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ORIGINAL ARTICLE

Circulating metabolites associated with objectively measured sleep duration and sleep variability in overweight/obese participants: a metabolomics approach within the SATIN study

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Abstract

Study Objectives: To investigate the associations of circulating metabolites with sleep duration and sleep variability. We also assessed the ability of metabolites to discriminate between sleep duration and sleep variability categories.

AQ2 **Methods:** Cross-sectional analysis of 205 participants with overweight/obesity from the "Satiety Innovation" (SATIN) study. A targeted metabolite profiling (n = 159 metabolites) approach was applied using three different platforms (nuclear magnetic resonance, liquid chromatography coupled to mass spectrometry, and gas chromatography coupled to mass spectrometry). Associations between circulating metabolite concentrations and accelerometer-derived sleep duration and variability in sleep duration were assessed using elastic-net regression analysis. Ten-fold cross-validation was used to estimate the discriminative accuracy of metabolites for sleep duration and sleep variability categories.

Results: A metabolite profile, including acyl-carnitines (C11:0/C5:1-DC/iso-C11:0, 2-M-C4:1/3-M-C4:1, C4:0), sphingomyelins (42:1, 33:1), glycerol, stearic acid, 2- and 3-hydroxyl-butyric acid, docosahexaenoic acid, serotonin, and phosphatidylcholine (34:2), was significantly associated with high sleep duration (4th plus 5th quintile). Ten metabolites, including acyl-carnitines (C18:1, C7:0, C6-OH), phosphatidylcholine (40:6, 37:4, 42:5), lyso-phosphatidylcholine (20:1), sucrose, glutamic acid, and triacylglycerol (52:4), were significantly associated with high sleep variability (4th plus 5th quintile). The area under the curve was 0.69 (95% CI: 0.62–0.75) and 0.63 (95% CI: 0.53–0.72) in the multimetabolite score for high sleep duration and sleep variability, respectively. The variance in sleep duration explained by metabolites was 7%. No metabolites were selected for prediction of sleep variability (continuous).

Conclusions: A small set of metabolites within distinct biochemical pathways were associated with high sleep duration and sleep variability. These metabolites appeared to moderately discriminate sleep duration and sleep variability categories.

Statement of Significance

Sleep restriction and irregular sleep patterns have been associated with metabolic impairments. However, the metabolic dysregulations associated with these sleep disturbances remain to be determined under "real life," free-living conditions. The present study provides data showing associations between circulating metabolites and objectively measured sleep duration and sleep variability in overweight/obese adults. These findings may provide a deeper understanding of metabolic responses to sleep. Future research should assess the involvement of the identified metabolites in sleep-related metabolic health.

Key words: metabolomics; sleep duration; sleep variability; SATIN

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Introduction

In humans, adequate sleep and a steady sleep regimen confer a wide range of health benefits, whereas both sleep deprivation and irregular sleep patterns have been associated with metabolic disorders, including obesity and type 2 diabetes [1–6]. The biological mechanisms linking sleep deprivation/disruption and long-term health consequences have not yet been fully elucidated [7].

Metabolic impairments associated with sleep loss have been observed in several experimental studies. Total (24 hr) sleep deprivation results in increased plasma levels of glycerophospholipids, acylcarnitines, sphingolipids, and amino acids (tryptophan, serotonin, and taurine) [8]. Partial [9, 10] sleep deprivation has also been shown to induce plasma and serum metabolite changes in various pathways. However, the effects of sleep deprivation under controlled laboratory conditions on human metabolism may not reflect the natural variation in sleep patterns observed in "real-life" conditions. In addition, previous studies suggest that irregular sleep–wake rhythms may be associated with metabolic dysfunction [11, 12]. Recently, a cross-sectional study found 64 plasma metabolites across multiple biochemical pathways significantly associated with sleep timing (midpoint between bedtime and wake up time) [13].

Studies combining objectively measured "real-life" levels of free-living sleep deprivation and high sleep variability with systemic metabolite profiling would provide a deeper understanding of metabolic responses to sleep and also help us to identify relevant metabolites able to predict sleep patterns.

Using targeted metabolite profiling, cross-sectional associations between plasma/serum concentrations of metabolites with sleep duration and sleep variability were examined in participants with overweight/obesity included in the EU project Satiety Innovation (SATIN) work package 5, prior to the implementation of a dietary intervention. The ability of metabolite markers to discriminate between sleep duration and sleep variability categories was also investigated.

Participants and Methods

Study design

A cross-sectional analysis of baseline data from the SATIN study, a medium-term clinical trial focusing on satiation/satiety enhancement, appetite control, and weight management in overweight and obese participants, was performed. All procedures were conducted in accordance with the ethical principles set forth in the current version of the Declaration of Helsinki (Fortaleza, Brazil, October 2013) and the International Conference on Harmonization E6 Good Clinical Practice (ICH-GCP). The protocol was approved by the local institutional review boards and Ethics Committees of all the recruiting centres and all participants provided written informed consent. This trial was registered in clinicaltrials.gov, with the identifier NCT02485743.

Study population

Eligible participants were community-dwelling adults (aged between 20 and 65 years), with an initial body mass index of 27.0 to 35.0 kg/m^2, fat mass ≥23%, and without comorbidities. Exclusion criteria included weight changes ±3 kg in the last 3 months and cardiometabolic diseases (type 1 or 2 diabetes, cardiovascular disease, and hypertension). Cardiovascular disease included previous diagnosis of angina pectoris, myocardial infarction, coronary revascularization procedures, stroke (either ischemic or hemorrhagic, including transient ischemic attacks), symptomatic peripheral artery disease that required surgery or was diagnosed with vascular imaging techniques, ventricular arrhythmia, uncontrolled atrial fibrillation, congestive heart failure (New York Heart Association Class II or IV), hypertrophic myocardiopathy, and history of aortic aneurism ≥5.5 cm in diameter or aortic aneurism surgery within the past 6 months, as diagnosed by a medical doctor. Other exclusion criteria were chronic kidney and liver pathologies, diseases which may affect energy expenditure, current or former (<3 months) smoking, and regular consumption of alcohol above the recommendations. A total of 236 participants from Reus (n = 122) and Copenhagen (n = 114) recruiting centers with blood samples available at baseline were considered. Thirty one participants were excluded due to the lack of actigraphy data.

Targeted metabolomics

The current study used three different platforms to analyze metabolites. Proton nuclear magnetic resonance (^{1}H NMR), liquid chromatography coupled to mass spectrometry (LC–MS), and gas chromatography coupled to mass spectrometry (GC–MS) were used to perform optimal profiling of a wide range of metabolites. Fasting blood samples for GC–MS analysis were dried and stored at −80°C until analysis, whereas supernatants of samples for LC–MS and NMR were directly analyzed and maintained at 4°C during their sequence run.

Automation of multiple plasma sample extraction

Aqueous extractions of 250 µL of plasma were performed with a methanol/water solution in a Bravo-automated liquid handling platform (Agilent Technologies, CA). Lipid extractions of 100 µL of plasma were performed by a biphasic extraction with methanol/methyl-tert-butyl ether (MTBE). Solvents were added automatically to the samples and after the appropriate shaking and centrifugation steps the supernatants were dispensed in 96-well plates and stored until analysis with GC–MS, LC–MS, and NMR. Internal standards for GC and LC were previously dispensed by the robot to the same plates where supernatants were collected. Quality controls (i.e. pool of samples) were used in both GC and LC to discard drift in the instrumental response.

Lipid ^{1}H-NMR profiling

Samples were prepared following the procedure previously published [14]. ^{1}H-NMR spectra were recorded at 300 K on an Avance III 600 spectrometer (Bruker, Germany) operating at a proton frequency of 600.20 MHz using a 5 mm PBBO gradient probe. Lipid samples were measured and recorded in PROCNO 11 using a simple presaturation sequence (recycle delay [RD]–90°–ACQ presaturation pulse [zgpr] program) to eliminate the residual water moisture of deuterated methanol. After preprocessing and visual checking of the NMR dataset, specific ^{1}H regions of diacylgycerols, triglycerides, and total lipids based

on terminal methyl and methylene signals were identified in the spectra using a comparison in the AMIX 3.9 software (Bruker, Germany). Curated identified regions across the spectra were integrated using the same AMIX 3.9 software package and exported to excel spreadsheets to obtain relative concentrations.

Lipid LC–MS profiling

The lipid species in plasma samples were determined by ultra-high performance liquid chromatography (UHPLC) coupled to quadrupole time-of-flight (qTOF) high-resolution mass spectrometry (MS; 6550 iFunnel series, Agilent Technologies, Spain). The ionization was performed in positive electrospray, and the mass calibration reference was used in all the analyses to keep the mass accuracy below 5 ppm. Lipids were separated in a C18 reversed phase column (Kinetex C18-EVO from Phenomenex) and a ternary mobile phase (water/methanol/2-propanol) was used. The lipid measurements were generated from specific RT, isotope peaks relation, and the most intense adduct form observed. Each lipid was quantified with an internal standard calibration method using one analytical standard and one deuterated internal standard for each lipid family. Specific vendor software was used (Quantitative Mass Hunter from Agilent).

Aqueous GC–MS profiling

Samples were analyzed in a 7890A Series GC coupled to a triple quadrupole (QqQ) (7000 series; Agilent Technologies, Barcelona, Spain). The chromatographic column was a J&W Scientific HP5-MS (30 m × 0.25 mm i.d., 0.25 μm film; Agilent Technologies, Barcelona, Spain), and helium (99.999% purity) was used as a carrier gas. Ionization was carried out with electronic impact recording data in "Full Scan" mode.

Metabolite measurements were based on specific RT plus ion fragmentation pattern. Quantification was performed by internal standard calibration, using the corresponding analytical standard for each determined metabolite and a deuterated internal standard depending on the family of metabolite. The internal standards used were succinic d_4 acid, glycerol $^{13}C_3$, norvaline, L-methionine-(carboxy-^{13}C, methyl-d_3), D-glucose $^{13}C_6$, myristic-d_{27} acid, and alpha-tocopherol d_6.

Sleep, physical activity, and sedentary time

Participants were asked to wear an ActiGraphTM tri-axis accelerometer monitor (GT3X+, Pensacola, FL) tightly on the right hip using an elastic belt for seven consecutive days and eight nights (entire 24 hr period); they were only allowed to remove it during water activities (i.e. showering or swimming). At the end of the observation period, data were reintegrated to 60 s epochs and analyzed using ActiLife6 (the ActiGraph 2012, ActiLife version 6). Participants were instructed to keep logs for bedtime ("lights off" and trying to sleep) and waking time ("lights on") during the week in which the monitor was worn. To estimate accelerometer-determined sleep duration, the self-reported bedtimes and waking times were used as the possible window of sleep and accelerometer data within this window were scored in ActiLife6 using the algorithm by Sadeh et al. [15]. The weekly average of sleep duration was calculated in the proportion of five to two between weekdays (Sunday to Thursday) and weekend days (Friday and Saturday). Sleep duration was only considered valid if it was measured for a minimum of one weekday and one weekend night. The intra-participant standard deviation of the sleep duration was used to represent sleep variability.

Before analysis of physical activity and sedentary time, self-reported sleep duration was removed as well as nonwear time defined as 60 min of consecutive zeros using vector magnitude, allowing for 2 min of nonzero interruptions with a maximum of 100 counts/min (cpm). Total physical activity was expressed as vector magnitude of the total tri-axial counts from monitor wear time divided by monitor wear time. Time spent in a sedentary state, doing light physical activity, and moderate-to-vigorous physical activity (MVPA) were defined as all minutes showing <200, 200 to 2689, and ≥2690 vector magnitude cpm, respectively [16]. The percentage of time spent in a sedentary state was calculated by dividing sedentary time with monitor wear time and multiplying by 100. The percentage of time spent in light physical activity and MVPA was calculated in a similar way. The weekly averages of total physical activity, light physical activity, MVPA, and sedentary time were calculated in the proportion of five to two between weekdays (Monday to Friday) and weekend days (Saturday and Sunday) and were only considered valid if monitor wear time was at least 10 hr/day (excluding sleep duration) for a minimum of one weekday and one weekend day.

Anthropometrical and biochemical measurements

Anthropometric measures (body mass index, waist circumference, and sagittal diameter) were determined by trained personnel with calibrated equipment. Body composition was assessed by dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy Primo, GEHealthcare, Little Chalfond, UK [in Reus participants] and GE Lunar iDXA, Encore software version 16.2 [in Copenhagen participants]). Blood pressure was measured using a validated semiautomatic oscillometer (Omron HEM-705CP; Omron Healthcare). Glucose and insulin concentrations were measured using standard enzymatic automated methods and insulin resistance was estimated [17]. Plasma CRP (C-reactive protein), IL-6, leptin, and adiponectin were determined using a MILLIPLEX MAP Plex Kit (Merck Millipore, Billerica, MA).

Dietary assessment

The dietary intake was based on 3 day dietary record (3DDR) including two workdays and a weekend day. The records were inspected for clarification immediately after receiving it by trained dietitians. All foods noted in these diaries were coded to foods listed in country specific-food databases [18–21].

Statistical analyses

Baseline characteristics of study participants were described as means and standard deviations (*SD*) (normally distributed continuous variables) or median and interquartile range (not normally distributed continuous variables), and percentages or numbers for categorical variables. Individual metabolites with more than 20% missing values were excluded and for the rest of metabolites missing values were imputed using the random forest imputation approach ("missForest" R package

version 3.3.1). The levels of 159 metabolites were normalized and scaled to multiples of 1 *SD* with the rank-based inverse normal transformation. Participants were grouped into two groups based on sleep duration and variability quintiles: "low sleep duration or variability" (1st–2nd quintile) and "high sleep duration or variability" (4th–5th quintile). Due to the number of metabolites exceeding the number of observations and the collinear nature of the data, logistic regression with elastic net penalty implemented in the "glmnet" R package version 3.3.3 (alpha = 0.5) was used to build a discrimination model for high sleep duration and high sleep variability. We performed 10-fold cross-validation to find the optimal value of the tuning parameter (λ [lambda]) that yielded the minimum mean-squared error (MSE) [22]. The values minMSE and minMSE + 1SE were estimated using the argument s = "lambda.min" in the cv.glmnet function. The discrimination model scores were computed as the weighted sum of all metabolites with weights equal to the regression coefficients from the discrimination models.

To estimate the prediction accuracy, we split the data into 90% set and 10% set. Within the 90% set, we used the same elastic net procedure to build the model. Another 10-fold cross-validation was used to tune the model parameters. Then, we used the outer 10% set to evaluate the model built at the previous step. This procedure guarantees that the outer 10% set is completely separated from the model building procedure, so the discriminative accuracy estimated in this step is unbiased. We then repeated all these steps for 10 times and averaged their discriminative accuracy in the 10% set. Since each of them is unbiased estimate of discriminative accuracy, the average is also unbiased. In addition, we calculated the receiver operator characteristic (ROC) curves to discriminate between sleep duration and sleep variability categories in the 10% set. The area under the curve (AUC) was calculated to estimate the discriminative accuracy.

We also treated sleep duration and sleep variability as continuous variables (sleep duration was normally distributed and sleep variability was log transformed prior to analyses), and linear regression with elastic net penalty was performed in order to examine associations with metabolites. The variance of aforementioned sleep variables explained by the metabolites was estimated from the adjusted R squared by including all selected metabolites in the model. All analyses were performed using R statistical package (version 3.1.1; R Development Core Team, 2011; http://cran.r-project.org).

Results

Participant characteristics

The general characteristics of study participants are shown in Table 1. The sleep duration and sleep variability groups were similar with respect to most characteristics with the exception of age and MVPA, which were higher in the group of low sleep duration when compared with the high sleep duration group, whereas in the latter group participants were more likely to be women ($p < 0.05$) (Table 1). In the high sleep duration group, sleep duration was also higher (8.14 ± 0.68 hr) than the other group (6.49 ± 0.48 hr) ($p < 0.001$), but sleep variability did not significantly differ. On the other hand, sleep variability was higher in the high sleep variability group (1.52 ± 0.42 hr) compared with the other group (0.61 ± 0.18 hr) ($p < 0.001$). However, sleep duration was not significantly different between the two sleep variability groups.

Associations between metabolites and high sleep duration

Supplementary Figure S1a shows both the number of metabolites in the models and MSE as functions of λ. The figures also show the location of minMSE and minMSE + 1SE. Table 2 shows selected metabolites (n = 12) ranked from the highest to the lowest elastic net positive and negative regression coefficients for high sleep duration. Undecanoylcarnitine/glutaconylcarnitine, isoundecanoylcarnitine, stearic acid, glycerol, and docosahexaenoic acid were positively associated, whereas tiglylcarnitine/3-methylcrotonylcarnitine, 2-hydroxybutyric acid, sphingomyelin 42:1, butyrylcarnitine, sphingomyelin 33:1, phosphatidylcholine 34:2, and 3-hydroxybutyric acid were negatively associated with high sleep duration.

Associations between metabolites and high sleep variability

Supplementary Figure S1b shows both the number of metabolites in the models, the MSE and the location of minMSE and minMSE + 1SE. Table 3 shows selected metabolites (n = 10) ranked from the highest to the lowest elastic net positive and negative regression coefficients for high sleep variability. Positive regression coefficients were found for six metabolites: one lysophosphatidylcholine (20:1), one phosphatidylcholine (42:5), sucrose, two acylcarnitines (octadecenyl-, hydroxyhexadecanoyl-), and glutamic acid, whereas negative for two phosphatidylcholine species (40:6, 37:4): heptanoylcarnitine and triacylglycerol (52:4).

Discrimination of sleep duration and sleep variability categories (high [4th plus 5th quintile] vs. low [1st plus 2nd quintile])

To explore the discriminative ability of the multimetabolite models, AUC analyses were carried out. The AUC was 0.69 (95% CI: 0.62–0.75) and 0.63 (95% CI: 0.53–0.72) in the multimetabolite score, for high sleep duration and sleep variability, respectively. Supplementary Figure S2a shows ROC curves for each of the 10 iterations together with the 10-fold cross-validated ROC curve for sleep duration categories and Supplementary Figure S2b shows ROC curves for each of the 7 iterations for sleep variability categories.

Associations between metabolites and sleep duration/sleep variability (continuous)

When we treated sleep duration as a continuous variable, three metabolites were selected from the elastic net regression with positive and negative regression coefficients (Table 4). These results were consistent with those of the primary analysis in relation to selected metabolites. In addition, the metabolites explained 7% of variance in sleep duration. On the other hand, the elastic regression model did not select any metabolites for sleep variability.

Table 1. Characteristics of the study population

	Low sleep duration (n = 82)	High sleep duration (n = 82)	Low sleep variability (n = 82)	High sleep variability (n = 82)
Sleep parameters				
Sleep duration (hr)	6.49 (0.48)	8.14 (0.68)*	7.27 (0.79)	7.28 (1.04)
Sleep variability (hr)	1.06 (0.46)	1.08 (0.59)	0.61 (0.18)	1.52 (0.42)*
Age (years)	48 (10)	43 (11)**	47 (10)	46 (11)
Sex (% Women)	71.9	86.6**	81.7	75.6
Body mass index (kg/m^2)	31.07 (2.23)	30.87 (1.91)	31.14 (2.26)	30.92 (1.91)
Waist circumference (cm)	101.94 (9.44)	99.85 (8.71)	100.74 (9.11)	101.16 (9.15)
Sagittal diameter	23.15 (2.46)	22.96 (2.29)	22.96 (2.29)	23.11 (2.33)
Total fat (kg)	36.32 (5.83)	37.04 (5.28)	36.81 (6.14)	36.04 (4.89)
Lean mass (kg)	47.32 (10.92)	46.30 (7.91)	47.03 (8.22)	47.21 (10.34)
Moderate-to-vigorous PA (min/day)	59.94 (29.42)**	49.70 (25.71)	54.72 (28.08)	51.34 (29.71)
Sedentary time (min/day)	509.35 (116.82)	503.55 (101.36)	509.99 (98.20)	517.21 (111.64)
Total body fat (%E)	35.50 (5.45)	35.24 (6.78)	35.43 (5.51)	35.38 (6.46)
Saturated fat (%E)	11.67 (0.34)	11.85 (0.37)	11.63 (3.39)	11.55 (3.00)
Monounsaturated fat (%E)	13.41 (0.37)	12.87 (0.44)	13.35 (3.01)	13.00 (4.13)
Polyunsaturated fat (%E)	5.05 (0.19)	4.96 (0.17)	4.96 (1.54)	5.01 (1.72)
Protein (%E)	19.43 (5.21)	18.04 (3.35)	18.43 (3.83)	19.06 (4.81)
Carbohydrate (%E)	42.90 (6.64)	44.21 (6.60)	44.49 (6.65)	43.37 (6.62)
Sugar intake (g)	83.61 (50.43)	80.60 (38.76)	84.76 (36.99)	79.41 (53.07)
HOMA-IR	1.93 (1.04; 3.72)	1.68 (1.05; 3.13)	2.07 (1.29; 3.62)	1.68 (1.04; 3.06)
IL-6 (pg/mL)	2.04 (1.02; 2.93)	1.93 (1.36; 2.84)	1.91 (0.95; 2.54)	1.88 (1.09; 2.84)
Adiponectin (μg/mL)	11.62 (8.56)	10.01 (5.18)	10.14 (6.01)	11.13 (6.70)
Leptin (ng/mL)	3.57 (1.33; 7.91)	2.97 (1.22; 8.00)	2.81 (1.33; 7.21)	3.47 (1.24; 8.76)

Data are presented as mean (*SD*), median (interquartile range) unless otherwise indicated.
The x^2 test is used for comparison of categorical variables and Student's t-test or Mann–Whitney U test is used for comparison of continuous variables.
*($p < 0.001$); **($p < 0.05$).
Low sleep duration: 1st plus 2nd quintile.
High sleep duration: 4th plus 5th quintile.
Low sleep variability: 1st plus 2nd quintile.
High sleep variability: 4th plus 5th quintile.
PA = physical activity; HOMA-IR = homeostasis model of insulin resistance; IL-6 = interleukin 6; E = total energy intake.

Table 2. Metabolites ranked from the highest to the lowest elastic net positive or negative regression coefficients for high sleep duration (4th plus 5th quintile)

Metabolite	Coefficient	Metabolite	Coefficient
Undecanoylcarnitine/Glutaconylcarnitine/isoundecanoylcarnitine	0.0862	Tiglylcarnitine/3-methylcrotonylcarnitine	−0.2791
Stearic acid	0.0814	2-Hydroxybutyric acid	−0.1169
Glycerol	0.0436	SM 42:1	−0.0971
Docosahexaenoic acid	0.0008	Butyrylcarnitine	−0.0772
Serotonin	0.0003	SM 33:1	−0.0217
		PC 34:2	−0.0183
		3-Hydroxybutyric acid	−0.0072

PC = phosphatidylcholine; SM = sphingomyelin.

Table 3. Metabolites ranked from the highest to the lowest elastic net positive or negative regression coefficients for high sleep variability (4th plus 5th quintile)

Metabolite	Coefficient	Metabolite	Coefficient
Octadecenylcarnitine	0.2809	PC 40:6	−0.2695
Sucrose	0.1529	Heptanoylcarnitine	−0.0561
Hydroxyhexadecanoylcarnitine	0.1313	PC 37:4	−0.0319
LPC 20:1	0.1143	TAG 52:4	−0.0019
PC 42:5	0.0277		
Glutamic acid	0.0201		

LPC = lysophosphatidylcholine; PC = phosphatidylcholine; PE = phosphatidylethanolamine; SM = sphingomyelin; TAG = triacylglycerol.

Table 4. Metabolites ranked from the highest to the lowest elastic net positive or negative regression coefficients for sleep duration (continuous)

Metabolite	Coefficient	Metabolite	Coefficient
Glycerol	0.0001	Tiglylcarnitine/3-methylcrotonylcarnitine	−0.0665
		3-Hydroxybutyric acid	−0.0122

Discussion

In the present study, specific metabolites associated with sleep duration and sleep variability were identified among adults of the SATIN study. It was also demonstrated that these metabolite traits, together, contribute information both distinct and complementary to each other, with potential implications for discriminating these sleep characteristics. Despite these associations, the discriminative ability of the multimetabolite model for high sleep duration and sleep variability was moderate.

Concerning metabolites positively associated with high sleep duration, acylcarnitines are known transmembrane transporters of fatty acids across the mitochondrial membrane [23]. The highest association with sleep duration concerning acylcarnitines was found for a monounsaturated short-chain species 5:1-DC: undecanoylcarnitine (mesaconylcarnitine) or glutaconylcarnitine, as well as an odd-numbered medium-chain species, is oundecanoylcarnitine (iso-C11:0). Changes in acylcarnitines during sleep or sleep deprivation indicate a role for the carnitine system and fatty-acids β-oxidation in sleep/wake regulation [8]. However, we cannot exclude that a potential increase in energy demands induced by low sleep duration leads a higher oxidation of fatty acids, may increase intermediary acylcarnitines production. It has been previously shown that 5 days of insufficient sleep (5 hr sleep loss condition) increase energy needs. Specifically, an increase of ~5% (~111 kcal/day) in 24 hr energy expenditure was observed during sleep loss compared with 9 hr control [24]. On the other hand, this physiologically meaningful difference was not detected in prior research [25, 26]. In our study, we have two sleep categories (8.14 vs. 6.49 hr), and it is unknown whether a habitual sleep of 6.49 hr would induce significant increases in energy demands when compared with 8.14 hr.

Stearic acid (C18:0), another metabolite positively associated with sleep duration in the present study, was among fatty acids (14:0, 16:0, 16:1, 18:1, 18:2, 18:3, 22:5, C22:6), found significantly reduced in chronically sleep-restricted rats and possibly associated with lower insulin secretion from pancreas islets [27]. Concerning glycerol, the backbone of triacylglycerol, on which fatty acids esterify, it was positively associated with sleep duration. Overall, glycerol and free fatty acids were reported to decrease progressively during sleep; however, they may decrease during early sleep and subsequently rise in a growth hormone-dependent manner [28].

Finally, docosahexaenoic acid (DHA), an omega-3 fatty acid and major component of neural tissues, is necessary for the transformation of serotonin into melatonin and it has been suggested to influence sleep physiology [29]. Higher maternal plasma DHA levels during pregnancy have been associated with more mature sleep patterns in newborn babies [30]. Lower blood concentrations of DHA can predict more serious sleep problems in otherwise healthy children, whereas dietary supplementation with DHA for 16 weeks can improve parent-rated sleep in children with such problems and increase sleep duration by almost 1 hr compared with placebo [31]. The evidence from these investigations indicates that DHA supplementation may play a positive role in improving sleep parameters, at least in children [31].

Concerning metabolites negatively associated with high sleep duration, among acylcarnitine species (tiglyl- and butyryl-), the main was tiglylcarnitine or 3-methylcrotonylcarnitine (C5:1), a molecule associated with beta-ketothiolase deficiency and ketoacidosis [32]. Another metabolite, 2-hydroxybutyric acid, was reported to be raised significantly in the blood following sleep deprivation, whereas it was also identified as an early metabolic marker of type 2 diabetes, inhibiting the release of insulin from β-cells [33]. On the other hand, elevated 2-hydroxybutyric acid may be a by-product of the breakdown of the sulphur amino-acid metabolite, cystathionine, formed in excess during oxidative stress [34]. These associations are consistent with the viewpoint that sleep deprivation contributes to metabolic stress and diabetes development.

Two sphingomyelins (SM 42:1, 33:1) and one phosphatidylcholine (PC 34:2) were negatively associated with sleep duration. SMs are hydrolyzed by sphingomyelinases activated by inflammatory cytokines and oxidative stress, to produce ceramides that further activate enzymes involved in stress signaling and apoptosis [35]. PCs are direct substrates for the formation of SMs by SM-synthase, which transfers the choline-phosphate group to ceramides [36]. Finally, 3-hydroxybutyric acid (beta hydroxybutyrate) is one of the ketone bodies and metabolism of these substances affects sleep homeostasis in mice [37] or is affected by ketosis due to low sleep duration.

Among metabolites positively associated with high sleep variability, acylcarnitines octadecenylcarnitine (acylcarnitine C18:1) and hydroxylhexadecanoyl-carnitine were positively associated, whereas heptanoylcarnitine was negatively associated. Phosphatidylcholines PC 42:5 and LPC 20:1 were positively associated, whereas PC 40:6 and PC 37:4 were negatively associated. The mechanisms linking the aforementioned carnitine, PC, and LPC molecules to sleep require further investigation.

Sucrose and glutamic acid were recorded to be positively associated with high sleep variability. The levels of sucrose in the blood may reflect gastric permeability to sucrose [38]. Finally, glutamate is associated with sleep/wake regulation and cortical projections of the glutamatergic system are important for cortical activation and wakefulness [39]. In the brain itself, in numerous regions, levels of glutamate are highest during wakefulness or rapid eye movement sleep [40]. Abnormal glutamate homeostasis may be an important contributor to a higher risk of developing diabetes [41].

The results of the present study should be interpreted in the context of its limitations and strengths. First, the cross-sectional design does not allow making any causal inference of the observed associations, and therefore, both directions are

plausible. Second, despite participants were not diagnosed for obstructive sleep apnea (OSA), we cannot completely discard it. An exponential pattern was previously observed with the hazard ratio for OSA development increasing with body mass index (BMI) [42]. Since BMI did not significantly differ between sleep duration or sleep variability categories, we assume that OSA prevalence is not substantially different between these categories and therefore may not influence the reported findings. Third, the lack of external validation in our study may have increased the risk of overfitting of the model. To decrease the risk of overfitting and get an unbiased estimate of prediction accuracy, we have internally cross-validated the models using a 10-fold cross-validation approach. Although we found a moderate discriminating performance, this multimetabolite model should be tested in a larger sample size with potentially better discriminating accuracy. Fourth, participants were overweight/obese individuals without comorbidities and this may limit the generalizability of the findings to other populations. Regarding strengths, we have used a multimetabolomics approach in order to cover a wide range of metabolites.

In conclusion, the present study revealed associations of specific metabolites with sleep duration and sleep variability in overweight/obese adults. The exact mechanisms of association for the selected metabolites and sleep patterns remain to be determined.

Further studies are needed to confirm our findings and assess the potential involvement of the identified metabolites in sleep-related metabolic health.

Supplementary Material

Supplementary material is available at *SLEEP* online.

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