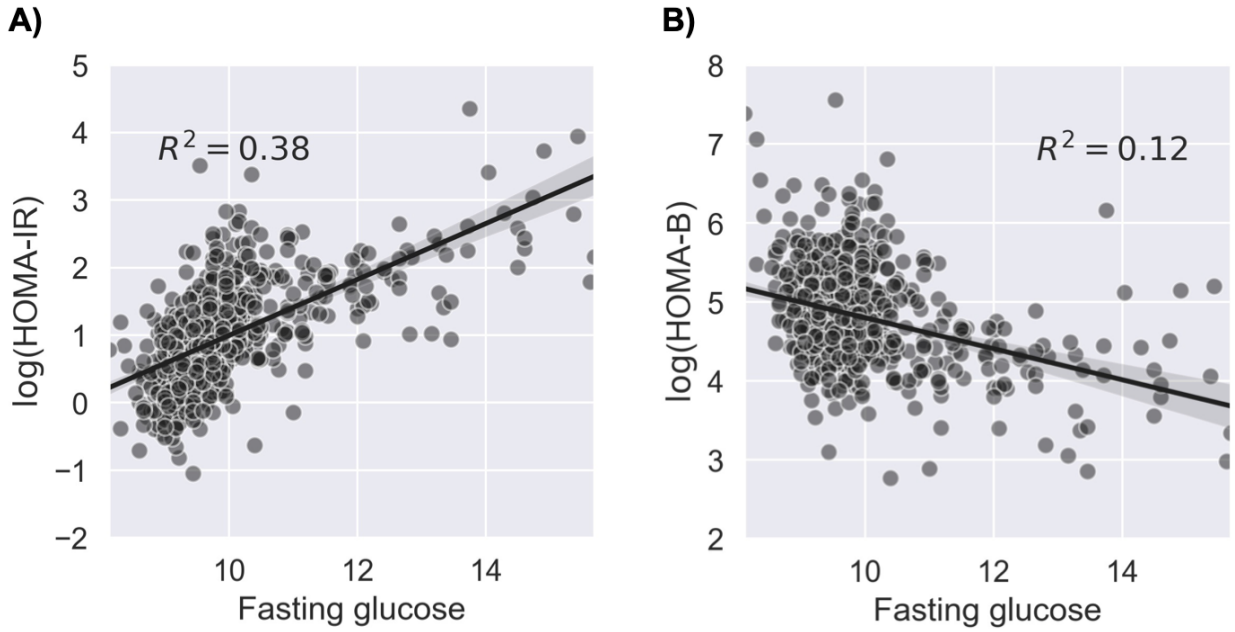


Figure S2. Mediation analysis demonstrated that the link between SO-spindle coupling and improved next-day fasting glucose is, in part, explained by increased heart rate variability, in the MESA dataset. A) A significant association between the proportion of SO-spindle coupling and increased heart rate variability (HRV), which in turn predicted lower (improved) fasting glucose values. B) A significant association between the strength of SO-spindle coupling and increased HRV, which in turn predicted lower (improved) fasting glucose values. Related to Figure 3.

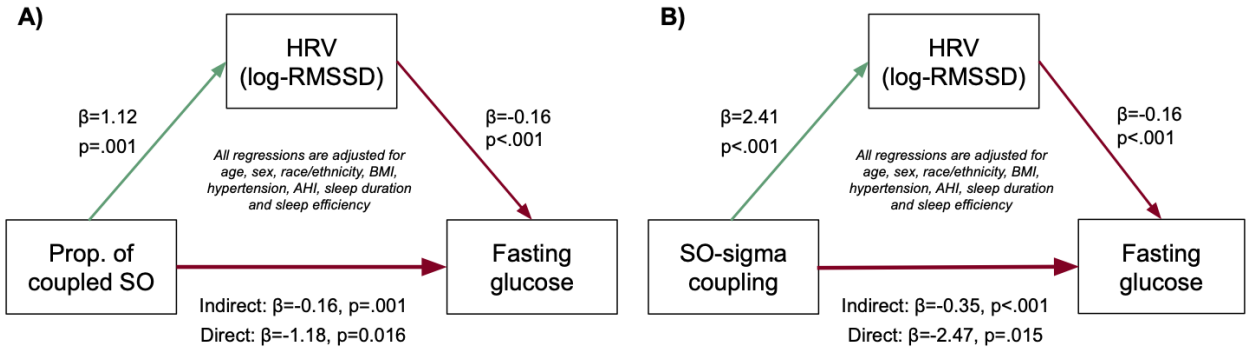


Figure S3. Mediation analysis demonstrated that the link between SO-spindle coupling and improved next-day fasting glucose is not, in part, explained by increased heart rate variability, in the CFS dataset. A) A significant association between the proportion of SO-spindle coupling and lower (improved) fasting glucose values is not mediated by HRV. B) A significant association between the strength of SO-spindle coupling and lower (improved) fasting glucose values is not mediated by HRV. Related to Figure 2.

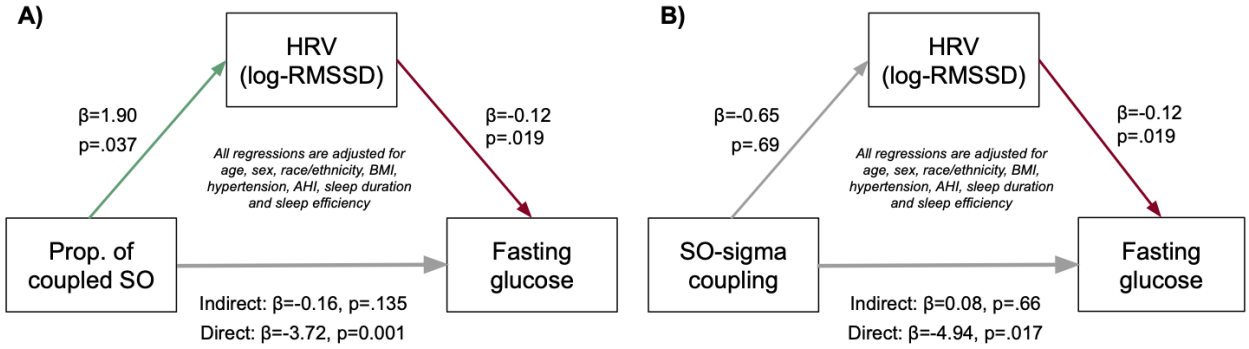
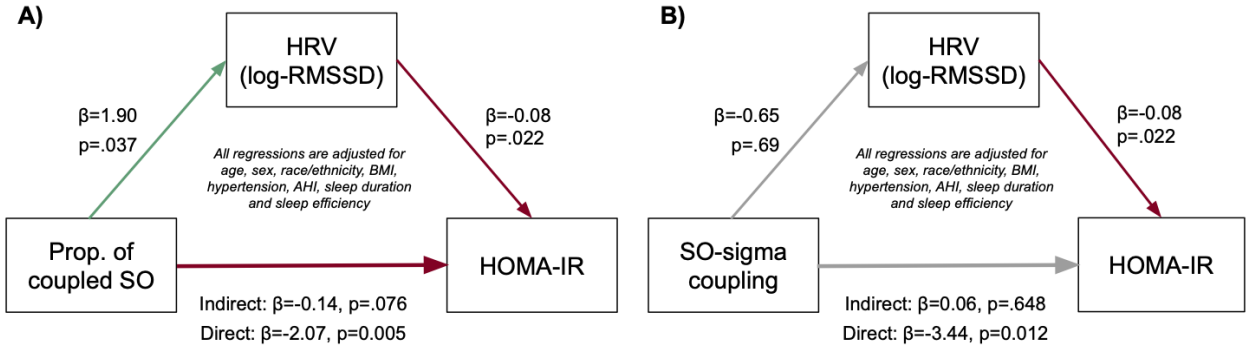


Figure S4. Mediation analysis demonstrated that the link between SO-spindle coupling and next-day insulin resistance is modestly explained by increased heart rate variability, in the CFS dataset. A) A trending significant association between the proportion of SO-spindle coupling increased heart rate variability (HRV), which in turn predicted lower (better) insulin resistance values. B) A significant association between the strength of SO-spindle coupling and lower (better) insulin resistance values is not mediated by HRV. Related to Figure 2.



General Conclusions

Taken together, this thesis provides strong support for the hypothesis that impairments in sleep are closely associated with impairments in cardiometabolic health. Cardiovascular disease and metabolic disease have shared etiology and interrelated pathophysiology. Cardiometabolic disease exists on a continuum [64,165], starting from the presence of risk factors and ending in complete organ failure and death. The presence of sleep as a biomarker, as well as a modifiable intervention, at any stage during this continuum is of therapeutic value, as it can disrupt the pathophysiology of fatal events, and confer cardiometabolic resilience.

Both, the domains of sleep and cardiovascular health, are multi-faceted. This thesis sheds light on the specificity of unique features of sleep as biomarkers for specific core metrics of cardiometabolic health. First, sleep fragmentation, a macro metric of sleep quality, is a biomarker for the build-up of plaque in one's arteries (Chapter 1). Second, unique features of an individual's sleep over a period of many days, are a biomarker for abnormal lipid profiles, which in turn lead to atherosclerosis. As discussed (Chapter 2), late clock timing for sleep is a biomarker for the homeostatic process of regulating fasting total triglycerides and VLDL cholesterol levels. Sleep efficiency is a biomarker for the allostatic process of regulated post-prandial total triglycerides and VLDL cholesterol levels. Last, and delving into more micro brainwave oscillation metrics and their coupling, both the amount and strength of NREM slow oscillation--spindle coupling are biomarkers for glucose homeostasis (Chapter 3). Therefore, different features of sleep provide a lens into the pathophysiology of unique aspects of the pathophysiology of cardiometabolic health, and act as specific biomarkers for different metrics of cardiometabolic health.

Given that cardiometabolic disease is a top killer of the human race, and having established sleep as a biomarker of cardiometabolic health, has significant implications for therapeutic interventions. First, sleep is a modifiable risk factor for cardiometabolic health, which could help inform public health guidelines that focus on societal sleep health, to lower cardiometabolic burden. Second, the multidimensionality of sleep as a biomarker for specific aspects of cardiometabolic health can be leveraged to develop personalized sleep interventions for patients with diabetes and/or dyslipidemia.

Insufficient sleep has long been linked with increased inflammation [11]. One mutual potential pathway through which poor sleep impaired two of the key cardiometabolic outcomes examined - that of glucose homeostasis, and fasting triglycerides - is increased inflammation. Collectively, this thesis supports the hypothesis that poor sleep increases inflammation, which in turn has multiple downstream effects on cardiometabolic health. Interventions with modifiable lifestyle changes such as dietary

changes can help decrease inflammation [166], and provide increased cardiometabolic protection.

In conclusion, this thesis strongly supports a sleeping-brain—body framework of inflammation-associated cardiometabolic homeostasis in humans, and re-emphasizes the importance of sleep in the clinical management of cardiometabolic health.

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