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Habitual Sleep and human plasma metabolomics

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Abstract

Introduction—Sleep plays an important role in cardiometabolic health. The sleep-wake cycle is partially driven by the endogenous circadian clock, which governs a range of metabolic pathways. The association between sleep and cardiometabolic health may be mediated by alterations of the human metabolome

Objectives—To better understand the biological mechanism underlying the association between sleep and health, we examined human plasma metabolites in relation to sleep duration and sleep timing.

Methods—Using an untargeted approach, 329 fasting plasma metabolites were measured in 277 Chinese participants. We measured sleep timing (midpoint between bedtime and wake up time) using repeated time-use surveys (4 weeks during one year) and previous night sleep duration from questionnaires completed before sample donation.

Results—We found 64 metabolites that were associated with sleep timing with a false discovery rate of 0.2 or lower, after adjusting for potential confounders. Notably, we found that later sleep timing was associated with higher levels of multiple metabolites in amino acid metabolism, including branched chain amino acids and their gamma-glutamyl dipeptides. We also found

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Compliance with Ethical Standards:

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widespread associations between sleep timing and numerous metabolites in lipid metabolism, including bile acids, carnitines and fatty acids. In contrast, previous night sleep duration was not associated with plasma metabolites in our study.

Conclusion—Sleep timing was associated with a large number of metabolites across a variety of biochemical pathways. Some metabolite associations are consistent with a relationship between late chronotype and adverse effects on cardiometabolic health.

Keywords

Sleep duration; sleep timing; metabolomics

Introduction

Growing evidence has suggested that sleep plays an important role in multiple cardiometabolic conditions: sleep deficiency has been repeatedly associated with higher risks of obesity (Wu et al., 2014), type 2 diabetes (Shan et al., 2015, Cappuccio et al., 2010), and cardiovascular diseases (CVD) (Cappuccio et al., 2011). Night-shift workers who commonly suffer circadian disruption experience larger weight gain (van Drongelen et al., 2011) and are more likely to develop metabolic syndrome (Wang et al., 2014), diabetes (Gan et al., 2015), and CVD (Vyas et al., 2012). Moreover, several recent studies also reported that a preference of later sleep schedules (late chronotype) and larger differences in weekday/weekend schedules (social jetlag) are associated with worse metabolic health (Wittmann et al., 2006, Wong et al., 2015).

The sleep-wake cycle is an important behavioral manifestation of the endogenous circadian clock, which governs a range of metabolic pathways. Therefore, the association between sleep and cardiometabolic health may be mediated by alterations of the human metabolome (Bass and Takahashi, 2010). To date, three studies examined the acute effects of total and partial sleep deprivation in controlled laboratory conditions on human metabolome (Bell et al., 2013, Davies et al., 2014, Weljie et al., 2015). All three studies reported that sleep restriction resulted in widespread changes in circulating metabolites, including reduction of carbohydrates and increased levels of certain lipids and amino acids. However, such effects may not reflect those caused by habitual sleep conditions, such as chronic sleep deprivation or general sleep timing. Moreover, previous studies suggested that late sleep timing may be associated with more severe circadian disruption and may be a risk factor for multiple cardiometabolic conditions independent of sleep duration (Wong et al., 2015). However, no study has examined sleep timing in relation to human metabolome.

In a group of Chinese men and women who completed daily time-use log for four separate weeks in a 1-year period, we used an untargeted approach to measure over 300 metabolites from fasting plasma samples. We examined metabolite levels in relation to multiple measures of sleep timing and sleep duration. The aim of our study is to identify metabolite markers that are associated with natural variations in sleep behavior in the general population, which may help elucidate the biological mechanisms driving the health effects of sleep and circadian rhythm.

Methods

Study population

Our study included subjects from the Shanghai Physical Activity Study. Details of this study have been previously reported (Peters et al., 2010). Briefly, a total of 619 participants were randomly selected from two population-based prospective studies, the Shanghai Women's Health Study (Zheng et al., 2005) and Shanghai Men's Health Study (Shu et al., 2015). Participants were asked to complete a daily activity log for seven consecutive days on four separate occasions during a one-year study period (roughly one administration in each season). On average, each participant provided 27.3 days of log data. They also donated blood samples at the beginning and at the end of the one-year period, but only the samples donated at the end of the study period were used for metabolomics assay. A previous study selected 339 men and women for metabolomics assay using the blood samples donated at the end of the study (Xiao et al., 2016), and among them we excluded subjects with non-fasting blood samples. The final analytic sample included 277 men and women. A diagram depicting the study design and sleep variables are presented in Supplementary figure 1.

Measurement of sleep variables

In the activity log, the participants reported the time they went to bed at night and got up in the morning. Additionally, before each blood donation, they completed a short questionnaire and reported sleep duration in the previous night. From these we calculated two main sleep variables: 1) yearly average of the midpoint between bedtime and wake-up time (midpoint of time in bed), a common measure of chronotype (Kantermann et al., 2015, Roenneberg et al., 2003); and 2) sleep duration during the night prior to end-of-study blood donation.

We also calculated several other sleep variables for additional analysis (Supplementary figure 1). These include yearly average of total time spent in bed as a proxy of habitual sleep duration; average midpoint on nights before weekends, difference between average midpoint in weekdays and weekends, standard deviation of repeated measures of midpoint over the 1-year period, and average previous night sleep duration between the two sample donations at the beginning and the end of the study. Moreover, to address the potential U-shaped association between sleep duration and metabolites, we also created two categorical variables to indicate short, normal, and long sleep for average total time in bed and previous-night sleep. For previous-night sleep, we defined <6 hr as short sleep and >9 hr as long sleep. For total time in bed, we designated the bottom 10% (<7.3 hr) to the short and the upper 10% (≥10 hr) to the long category.

Measurement of metabolites

We used EDTA treated fasting plasma samples donated at the end of study year. Metabolite levels were measured by Metabolon, Inc. whose platform and procedures have been described previously (Evans et al., 2009, DeHaven et al., 2010). Briefly, samples were analyzed using ultra high performance liquid-phase chromatography coupled with mass spectrometry and tandem mass spectrometry (LC/MS and LC/MS^2) and gas chromatography coupled with mass spectrometry (GC/MS). For each sample, the batch and position within a batch of 32 samples were randomly assigned, and the value for each metabolite was e

normalized metabolite to the median for that batch. Individual metabolites were identified by comparing the mass spectra peaks to a chemical reference library. Of the 445 metabolites that were detected, 329 were of known identity. Identified metabolites were grouped into 8 chemical classes (amino acids, carbohydrates, cofactors and vitamins, energy metabolites, lipids, nucleotide metabolites, peptides, and xenobiotics) and 55 sub-pathways. Previous studies have reported a high level of reliability for the metabolomics platform used in this study (Sampson et al., 2013). Detailed methods for LC/MS, LC/MS^2, and /GC-MS, as well as compound identification and curation are reported in Supplementary materials. Reference spectral data including retention time and m/z for the 329 metabolites detected in our study are presented in Supplementary table 1.

Covariates

The baseline questionnaire of the Shanghai Women's Health Study and Shanghai Men's Health Study collected demographic information including age and gender. Follow-up in-person interviews were conducted every two years. Height was measured at baseline and weight was measured repeatedly in follow-up interviews. To calculate body mass index (BMI, weight (kg)/height$(m)^2$), we used baseline height and weight from the interview that was the closest to the date of sample collection (<2 years). Smoking status (current smoker or non-smoker) was reported when the plasma samples were taken. We calculated day-time napping duration based on activity log. In the same periods when activity log was completed, the participants were asked to wear an Actigraph accelerometer on the left hip at all times except when sleeping, showering and swimming. Total physical activity was calculated using actigraphy data using previously described methods (Peters et al., 2010). The time of sample donation was recorded in the study. The distribution of these covariates in the overall study and by sex is presented in Supplementary table 2.

Statistical analysis

Metabolite levels were first batch normalized and then log-transformed. Values below the detection threshold were set to the minimum observed value of the metabolite. The median level of "missingness" before imputation was 1%. Metabolites that were observed in ≤90% of the samples were excluded from the analysis. Pairwise correlations among metabolites were determined using the Pearson correlation coefficient, and correlations among sleep variables were determined using the Spearman correlation coefficient. Linear regression was used to estimate the association between each metabolite and sleep variables, adjusted for age (continuous), clock time at sample collection (continuous), sex (male, female), smoking status (yes, no), BMI (continuous), napping time (continuous), and physical activity energy expenditure (continuous). Moreover, because age and time of sample collection are important confounders and may have a nonlinear relationship with metabolites, we explored using spline terms for age and time at sample collection, but found the additional flexibility had little impact on the results. To account for multiple comparisons, we primarily used a false discovery rate (FDR) <0.2 to define statistical significance for purposes of reporting associations, but we also reported statistical significance using more stringent Bonferroni correction method (0.05/329=0.00015). We used the likelihood-ratio test to determine whether is a statistically significant interaction between sex and sleep in relation to

metabolites. Because a statistically significant interaction was detected for some metabolites, we also performed stratified analysis in men and women separately.

We also evaluated the association between sleep variables and metabolomic patterns. Specifically, we used sparse principal component analysis (Zou et al., 2006) to create 10 principal components (PCs) with 10 non-zero loadings. We then performed step-wise regression between sleep variables and 10 PCs adjusted for the same set of covariates. We calculated the additional percent of variance in sleep variables explained by each of the PCs. We used Bonferroni correction to evaluate the statistical significance for interaction with sex ($p<0.0078$ (0.05/64)). All analyses were performed with SAS (version 9.1.3, SAS Institute, Cary, NC) and the R statistical language package (version 3.1.2).

Results

The distribution of the two main sleep variables by study characteristics is presented in Supplementary table 3. Participants with less than elementary school education had earlier average midpoint of time in bed, and time at sample donation was positively associated with midpoint. In contrast, sleep duration in the previous night before sample collection was only associated with clock time at sample collection.

After controlling for all covariates, 64 of the 329 metabolites with known identity were found to be associated with average midpoint of time in bed with an FDR<0.2 (Table 1, Figure 1), and they represented all 8 biochemical classes and 29 out of the 55 sub-pathways. Late midpoint was associated with higher levels of amino acids, carbohydrates, bile acids, steroids, dipeptides and several xenobiotics. In contrast, midpoint point was inversely associated with all the carnitines and fatty acids (except for CMPF), as well as bilirubin and AMP. When we used Bonferroni correction, only three metabolites had a significant association with midpoint of sleep, and these include cis-4-decenoyl carnitine, laurylcarnitine, and 1,6-anhydroglucose. Detailed information on the specific metabolites as well as their effect estimates and p-values are presented in Figure 2, Table 2 (for the 64 metabolites associated with midpoint) and Supplementary table 4 (full list). When we conducted subgroup analysis by sex focusing on the 64 metabolites significantly associated with midpoint, the metabolite associations were the same in direction for both men and women and we did not find any statistically significant interaction with sex (Supplementary table 5). Additional adjustment of previous night sleep duration or average time spent in bed had little impact on the results (data not shown).

An individual’s sleep schedule often differs between weekdays and weekends (Wong et al., 2015). However, in our population the average differences between midpoints on weekdays and weekends were small (<30 minutes for over 90% subjects and <10 minutes for over 50%). When we conducted a sensitivity analysis focusing on average midpoint on weekend evenings (Friday and Saturday nights) alone, we found that the results were largely similar to those for average of all nights (Supplementary table 6). Moreover, we did not find any metabolite that was associated with standard deviation of repeated measures of midpoint over the one-year period or the difference between averaged midpoints in weekdays and weekends (data not shown).

We investigated the clustering of the 64 metabolites that were significantly associated with midpoint of time in bed. The overall correlations were moderate to low (Pearson correlation coefficient<0.4). However, we observed very high correlations (Pearson correlation coefficient>0.8) among carnitines, branched chain amino acids and dipeptides. We also found moderate to high correlations (Pearson correlation coefficient, 0.4–0.8) among various fatty acids, between fatty acids and carnitines, and between amino acids and their dipeptide derivatives (Figure 3).

We examined metabolites in relation to previous night sleep duration, and we did not find any metabolite with a significantly (FDR<0.2) association (full list of metabolites associations are shown in Supplementary table 7). We also did not find any metabolite associations with yearly average of total time spent in bed as a proxy of habitual sleep duration (Supplementary table 8). Further analysis showed no metabolite associations with the short or long categories of total time in bed or previous night sleep duration (data not shown). Finally, we further compared the metabolite associations for sleep timing, previous night sleep duration and average time in bed (Figure 4). For a more complete comparison, we present all the metabolites that were associated with at least one of the sleep variables at p<0.05. Overall we found the pattern of metabolite associations for the three variables was quite distinct. Metabolites that showed stronger association with total time in bed and previous night sleep duration tended to be only weakly associated with midpoint of time in bed.

Discussion

Our study is the first to investigate the human metabolome in relation to habitual sleep. The sleep-wake cycle is driven by the internal circadian clock, which orchestrates a wide range of metabolic pathways, including carbohydrate, protein and amino acid, and lipid metabolism (Bailey et al., 2014). We found that sleep timing was associated with the fasting levels of a large number of metabolites across multiple biochemical pathways, highlighting the central role of circadian rhythms and sleep in human metabolism.

Amino acids and peptides

Both protein degradation and synthesis are regulated by the circadian system, with increased protein degradation during sleep and higher protein synthesis during awake time. (Bailey et al., 2014). Microarray studies have found diurnal variations in genes involved in protein turnover (Duffield et al., 2002), and previous studies have reported daily fluctuations in circulating amino acid levels (Dallmann et al., 2012, Weljie et al., 2015, Ang et al., 2012). We found sleep timing was associated with multiple amino acids. Notably, later sleep timing was associated higher circulating levels of valine, leucine, isoleucine (branched chain amino acids (BCAA)) and their gamma-glutamyl dipeptides, as well as several metabolites from the sulfur amino acids metabolism, and tyrosine and phenylalanine metabolism (alpha-hydroxybutyrate, alpha-ketobutyrate, Phenylacetate and 3-(4-hydroxyphenyl)lactate).

A growing body of research has linked BCAA metabolism, sulfur amino acids metabolism, and tyrosine and phenylalanine metabolism pathways to impaired cardiometabolic health (Adams, 2011, Lynch and Adams, 2014). For example, it has been suggested that higher

concentrations of circulating BCAAs, alpha-hydroxybutyrate and alpha-ketobutyrate may serve as markers of insulin resistance (Batch et al., 2014, Tom and Nair, 2006, Ferrannini et al., 2013, Gall et al., 2010). Moreover, circulating levels of BCAAs and 3-(4-hydroxyphenyl)lactate were found to be associated with higher BMI (Moore et al., 2014). Interestingly, a recent study found fasting isoleucine level was elevated after eight nights of sleep restriction (5.5 hour) in 12 healthy adults (Bell et al., 2013), and another study found higher levels of leucine and valine following one night sleep restriction (4 hour) in rats (Weljie et al., 2015). It is worth noting that the magnitude of the associations between sleep timing and amino acids observed in our study was relatively small – one hour of delay in midpoint was associated with only <0.1 SD increase in many amino acids in this population, which may not lead to clinically meaningful difference. However, on average, our study subjects had an early sleep timing: the average midpoint of sleep of our study population was 2:30 am, 1 hour earlier than what was reported in previous literature for the same age group on work-free days (Roenneberg et al., 2007). We cannot exclude the possibility that the effect of sleep timing on metabolites may be nonlinear, and the potentially adverse effect of sleep timing may become more pronounced when sleep timing becomes more extremely late.

Together with the previous findings, our findings suggest that late sleep timing was associated with systematic changes in several amino acid pathways, some of which were previously indicated in cardiometabolic disease risk. More future studies are needed to directly examine the potential role of amino acid metabolism in mediating the health effects of sleep timing.

Lipids

Internal circadian clocks control multiple aspects of lipid metabolism (Bailey et al., 2014). Several recent metabolomics studies reported 24-hr oscillations of multiple lipid metabolites (Dallmann et al., 2012, Ang et al., 2012, Davies et al., 2014), even when the subjects followed a constant routine that removed external stimuli such as changes in light, physical activities and food intakes (Dallmann et al., 2012). In our study, we found that a large number of compounds in multiple lipid pathways were associated with sleep timing. However, it is worth noting that because the circulating levels of many lipids show circadian variation, the associations found in our study may reflect changes in average metabolite levels, alterations in the amplitude of the fluctuation, or a phase shift in the fluctuation patterns. Unfortunately we only measured plasma metabolites at one time point during the day, and therefore we cannot determine which of the aforementioned mechanisms drove the findings observed in our study.

Most notably, we found that late sleep timing was associated with lower levels of multiple fatty acids. Moreover we found that sleep timing was also inversely associated with several acylcarnitines, which play an essential role in transporting fatty acids into mitochondria, where fatty acid β-oxidation takes place (McCoin et al., 2015). The molecular clock regulate multiple enzymes involved in fatty acid metabolism (Gooley and Chua, 2014), including CPT-1, the enzyme that converts acyl-CoA to acylcarnitine (Panda et al., 2002, Hughes et al., 2009). Interestingly, in a recent study by Weljie et al., human subjects were subject to 5-

day sleep restriction (4 hours of sleep per night) and then a night of recovery sleep. The study found reduced levels of acylcarnitines among subjects who did not recover after sleep restriction (Weljie et al., 2015). However, another study reported higher levels of acylcarnitines during total sleep deprivation (Davies et al., 2014). The discrepancy in these two studies indicates that acute effects induced by sleep deprivation may be different from chronic sleep debt. Our findings suggest that in addition to chronic sleep debt, habitually late sleep timing may also be associated with systematic alterations in fatty acid metabolism, and more future studies are needed to investigate the long-term impact of circadian dysfunction in real-world situations on lipid metabolism.

Higher levels of bile acids were associated with late sleep timing in our study. Bile acids are major cholesterol metabolites that are synthesized in the liver. Elevated bile acid levels have been linked with dyslipidemia and hyperglycemia, and recent studies suggested that bile acid sequestrants that aim at reducing circulating bile acid levels can cause markedly improvement in these two conditions (Brinton, 2008). Moreover, we found that higher levels of lathosteral, an important maker for impaired cholesterol metabolism (Farkkila et al., 1996, Matthan et al., 2013), was also associated with late sleep timing. Taken together, our findings are consistent with a disruptive role of late sleep timing on bile acid and cholesterol homeostasis.

Other metabolites and pathways

A number of xenobiotic metabolites were positively associated with late sleep timing in our study. Higher levels of markers of coffee consumption such as caffeine, hippurate, theobromine, and cinnamoylglycine (Guertin et al., 2015) were strongly associated with late midpoint of time in bed, which is consistent with the established association between coffee consumption and late sleep timing (Roehrs and Roth, 2008). Additionally, late sleep timing was also associated with salicylate, a major ingredient in pain medications, suggesting that certain medical conditions may be in play. Finally, several other metabolites associated with sleep timing are also potential markers of certain dietary exposures, including glucurionide (alcohol) (Dinis-Oliveira, 2016), xylitol (sweetner), CMPF (fish intake), 1-linoleoylglycerol (plant lipids), and prolylhydroxyproline (collagen supplement), stachydrine and chiro inositol (citrus)) (Guertin et al., 2014). Taken together, lifestyle factors may be responsible for some of the observed metabolite associations with sleep timing.

Sleep duration

We did not find any metabolite associations with total time in bed or previous night sleep duration, which may be explained by a number of factors. First, total time in bed may include other non-sleep-related activity such as reading and watching television. Previous work showed that total time in bed is a poor measure of sleep behavior and does not distinguish between controls and patients with insomnia (Natale et al., 2009). On the other hand, self-reported previous night sleep duration may be more accurate. However, it does not necessarily reflect habitual sleep duration. Moreover, we had a relatively narrow distribution of sleep duration and were underpowered to detect meaningful associations with short sleep: ~90% participants reported between 6 and 9 hours of sleep and only 22 participants reported less than 6 hours of sleep. We believe future studies with better measurement of sleep

duration and larger sample size will be needed to investigate the metabolic profiles associated with quantity of sleep.

Strengths and limitations

A major strength of our study is that we have repeated measures (~28 days) of sleep over a one-year period, which allowed us to more accurately assess habitual sleep. Moreover, using an untargeted approach to examine the metabolome, we were able to measure a large number of metabolites across a broad range of biochemical pathways, many of which have never been examined before in relation to sleep. However, there are also several limitations of our study. First, as mentioned above, we did not have information on habitual sleep duration. Second, our population has a fairly stable sleep routine with small overall variation and little difference between weekdays and weekends, probably due to their older age and the fact that most of our study participants were retired at the time of the study. This limited our ability to examine metabolites in relation to shift sleep schedule between weekday and weekends, which has been previously shown to be an important cardiometabolic risk factor (Wong et al., 2015). However, the stability in sleep routines also has important advantage, because it suggests that in this population, day-time schedule might have little impact on sleep timing. Therefore, our measurement may well reflect the intrinsic preference of sleep timing (chronotype). Third, we have identified a number of metabolites that were markers of diet, coffee drinking and alcohol consumption, which suggest that uncontrolled lifestyle factors may had an impact on our results. Particularly, dietary intake and timing of the last meal may be associated with both sleep timing and plasma levels of certain metabolites. Unfortunately we don't have information on the last meal before blood donation and could not control for its confounding effects. Moreover, although we adjusted for several health behaviors, including napping, physical activity and smoking, confounding due to other lifestyle factors and environmental factors may also have an impact on our results. Finally, we did not have multiple measurements of metabolites at different times of the day, and therefore we were unable to assess the diurnal fluctuation patterns of metabolite levels.

Conclusion

In summary, we found late sleep timing was associated with a large number of metabolites across a variety of biochemical pathways. Although we cannot rule out confounding completely, many metabolites associated with sleep timing in our study were also previously linked with cardiometabolic health and warrant further investigation, particularly in lipid and amino acid metabolism. Overall, our study provides new insight into the biological mechanisms underlying the health effects of sleep and points to the need for future studies to better understand the role of circadian rhythms in metabolic regulation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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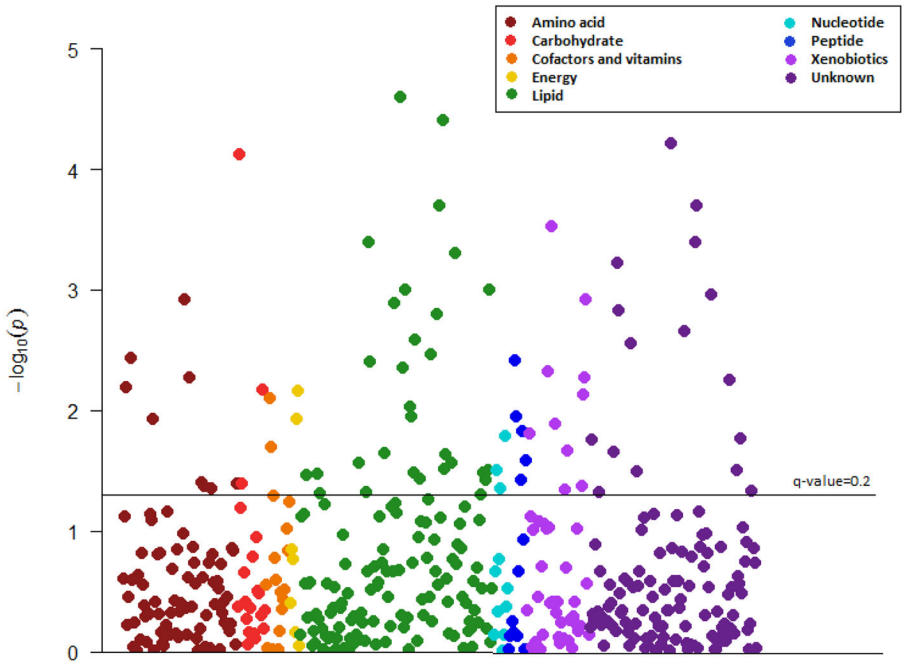

Figure 1.
Manhattan plot of metabolites associations with midpoint of time spent in bed

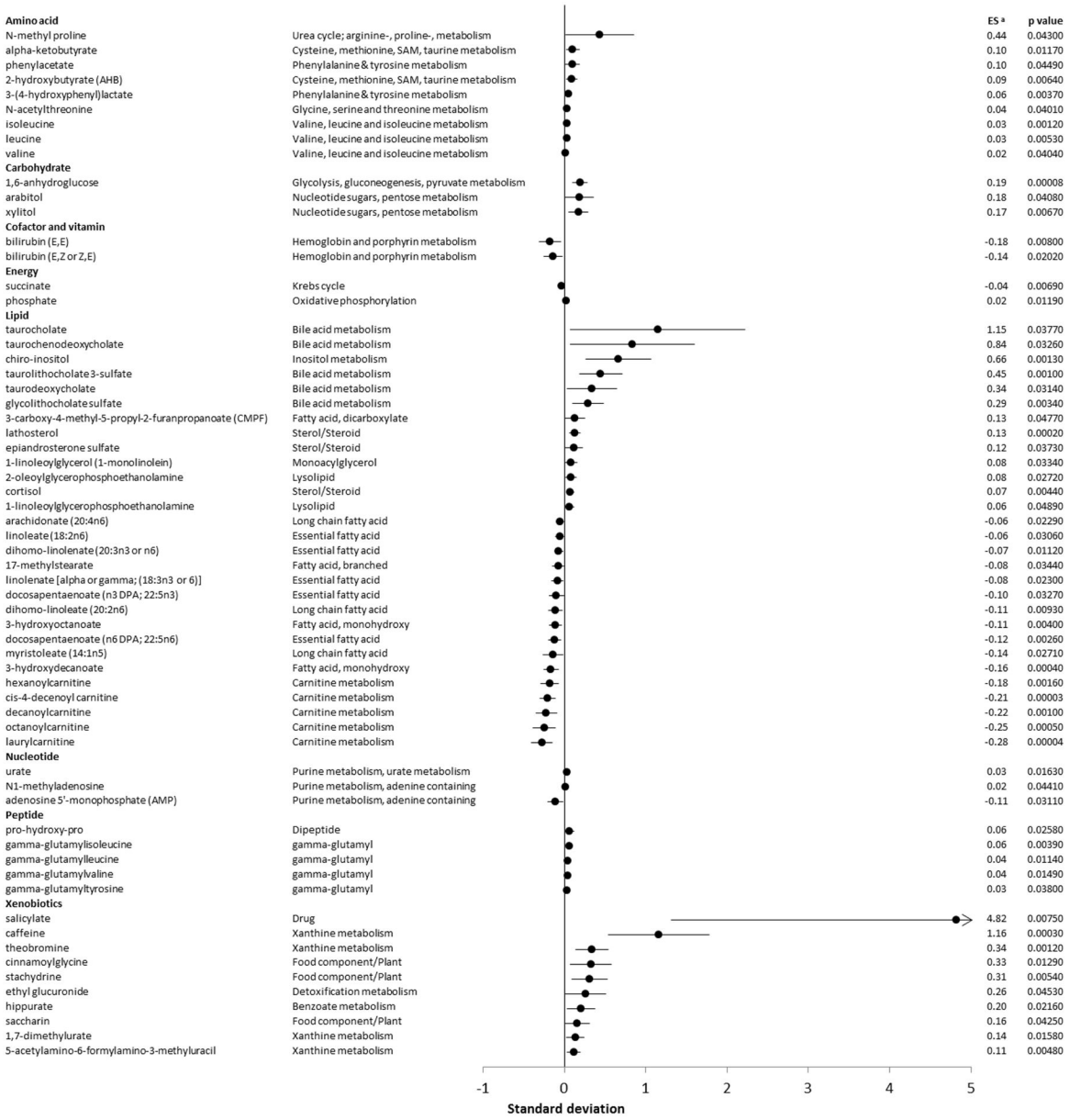

Figure 2.

Forest plot of metabolites associations with midpoint of time spent in bed. Associations shown on the plot are statistically significant, defined as false discovery rate<0.2. [a] Effect estimate expressed as changes in metabolite level (standard deviation, log scale) per 1 hour delay in midpoint of time. Results adjusted for age (continuous), sex (male, female), body mass index (continuous), smoking status (nonsmoker, smoker), napping (continuous), physical activity (continuous) and sampling time (continuous). Abbreviation: ES, effect size.

Metabolite	Super Pathways	Sub Pathways
2-hydroxybutyrate (AHB)	Amino acid	Cysteine, methionine, SAM, taurine metabolism
alpha-ketobutyrate	Amino acid	Cysteine, methionine, SAM, taurine metabolism
N-acetylthreonine	Amino acid	Glycine, serine and threonine metabolism
3-(4-hydroxyphenyl)lactate	Amino acid	Phenylalanine & tyrosine metabolism
phenylacetate	Amino acid	Phenylalanine & tyrosine metabolism
N-methyl proline	Amino acid	Urea cycle; arginine-, proline-, metabolism
isoleucine	Amino acid	Valine, leucine and isoleucine metabolism
leucine	Amino acid	Valine, leucine and isoleucine metabolism
valine	Amino acid	Valine, leucine and isoleucine metabolism
1,6-anhydroglucose	Carbohydrate	Glycolysis, gluconeogenesis, pyruvate metabolism
xylitol	Carbohydrate	Nucleotide sugars, pentose metabolism
arabitol	Carbohydrate	Nucleotide sugars, pentose metabolism
bilirubin (E,E)	Cofactors and vitamins	Hemoglobin and porphyrin metabolism
bilirubin (E,Z or Z,E)	Cofactors and vitamins	Hemoglobin and porphyrin metabolism
succinate	Energy	Krebs cycle
phosphate	Energy	Oxidative phosphorylation
taurolithocholate 3-sulfate	Lipid	Bile acid metabolism
glycolithocholate sulfate	Lipid	Bile acid metabolism
taurodeoxycholate	Lipid	Bile acid metabolism
taurochenodeoxycholate	Lipid	Bile acid metabolism
taurocholate	Lipid	Bile acid metabolism
cis-4-decenoyl carnitine	Lipid	Carnitine metabolism
laurylcarnitine	Lipid	Carnitine metabolism
octanoylcarnitine	Lipid	Carnitine metabolism
decanoylcarnitine	Lipid	Carnitine metabolism
hexanoylcarnitine	Lipid	Carnitine metabolism
docosapentaenoate (n6 DPA; 22:5n6)	Lipid	Essential fatty acid
dihomo-linolenate (20:3n3 or n6)	Lipid	Essential fatty acid
linolenate [alpha or gamma; (18:3n3 or 6)]	Lipid	Essential fatty acid
linoleate (18:2n6)	Lipid	Essential fatty acid
docosapentaenoate (n3 DPA; 22:5n3)	Lipid	Essential fatty acid
17-methylstearate	Lipid	Fatty acid, branched
3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)	Lipid	Fatty acid, dicarboxylate
3-hydroxydecanoate	Lipid	Fatty acid, monohydroxy
3-hydroxyoctanoate	Lipid	Fatty acid, monohydroxy
chiro-inositol	Lipid	Inositol metabolism
dihomo-linoleate (20:2n6)	Lipid	Long chain fatty acid
arachidonate (20:4n6)	Lipid	Long chain fatty acid
myristoleate (14:1n5)	Lipid	Long chain fatty acid
2-oleoylglycerophosphoethanolamine	Lipid	Lysolipid
1-linoleoylglycerophosphoethanolamine	Lipid	Lysolipid
1-linoleoylglycerol (1-monolinolein)	Lipid	Monoacylglycerol
lathosterol	Lipid	Sterol/Steroid
cortisol	Lipid	Sterol/Steroid
epiandrosterone sulfate	Lipid	Sterol/Steroid
adenosine 5'-monophosphate (AMP)	Nucleotide	Purine metabolism, adenine containing
N1-methyladenosine	Nucleotide	Purine metabolism, adenine containing
urate	Nucleotide	Purine metabolism, urate metabolism
pro-hydroxy-pro	Peptide	Dipeptide
gamma-glutamylisoleucine	Peptide	gamma-glutamyl
gamma-glutamylleucine	Peptide	gamma-glutamyl
gamma-glutamylvaline	Peptide	gamma-glutamyl
gamma-glutamyltyrosine	Peptide	gamma-glutamyl
hippurate	Xenobiotics	Benzoate metabolism
ethyl glucuronide	Xenobiotics	Detoxification metabolism
salicylate	Xenobiotics	Drug
stachydrine	Xenobiotics	Food component/Plant
cinnamoylglycine	Xenobiotics	Food component/Plant
saccharin	Xenobiotics	Food component/Plant
caffeine	Xenobiotics	Xanthine metabolism
theobromine	Xenobiotics	Xanthine metabolism
5-acetylamino-6-formylamino-3-methyluracil	Xenobiotics	Xanthine metabolism
1,7-dimethylurate	Xenobiotics	Xanthine metabolism

Figure 3.

This heat map shows the correlation among the known metabolites significantly [a] associated with midpoint of time in bed. The colors represent Pearson correlation coefficient: very dark red, ≥0.8; dark red, 0.6-<0.8; medium red, 0.4-<0.6; light red, 0-<0.4; light green, −0.4 - <0.

[a] Statistical significance was determined using a false discovery rate threshold of 0.2.

Figure 4.

This heat map shows the associations between metabolites and three sleep variables, average sleep timing, average time in bed and previous night sleep duration. Metabolites that are included in this figure have at least one association with a p-value<0.05. The 8 color codes represent different z-scores: very dark green, <−3.29 (p-value <0.001); dark green, −3.29 – < −2.58 (0.001-<0.01); medium green, −2.58 – <−1.96 (0.01-<0.05); light green, −1.96 – <0 (0.05–1); light red, >0 – 1.96 (0.05–1); medium red, >1.96 – 2.58 (0.01–<0.05); dark red, >2.58 – 3.29 (0.001–<0.01); very dark red >3.29 (<0.001). Positive z-scores suggest positive association (higher levels of metabolites with later midpoint) while negative z-scores suggest inverse association (higher levels of metabolites with earlier midpoint). All models were adjusted for age (continuous), sex (male, female), body mass index (continuous), smoking status (nonsmoker, smoker), napping (continuous), physical activity (continuous) and sampling time (continuous).

Table 1

Number of metabolites with known identity detected by chemical class, and the number of metabolites that were significantly [a] associated with midpoint of time in bed

Chemical class	Total number of known metabolites	Number (%) of known metabolites with significant association
All	329	64 (19.5)
Amino acids	81	9 (11.1)
Carbohydrates	20	3 (15.0)
Cofactors and vitamins	17	2 (11.8)
Energy metabolites	7	2 (28.6)
Lipids	136	29 (21.3)
Nucleotide metabolites	11	3 (27.3)
Peptides	13	5 (38.5)
Xenobiotics	44	10 (22.7)

[a] Statistical significance defined as false discovery rate (FDR) <0.2

Table 2

Metabolites associated [a] with midpoint of time in bed in the Shanghai Physical Activity Study

Metabolite	Pathway	Effect estimate [b]	Standard error	P value	Q value	HMDB ID	KEGG ID
	Amino acid						
isoleucine	Valine, leucine and isoleucine metabolism	0.03	0.01	0.0012	0.02	HMDB00172	C00407
3-(4-hydroxyphenyl)lactate	Phenylalanine & tyrosine metabolism	0.06	0.02	0.0037	0.05	HMDB00755	C03672
leucine	Valine, leucine and isoleucine metabolism	0.03	0.01	0.0053	0.06	HMDB00687	C00123
2-hydroxybutyrate (AHB)	Cysteine, methionine, SAM, taurine metabolism	0.09	0.03	0.0064	0.06	HMDB00008	C05984
alpha-ketobutyrate	Cysteine, methionine, SAM, taurine metabolism	0.1	0.04	0.0117	0.09	HMDB00005	C00109
N-acetylthreonine	Glycine, serine and threonine metabolism	0.04	0.02	0.0401	0.19		C01118
valine	Valine, leucine and isoleucine metabolism	0.02	0.01	0.0404	0.19	HMDB00883	C00183
N-methyl proline	Urea cycle; arginine-, proline-, metabolism	0.44	0.21	0.043	0.19		
phenylacetate	Phenylalanine & tyrosine metabolism	0.1	0.05	0.0449	0.19	HMDB00209	C07086
	Carbohydrate						
1,6-anhydroglucose	Glycolysis, gluconeogenesis, pyruvate metabolism	0.19	0.05	0.00008	0.01	HMDB00640	
xylitol	Nucleotide sugars, pentose metabolism	0.17	0.06	0.0067	0.06	HMDB00568	C00379
arabitol	Nucleotide sugars, pentose metabolism	0.18	0.09	0.0408	0.19	HMDB01851	C00474
	Cofactor and vitamin						
bilirubin (E,E)	Hemoglobin and porphyrin metabolism	−0.18	0.07	0.008	0.07		
bilirubin (E,Z or Z,E)	Hemoglobin and porphyrin metabolism	−0.14	0.06	0.0202	0.13		
	Energy						
succinate	Krebs cycle	−0.04	0.01	0.0069	0.06	HMDB00254	C00042
phosphate	Oxidative phosphorylation	0.02	0.01	0.0119	0.09	HMDB01429	C00009
	Lipid						
cis-4-decenoyl carnitine	Carnitine metabolism	−0.21	0.05	0.00003	0.01		
laurylcarnitine	Carnitine metabolism	−0.28	0.07	0.00004	0.01	HMDB02250	
lathosterol	Sterol/Steroid	0.13	0.03	0.0002	0.01	HMDB01170	C01189
3-hydroxydecanoate	Fatty acid, monohydroxy	−0.16	0.05	0.0004	0.01	HMDB02203	
octanoylcarnitine	Carnitine metabolism	−0.25	0.07	0.0005	0.02		

Metabolite	Pathway	Effect estimate [b]	Standard error	P value	Q value	HMDB ID	KEGG ID
decanoylcarnitine	Carnitine metabolism	−0.22	0.07	0.001	0.02	HMDB00651	
taurolithocholate 3-sulfate	Bile acid metabolism	0.45	0.13	0.001	0.02	HMDB02580	C03642
chiro-inositol	Inositol metabolism	0.66	0.2	0.0013	0.02		
hexanoylcarnitine	Carnitine metabolism	−0.18	0.06	0.0016	0.03	HMDB00705	C01585
docosapentaenoate (n6 DPA; 22:5n6)	Essential fatty acid	−0.12	0.04	0.0026	0.04	HMDB13123	C06429
glycolithocholate sulfate	Bile acid metabolism	0.29	0.1	0.0034	0.05		
3-hydroxyoctanoate	Fatty acid, monohydroxy	−0.11	0.04	0.004	0.05	HMDB01954	
cortisol	Sterol/Steroid	0.07	0.03	0.0044	0.05	HMDB00063	C00735
dihomo-linoleate (20:2n6)	Long chain fatty acid	−0.11	0.04	0.0093	0.08		C16525
dihomo-linolenate (20:3n3 or n6)	Essential fatty acid	−0.07	0.03	0.0112	0.09	HMDB02925	C03242
arachidonate (20:4n6)	Long chain fatty acid	−0.06	0.02	0.0229	0.14	HMDB01043	C00219
linolenate [alpha or gamma; (18:3n3 or 6)]	Essential fatty acid	−0.08	0.04	0.023	0.14	HMDB01388	C06427
myristoleate (14:1n5)	Long chain fatty acid	−0.14	0.06	0.0271	0.16	HMDB02000	C08322
2-oleoylglycerophosphoethanolamine	Lysolipid	0.08	0.04	0.0272	0.16		
linoleate (18:2n6)	Essential fatty acid	−0.06	0.03	0.0306	0.17	HMDB00673	C01595
taurodeoxycholate	Bile acid metabolism	0.34	0.16	0.0314	0.17	HMDB00896	C05463
taurochenodeoxycholate	Bile acid metabolism	0.84	0.39	0.0326	0.17	HMDB00951	C05465
docosapentaenoate (n3 DPA; 22:5n3)	Essential fatty acid	−0.1	0.05	0.0327	0.17	HMDB01976	C16513
1-linoleoylglycerol (1-monolinolein)	Monoacylglycerol	0.08	0.04	0.0334	0.17		
17-methylstearate	Fatty acid, branched	−0.08	0.04	0.0344	0.17		
epiandrosterone sulfate	Sterol/Steroid	0.12	0.06	0.0373	0.18	HMDB00365	C07635
taurocholate	Bile acid metabolism	1.15	0.55	0.0377	0.18	HMDB00036	C05122
3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)	Fatty acid, dicarboxylate	0.13	0.06	0.0477	0.2		
1-linoleoylglycerophosphoethanolamine	Lysolipid	0.06	0.03	0.0489	0.2		
	Nucleotide						
urate	Purine metabolism, urate metabolism	0.03	0.01	0.0163	0.12	HMDB00289	C00366
adenosine 5′-monophosphate (AMP)	Purine metabolism, adenine containing	−0.11	0.05	0.0311	0.17	HMDB00045	C00020
N1-methyladenosine	Purine metabolism, adenine containing	0.02	0.01	0.0441	0.19	HMDB03331	C02494
	Peptide						
gamma-glutamylisoleucine	gamma-glutamyl	0.06	0.02	0.0039	0.05		
gamma-glutamylleucine	gamma-glutamyl	0.04	0.02	0.0114	0.09	HMDB11171	

Metabolite	Pathway	Effect estimate [b]	Standard error	P value	Q value	HMDB ID	KEGG ID
gamma-glutamylvaline	gamma-glutamyl	0.04	0.02	0.0149	0.11	HMDB11172	
pro-hydroxy-pro	Dipeptide	0.06	0.03	0.0258	0.16	HMDB06695	
gamma-glutamyltyrosine	gamma-glutamyl	0.03	0.02	0.038	0.18		
	Xenobiotics						
caffeine	Xanthine metabolism	1.16	0.32	0.0003	0.01	HMDB01847	C07481
theobromine	Xanthine metabolism	0.34	0.1	0.0012	0.02	HMDB02825	C07480
5-acetylamino-6-formylamino-3-methyluracil	Xanthine metabolism	0.11	0.04	0.0048	0.05	HMDB11105	C16365
stachydrine	Food component/Plant	0.31	0.11	0.0054	0.06	HMDB04827	C10172
salicylate	Drug	4.82	1.79	0.0075	0.07	HMDB01895	C00805
cinnamoylglycine	Food component/Plant	0.33	0.13	0.0129	0.1		
1,7-dimethylurate	Xanthine metabolism	0.14	0.06	0.0158	0.11	HMDB11103	C16356
hippurate	Benzoate metabolism	0.2	0.09	0.0216	0.14	HMDB00714	C01586
saccharin	Food component/Plant	0.16	0.08	0.0425	0.19		D01085
ethyl glucuronide	Detoxification metabolism	0.26	0.13	0.0453	0.19		

[a] Associations presented in this table are statistically significant using a false discovery rate threshold of 0.2

[b] Expressed as changes in metabolite level (standard deviation, log scale) per 1 hour delay in midpoint of time. Results adjusted for age (continuous), gender (male, female), body mass index (continuous), smoking status (nonsmoker, smoker), napping (continuous), physical activity (continuous) and sampling time (continuous).