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The association of sleep with metabolic pathways and metabolites: evidence from the Dietary Approaches to Stop Hypertension (DASH) - Sodium Feeding Study

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Abstract

Introduction—Sleep is increasingly being viewed as an issue of public health concern, yet few epidemiologic studies have explored associations between sleep habits and metabolomic profile.

Objectives—To assess the association between sleep and blood metabolites.

Methods—We examined the association between sleep and 891 fasting plasma metabolites in a subgroup of 106 participants from the Dietary Approaches to Stop Hypertension (DASH)-Sodium feeding trial (1997–1999). We produced two sleep variables to analyze, sleep midpoint (median time between bedtime and waketime) and sleep duration, as well as bedtime and wake time. Metabolites were measured using liquid and gas chromatography, coupled with mass spectrometry. We assessed associations between sleep variables and log transformed metabolites using linear mixed-effects models. We combined the resulting p-values using Fisher's method to calculate associations between sleep and 38 metabolic pathways.

Results—Sixteen pathways were associated ($p < 0.05$) with midpoint. Only the γ-glutamyl amino acid metabolism pathway reached Bonferroni-corrected threshold (0.0013). Eighty-three metabolites were associated with midpoint (FDR<0.20). Similar associations were found for wake time. Neither bed time nor duration were strongly associated. The top metabolites (pathways given in brackets) associated with sleep were erythrulose (advanced glycation end-product) (positive association) and several γ-glutamyl pathway metabolites, including CMPF (fatty acid, dicarboxylate), isovalerate (valine, leucine and isoleucine and fatty acid metabolism) and HWESASXX (polypeptide) (inverse association).

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Author Contributions

Rachael Stolzenberg-Solomon conceived of the project idea. Vanessa Gordon-Dseagu and Ishmael Williams undertook a literature review to support the study. All authors contributed to the analysis plan, the analysis undertaken, and the writing of the manuscript (as well as commenting upon and amending the final manuscript).

Compliance with Ethical Requirements

Disclosure of potential conflicts of interest.

Conclusion—Within our study, several metabolites that have previously been linked to inflammation and oxidative stress (processes involved in diseases such as cardiovascular disease and cancer) were found to be associated with sleep.

Keywords

Sleep; Metabolites; Lifestyle; Diet

1. Introduction

In recent years sleep deficiency has been increasingly acknowledged as an issue of public health concern. In the US, increasing numbers of individuals are achieving insufficient levels of sleep – the CDC estimates that around a third of American adults attained less than 7 hours of sleep per night in 2016 (Yong Liu et al. 2016). Since the 1960s a number of studies have observed a U-shaped relationship between sleep duration and all-cause mortality and mortality from several causes including cardiovascular disease and accidents, although the results have been somewhat heterogeneous, with some studies finding no association (Cappuccio et al. 2010; Gallicchio and Kalesan 2009; Iftikhar et al. 2015; Rod et al. 2011; Xiao et al. 2014). Lack of sleep has further been associated with increased incidence of a number of health outcomes including metabolic syndrome, (Iftikhar et al. 2015) diabetes, (Shan et al. 2015), cardiovascular disease (Grandner et al. 2013) and several site-specific cancers (Zhao et al. 2013). There is also a growing body of evidence that sleep duration has an impact upon lipid/lipoprotein levels, (Kaneita et al. 2008; Kinuhata et al. 2014), the proinflammatory state (Grandner et al. 2013; Irwin et al. 2010) and overweight/obesity (Beccuti and Pannain 2011; Fatima et al. 2015). Sleep timing is also associated with increased calorie intake, consumption of free sugars, BMI, insulin resistance and diabetes (Al Khatib et al. 2018; Baron et al. 2011; Knutson et al. 2017; Merikanto et al. 2013).

Few studies have explored the association between sleep and metabolomic profiles (Aho et al. 2016). In small, experimental and epidemiological studies, sleep deprivation or sleep fragmentation resulted in alterations in lipid profiles (Aho et al. 2016) and glucose metabolism (Stamatakis and Punjabi 2010), increased insulin resistance (van den Berg et al. 2016) and elevated blood concentrations of acylcarnitines (van den Berg et al. 2016), formate (an intermediate of several metabolic processes, including indirect glycolysis stimulation) (Tulpule et al. 2013) and citrate (Giskeødegård et al. 2015). In larger epidemiological studies, increased sleep duration and sleep quality were associated with lipid profiles reflective of cardiovascular disease (Xiao et al. 2017) (Lemke et al. 2017). In experimental animal studies, sleep deprivation caused marked impairment in insulin secretion (S. Zhan et al. 2016), adverse impact upon cognitive performance (Feng et al. 2016) and hormonal imbalance (Martins et al. 2011).

Our study examines the association between metabolites and sleep in a well-phenotyped set of participants from the Dietary Approaches to Stop Hypertension (DASH)-Sodium Trial. Importantly, their diet – a factor that is normally highly correlated with both sleep and serum metabolite levels (Crispim et al. 2011) – was strictly controlled (Sacks et al. 2001). We aim

to provide further insight into the potentially mediating role that metabolites play within the relationship between sleep and health.

2. Methods

2.1 Study population

The study utilized participant data collected from the Dietary Approaches to Stop Hypertension (DASH) - Sodium Trial, which has been described previously (Sacks et al. 2001; Svetkey et al. 1999). Briefly, the DASH-Sodium trial aimed to assess the impact of two dietary patterns and three sodium levels upon blood pressure in individuals of European and African American ancestry. Participants were randomized to one of the two dietary patterns (parallel design) for 12 weeks and, within each dietary pattern arm, received each of three sodium levels in random order (cross-over design) for 30 days (Sacks et al. 2001). DASH-Sodium was a feeding study, therefore diet was strictly controlled. The two possible diets were 1) the DASH diet – high in fruits and vegetables, low-fat dairy products, whole grains, fish, nuts and poultry while being low in saturated fat or 2) the control diet, a diet typical to that of many Americans – including red meat, sweets and sugary drinks. The three sodium levels were 1) low (1150mg/d – representing optimal sodium levels); 2) medium (2300mg/d – representing the upper limit of US sodium recommendations); and 3) high (3450 mg/d - representing current consumption within the US) (Svetkey et al. 1999). Each participant's energy intake was adjusted to ensure that their weight remained constant during the study (Svetkey et al. 1999; Vogt et al. 1999).To be included in the study, participants had to be over the age of 22 years with a systolic blood pressure (SBP) of 120–159 mm/Hg and a diastolic blood pressure (DBP) of 80–95 mm/Hg. Exclusion criteria included the presence of comorbidities (insulin dependent diabetes, hyperlipidemia, use of medication for hypertension, renal insufficiency) as well as several factors related to diet and alcohol use. All participants signed an informed consent and the study was approved by the human subject committees of each center (Sacks et al. 2001). The study was further approved by the Office of Human Subjects Research at the National Institutes of Health.

2.2 Data collection and variables

Participant information (demographics, height, exercise, weight, blood samples and lifestyle factors such as smoking and alcohol consumption) were gathered by trained staff during visits to the study center at baseline and during follow-up (Sacks et al. 2001; Svetkey et al. 1999). Blood pressure was measured using a random-zero sphygmomanometer while participants were seated (Sacks et al. 2001). Body mass index (BMI kg/m^2) was calculated using the height and weight measurements (weight in kg/height in m^2). Activity was queried as the number of times that a participant undertook either moderate or vigorous exercise per week/month (Svetkey et al. 1999). Smoking was categorized as never, former or current. At the end of each sodium intervention, fasting EDTA plasma samples were collected from participants. The samples were stored at −80 degrees Celsius at the National Heart, Lung, and Blood Institute repository. As metabolite measures are affected by sample handling, we only used never-thawed samples. We preferred to measure metabolite levels in the samples collected during the high- and low-sodium interventions, but when not possible (Derkach et

al. 2017) we used the sample collected after the medium-sodium intervention in place of an unavailable sample.

Participants recorded times they went to sleep and woke up while wearing 24-hour ambulatory blood pressure monitors during the final week of each sodium intervention and the same week as their blood draw. This provided us with bed and wake time for a single day during the trial period. From this, we created two variables. The first was 'midpoint', which we defined as the time point between when an individual went to bed and when they awoke. The second was sleep duration, the amount of time the individual reported being asleep.

2.3 Metabolites and Metabolomic Sub-study

The methods and procedures used by Metabolon Inc. to measure the metabolites have been described previously (Bridgewater BR 2014; Evans et al. 2009). Untargeted ultra-HPLC coupled to tandem mass spectrometry (MS) and gas chromatography (GC)–MS were used to assay the samples. The platform portion for Liquid chromatography (LC)–MS utilized Waters ACQUITY ultra-performance liquid chromatographery, coupled with a Thermo Scientific Q-Exactive high-resolution accurate mass spectrometer. This was further integrated with a heated electrospray ionization source and an Orbitrap mass analyzer (35,000 mass resolution). A Thermo Finnigan Trace DSQ fast-scanning single quadruple mass spectrometer using electron impact ionization, and operated at unit mass resolving power, was used for the GC–MS portion (Bridgewater BR 2014). Metabolite peak intensity was normalized according to run-day by dividing each metabolite observation by the median for that metabolite on that run-day. Peaks were identified using Metabolon Inc.'s chemical reference library (Evans et al. 2009). Metabolon grouped the metabolites into the chemical classes and metabolic sub-pathways based on the Kyoto Encyclopedia of Genes and Genomes classifications.

For our sub-study, all blood samples were thawed, aliquoted and processed in a controlled and consistent manner. Samples from the same participant were analyzed consecutively within batches, and we included 24 blinded replicate samples for quality control. The median (inter-quartile range) intra-class correlation coefficient for the metabolites was 0.84 (0.62–0.91) (Derkach et al. 2017).

We started with a subset of 120 participants of the trial with serum metabolites measured at two time points (Derkach et al. 2017). These participants were equally divided between the two dietary intervention patterns. Among these 120 participants, 73 participants had metabolites measured after the high- and low-sodium interventions, 46 after high- and medium-interventions, and 1 after medium- and low-sodium interventions. We excluded participants if they had missing data related to the sleep variables (bed time, wake time, sleep duration and sleep midpoint, n=5) or their sleep patterns indicated disturbed sleep (they slept during the day/early evening only or for < 5 hours a night, n= 9). In total, we included 106 individuals. Seventeen participants had a single metabolite measurement, while 89 had two.

For our analysis, we focused upon only those metabolites that exceeded the limit of detection within ≥50% of our study sample. In total, 891 plasma metabolites were found:

545 were chemically identified and 346 were unknown. We report the results of the unknown metabolites in the supplemental information.

2.4 Statistical Analysis

We evaluated the relationship between the sleep variables (i.e. bed time, wake time, sleep duration and sleep midpoint) and metabolite levels using the following mixed-effects model. Let Y_{ijk} be the level of metabolite i in the k^{th} sample (k=1,2) of subject j; X_{jk} be the sleep variable and $\mathbf{C}_{jk}$ be a vector of covariates that includes categorical age (≤55, >55 years old), sex, race (African American, non-Hispanic white or other), dietary pattern (DASH or control) and sodium intervention (low, medium, high). Inclusion of BMI, smoking and other variables in Table 1, difference (in days) between sleep measures and blood draw and season of blood draw in the models did not substantially change the associations, therefore were not included in our final model. We then fit the model

$$\log(Y_{ijk}) = (\beta_{i0} + \gamma_{ij0}) + \beta_{i1}X_{jk} + \sum_l \beta_{il}C_{jkl} \quad (1)$$

using mixed-effects linear regression with a subject-specific random intercept (i.e. $\gamma_{ij0} \sim N(0, \sigma^2)$). We report an estimate of the relative change, $R_i = \exp(\beta_{i1})$, in metabolite level when increasing the value of the sleep variable by 1 hour. For example, $R_i = 1.2$ would suggest that increasing the sleep variable by 1 hour increased the metabolite level by a factor of 1.2 or 20%. We report both the p- and q-values from the likelihood ratio test for $\beta_{i1} = 0$. Here, q_i effectively estimates the proportion of associations with a p-value less than or equal to p_i that are likely to be false positives (Bass et al. n.d.; Storey 2002).

We determined the association between metabolic pathways and the sleep variables. The metabolites were divided into defined pathways (Supplementary Table 1). For each pathway, we combined the p-values of the included metabolites by Fisher's method (Fisher's Statistic = $\sum_i -2\ln(p_i)$).

Because the metabolites are correlated, the standard transformation of Fisher's statistic does not follow a chi-squared distribution. We therefore assessed the statistical significance and assigned a pathway-level p-value by permutation. For the permutation test, we first obtained residuals from fitting the mixed effects model without sleep exposure, and then randomly permuted the pairs of residual vectors across participants. We reported the pathway-level p-value as the proportion of the 10^4 permutations where the Fisher combined p-value was below the observed value.

We also performed secondary/sensitivity analyses to test whether covariates (e.g. race, gender, diet, age, BMI, smoking) modified the association between the metabolites and sleep variables. We considered

$$\log(Y_{ijk}) = (\beta^*_{i0} + \gamma^*_{ij0}) + \beta^*_{i1}X_{jk} + \sum_l \beta^*_{il}C_{jkl} + \beta^*_{iI}C_{jkL}X_{jk} \quad (2)$$

and tested whether each covariate modified the effect of intervention (i.e. $\beta_{jI} = 0$). We report the relative change in effect size, $R_j^* = \exp(\beta_{jI}^*)$, per 1-unit increase in the covariate.

We used a false discovery rate (FDR) level of 0.20 for statistical significance (q-value < 0.2). However, we note that the Bonferroni-adjusted α-level is 5.75×10^{-5} (0.05/870) for individual metabolites and 0.0013 (0.05/38) for metabolic pathways. All statistical analyses were performed using R programming language (R Core Team 2017).

3. Results

The demographic characteristics of the study sample are given in Table 1 overall and stratified by median midpoint (early: <2.40 hour and late: ≥ 2.40 hour). Mean bedtime was 23.12 hour, mean wake time was 6.24 hour and the median sleep duration was 7.23-hrs (SD: 1.28). Midpoint and wake time, and midpoint and bedtime, were correlated (r: 0.89 and 0.82, respectively). The remaining pairs of sleep variables were less strongly correlated: r=0.68 for duration and wake time, r=0.48 for bedtime and waketime, r=0.32 for duration and bedtime and r=0.28 for midpoint and sleep duration. Across the two sleep midpoint groups, participants had a similar distribution of characteristics, although participants with later sleep midpoint tended to have lower income.

Sleep midpoint was most strongly associated with blood metabolite levels. Sixteen pathways were significantly associated with midpoint using a p-value of <0.05 (Table 2). In general, the results for wake time mirrored those for sleep midpoint with 15 pathways associated (p-value <0.05). The three most strongly associated pathways were γ-glutamyl amino acid, phenylalanine and tyrosine metabolism and glutamate metabolism. Of the 11 metabolites within the γ-glutamyl amino acid pathway, seven metabolites (γ-glutamylisoleucine, γ-glutamylphenylalanine, γ-glutamylleucine, γ-glutamylvaline, γ-glutamylalanine, γ-glytamyltyrosine, γ-glutamylglutamate) were positively (q-value < 0.2) associated with sleep midpoint. For these seven metabolites (Table 3), a 1-hour increase in sleep midpoint increased the metabolite level by factor between 1.04 and 1.19. Of the five metabolites positively associated with sleep midpoint in the phenylalanine and tyrosine metabolism pathway, a 1-hr increase in sleep midpoint increased the metabolite level by a factor between 1.02 and 1.12. A 1-hr increase in sleep midpoint also increased the metabolite level of glutamate (within the glutamate metabolism pathway) by a factor of 1.06.

Eighty-three known metabolites (out of 545) were associated with sleep midpoint at an FDR of ≤ 0.20 (Table 3 and Supplemental Table 2), although none of them reached Bonferroni-corrected significance (p-value $<10^{-5}$). In addition to those above, the most strongly associated metabolites (p-value ≤ 0.001, Q-value < 0.05) were erythrulose (advanced glycation end-product), which decreased 0.94 per 1- hr increase in sleep mid-point and CMPF (fatty acid, dicarboxylate), isovalerate (both an amino acid and short chain fatty acid - valine, leucine and isoleucine and fatty acid metabolism) and HWESASXX (polypeptide), which increased 1.06 to 1.15 per 1-hr increase.

Wake time showed a similar pattern of metabolic associations to sleep midpoint. Forty-one metabolites were associated with wake time (Table 3 and supplemental table 2), with

the metabolites within the γ-glutamyl amino acid metabolism pathway among the most significantSeven γ-glutamyl amino acid pathway metabolites were positively associated with sleep wake time, a 1-hr increase in wake time increased the metabolite level by a factor between 1.03 and 1.17. The corresponding metabolite levels for the phenylalanine and tyrosine metabolism pathway were 1.02 to 1.10, while for glutamate it was 1.05. Two metabolites (ergothioneine and palmitoycarnitine (C16)) were inversely associated with wake time but were not associated with sleep midpoint.

Neither bedtime nor total sleep duration were strongly associated with metabolite levels. Only the metabolite erythrulose was, inversely, associated (FDR = 0.12) with time of going to sleep, while no metabolites were associated with sleep duration at an FDR ≤ 0.20. There was no significant interaction for the sleep associations by sex, race, BMI or dietary intervention.

Among the unknown metabolites, 64 were associated with sleep midpoint (supplemental table 3), 21 with wake time, three with bedtime and none with sleep duration (not shown) at an FDR <0.2.

4. Discussion

We found sleep midpoint to be associated with the highest proportion of serum metabolites compared with the other sleep measures under investigation. Among the 38 pathways, 16 were associated with sleep midpoint, as were 83 of the 545 known metabolites. Forty-one metabolites were associated with wake time, while only one (erythrulose) was associated with time of going to sleep and none were associated with sleep duration.

To our knowledge, only one epidemiologic study has examined metabolites and sleep habits. Xiao et. al. examined sleep habits ascertained from self-recorded logs (~28 days) over a year period and 329 fasting plasma metabolites in 277 Chinese adults (Xiao et al. 2017), and similarly found sleep midpoint, but not sleep duration, to be associated with a large number of metabolites (Xiao et al. 2017). The specific pathways and metabolites overlapped with our own discoveries. For example, both studies found metabolites within the γ-glutamyl amino acid metabolism pathway to be positively associated with sleep midpoint. These metabolites are formed through the mechanism of amino acid translocation across the cell membrane with the involvement of γ-glutamyl transpeptidase action upon glutathione within the cell and amino acids outside the cell (O. W. Griffith et al. 1979). γ-glutamyl transferase (GGT), a membrane-bound enzyme involved within Meister's γ-glutamyl cycle of moving γ-glutamyl amino acids across the cell membrane and the metabolism of glutathione, has been found to be positively associated with oxidative stress, alcohol consumption, diabetes, the presence of heavy metals, cardiovascular disease and cancer risk (Corti et al. 2010; Fentiman 2012; Kazemi-Shirazi et al. 2007). Elevated levels of GGT have also been found to be associated with obstructive sleep apnea and severity of the condition, which is further suggestive of a link between GGT and sleep disturbances (Kanbay et al. 2011; Sánchez-Armengol et al. 2015).

Xiao et al. found the branch chain amino acid (BCAA) metabolites leucine, valine and isoleucine to be positively associated with later sleep midpoint (Xiao et al. 2017), while our study found significant associations with isoleucine, dipeptides that contain BCAAs, and the valine, leucine and isoleucine pathway group. The magnitude of the associations was similar across the two studies. Higher concentrations of BCAA have been associated with obesity (Lynch and Adams 2014), insulin resistance and cardiovascular health (Ferguson and Wang 2016). These metabolites play a role in the synthesis of proteins, alanine and glutamine (Blomstrand et al. 2006). Isoleucine has also been found to prevent tumor growth and metastasis within mouse models (Murata and Moriyama 2007) and, when orally administered, to have anti-inflammatory properties (Saxena et al. 1984).

We found glutamate metabolism, involved in several metabolic pathways and stress responses, to be associated with sleep. Glutamate, an amino acid, serves as a precursor molecule for individual γ-glutamyl metabolites discussed above and is synthesized from glutamine. It acts as a neurotransmitter in the central nervous system (Yelamanchi et al. 2016). Altered glutamate levels have been associated with obesity and type-2 diabetes, (Davalli et al. 2012) as well as neurologic diseases (i.e. epilepsy, Parkinson's/Alzheimer's and stroke), (H. R. Griffith et al. 2008) with glutamate related to increased apoptosis of cells within the nervous system (Stepulak et al. 2014). A growing body of evidence suggests that the metabolite, functioning as both a growth factor and signal mediator, is involved in tumor development (Stepulak et al. 2014). Finally, a previous study is suggestive of exogenous glutamate exacerbating inflammation of the nervous system after cerebral ischemia injury (Xu 2004).

Metabolites in the phenylalanine and tyrosine metabolism pathway were associated with later sleep midpoint and wake time within our study. Phenylalanine is known to play a role in phenylketonuria, a rare familial condition defined by an inability to metabolize phenylalanine (Romani et al. 2017), but may also be associated with obesity and insulin resistance, both conditions associated with inflammation (Adams 2011; de Luca and Olefsky 2008).

Several lipid pathways and individual lipids were positively associated with sleep midpoint and wake time within our study. CMPF, a metabolite of furan fatty acids, was among our top associated metabolites and was positively associated with sleep mid-point in the previous study (Xiao et al. 2017). Furan fatty acids are found in a wide range of food (fish, butter, fruits, and vegetables) and derived from gut microbiota (Xu et al 2017). Increased CMPF levels are associated with chronic renal failure (Zhang et al. 2017), as well as, with impaired glucose tolerance, type 2 diabetes, and progression of pre-diabetes to type-2 diabetes in both humans (Koppe and Poitout 2016) and mouse models (Ying Liu et al. 2016). CMPF has been inversely associated with chronic fatigue among women with chronic widespread musculoskeletal pain which was mediated through BMI (Freidin et al. 2018). Interestingly, urinary excretion of CMPF has a circadian rhythm (Dietel et al. 1987). Previous studies have found longer sleep duration to be associated with lipid perturbation (Petrov et al. 2013) and sleep disruption appears to impact upon lipid levels (Wan Mahmood et al. 2013). The results for sleep timing appear to be inconsistent with some studies finding late sleep time to be associated with triglyceride/lower high-density lipoprotein levels (Berentzen et al.

2014; Rey-López et al. 2014; Wong et al. 2015). The relationship between lipid levels and atherosclerotic cardiovascular conditions is well established (Chait and Eckel 2016).

Several individual metabolites that were strongly associated with habitual sleep deserve mention. HWESASXX was positively, while erythrulose was inversely associated with wake time and sleep mid-point. Although little is known about the role of HWESASXX or erythrulose in disease development, the former is a peptide associated with the presence of an inflammatory state and acts to increase blood pressure as well as several actions inherent to insulin-like growth factor (Menni et al. 2016). Erythrulose is an advanced glycation end-product synthesized from reducing sugars, especially glucose and a Mallard reaction degradation product of ascorbic acid (Knight et al. 2016; Smuda et al. 2015). More research is needed to understand these associations with sleep timing.

A strength of our analysis its use of DASH-Sodium trial data, a strictly controlled feeding study of well-phenotyped participants, enabling evaluation of the associations between sleep and metabolites while avoiding confounding by diet and potential changes in weight. The metabolomic measures and agnostic approach permitted evaluation of metabolite associations across a broad range of biochemical pathways not previously investigated. Finally, we used fasting blood samples and included repeated measures of sleep habits and metabolites for most participants, increasing validity of our measures and statistical power.

Our study also has limitations. We did not have information related to sleep quality, which a number of studies have hypothesized is a factor related to metabolic health and metabolism (Okubo et al. 2014; Y. Zhan et al. 2014). As with other self-reported exposures, self-reported sleep may have issues with accuracy, however previous studies have found it reliable and valid (Biddle et al. 2015; Cespedes et al. 2016). Further, the DASH-Sodium participants recorded their sleep times while wearing 24-hour ambulatory blood pressure monitors which may have increase the precision of reported sleep. We were also unable to gather exact information about time of blood draw, although it was taken after an over-night fast. This information may have given us a better understanding of the confounding role of the circadian rhythm, and it may be that our results for midpoint and wake time are further associated with circadian rhythm. Our study sample size was relatively small; therefore, we may be underpowered to detect weak associations or heterogeneity across subgroups including the interaction of the dietary intervention on the association of sleep habits and metabolite levels. In addition, we did not have an independent population to replicate our results. Our study is cross-sectional therefore, we were also unable to determine whether sleep was impacting upon the metabolites associated with it or vice versa.

We did not observe associations between sleep duration and metabolites, and although there is some evidence that sleep duration may affect metabolism and the risk of a number of diseases (Sharma and Kavuru 2010). It appeared that sleep midpoint was most strongly correlated with wake time, followed by bed time. Why our results for wake time closely match those for midpoint, but those for bedtime do not is currently unclear – although similar results were found within the early study (Xiao et al. 2017).

Overall, we found sleep midpoint and, to a lesser extent, wake time, were associated with several metabolic pathways and individual metabolites. Evidence indicates that several of the metabolites are associated with inflammation and oxidative stress, processes that are associated with health outcomes such as diabetes, cardiovascular disease and cancer (Adams 2011; Fentiman 2012; Ferguson and Wang 2016; Kazemi-Shirazi et al. 2007; Koppe and Poitout 2016).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Author Conflict of Interest Statement

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Data Availability Statement

The metabolomic data reported in this paper are available upon request.

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Table 1:

Baseline Characteristics of DASH-Sodium Study Participants (Total and by Early/Late Sleep Midpoint)[1,2]

Characteristic	Overall	Early Midpoint (<2.40am)	Late Midpoint (>=2.40am)
Number of participants, n (%)	106	47 (44)	59 (56)
Sleep - Hours			
Sleep Midpoint: Median (interquartile range)	2.40 (1.30)	2.00 (0.75)	3.30 (0.85)
Sleep Midpoint: Mean (SD)	2.40 (1.08)	1.52 (0.49)	3.35 (0.75)
Sleep Duration: Mean (SD)	7.23 (1.28)	6.53 (1.01)	7.30 (1.42)
Bedtime: Mean (SD)	23.12 (1.1)	22.30 (0.75)	23.54 (0.9)
Waketime: Mean (SD)	6.24(1.4)	5.24 (0.75)	7.30 (1.1)
Age-group (n, %)			
18 to 30	0 (0)	0 (0)	0 (0)
31 to 55	69 (65)	31 (66)	38 (64)
56 to 65	28 (26)	13 (28)	15 (25)
>65	9 (8)	3 (6)	6 (10)
Sex (n, %)			
Male	46 (43)	18 (38)	28 (47)
Race/ethnicity (n, %)			
Black/African-American	54 (51)	24 (51)	30 (51)
Non-Hispanic White or Other	52 (49)	23 (49)	29 (49)
Body mass index (kg/m^2)			
Mean (SD)	29.28 (4.13)	29.52 (4.68)	29.08 (3.67)
World Health Organization Classification n (%)			
<= 18.5	0 (0)	0 (0)	0 (0)
18.5 – <= 25	12 (11)	7 (15)	5 (8)
>25	94 (89)	40 (85)	54 (92)
Physical activity: moderate n (%)			
> Four Times a Week	21 (20)	6 (13)	15 (25)
2–4 Times a Week	40 (38)	22 (47)	18 (31)
About Once a Week	16 (15)	8 (17)	8 (14)
2–3 Times a Month	13 (12)	4 (9)	9 (15)
Rarely or Never	16 (15)	7 (15)	9 (15)
Physical activity: vigorous n (%)			
> Four Times a Week	2 (2)	0 (0)	2 (3)
2–4 Times a Week	20 (19)	9 (19)	11 (19)
About Once a Week	9 (8)	1 (2)	8 (14)
2–3 Times a Month	10 (9)	3 (6)	7 (12)
Rarely or Never	65 (61)	34 (72)	31 (53)
Blood Pressure (mm Hg), Mean (SD) [4]			
Systolic	142.51 (14.80)	142.98 (15.19)	142.14 (14.61)
Diastolic	94.13 (10.33)	94.68 (10.94)	93.69 (9.88)
Hypertension, n (%)	8 (8)	4 (9)	4 (7)

Characteristic	Overall	Early Midpoint (<2.40am)	Late Midpoint (>=2.40am)
Education, n (%)			
High School Graduate or less	19 (18)	8 (17)	11 (19)
Some College	43 (41)	19 (40)	24 (41)
College Degree	18 (17)	7 (15)	11 (19)
Post-graduate Work/Degree	26 (25)	13 (28)	13 (22)
Household income (n, %)			
< $30,000	30 (28)	9 (19)	21 (36)
$30,000–$60,000	44 (42)	19 (40)	25 (42)
> $ 60,000	30 (28)	18 (38)	12 (20)
Missing	2 (2)	1 (2)	1 (2)
Smoker (n, %)			
Never Smoked	59 (56)	28 (60)	31 (53)
Former Smoker	36 (34)	15 (32)	21 (36)
Current Smoker	11 (9)	4 (9)	7 (12)
Diet Intervention n (%)			
Dietary pattern			
Control	53 (50)	21 (45)	32 (54)
DASH-Combination Diet	53 (50)	26 (55)	27 (46)
Sodium Level			
Repeated Measures			
High and Low/Medium	88 (83)	42 (89)	46 (78)
Medium and Low	1 (1)	0 (0)	1 (2)
One measure			
High	11 (10)	4 (9)	7 (12)
Medium	4 (4)	1 (2)	3 (5)
Low	2 (2)	0 (0)	2 (3)
Alcohol: No. of Drinks per Week, n (%)			
0	67 (63)	29 (62)	38 (64)
≤ 5	28 (26)	15 (32)	13 (22)
6 +	11 (10)	3 (6)	8 (14)
No. of Metabolite Measurements, n (%)			
1	17 (16)	5 (11)	12 (20)
2	89 (84)	42 (89)	47 (80)

Values are means (SD) or n (%). DASH, Dietary Approaches to Stop Hypertension Trial.

[1]Early and late midpoint defined by median midpoint sleep value for cohort.

[2]Totals may not sum to 100 percent due to rounding.

[3]Hypertension defined as an average systolic blood pressure of 140 to 159 mm Hg or an average diastolic blood pressure of 90 to 95 mm Hg during the three screening visits.

[4]Blood pressure was the average of 3 screening measurements and 2 measurements during the run-in period.

Table 2:

Pathways Sub Organized by Midpoint

	Sleep Variable				
	Number of Metabolites	**Bedtime**	**Waketime**	**Sleep Duration**	**Sleep Midpoint**
Pathway Group		**P-value**	**P-value**	**P-value**	**P-value**
Gamma-glutamyl amino acid	11	0.02	0.0008	0.14	0.0004
Phenylalanine & tyrosine metabolism	27	0.02	0.0072	0.58	0.0017
Glutamate metabolism	6	0.22	0.0023	0.03	0.0058
Dipeptide	9	0.06	0.01	0.40	0.0076
Pyrimidine metabolism group	12	0.06	0.04	0.89	0.01
Krebs cycle / TCA cycle	6	0.08	0.03	0.77	0.02
Methionine, cysteine, SAM and taurine metabolism	14	0.26	0.01	0.32	0.02
Glycolysis, gluconeogenesis, pyruvate metabolism group	7	0.37	0.01	0.26	0.02
Lysolipid	31	0.19	0.02	0.21	0.02
Others	56	0.10	0.03	0.87	0.02
Tocopherol metabolism	6	0.02	0.12	0.89	0.02
Tryptophan metabolism group	18	0.07	0.10	0.90	0.03
Leucine, Isoleucine and Valine Metabolism	30	0.26	0.04	0.66	0.03
Fatty acid group	15	0.10	0.04	0.20	0.04
Alanine and Aspartate Metabolism	8	0.27	0.05	0.69	0.04
Long chain fatty acid; polyunsaturated fatty acid (n3 and n6)	16	0.32	0.03	0.23	0.05
Fatty acid, monohydroxy	13	0.33	0.05	0.66	0.06
Drug	5	0.35	0.08	0.44	0.08
Benzoate metabolism	15	0.79	0.03	0.15	0.09
Lysine metabolism	10	0.11	0.13	0.16	0.10
Monoacylglycerol	10	0.08	0.13	0.21	0.11
Pentose metabolism	9	0.46	0.20	0.65	0.16
Purine metabolism	17	0.28	0.18	0.58	0.16
Food component/plant	27	0.13	0.38	0.59	0.18
Glycine, Serine and Threonine Metabolism	10	0.62	0.05	0.05	0.19
Long chain fatty acid	15	0.42	0.19	0.41	0.19
Sterol/steroid	35	0.30	0.34	0.73	0.21
Xanthine metabolism	12	0.02	0.78	0.06	0.23
Chemical	15	0.47	0.42	0.77	0.29
Fatty acid, dicarboxylate	13	0.53	0.27	0.40	0.30
Urea cycle group	13	0.28	0.53	0.69	0.33
Carnitine metabolism group	12	0.56	0.38	0.44	0.40

	Sleep Variable				
	Number of Metabolites	**Bedtime**	**Waketime**	**Sleep Duration**	**Sleep Midpoint**
Pathway Group		**P-value**	**P-value**	**P-value**	**P-value**
Fructose, mannose, galactose, starch, and sucrose metabolism	7	0.67	0.44	0.66	0.55
Primary bile acid metabolism	6	0.69	0.34	0.15	0.66
Medium chain fatty acid group	9	0.48	0.73	0.66	0.70
Sphingolipid metabolism	10	0.63	0.87	0.98	0.70
Histidine metabolism	7	0.57	0.88	0.44	0.77
Secondary bile acid metabolism	12	0.37	0.91	0.40	0.89

[1]The P-value was calculated using Fisher's method to combined p-values of individual metabolite associations from linear random effect models adjusted for age, sex, race, sodium level, dietary pattern and visit.

Pathways are based on the Kyoto Encyclopedia of Genes and Genomes.

The reported pathway-level values combine metabolite-level values obtained from adjusted random effects models.

The Bonferroni-corrected significance for the 38 pathways was 0.05/38 = 0.0013.

Table 3:

Sleep Wake Time, Midpoint and Associated Metabolites by Pathway[1]

Metabolite	Wake Time				Sleep Midpoint			
	Relative change[2]	95% CI[3]	P-value	Q-value[4]	Relative Change[2]	95% CI[3]	P-value	Q-value[4]
Gamma-glutamyl amino acid								
Gamma-glutamylisoleucine	1.11	1.05–1.16	0.00014	0.036	1.14	1.06–1.21	0.0002	0.035
Gamma-glutamylphenylalanine	1.04	1.02–1.07	0.00038	0.036	1.06	1.03–1.09	0.0003	0.035
Gamma-glutamylleucine	1.08	1.04–1.13	0.00037	0.036	1.10	1.05–1.17	0.0004	0.036
Gamma-glutamylvaline	1.10	1.04–1.15	0.00054	0.040	1.13	1.06–1.20	0.0005	0.038
Gamma-glutamylalanine	1.06	1.01–1.11	0.012	0.159	1.09	1.03–1.16	0.0053	0.078
Gamma-glutamyltyrosine	1.03	1.01–1.05	0.0083	0.122	1.04	1.01–1.07	0.0057	0.078
Gamma-glutamylglutamate	1.17	1.05–1.29	0.0041	0.099	1.19	1.04–1.36	0.0137	0.108
Phenylalanine and tyrosine metabolism								
N-acetyltyrosine	1.02	1.00–1.03	0.010	0.132	1.03	1.01–1.05	0.003	0.075
N-acetylphenylalanine	1.02	1.01–1.03	0.005	0.100	1.02	1.01–1.04	0.006	0.078
3-[3-(sulfooxy)phenyl]propanoic acid	1.10	1.03–1.17	0.004	0.099	1.12	1.03–1.22	0.009	0.095
3-methoxytyramine sulfate	1.02	0.98–1.06	0.314	0.540	1.07	1.01–1.12	0.016	0.117
Thyroxine	0.94	0.89–0.99	0.013	0.168	0.93	0.87–0.99	0.022	0.134
4-hydroxyphenylpyruvate	0.94	0.88–1.00	0.056	0.314	0.91	0.83–0.99	0.027	0.152
P-cresol sulfate	1.03	1.00–1.06	0.081	0.333	1.04	1.00–1.08	0.046	0.194
Glutamate metabolism								
Glutamine	0.97	0.95–0.99	0.002	0.092	0.96	0.94–0.99	0.008	0.088
Glutamate	1.05	1.02–1.09	0.003	0.092	1.06	1.02–1.11	0.009	0.100
Dipeptide group								
N-acetylcarnosine	1.02	1.00–1.05	0.021	0.213	1.04	1.01–1.07	0.004	0.075
Isoleucylglycine	1.03	1.01–1.06	0.006	0.101	1.04	1.01–1.07	0.009	0.100
Valylleucine	1.04	0.99–1.10	0.092	0.342	1.07	1.00–1.14	0.039	0.183
Valylglycine	1.05	0.99–1.10	0.096	0.344	1.07	1.00–1.14	0.051	0.200
Pyrimidine metabolism								

Metabolite	Wake Time				Sleep Midpoint			
	Relative change[2]	95% CI[3]	P-value	Q-value[4]	Relative Change[2]	95% CI[3]	P-value	Q-value[4]
5-methyluridine (ribothymidine)	1.01	1.00–1.03	0.016	0.187	1.02	1.01–1.04	0.003	0.075
Orotidine	1.02	1.01–1.04	0.006	0.101	1.03	1.01–1.05	0.006	0.078
Pseudouridine	1.01	1.00–1.02	0.068	0.319	1.02	1.00–1.03	0.013	0.108
2’-deoxyuridine	1.02	1.00–1.05	0.068	0.319	1.03	1.00–1.07	0.050	0.200
Krebs cycle / TCA cycle								
Succinate	1.03	1.01–1.05	0.002	0.092	1.04	1.02–1.07	0.002	0.071
Citrate	0.95	0.89–1.01	0.117	0.379	0.92	0.84–0.99	0.036	0.175
Alpha-ketoglutarate	0.96	0.92–1.00	0.044	0.280	0.95	0.90–1.00	0.050	0.200
Methionine, cysteine, SAM, and taurine metabolism group								
Methionine sulfoxide	1.10	1.04–1.16	0.001	0.043	1.12	1.04–1.20	0.002	0.070
Methionine sulfone	1.02	1.00–1.04	0.052	0.302	1.03	1.01–1.06	0.007	0.081
N-acetyltaurine	1.03	1.01–1.05	0.003	0.099	1.03	1.01–1.06	0.011	0.105
Glycolysis, gluconeogenesis, pyruvate metabolism group								
1,5-anhydroglucitol (1,5-ag)	1.02	1.01–1.03	0.001	0.060	1.02	1.01–1.04	0.004	0.075
Glucuronate	1.03	1.01–1.05	0.017	0.192	1.04	1.01–1.07	0.005	0.075
Lysolipid								
1-stearoylglycerophosphoinositol	1.06	1.03–1.09	0.001	0.043	1.08	1.03–1.12	0.001	0.040
1-oleoylglycerophosphoinositol*	1.04	1.00–1.08	0.063	0.318	1.07	1.02–1.13	0.012	0.108
1-palmitoylplasmenylethanolamine*	1.04	1.01–1.07	0.019	0.201	1.05	1.01–1.09	0.013	0.108
1-palmitoylglycerophosphoinositol*	1.07	1.02–1.13	0.005	0.101	1.08	1.02–1.15	0.015	0.115
1-oleoylplasmenylethanolamine*	1.05	1.01–1.09	0.022	0.213	1.06	1.01–1.11	0.021	0.133
1-stearoylglycerophosphoethanolamine	1.02	1.00–1.04	0.101	0.352	1.03	1.00–1.06	0.035	0.175
1-stearoylplasmenylethanolamine*	1.03	1.00–1.06	0.049	0.294	1.04	1.00–1.08	0.036	0.175
1-arachidonoylglycerophosphoinositol*	1.01	0.99–1.03	0.191	0.456	1.02	1.00–1.05	0.048	0.198
Other								
Erythrulose (Advance glycation end-product)	0.96	0.94–0.99	0.004	0.099	0.94	0.91–0.97	0.0002	0.035
HWESASXX (Polypeptide)	1.08	1.02–1.14	0.007	0.113	1.13	1.05–1.21	0.001	0.049
5-Hete (Eicosanoid)	1.06	1.02–1.10	0.003	0.099	1.08	1.03–1.13	0.002	0.075

Metabolite	Wake Time				Sleep Midpoint			
	Relative change[2]	95% CI[3]	P-value	Q-value[4]	Relative Change[2]	95% CI[3]	P-value	Q-value[4]
Leukotriene B4 (Eicosanoid)	1.20	1.04–1.39	0.014	0.175	1.32	1.09–1.59	0.005	0.075
Prostaglandin E2 (Eicosanoid)	1.13	1.03–1.24	0.008	0.122	1.16	1.03–1.31	0.013	0.108
Erythronate (Aminosugar metabolism) *	1.02	1.01–1.03	0.003	0.099	1.02	1.00–1.03	0.015	0.115
Tartronate (hydroxymalonate) (Bacterial/fungal)	1.06	1.00–1.12	0.042	0.274	1.09	1.01–1.17	0.023	0.141
Myo-inositol (Inositol metabolism)	1.01	1.00–1.03	0.030	0.248	1.02	1.00–1.04	0.043	0.189
4-guanidinobutanoate (Guanidino and acetamido metabolism)	1.08	0.99–1.17	0.075	0.327	1.12	1.00–1.24	0.049	0.200
Bradykinin, Des-Arg(9)(Polypeptide)	1.08	0.96–1.22	0.222	0.482	1.17	1.00–1.37	0.051	0.200
Tocopherol metabolism								
Delta-tocopherol	1.04	1.00–1.09	0.072	0.323	1.09	1.03–1.15	0.005	0.078
Tryptophan metabolism								
Indole-3-carboxylic acid	1.03	1.00–1.06	0.034	0.250	1.05	1.01–1.09	0.007	0.081
Kynurenine	1.01	1.00–1.02	0.032	0.248	1.01	1.00–1.02	0.016	0.118
Leucine, isoleucine and valine metabolism								
Isovalerate[5]	1.12	1.06–1.19	0.0002	0.036	1.15	1.06–1.24	0.001	0.047
N-acetylvaline	1.01	1.00–1.02	0.023	0.214	1.01	1.00–1.03	0.013	0.108
3-methyl-2-oxobutyrate	0.95	0.91–0.99	0.018	0.196	0.94	0.89–0.99	0.014	0.108
Isoleucine	1.01	1.00–1.01	0.042	0.274	1.01	1.00–1.02	0.041	0.184
N-acetylisoleucine	1.02	1.00–1.03	0.064	0.318	1.02	1.00–1.04	0.041	0.184
Fatty acid metabolism group								
Butyrylcarnitine	0.96	0.93–0.99	0.017	0.196	0.95	0.91–0.99	0.013	0.108
Palmitoylcarnitine (C16)	0.96	0.93–0.99	0.018	0.196	0.96	0.92–1.01	0.114	0.266
Alanine and aspartate metabolism								
N-acetylalanine	1.01	1.00–1.02	0.006	0.103	1.01	1.00–1.02	0.006	0.078
Fatty acid, monohydroxy								
13-Hode + 9-Hode	1.10	1.03–1.18	0.004	0.099	1.14	1.05–1.24	0.004	0.075
Drug								
4-acetylphenol sulfate	1.05	1.00–1.10	0.035	0.250	1.08	1.01–1.14	0.023	0.141
2-acetamidophenol sulfate	1.06	0.99–1.13	0.089	0.342	1.09	1.01–1.19	0.040	0.184

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Metabolite	Wake Time				Sleep Midpoint			
	Relative change[2]	95% CI[3]	P-value	Q-value[4]	Relative Change[2]	95% CI[3]	P-value	Q-value[4]
Benzoate metabolism								
4-ethylphenylsulfate	1.05	1.02–1.09	0.006	0.101	1.06	1.01–1.11	0.021	0.133
Catechol sulfate	1.03	1.00–1.07	0.048	0.294	1.05	1.01–1.09	0.027	0.154
O-methylcatechol sulfate	1.03	1.00–1.07	0.085	0.339	1.05	1.00–1.10	0.045	0.192
Lysine metabolism								
N6-acetyllysine	1.01	1.00–1.02	0.056	0.314	1.02	1.00–1.03	0.021	0.133
Glutarylcarnitine (C5)	0.99	0.99–1.00	0.166	0.429	0.99	0.98–1.00	0.045	0.192
Monoacylglycerol								
1-myristoylglycerol (1-monomyristin)	1.03	1.00–1.06	0.024	0.217	1.05	1.02–1.09	0.005	0.075
Pentose metabolism group								
Ribitol	1.02	1.01–1.03	0.002	0.092	1.03	1.01–1.04	0.003	0.075
Purine metabolism group								
N6-succinyladenosine	1.02	1.00–1.03	0.006	0.101	1.02	1.00–1.03	0.029	0.156
7-methylguanine	1.01	1.00–1.02	0.078	0.331	1.02	1.00–1.03	0.010	0.104
N2,N2-dimethylguanosine	1.01	1.00–1.02	0.036	0.250	1.02	1.00–1.03	0.013	0.108
Food component/plant								
Saccharin	0.93	0.86–1.01	0.106	0.360	0.88	0.78–0.98	0.025	0.145
2-oxindole-3-acetate	1.05	0.99–1.11	0.104	0.357	1.09	1.01–1.17	0.028	0.156
Ergothioneine	0.93	0.88–0.98	0.014	0.171	0.94	0.87–1.01	0.073	0.231
Glycine, serine and threonine metabolism								
N-acetylthreonine	1.01	1.00–1.03	0.090	0.342	1.02	1.00–1.05	0.037	0.180
N-acetylserine	.	.	.	.	1.01	1.00–1.02	0.045	0.192
Long chain fatty acid								
Cis-vaccenate (18:1n7)	0.98	0.97–1.00	0.062	0.318	0.97	0.95–0.99	0.015	0.113
Myristoleate (14:1N5)	0.98	0.96–1.00	0.063	0.318	0.97	0.94–1.00	0.029	0.156
Xanthine metabolism								
3,7-dimethylurate	1.04	0.98–1.11	0.193	0.456	1.10	1.02–1.19	0.020	0.133
Chemical								

Metabolite	Wake Time				Sleep Midpoint			
	Relative change[2]	95% CI[3]	P-value	Q-value[4]	Relative Change[2]	95% CI[3]	P-value	Q-value[4]
4-Hydroxychlorothalonil	1.01	1.00–1.02	0.094	0.342	1.02	1.00–1.03	0.018	0.128
Fatty acid, dicarboxylate								
3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)	1.04	1.02–1.07	0.0003	0.036	1.06	1.03–1.09	0.0004	0.035
Sphingolipid metabolism								
Oleoyl sphingomyelin	1.01	1.00–1.02	0.094	0.342	1.02	1.00–1.03	0.035	0.175

[1] The relative change and its associated 95% confidence interval, P -value and Q-value were calculated using linear random effect models adjusted for age, sex, race, sodium level, dietary pattern and visit. The table is ranked by p-value of pathway analysis.

[2] The relative change has been exponentiated. The relative change in metabolite per 1-hour increase in sleep variable.

[3] Lower and upper 95% confidence intervals.

[4] Metabolites with a Q-value of ≥ 0.2 for either wake time or sleep mid-point are included.

˙ Result not available.

[5] Isovalerate is classified as both a short chain fatty acid and amino acid