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Effects of Sleep Restriction on the Human Plasma Metabolome

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Abstract

This study examined the effects of recurrent sleep restriction on the plasma metabolome of adults with familial risk of type 2 diabetes. Eleven healthy adults (6M/5F; mean [SD] age: 26 [3] years; BMI 23.5 [2.3] kg/m^2) with parental history of type 2 diabetes participated in a two-condition, two-period randomized crossover study at the Clinical Resource Center at an academic hospital. Each participant completed two 8-night inpatient sessions with restricted (5.5-h time-in-bed) vs. adequate (8.5-h time-in-bed) sleep opportunity while daily food intake and physical activity were carefully controlled. A combination of two UHPLC/MS/MS platforms and one GC/MS platform was used to measure 362 biochemicals in fasting plasma samples collected from study participants the morning after each 8-night sleep treatment. Relative concentrations of 12 amino acids and related metabolites were increased when sleep was curtailed. Sleep restriction also induced elevations in several fatty acid, bile acid, steroid hormone, and tricarboxylic acid cycle intermediates. In contrast, circulating levels of glucose, some monosaccharides, gluconate, and five-carbon sugar alcohols tended to decline when sleep was reduced. Recurrent sleep curtailment affected multiple pathways of intermediary metabolism in adults at risk for type 2 diabetes. An elevation in plasma amino acids and related biochemicals was the most pronounced metabolic signature seen in response to 8 nights of sleep restriction.

Keywords

Metabolome; Diabetes; Sleep; Metabolism

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1. Introduction

Changes in sleep duration have been related to alterations in human energy and substrate metabolism.[1] In addition, epidemiological data raise the possibility that insufficient sleep may increase the risk of diabetes,[2] but the biochemical pathways which underlie these findings are poorly understood.

Individuals with parental history of type 2 diabetes have increased risk of developing the disease, particularly in the setting of weight gain and physical inactivity,[3] and may be more susceptible to the metabolic effects of insufficient sleep.[4] Metabolomic profiling can identify biochemical signatures involved in the pathogenesis of type 2 diabetes.[5-9] However, this promising methodology has not been used to assess the impact of recurrent sleep restriction on human intermediary metabolism. To identify biochemical signatures that may reflect the effects of sleep curtailment on metabolic risk, we compared the plasma metabolite profiles of healthy adults with parental history of type 2 diabetes following experimental exposure to restricted and adequate sleep opportunity in metabolic-ward settings with controlled food intake and physical activity.

2. Methods

2.1 Subjects and Experimental Procedures

Participants were part of a larger study on sleep loss and daily physical activity.[10] Briefly, men and women between the ages of 21 and 40 y with body mass index between 20 and 27 kg/m2 who lived in the greater Chicago area and had at least one parent with type 2 diabetes were recruited through local media advertisements. We excluded subjects who had: any acute or chronic medical condition; self-reported sleep problems (Pittsburgh Sleep Quality Index score >7), night work or habitual daytime naps; recent (<4 weeks) travel across time zones; history of irregular menstrual periods or pregnancy during the past year; depressed mood (Center for Epidemiologic Studies Depression Scale score >15 confirmed by clinical interview); excessive alcohol intake (>14 drinks/week for men; >7 for women); use of tobacco, prescription, over-the-counter, and illicit drugs or supplements that can affect sleep or metabolism; and abnormal findings on physical exam or laboratory testing. All subjects were screened by full overnight polysomnography to exclude sleep pathology.[10] To ensure comparable daily activity during each study period,[10] only subjects who did not exercise were included in this analysis (n=11; 5 women and 6 men; mean [SD] age 26 [3] y; BMI 23.5 [2.3] kg/m^2). The study protocol was registered (ClinicalTrials.gov Identifier NCT00721019) and approved by the Institutional Review Board of the University of Chicago. Participants gave written informed consent and were paid for their participation.

Each participant completed two 8-night inpatient sessions with restricted (5.5-h time-in-bed) vs. adequate (8.5-h time-in-bed) sleep opportunity in random order at least 3 weeks apart (6 participants were studied in the 5.5-h time-in-bed condition first and 5 in the 8.5-h time-in-bed condition first).[10] Women completed each study session during the same phase of their menstrual cycle. To expose participants to comparable “occupational” activity during each study, they performed office work tasks for 6 h/day and spent most of the remaining waking time engaged in indoor leisure activities.[10] No naps or exercise were allowed and a member of the research staff monitored participant safety and compliance during all waking hours. Continuous wrist actigraphy (Actiwatch-64; Mini-Mitter Respironics, Bend, OR) and waist accelerometry (Actical; Mini-Mitter Respironics) were used to measure sleep and physical activity during each session.[10]

Participants received the same 3d-cycle rotating menu customized to their individual food preferences during each study session.[10] This nutritionally balanced diet had initial caloric

content equal to 1.5 times the resting metabolic rate of the participants at the time of screening. Participants were weighed each morning before breakfast and energy intake was adjusted as needed to avoid >1% changes in body weight. Daily calories were divided among breakfast (~25%, 8:00-9:00), lunch (~30%, 12:30-13:30), dinner (~35%, 18:30-19:30) and an evening snack (~10%, 21:00). Participants were allowed a caffeinated beverage with breakfast and lunch as needed to match their usual caffeine intake at home. The caloric content and macronutrient composition of consumed meals, snacks, and beverages were calculated using Food Processor SQL (version 10.10, ESHA Research, Salem, OR). Energy intake records of one participant were incomplete and were not used for analysis.

Fasting plasma samples were collected at the end of each study session in the morning between 9:30-10:00 after 8 nights with 8.5 vs. 5.5-h time-in-bed. Samples were stored at -20°C until the end of the study and shipped on dry ice to Metabolon, Inc., Durham, NC.

2.2 Metabolic profiling

The non-targeted metabolic profiling approach combined three independent platforms: ultrahigh performance liquid chromatography/tandem mass spectrometry (UHPLC/MS/MS) optimized for positive ionization, UHPLC/MS/MS optimized for negative ionization, and gas chromatography/mass spectrometry (GC/MS).[11] For each 100μL biosample, protein was precipitated from plasma with methanol that contained standards to report on extraction efficiency. The resulting supernatant was split into equal aliquots for analysis on each platform. Aliquots, dried under nitrogen and vacuum-desiccated, were subsequently either reconstituted in 50μL 0.1% formic acid in water (acidic conditions) or in 50μL 6.5mM ammonium bicarbonate in water, pH 8 (basic conditions) for the two UHPLC/MS/MS analyses or derivatized to a final volume of 50μL for GC/MS analysis using equal parts bistrimethyl-silyl-trifluoroacetamide and solvent mixture acetonitrile: dichloromethane: cyclohexane (5:4:1) with 5% triethylamine at 60°C for one hour.

For UHPLC/MS/MS analysis, aliquots were separated using a Waters Acquity UPLC (Waters, Millford, MA) and analyzed using an LTQ mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA). Derivatized samples for GC/MS were separated on a 5% phenyldimethyl silicone column with helium as the carrier gas and a temperature ramp from 60°C to 340°C and then analyzed on a Thermo-Finnigan Trace DSQ MS (Thermo Fisher Scientific, Inc., Waltham, MA).

Metabolites were identified by automated comparison of the ion features in the experimental samples to a reference library of chemical standard entries that included retention time, molecular weight (*m/z*), preferred adducts, and in-source fragments as well as associated MS spectra, and were curated by visual inspection for quality control using software developed at Metabolon, Inc.[12]

2.3 Data analysis and statistics

The effect of bedtime restriction on sleep and physical activity was examined using repeated-measures analysis of variance controlling for sampling sequence, order-of-treatment, and gender (SPSS 19.0, 2011, SPSS Inc. IBM, Chicago, IL). Weight maintenance parameters during each sleep condition were compared using paired t-tests.

To assist with data visualization, raw area counts for each metabolite were rescaled by dividing all sample values by the median value for each individual metabolite. Each individual determination was then expressed as a ratio relative to this median value, to determine fold-changes in metabolite concentrations. Missing metabolite values were

assumed to be below the limits of detection and were imputed with the compound minimum (minimum value imputation).

Statistical analysis of metabolomics data was performed using "R" software (http://cran.r-project.org/). Relative observed concentrations for each metabolite were log transformed prior to statistical analysis to produce a more normal data distribution. A crossover model controlling for sampling sequence, order-of-treatment, and gender was used to compare the two sleep conditions. Multiple comparisons were accounted for by estimating the false discovery rate using *q*-values.[13] P-values ≤0.05 were considered statistically significant and values <0.10 were reported as trends.

3. Results

As expected, participants fell asleep faster and had higher sleep efficiency during the 5.5-h time-in-bed condition (P<0.001) when daily sleep was reduced by 2 h 12 min (SD 17 min) (Table 1). Energy and macronutrient intake was comparable, and body weight was well maintained without differences in pre- and post-treatment measurements between sleep conditions (Table 1). The amount of total daily movement recorded during each study session was also well-matched (Table 1).

3.1 Global metabolic profiling

Using a combination of two UHPLC/MS/MS platforms and one GC/MS platform, 362 biochemicals were identified in fasting plasma samples obtained from study participants at the end of each sleep condition (see Tables 3, 4, and 5). Overall, 16 biochemicals changed significantly (P≤0.05) after sleep was curtailed (13 metabolites increased and 3 decreased). In addition, 17 biochemicals showed trends towards change ($0.05< p <0.10$) when comparing metabolic profiles at the end of the 5.5 and 8.5-h time-in-bed condition (12 biochemicals increased and 5 decreased). The greatest number of significant and trending biochemical changes when comparing the two sleep conditions was related to amino acid and peptide metabolism (12 biochemicals), followed by lipid metabolism (8 biochemicals), carbohydrate and energy metabolism (6 biochemicals), xenobiotics (4 biochemicals), cofactors and vitamins (2 biochemicals), and nucleotide metabolism (1 biochemical) (Table 2).

3.2 Changes in amino acid and peptide metabolism

All amino acids and associated degradation products that changed in response to sleep curtailment showed elevated concentrations at the end of the 5.5-h time-in-bed condition (Tables 2 and 3).

3.3 Changes in lipid metabolism

Sleep restriction was accompanied by elevations in biochemicals involved in free fatty acid, bile acid, and steroid hormone metabolism, including increased levels of medium-chain fatty acids (caproate), fatty acids conjugated to carnitine (2-decenoyl carnitine), secondary (deoxycholate) and sulfate-conjugated (glycocholenate sulfate) bile acids, circulating lysolipids (2-myristoylglycerophosphocholine), cholesterol, and the major bile acid precursor 7-α-hydroxy-3-oxo-4-cholestenoate (7-Hoca) (Table 2). Only β-sitosterol - a plant-derived sterol that reduces cholesterol absorption in the intestine and lowers plasma cholesterol levels - was significantly decreased following sleep restriction (Table 4).

3.4 Changes in glucose and energy metabolism

There were several significant and trending changes in metabolites related to carbohydrate metabolism and mitochondrial energy production via the Krebs cycle between the two sleep

conditions (Table 2). In general, circulating levels of glucose and related monosaccharides/sugar alcohols (mannose) were lower at the end of the restricted sleep condition, as were gluconate and five-carbon sugar alcohols associated with the pentose phosphate pathway (xylonate). In addition, a shift towards elevated Krebs cycle intermediates, including malate, seemed to emerge when sleep was curtailed (Table 5). 3.5 Other changes.

Experimental sleep restriction was accompanied by additional changes in several biochemicals related to cofactor/vitamin (pantothenate and γ-CEHC) and xenobiotic (benzoate, 2-hydroxyisobutyrate, piperine, and theophylline) metabolism, as well as the processing and/or excretion of some endogenous metabolites that can also be obtained from outside sources (e.g. creatine, 1,6-anhydroglucose, gluconate, β-sitosterol, and cholesterol) (Table 2).

4. Discussion

Self-reported lack of sufficient sleep has been hypothesized to contribute to the development of insulin resistance and type 2 diabetes.[2, 14-17] Although experimental sleep deprivation is known to alter substrate utilization and energy metabolism,[1] a detailed description of the specific biochemical changes resulting from insufficient sleep is still lacking. The purpose of this pilot study was to globally profile the plasma metabolome of healthy adults at risk for type 2 diabetes exposed to recurrent sleep restriction (time-in-bed 5.5h/night) vs. adequate sleep opportunity (time-in-bed 8.5h/night) in a carefully controlled metabolic-ward setting. We were successful in changing daily sleep duration from ∼7h to ∼5h (which approximates the epidemiologic sleep categories with low vs. high metabolic risk) while participants maintained comparable levels of food intake, total daily activity, and body weight during each study session (Table 1). The most pronounced metabolic signature when sleep was curtailed was an elevation in multiple plasma amino acids and related metabolites (Table 2). Despite previous reports of detrimental effects of sleep restriction on insulin sensitivity and glucose tolerance,[14-17] fasting plasma concentrations of glucose, some monosaccharides, gluconate, and five-carbon sugar alcohols tended to be lower when sleep was curtailed (Table 2). Also, a strong signature of atherogenic changes in lipid metabolism was not seen at the end of the 5.5-h time-in-bed condition (Table 2). Overall, this pilot study indicates that sleep-loss-induced changes in human plasma metabolome can be detected with a small sample size under well-controlled experimental conditions.

Observed changes in plasma histidine, serotonin, isoleucine, γ-glutamylglutamine, and metabolites related to energy metabolism (creatine) may be related to some of the known catabolic effects of sleep loss. Previous studies in rodents[18] and humans[19-21] have suggested that sleep loss has catabolic effects on whole-body protein metabolism and lean body mass. For example, obtaining less sleep at times of reduced food intake may require additional support of the energy needs of glucose-dependent tissues via increased protein breakdown and loss of lean body mass.[21] Together with the concomitant reduction in overnight insulin secretion and fasting blood glucose concentrations in this setting,[22] such findings suggest a metabolic adaptation to limited carbohydrate availability in the face of increased demand induced by extended wakefulness. The pattern of declining fasting plasma glucose and pentose phosphate pathway intermediates (gluconate, xylonate) in the present study suggests that postabsorptive carbohydrate availability may be reduced even when short sleep occurs in combination with a weight-maintenance diet. Furthermore, we did not find a strong signature of sleep-loss-related changes in glycolysis, gluconeogenesis, or glycogen metabolism. Instead, declines in fasting glucose coupled with a pattern of elevations in amino acids, related catabolites, and some tricarboxylic acid cycle intermediates (malate) suggest enhanced anaplerotic contribution of amino acids to the Krebs cycle raising the possibility that increased postabsorptive protein catabolism may contribute to overall energy

homeostasis under short sleep conditions. In addition, plasma creatine - a nitrogenous compound synthesized from the amino acids arginine, glycine, and methionine - was elevated when sleep was curtailed. This may also have relevance to energy metabolism as phosphorylated creatine synthesized primarily by the liver and kidneys is taken up by peripheral tissues and serves as a reservoir for rapid generation of adenosine-triphosphate (ATP) to satisfy acute elevations in local energy needs.

Large-scale metabolomic studies have consistently linked systemic insulin resistance with a set of elevated amino acid concentrations in serum and plasma.[6-9, 23, 24] Observational and experimental data also raise the possibility that increased insulin resistance may contribute to the increased metabolic risk related to short sleep.[14-17] Preliminary measurements in our laboratory indicate that fasting insulin concentrations and homeostatic model assessment of insulin resistance (HOMA-IR) estimates tend to be lower at the end of the 5.5-h time-in-bed condition. This is consistent with observations in sleep-restricted overweight dieters[22] and free-living participants in the CARDIA study with reduced quantity and quality of sleep who also had reduced fasting insulin concentrations and lower HOMA-IR.[25] In agreement with the metabolic profiles associated with insulin resistance,[6-9, 23, 24] the elevations in plasma amino acids and related metabolites induced by sleep loss in the present study may be related to systemically decreased insulin signaling, albeit as a result of reduced insulin secretion and not sensitivity.[26]

Some of the sleep-loss-induced alterations in amino acids and related metabolites identified via global metabolomic profiling are known to have biological activities relevant to the control of the sleep-wake cycle. For example, the hypothalamic histaminergic system is involved in the regulation of wakefulness and rapid-eye-movement sleep,[27] and levels of plasma histidine, which crosses the blood-brain barrier and can be metabolized to histamine,[28] were significantly increased by sleep restriction. In addition, sleep restriction was accompanied by alterations in tryptophan metabolism including elevations in glycosylated tryptophan and the neurotransmitter serotonin which is derived from tryptophan. In the pineal gland, which is not protected by the blood-brain-barrier, serotonin can be further metabolized to melatonin and once again contribute to the regulation of the sleep-wake cycle.[29] Finally, the most abundant amino acid in plasma, glutamine, can be transported across the blood-brain-barrier[30] and undergo conversion into glutamate - a neurotransmitter which is increased in the posterior hypothalamic region of sleep-deprived rodents.[31] Interestingly, γ-glutamylglutamine was elevated at the end of the short-sleep condition of our experiment and addition of a γ-glutamyl group to amino acids is often used to enhance the transport of these metabolites.

A few significant or trending elevations in biochemicals related to lipid metabolism at the end of the 5.5-h time-in-bed condition were consistent with previous reports of increased fasting free fatty acid concentrations in sleep-restricted individuals.[17, 22] However, while overweight individuals placed on a reduced-calorie diet had lower fasting cholesterol concentrations when their sleep was curtailed,[22] plasma cholesterol was higher when participants in the present study receiving a weight-maintenance diet obtained less sleep. In addition, short sleep was accompanied by elevations in bile acids and the major bile acid precursor 7-Hoca, whereas the diet-derived cholesterol-lowering phytochemical β-sitosterol was reduced. Since bile acid synthesis is a primary route for cholesterol excretion and can affect intestinal absorption of dietary fats, these observations suggest that sleep duration may interact with various dietary and intrinsic gastrointestinal factors to influence systemic lipid metabolism.

Due to the high cost and technical difficulty of such inpatient sleep restriction studies, our proof-of-concept analysis had a small sample size and expectedly high *q*-values. It should

also be noted that despite the carefully controlled living environment and dietary oversight of the study, some of the biochemical changes in the cofactor, vitamin, and xenobiotic superpathways may have been related not only to sleep-loss-induced differences in their metabolism and clearance, but also to potential variability in food intake and environmental exposure during the home free-living period before each treatment. Despite its limitations, this study was the first to perform global plasma metabolite profiling in healthy individuals at risk for type 2 diabetes under restricted vs. adequate sleep conditions.

5. Conclusions

The results of this pilot analysis indicate that global plasma metabolite profiling in adults at risk for type 2 diabetes can detect novel and potentially informative metabolic signatures induced by recurrent sleep restriction. After 8 nights of recurrent sleep curtailment, multiple pathways of intermediary metabolism were affected, particularly elevation in plasma amino acids and related biochemicals. Larger discovery and validation studies should be considered to expand our understanding of the metabolic consequences of insufficient sleep.

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Highlights

- We examine the effects of recurrent sleep restriction on the plasma metabolome of adults with familial risk of type 2 diabetes.
- When sleep was curtailed the most pronounced metabolic signature was an elevation in multiple plasma amino acids and related metabolites.
- Sleep restriction also induced elevations in several fatty acid, bile acid, steroid hormone, and tricarboxylic acid cycle intermediates.
- Circulating levels of glucose, some monosaccharides, gluconate, and five-carbon sugar alcohols tended to decline when sleep was reduced.

Table 1

Sleep, diet, physical activity, and weight maintenance during each condition [a]

	8.5-h TIB [e]	5.5-h TIB [e]
8-night average sleep data [b]		
Going-to-bed time (h:min)	23:54 (0:20)	1:03 (0:02) **
Out-of-bed time (h:min)	8:26 (0:20)	6:32 (0:02) **
Total sleep time (h:min/day)	6:56 (0:34)	4:44 (0:17) **
Sleep onset latency (min) [d]	0:18 (0:20)	0:09 (0:11) **
Sleep efficiency (%)	81 (7)	86 (5) **
7-day average food intake [b]		
Energy consumption (kcal/kg)	32 (2)	32 (3)
Carbohydrate (% of energy)	50 (2)	50 (2)
Protein (% of energy)	16 (1)	16 (1)
Fat (% of energy)	34 (1)	34 (1)
7-day average physical activity [b]		
Total activity count (thousands/day)	70.3 (39.1)	71.0 (45.2)
Body weight maintenance [c]		
Pre-treatment body weight (kg)	73.5 (12.7)	73.1 (12.4)
Post-treatment body weight (kg)	73.4 (12.6)	73.0 (12.7)
7-day change in body weight (kg)	-0.1 (0.5)	0.0 (0.6)
Body weight coefficient of variability (%)	0.5 (0.2)	0.4 (0.1)

[a] Data are mean (SD).

[b] Measures of sleep, food intake, and physical activity were compared using repeated-measures analysis of variance with order of treatment and gender as between-subject factors.

[c] Weight maintenance was assessed using paired t-tests.

[d] Square root transformed data used for comparison;

[e] TIB: time-in-bed (h/day).

** P<0.01.

Table 2

Summary of detected biochemical changes following experimental sleep restriction.

Super pathway	Sub-pathway	Biochemical name	TIB 5.5h/8.5h ratio	p value	q value
Amino acid	Glycine, serine & threonine metabolism	N-acetylthreonine	**1.30**	<0.001	0.186
		betaine	**1.04**	0.051	0.808
	Histidine metabolism	histidine	**1.06**	0.041	0.808
	Lysine metabolism	glutaroyl carnitine	**1.12**	0.028	0.808
	Phenylalanine & tyrosine metabolism	phenyllactate	**1.35**	0.010	0.478
	Tryptophan metabolism	5-hydroxytryptophan (serotonin)	**1.08**	0.073	0.808
		C-glycosyltryptophan	**1.07**	0.036	0.808
	Valine, leucine & isoleucine metabolism	isoleucine	**1.10**	0.003	0.302
	Cysteine, methionine, SAM & taurine metabolism	N-formylmethionine	**1.17**	0.099	0.808
		N-acetylmethionine	**1.24**	0.055	0.808
	Creatine metabolism	creatine	**1.13**	0.086	0.808
Peptide	gamma-glutamyl	gamma-glutamylglutamine	**1.16**	0.087	0.808
Carbohydrate	Fructose, mannose, galactose, starch & sucrose	mannose	**0.80**	0.039	0.808
	Glycolysis, gluconeogenesis, & pyruvate metabolism	glucose	**0.89**	0.086	0.808
		1,6-anhydroglucose	**1.51**	0.001	0.186
	Nucleotide sugars, pentose metabolism	gluconate	**0.66**	0.086	0.808
		xylonate	**0.74**	0.088	0.808
Energy	Krebs cycle	malate	**1.27**	0.075	0.808
Lipid	Medium-chain fatty acid	caproate (6:0)	**1.11**	0.076	0.808
	Carnitine metabolism	2-decenoyl carnitine	**1.29**	0.095	0.808
	Bile acid metabolism	deoxycholate	**1.38**	0.059	0.808
		glycocholenate sulfate	**1.23**	0.008	0.465
	Lysolipid	2-myristoylglycerophosphocholine	**1.77**	0.066	0.808
	Sterol/steroid	cholesterol	**1.11**	0.030	0.808
		beta-sitosterol	**0.73**	0.030	0.808
		7-alpha-hydroxy-3-oxo-4-cholestenoate	**1.15**	0.030	0.808
Nucleotide	Pyrimidine metabolism, uracil containing	pseudouridine	**1.06**	0.080	0.808

Super pathway	Sub-pathway	Biochemical name	TIB 5.5h/8.5h ratio	p value	q value
Cofactors & vitamins	Pantothenate & CoA metabolism	pantothenate	**1.12**	0.023	0.808
	Tocopherol metabolism	gamma-CEHC	**1.32**	0.038	0.808
Xenobiotics	Benzoate metabolism	benzoate	**1.10**	0.009	0.465
	Chemical	2-hydroxyisobutyrate	**0.87**	0.058	0.808
	Food component/plant	piperine	**0.64**	0.006	0.465
	Xanthine metabolism	theophylline	**0.83**	0.090	0.808

Sleep-loss-related significant and trending elevations in plasma metabolites are highlighted respectively in red and orange, whereas significant and trending declines are highlighted respectively in green and light green.

Table 3

Summary of identified metabolites related to amino acid and peptide metabolism

Super pathway	Sub-pathway	Biochemical name	TIB 5.5h/8.5h ratio	p value	q value
Amino acid	Glycine, serine & threonine metabolism	Glycine	**1.02**	0.863	0.897
		dimethylglycine	**0.93**	0.459	0.824
		N-acetylglycine	**0.96**	0.376	0.817
		beta-hydroxypyruvate	**0.92**	0.671	0.878
		Serine	**1.00**	0.956	0.913
		N-acetylserine	**0.91**	0.627	0.855
		Threonine	**0.98**	0.669	0.878
		N-acetylthreonine	**1.30**	<0.001	0.186
		Betaine	**1.04**	0.051	0.808
	Histidine metabolism	Histidine	**1.06**	0.041	0.808
		trans-urocanate	**1.17**	0.187	0.808
		3-methylhistidine	**1.07**	0.769	0.881
	Lysine metabolism	glutarate (pentanedioate)	**1.16**	0.877	0.897
		Lysine	**1.19**	0.279	0.817
		Pipecolate	**1.25**	0.306	0.817
		glutaroyl carnitine	**1.12**	0.028	0.808
	Phenylalanine & tyrosine metabolism	phenyllactate	**1.35**	0.010	0.478
		phenylalanine	**1.06**	0.242	0.808
		phenylacetate	**0.94**	0.626	0.855
		p-cresol sulfate	**1.13**	0.182	0.808
		Tyrosine	**1.02**	0.660	0.878
		3-(4-hydroxyphenyl)lactate	**1.07**	0.169	0.808
		3-methoxytyrosine	**1.17**	0.169	0.808
		phenylacetylglutamine	**1.02**	0.787	0.881
		3-phenylpropionate (hydrocinnamate)	**0.81**	0.281	0.817
		phenol sulfate	**0.84**	0.150	0.808
	Tryptophan metabolism	Kynurenine	**1.07**	0.368	0.817

Super pathway	Sub-pathway	Biochemical name	TIB 5.5h/8.5h ratio	p value	q value
		Tryptophan	**1.05**	0.188	0.808
		indolelactate	**0.93**	0.328	0.817
		indoleacetate	**1.04**	0.763	0.881
		tryptophan betaine	**1.06**	0.968	0.913
		serotonin (5HT)	**1.08**	0.073	0.808
		C-glycosyltryptophan	**1.07**	0.036	0.808
		3-indoxyl sulfate	**1.06**	0.395	0.817
		indolepropionate	**1.03**	0.932	0.911
	Valine, leucine & isoleucine metabolism	3-methyl-2-oxobutyrate	**0.96**	0.537	0.848
		3-methyl-2-oxovalerate	**0.98**	0.705	0.878
		levulinate (4-oxovalerate)	**0.86**	0.134	0.808
		beta-hydroxyisovalerate	**1.02**	0.514	0.836
		alpha-hydroxyisocaproate	**1.08**	0.688	0.878
		Isoleucine	**1.10**	0.003	0.302
		Leucine	**1.08**	0.103	0.808
		Valine	**1.05**	0.202	0.808
		3-hydroxyisobutyrate	**0.95**	0.330	0.817
		4-methyl-2-oxopentanoate	**0.95**	0.583	0.855
		3-hydroxy-2-ethylpropionate	**1.12**	0.572	0.855
		alpha-hydroxyisovalerate	**0.97**	0.577	0.855
		isobutyrylcarnitine	**1.07**	0.255	0.817
		2-methylbutyroylcarnitine	**1.05**	0.782	0.881
		isovalerylcarnitine	**1.16**	0.625	0.855
		tiglyl carnitine	**1.14**	0.407	0.822
		methylglutaroylcarnitine	**1.00**	0.834	0.897
	Cysteine, methionine, SAM, & taurine metabolism	Cysteine	**1.08**	0.729	0.881
		S-methylcysteine	**1.04**	0.843	0.897
		N-formylmethionine	**1.17**	0.099	0.808
		Taurine	**1.11**	0.370	0.817

Super pathway	Sub-pathway	Biochemical name	TIB 5.5h/8.5h ratio	p value	q value
		Methionine	**1.04**	0.411	0.822
		N-acetylmethionine	**1.24**	0.055	0.808
		2-hydroxybutyrate	**0.92**	0.215	0.808
	Creatine metabolism	Creatine	**1.13**	0.086	0.808
		Creatinine	**0.98**	0.463	0.824
Peptide	gamma-glutamyl	gamma-glutamylvaline	**1.02**	0.538	0.848
		gamma-glutamylleucine	**1.06**	0.354	0.817
		gamma-glutamylisoleucine	**1.11**	0.168	0.808
		gamma-glutamylmethionine	**1.13**	0.297	0.817
		gamma-glutamylglutamate	**0.99**	0.486	0.833
		gamma-glutamylglutamine	**1.16**	0.087	0.808
		gamma-glutamylphenylalanine	**1.00**	0.885	0.897
		gamma-glutamyltyrosine	**1.04**	0.508	0.836
		gamma-glutamylthreonine	**1.12**	0.203	0.808
		gamma-glutamylalanine	**1.13**	0.239	0.808

Sleep-loss-related significant and trending elevations in plasma metabolites are highlighted respectively in red and orange.

Table 4

Summary of identified metabolites related to lipid metabolism

Super pathway	Sub-pathway	Biochemical name	TIB 5.5h/8.5h ratio	p value	q value
Lipid	Medium-chain fatty acid	caproate (6:0)	**1.11**	0.076	0.808
		heptanoate (7:0)	**1.07**	0.221	0.808
		caprylate (8:0)	**1.06**	0.358	0.817
		pelargonate (9:0)	**1.05**	0.325	0.817
		caprate (10:0)	**1.04**	0.421	0.822
		undecanoate (11:0)	**1.13**	0.137	0.808
		10-undecenoate (11:1n1)	**1.18**	0.271	0.817
		laurate (12:0)	**1.07**	0.239	0.808
		5-dodecenoate (12:1n7)	**1.15**	0.515	0.836
	Carnitine metabolism	Deoxycarnitine	**1.02**	0.911	0.907
		Carnitine	**0.99**	0.584	0.855
		3-dehydrocarnitine	**1.08**	0.261	0.817
		Acetylcarnitine	**1.09**	0.426	0.822
		hexanoylcarnitine	**1.22**	0.624	0.855
		octanoylcarnitine	**1.38**	0.301	0.817
		decanoylcarnitine	**1.38**	0.376	0.817
		2-decenoyl carnitine	**1.29**	0.095	0.808
		cis-4-decenoyl carnitine	**1.17**	0.499	0.836
		Laurylcarnitine	**1.36**	0.155	0.808
		palmitoylcarnitine	**1.15**	0.382	0.817
		stearoylcarnitine	**1.18**	0.426	0.822
		Oleoylcarnitine	**1.24**	0.197	0.808
	Bile acid metabolism	Cholate	**2.41**	0.355	0.817
		Glycocholate	**1.15**	0.796	0.881
		Deoxycholate	**1.38**	0.059	0.808
		glycodeoxycholate	**1.03**	0.776	0.881
		glycochenodeoxycholate	**1.18**	0.606	0.855

Super pathway	Sub-pathway	Biochemical name	TIB 5.5h/8.5h ratio	p value	q value
		glycolithocholate sulfate	**0.92**	0.890	0.897
		taurolithocholate 3-sulfate	**0.90**	0.996	0.913
		glycocholenate sulfate	**1.23**	0.008	0.465
		Taurocholenate sulfate	**1.22**	0.602	0.855
		glycoursodeoxycholate	**0.92**	0.510	0.836
	Lysolipid	1-palmitoylglycerophosphoethanolamine	**1.17**	0.101	0.808
		2-palmitoylglycerophosphoethanolamine	**1.20**	0.295	0.817
		1-stearoylglycerophosphoethanolamine	**1.18**	0.107	0.808
		1-oleoylglycerophosphoethanolamine	**1.01**	0.479	0.833
		2-oleoylglycerophosphoethanolamine	**0.93**	0.397	0.817
		1-linoleoylglycerophosphoethanolamine	**1.21**	0.709	0.878
		2-linoleoylglycerophosphoethanolamine	**1.15**	0.242	0.808
		1-arachidonoylglycerophosphoethanolamine	**0.93**	0.609	0.855
		2-arachidonoylglycerophosphoethanolamine	**1.01**	0.891	0.897
		1-myristoylglycerophosphocholine	**1.25**	0.390	0.817
		2-myristoylglycerophosphocholine	**1.77**	0.066	0.808
		1-pentadecanoylglycerophosphocholine	**1.30**	0.201	0.808
		1-palmitoylglycerophosphocholine	**1.22**	0.154	0.808
		2-palmitoylglycerophosphocholine	**1.29**	0.327	0.817
		1-palmitoleoylglycerophosphocholine	**1.24**	0.388	0.817
		2-palmitoleoylglycerophosphocholine	**1.07**	0.916	0.907
		1-heptadecanoylglycerophosphocholine	**1.24**	0.145	0.808
		1-stearoylglycerophosphocholine	**1.22**	0.237	0.808
		2-stearoylglycerophosphocholine	**1.21**	0.155	0.808
		1-oleoylglycerophosphocholine	**1.18**	0.209	0.808
		2-oleoylglycerophosphocholine	**1.26**	0.279	0.817
		1-linoleoylglycerophosphocholine	**1.22**	0.419	0.822
		2-linoleoylglycerophosphocholine	**1.22**	0.580	0.855
		1-eicosadienoylglycerophosphocholine	**1.19**	0.178	0.808

Super pathway	Sub-pathway	Biochemical name	TIB 5.5h/8.5h ratio	p value	q value
		1-eicosatrienoylglycerophosphocholine	**1.30**	0.734	0.881
		1-arachidonoylglycerophosphocholine	**1.29**	0.359	0.817
		2-arachidonoylglycerophosphocholine	**1.16**	0.789	0.881
		1-docosapentaenoylglycerophosphocholine	**1.21**	0.776	0.881
		1-docosahexaenoylglycerophosphocholine	**1.24**	0.456	0.824
		1-palmitoylglycerophosphoinositol	**1.04**	0.707	0.878
		1-stearoylglycerophosphoinositol	**0.92**	0.249	0.817
		1-arachidonoylglycerophosphoinositol	**0.91**	0.523	0.836
		1-palmitoylplasmenylethanolamine	**0.98**	0.357	0.817
	Sterol & Steroid	Lathosterol	**0.92**	0.351	0.817
		Cholesterol	**1.11**	0.030	0.808
		7-alpha-hydroxycholesterol	**1.12**	0.770	0.881
		7-beta-hydroxycholesterol	**1.13**	0.580	0.855
		dehydroisoandrosterone sulfate (DHEA-S)	**1.10**	0.267	0.817
		epiandrosterone sulfate	**1.00**	0.793	0.881
		androsterone sulfate	**1.03**	0.995	0.913
		Cortisol	**1.07**	0.525	0.836
		Cortisone	**0.94**	0.375	0.817
		beta-sitosterol	**0.73**	0.030	0.808
		7-alpha-hydroxy-3-oxo-4-cholestenoate (7-Hoca)	**1.15**	0.030	0.808
		4-androsten-3beta,17beta-diol disulfate 1	**1.01**	0.232	0.808
		4-androsten-3beta,17beta-diol disulfate 2	**1.01**	0.718	0.881
		5alpha-androstan-3beta,17beta-diol disulfate	**0.96**	0.734	0.881
		5alpha-pregnan-3beta,20alpha-diol disulfate	**1.08**	0.852	0.897
		pregnen-diol disulfate	**1.10**	0.481	0.833
		pregn steroid monosulfate	**1.06**	0.983	0.913
		andro steroid monosulfate 1	**0.98**	0.503	0.836
		andro steroid monosulfate 2	**1.02**	0.762	0.881
		21-hydroxypregnenolone disulfate	**1.08**	0.379	0.817

Super pathway	Sub-pathway	Biochemical name	TIB 5.5h/8.5h ratio	p value	q value
		pregnenolone sulfate	**1.03**	0.556	0.855
		pregnanediol-3-glucuronide	**1.05**	0.884	0.897

Sleep-loss-related significant and trending elevations in plasma metabolites are highlighted respectively in red and orange, whereas significant and trending declines are highlighted respectively in green and light green.

Table 5

Summary of identified metabolites related to carbohydrate and energy metabolism

Super pathway	Sub-pathway	Biochemical name	TIB 5.5h/8.5h ratio	p value	q value
Carbohydrate	Fructose, mannose, galactose, starch & sucrose metabolism	fructose	**0.76**	0.369	0.817
		mannitol	**0.73**	0.352	0.817
		mannose	**0.80**	0.039	0.808
		sorbitol	**0.90**	0.338	0.817
	Glycolysis, gluconeogenesis & pyruvate metabolism	1,5-anhydroglucitol (1,5-AG)	**0.96**	0.451	0.824
		glycerate	**0.96**	0.350	0.817
		glucose	**0.89**	0.086	0.808
		1,6-anhydroglucose	**1.51**	0.001	0.186
		pyruvate	**0.90**	0.364	0.817
		lactate	**1.07**	0.440	0.824
	Nucleotide sugars & pentose metabolism	arabitol	**0.93**	0.449	0.824
		ribitol	**1.02**	0.817	0.895
		threitol	**0.91**	0.387	0.817
		gluconate	**0.66**	0.086	0.808
		ribose	**0.71**	0.360	0.817
		ribulose	**0.88**	0.573	0.855
		xylitol	**0.96**	0.884	0.897
		arabinose	**1.01**	0.804	0.887
		xylose	**0.80**	0.244	0.808
		xylonate	**0.74**	0.088	0.808
		xylulose	**0.84**	0.488	0.833
Energy	Krebs cycle	citrate	**1.07**	0.525	0.836
		cis-aconitate	**1.11**	0.205	0.808
		alpha-ketoglutarate	**0.83**	0.625	0.855
		succinate	**1.19**	0.257	0.817
		succinylcarnitine	**1.14**	0.115	0.808
		fumarate	**1.17**	0.240	0.808

Super pathway	Sub-pathway	Biochemical name	TIB 5.5h/8.5h ratio	p value	q value
		malate	**1.27**	0.075	0.808

Sleep-loss-related significant and trending elevations in plasma metabolites are highlighted respectively in red and orange, whereas significant and trending declines are highlighted respectively in green and light green.