SI Appendix

Materials and Methods

Clinical study. All participants provided written consent prior to any procedures being performed. Male subjects (n = 15) aged between 18 and 35 years (24 ± 5 years; mean ± SD) with a BMI of 24.9 ± 2.6 kg/m^2 were enrolled into the study, and plasma samples from 12 subjects were analysed (23 ± 5 years, BMI 24.5 ± 2.3 kg/m^2).

Screening assessments and the in-laboratory session were conducted at the Surrey Clinical Research Centre. A complete description of the screening process and the study protocol is given in Ackermann et al., 2012 (1). Briefly, to determine eligibility potential participants were required to undergo a medical screening assessment and complete a range of questionnaires including Horne-Östberg Questionnaire (MEQ), Pittsburgh Sleep Quality Index (PSQI), Epworth Sleepiness scale (ESS) and Beck Depression Index (BDI). Exclusion criteria included the taking of any regular medication, pre-existing medical conditions, smoking, any self-reported sleep problems or depression, extreme morning and evening types (as determined by MEQ), PSQI score > 5 and ESS score > 9.

Subsequently, subjects maintained a regular sleep/wake schedule (23:00 – 07:00 h) for one week prior to the in-laboratory session which was confirmed by wrist actigraphy (Actiwatch-L, Cambridge Neurotechnology Ltd., UK), sleep diaries and subjects calling a time-stamped voicemail upon waking and before going to sleep. For 72 hours before the in-laboratory session, subjects were requested to abstain from alcohol, caffeine, heavy exercise, non-steroidal anti-inflammatory drugs and bright light in the evenings and at night. This baseline-at-home period minimised exogenous confounding factors, stabilised circadian phase and ensured participants were not sleep deprived.

In-laboratory session. Subjects remained in controlled conditions with respect to environmental light, sleep, meals and posture throughout the in-laboratory session: 18:00 - 23:00 h and 07:00 – 09:00 h: semi-recumbent, < 5 lux; 23:00 - 07:00 h: recumbent (sleep opportunity), 0 lux on N1, semi-recumbent (continual wakefulness) in < 5 lux on N2; 09:00 - 18:00 h: freely moving, 100 lux (Fig. S5). Standardised meals were given at 07:00, 13:00, and 19:00 h with a snack at 22:00 h; water was available *ad libitum*. The target was 2500 calories per day, which was divided into 3 x 30% (meals) and 1 x 10% (snack). The macronutrient composition of the daily food intake was designed to reflect the UK dietary guidelines (fat 35%, carbohydrate 50%, protein 15% of energy), calculated daily values were fat 35%, carbohydrate 49% and protein 12%. The breakfast, lunch, dinner and snack were identical in composition on all days of

the study with some variation across the meals reflecting the different meal types (breakfast fat 35%, carbohydrate 54%, protein 9%; lunch fat 47%, carbohydrate 38%, protein 13%; dinner fat 35%, carbohydrate 43%, protein 16%; snack fat 25%, carbohydrate 64%, protein 7%; the remaining % energy contribution coming from dietary fibre, ≤ 3%). During the sleep period on N1 (23:00 – 07:00 h), samples were taken through a portal in the wall of the individual sleep rooms so that sleep disturbance was minimised. Sleep was monitored by polysomnography on N1 and N2 from 22:00 – 09:00 h. The N1 data were analysed to assess the timing and duration of the different sleep states and the N2 data were used to ensure that subjects remained awake throughout the night of sleep deprivation.

Across the 48 h study period, blood samples for metabolomics analysis (2-hourly) and those for melatonin measurement (hourly) were collected into lithium heparin tubes and separated by centrifugation. Plasma fractions for metabolomics analysis were stored at -80°C until extraction; plasma for melatonin measurement was stored at -20°C until analysis.

Melatonin measurement. To determine concentrations of melatonin, radioimmunoassay analysis was performed on hourly plasma samples (Stockgrand Ltd., University of Surrey) as previously described (2). The limit of detection for the plasma melatonin assay was 2.6 ± 1.3 pg/ml (mean ± SEM). The interassay coefficients of variation (CVs) were 12% at 7.9 pg/ml, 9% at 29 pg/ml, 5% at 87 pg/ml and 8% at 124 pg/ml (n = 14 at each concentration).

Untargeted metabolomic analysis. Untargeted metabolomics LC-MS analysis was carried out on a Waters Acquity UPLC coupled to a QToF Premier mass spectrometer (Waters Corporation, Milford, MA, USA) with electrospray ionisation in positive ionisation mode as previously described by Pandher et al. (3, 4). Raw data were detected, aligned and processed using XCMS (5). Two-hourly plasma samples were thawed on ice before extraction using 100 µL plasma and 400 µL solvent (methanol/ethanol (1:1)) with a 30 s vortex step before centrifugation at 1500 *g*. Extracts were transferred to 384-well plates for LC-MS analysis using a Biomek 2000 (Beckman Coulter, Inc., High Wycombe, UK).

A Waters Acquity HSS T3 C18 column (100 x 2.1 mm, internal diameter 1.8 µm) and a mobile phase consisting of water (A) and acetonitrile (B) (both containing 0.1% formic acid) were used for chromatographic separation, with a flow rate of 0.6 mL/min. The 14 minute elution gradient comprised the following: 100% A for 0.5 min, followed by a change to 100% B over 7 min, holding at 100%

B for 2 min, changing to 100 % A over 0.5 min before holding at 100% A for 4 minutes. The autosampler was maintained at 4°C, and the column was maintained at 50°C throughout the experiment. The injection volume was 10 µL. The MS instrument and data acquisition parameters were as described by Pandher et al. (3, 4). Quality control (QC) samples consisted of pooled plasma from all analysed samples and were run approximately 1 in every 10 injections in order to assess the analytical variability. Batch variation and within-run variation in the resulting dataset was corrected using QC sample data for each feature in a method adapted from Kamleh et al. (6). The correction factor for a given feature in a group of approximately 30 samples was the mean intensity of that feature in three QC injections included in that group. Probabilistic quotient normalisation (PQN) (7) and unit variance scaling was carried out on the batch-corrected dataset prior to multivariate data analysis.

Metabolite identification (untargeted analysis). Tandem MS (MS/MS) analysis was used to characterise the fragmentation pattern of each metabolite feature of interest (as determined by univariate and multivariate techniques) for identification using fragmentation patterns from our previous study (8), online database searching (METLIN, Human Metabolome Database) and/or with comparison with pure standards. MS/MS was carried out on the Waters QToF system described above with a 5 – 30V collision energy ramp and a 50 – 800 Da mass range.

Targeted metabolomic analysis. To identify and quantify metabolite concentrations, two-hourly plasma samples were measured using the Absolute*IDQ* p180 targeted metabolomics kit (Biocrates Life Sciences AG, Innsbruck, Austria), and a Waters Xevo TQ-S mass spectrometer coupled to an Acquity H LC system (Waters Corporation, Milford, MA, USA). The kit provided absolute concentrations for acylcarnitines, amino acids, biogenic amines, hexose, glycerophospholipids and sphingolipids. Plasma samples were prepared according to the manufacturer's instructions. To correct for variation between batches in the targeted analysis, a correction factor was applied to each metabolite concentration value. The correction factor was calculated as the ratio of the geometric mean of the plate-specific geometric means for each metabolite to the relevant plate-specific geometric mean (9).

Statistical analysis. For both untargeted and targeted analyses, Principal Component Analysis (PCA) was carried out using all data and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) was carried out on selected time points (SIMCA-P v11.0 software,

Umetrics, Sweden). To examine changes in metabolite levels across different time periods and time of day (study and analysis protocol in SI Appendix; Table S1), a two-way repeated ANOVA was carried out on selected time periods and their corresponding times of day for all 171 metabolites using MATLAB and Bioinformatics Toolbox Release 2013b (The MathWorks, Inc., Natick, Massachusetts, United States). In order to account for one missing sample when carrying out the two-way repeated ANOVA, simulated metabolite levels were generated using linear interpolation based on data from the same subject and metabolite. To correct for multiple hypotheses testing, false discovery rates and q-values were calculated using the mafdr function based on the Storey et al. method (10). Values of p and $q < 0.05$ and 0.05, respectively, were considered significant.

To assess 24 h metabolite rhythmicity, cosinor analysis (11) was carried out for each metabolite quantified in targeted analysis for both the first 24 h (day 1/N1, wake/sleep) and the subsequent 24 h (day 2/N2, wake/wake). For each metabolite profile, cosinor analysis was performed on the mean z-score values (z-score calculated across 48 h) across all subjects. Peak time (acrophase), amplitude and significance of fit ($p < 0.05$) were determined in each case. The time of melatonin onset (dim light melatonin onset, DLMO) on both day 1 (sleep) and day 2 (sleep deprivation) was calculated for each participant using the 25% threshold method as previously described (2).

Over-representation analysis was performed with the IMPaLA web tool (impala.molgen.mpg.de) (10), using the 27 metabolites significantly increased in sleep deprivation as the dataset of interest, and all 171 metabolites identified using targeted analysis as the background dataset. The IMPaLA tool provided p-values and corresponding false discovery rates (calculated using the Benjamini Hochberg method) for each pathway identified as over-represented.

Results and Discussion

Comparison of untargeted and targeted LC-MS analysis. Twenty metabolites were common to both untargeted and targeted analyses (SI Appendix; Table S8). Pearson's correlation coefficients were calculated using all samples for each common metabolite. There was a significant correlation between untargeted and targeted analysis for each common metabolite ($p < 0.001$ in all cases) (SI Appendix; Table S8).

Over-representation analysis. Over-representation analysis was performed with the IMPaLA web tool (impala.molgen.mpg.de) (10). The top ten pathways were, in order of decreasing significance: metabolism of lipids and lipoproteins; signal transduction; glycerophospholipid metabolism; acyl chain remodelling of phosphatidylcholine; acyl chain remodelling of cardiolipin; serotonin transporter activity; opioid signalling; monoamine transport; linoleate metabolism; and HDL-mediated lipid transport. However, no pathways were identified as being significantly over-represented after the application of a false discovery rate correction.

Comparison of identified rhythmic metabolites in this study to other studies. We found that 25/40 acylcarnitines quantified with targeted analysis were rhythmic on day 1 (wake/sleep), with 11 of these acylcarnitines maintaining rhythmicity on day 2 (wake/wake). Acylcarnitine rhythmicity is in agreement with the study of Dallmann et al. (13) that reported some rhythmic acylcarnitines: laurylcarnitine (dodocanoylcarnitine), octanoylcarnitine and cis-4-decanoylcarnitine. We found both dodecanoylcarnitine and octanoylcarnitine to be rhythmic on day 1 (wake/sleep) only, and both of these acylcarnitines were significantly increased during sleep deprivation (00:00 – 06:00 h, day 2) compared with during sleep (00:00 – 06:00 h, day 1). Consistent with Dallmann et al. (13), we found no rhythmicity on either day 1 or day 2 in hexanoylcarnitine, hexadecenoylcarnitine (palmitoylcarnitine) or tigylcarnitine. Some acylcarnitines that were not rhythmic during their constant routine protocol were rhythmic in our protocol. These were butyrylcarnitine and carnitine (rhythmic on day 1 and day 2); acetylcarnitine, decanoylcarnitine, octadecanoylcarnitine (stearoylcarnitine) and octanoylcarnitine (rhythmic on day 1 only); and propionylcarnitine (rhythmic on day 2 only).

Dallmann et al. (13) found only one amino acid, glutamate, to be rhythmic in plasma. Similarly glutamate was found to be rhythmic in our protocol on both days. Kasukawa et al. (14) identified three rhythmic amino acids in their constant routine protocol, namely phenylalanine, tryptophan and leucine, none of which were found to be rhythmic in plasma by Dallmann et al. (13) or in our protocol. Dallmann et al. (13) reported rhythmicity in arginine, histidine, serine, tyrosine and ornithine in saliva. In our protocol we also identified rhythmicity in ornithine on both days and in tyrosine only during continual wakefulness (day 2), but no rhythmicity in arginine, histidine or serine on either day.

Both constant routine studies (13, 14) reported the oscillation of many lipids during their respective protocols, and this rhythmicity is consistent with our study, in which 45/86 phospholipids and 13/14 sphingolipids maintained rhythmicity in the presence and absence of sleep. This finding is supported by transcriptomic data, with Archer et al. (15) reporting that transcripts

associated with lipid metabolism were robustly rhythmic, and not affected by altered sleep. Möller-Levet at al. (16) showed that genes associated with phospholipid transporter activity only exhibited circadian variation with sufficient sleep, which may be linked with the loss in rhythmicity of some phospholipids observed during continual wakefulness in our protocol. The authors also reported that lipid metabolism transcripts have maximum transcription levels during the day. This is supported by the metabolomics data, with the levels of most lipids peaking during the afternoon in our study and most lipid products peaking during mid-morning to noon in Dallmann's constant routine protocol (13). The differences in lipid peak timing during the day may reflect differences between the constant routine protocols (13, 16) and our protocol (sleep/wake; light/dark; meals).

Comparison of metabolites changed significantly during sleep deprivation in this study to other studies. Dallmann et al. (13) identified 34 metabolites in plasma and 27 in saliva that demonstrated a monotonic increase or decrease across their 40 h constant routine protocol and suggested that some of these metabolites may be associated with sleep pressure. One of these metabolites was serotonin which increased monotonically during the constant routine protocol. The significantly increased serotonin levels we observed during sleep deprivation compared to during sleep adds weight to this indoleamine being associated with high sleep pressure.

Our findings suggest that the increased levels of acylcarnitines observed during sleep deprivation may point to changes in the beta-oxidation of fatty acids, and the monotonic increase in plasma 3-hydroxydecanoate, an intermediate in the beta-oxidation of fatty acids, observed by Dallmann et al. (13) may support this hypothesis. By contrast, the monotonic changes in many of the plasma and saliva amino acids reported by Dallmann et al. (13) were not seen between the sleep and sleep deprivation periods in our protocol, likely reflecting differences in the feeding regimen between the two protocols. While Dallmann et al. (13) did not report an increase in tryptophan across their protocol, the authors showed a monotonic increase in plasma 3-indoxyl sulphate, a metabolite of tryptophan, lending support to the significantly increased tryptophan levels we observed during sleep deprivation.

SI Appendix; References

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Figure S1

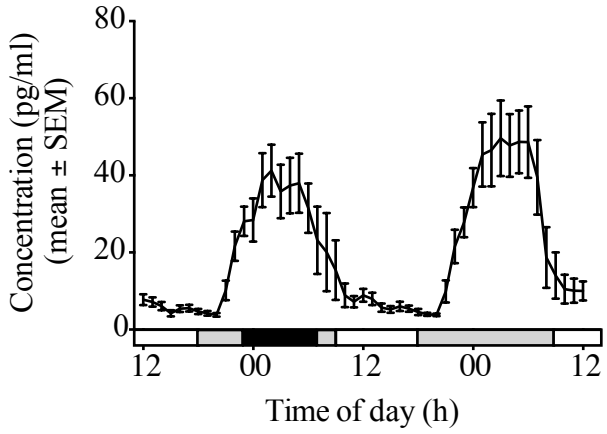

Figure S1. Mean (± SEM) melatonin concentrations (n = 12) over the 48-h sampling protocol. The black bar indicates the sleep period, 0 lux, supine; grey bars, wake periods, semi-recumbent position, < 5 lux; white bars, awake and free movement, 100 lux.

Figure S2

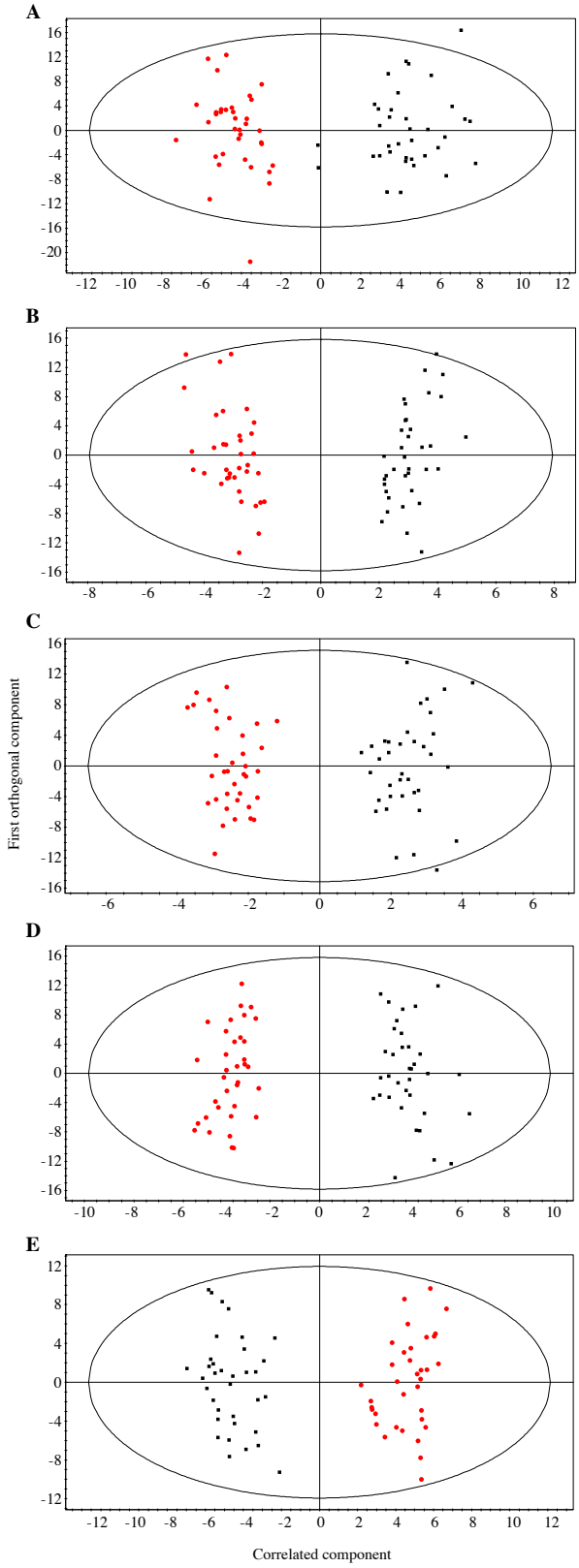

Figure S2. OPLS-DA models (validated by permutation analysis) of targeted metabolomics data in which selected time points on day 1 were separated according to time of day. A. Black squares – 12:00 – 16:00 h; red circles – 00:00 – 04:00 h; R^2X (cumulative) = 0.606, R^2Y (cumulative) = 0.897, Q^2 (cumulative) = 0.789. B. Black squares – 16:00 – 20:00 h; red circles – 04:00 – 08:00 h; R^2X (cumulative) = 0.640, R^2Y (cumulative) = 0.951, Q^2 (cumulative) = 0.852. C. Black squares – 18:00 – 22:00 h; red circles – 06:00 – 10:00 h; R^2X (cumulative) = 0.627, R^2Y (cumulative) = 0.937, Q^2 (cumulative) = 0.757. D. Black squares – 20:00 – 00:00 h; red circles – 08:00 – 12:00 h; R^2X (cumulative) = 0.632, R^2Y (cumulative) = 0.954, Q^2 (cumulative) = 0.828. E. Black squares – 22:00 – 02:00 h; red circles – 10:00 – 14:00 h; R^2X (cumulative) = 0.636, R^2Y (cumulative) = 0.943, Q^2 (cumulative) = 0.847.

Figure S3

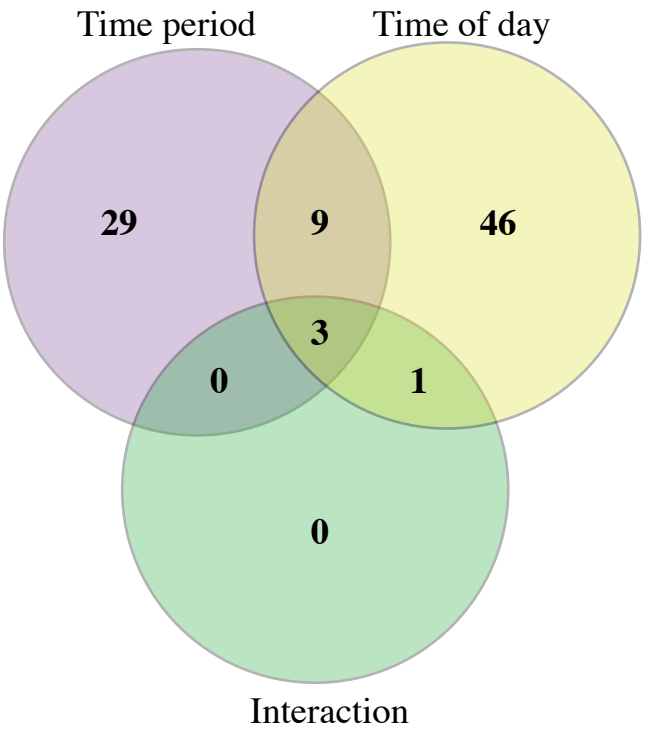

Figure S3. A Venn diagram showing the number of significant metabolites ($p < 0.05$ and $q < 0.05$) derived from repeated two-way ANOVA of the time period (sleep 00:00 – 06:00 h; sleep deprivation 00:00 – 06:00 h), time of day (00:00, 02:00, 04:00, 06:00 h) and time period*time of day interaction.

Figure S4

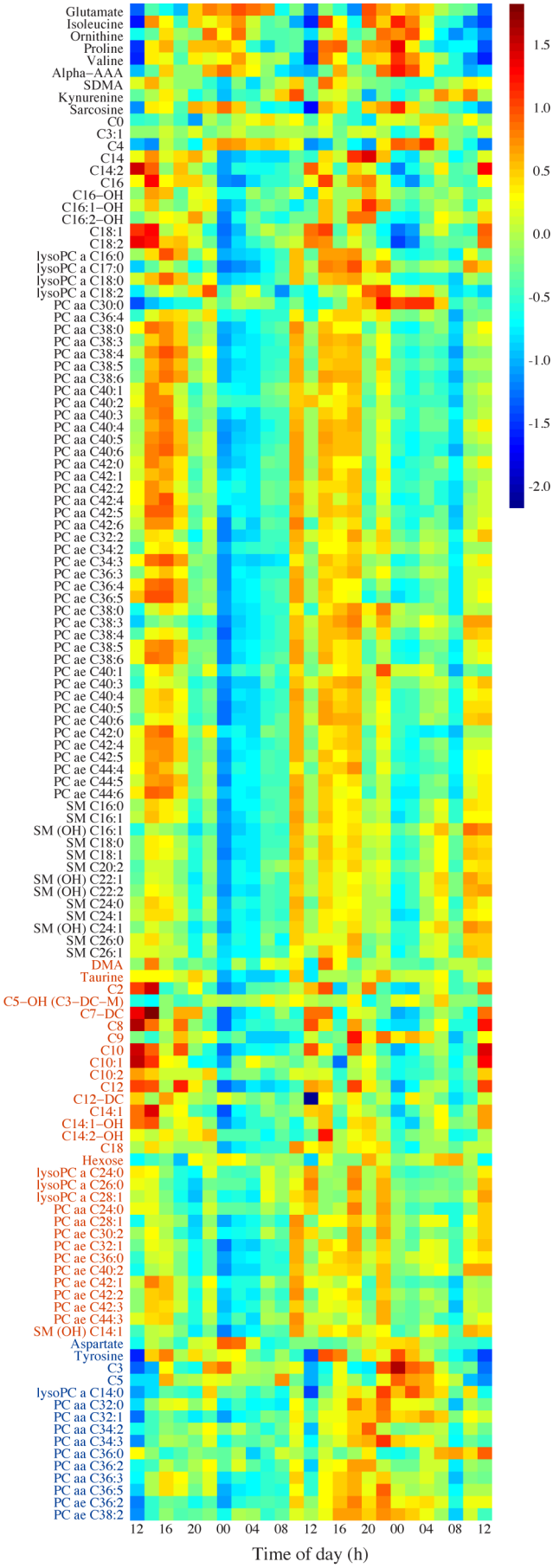

Figure S4. A heat map of all metabolites showing diurnal rhythms with significant fits to a cosine curve (n = 109). Mean z-score values for all subjects (n = 12) are shown (z-scores calculated across 48 h). High levels of metabolites are shown in red and low levels are shown in blue. The metabolites are divided into those that exhibited significant cosinor rhythms on both day 1 and day 2 (n=78, black labels), on day 1 only (n = 31, red labels) and on day 2 only (n = 15, blue labels). A full description of the cosinor analysis (acrophase, amplitude) for each metabolite is detailed in Table S6.

Figure S5

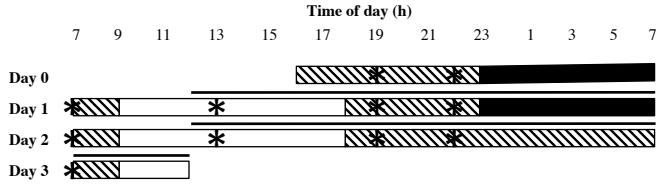

Figure S5. In-laboratory study protocol comprising 24 h wake/sleep followed by 24 h of wakefulness. White sections indicate wake periods (wake, 100 lux, free movement); hatched sections – wake, semi-recumbent position, < 5 lux; black sections – sleeping with eye masks, 0 lux, supine. Asterisks represent standardized meals; black bars represent hourly blood sampling across 48 h.

Figure S6

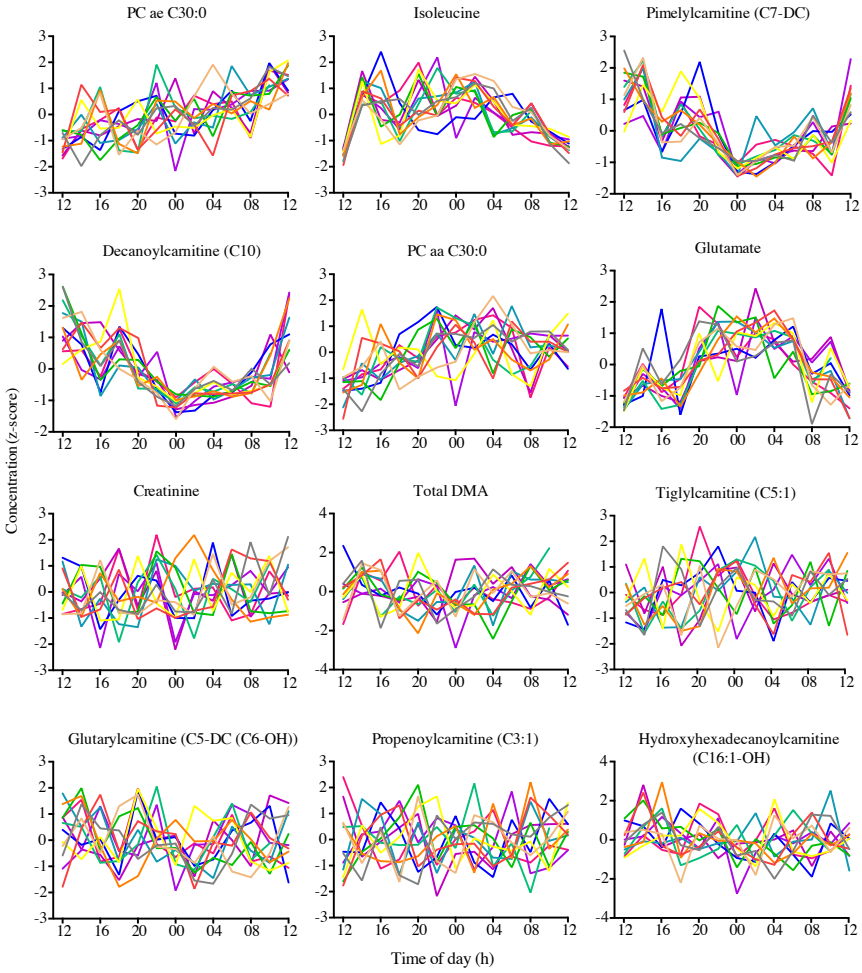

Figure S6. Concentrations of the six metabolites that vary least (PC ae C30:0, isoleucine, pimelylcarnitine, decanoylcarnitine, PC aa C30:0, glutamate) and the six that vary most (creatinine, total DMA, tiglylcarnitine, glutarylcarnitine, propenoylcarnitine, hydroxyhexadecanoylcarnitine) between individuals across day 1. Z-scored concentrations for each subject (n = 12) are shown.

Table S1: Study and analysis protocol illustrating the time periods and time of day. Two-way repeated ANOVA was carried out on the time periods shown and their corresponding times of day for all 171 metabolites measured.

Time of day		Time period			
Clock time (h)	Time point	1	2	3	4
12:00	1				
14:00	2	Level 1			Level 1
16:00	3	Level 1			Level 1
18:00	4	Level 1			Level 1
20:00	5	Level 1			Level 1
22:00	6	Level 1			Level 1
00:00	7	Level 1	Level 1	Level 1	
02:00	8	Level 1	Level 1	Level 1	
04:00	9	Level 1	Level 1	Level 1	
06:00	10	Level 1	Level 1	Level 1	
08:00	11	Level 1	Level 1		
10:00	12	Level 1	Level 1		
12:00	13	Level 1	Level 1		
14:00	14	Level 2			Level 2
16:00	15	Level 2			Level 2
18:00	16	Level 2			Level 2
20:00	17	Level 2			Level 2
22:00	18	Level 2			Level 2
00:00	19	Level 2	Level 2	Level 2	
02:00	20	Level 2	Level 2	Level 2	
04:00	21	Level 2	Level 2	Level 2	
06:00	22	Level 2	Level 2	Level 2	
08:00	23	Level 2	Level 2		
10:00	24	Level 2	Level 2		
12:00	25	Level 2	Level 2		

Time point 1 was not used in the two-way repeated ANOVA analysis in order to balance the number of time points compared between time periods.

Table S2: OPLS-DA models (targeted analysis data) p(corr) values of the measured metabolites in models comparing sleep (00:00 – 06:00 h, N1) with sleep deprivation (00:00 – 06:00 h, N2) (sleep status); 14:00 – 18:00 h with 02:00 - 06:00 h, day 1; 14:00 – 18:00 h with 02:00 - 06:00 h, day 2.

Sleep status		**Day 1** **14:00 - 18:00 vs 02:00 - 06:00 h**		**Day 2** **14:00 - 18:00 vs 02:00 - 06:00 h**	
Metabolite	**p(corr)**	**Metabolite**	**p(corr)**	**Metabolite**	**p(corr)**
Glutamate	-0.294	Octadecadienylcarnitine (C18:2)	-0.661	Butyrylcarnitine (C4)	-0.551
Decenoylcarnitine (C10:1)	-0.185	Dodecanoylcarnitine (C12)	-0.657	Glutamate	-0.505
Decadienylcarnitine (C10:2)	-0.104	Hexadecanoylcarnitine (C16)	-0.652	Propionylcarnitine (C3)	-0.444
Propenoylcarnitine (C3:1)	-0.100	Tetradecanoylcarnitine (C14)	-0.640	PC aa C30:0	-0.416
Octadecadienylcarnitine (C18:2)	-0.091	Decanoylcarnitine (C10)	-0.635	Valerylcarnitine (C5)	-0.282
Hydroxybutyrylcarnitine (C3-DC (C4-OH))	-0.089	Tetradecadienylcarnitine (C14:2)	-0.583	Carnitine (C0)	-0.242
lysoPC a C24:0	-0.087	PC aa C42:5	-0.549	PC ae C30:0	-0.241
Citrulline	-0.085	Octanoylcarnitine (C8)	-0.536	PC ae C34:0	-0.227
PC aa C26:0	-0.060	Pimelylcarnitine (C7-DC)	-0.535	Asymmetric dimethylarginine (ADMA)	-0.200
Aspartate	-0.033	PC aa C38:0	-0.535	Hydroxybutyrylcarnitine (C3-DC (C4-OH))	-0.192
Hydroxypropionylcarnitine (C3-OH)	-0.025	PC ae C40:5	-0.529	lysoPC a C14:0	-0.183
Total Dimethylarginine (DMA)	-0.025	PC ae C42:5	-0.526	Butenylcarnitine (C4:1)	-0.165
PC aa C24:0	-0.024	Hydroxytetradecenoylcarnitine C14:1-OH	-0.521	Hexose	-0.160
Butenylcarnitine (C4:1)	-0.023	PC ae C38:5	-0.514	Taurine	-0.142
PC aa C40:2	-0.020	PC aa C38:6	-0.507	Nonaylcarnitine (C9)	-0.115
Dodecanedioylcarnitine (C12-DC)	-0.018	Phenylalanine	-0.498	Citrulline	-0.114
Methylglutarylcarnitine (C5-M-DC)	-0.017	PC ae C38:6	-0.496	Serotonin	-0.108
Hexose	-0.015	Alanine	-0.496	Valine	-0.099
Hydroxyvalerylcarnitine (C5-OH (C3-DC-M))	-0.014	PC aa C42:4	-0.492	Hydroxypropionylcarnitine (C3-OH)	-0.090
Glutamine	-0.009	Taurine	-0.490	Threonine	-0.086
lysoPC a C26:0	-0.008	Octadecenoylcarnitine (C18:1)	-0.486	PC aa C34:4	-0.082
Histidine	-0.005	Hexadecenoylcarnitine (C16:1)	-0.482	Glycine	-0.078
Hexadecadienylcarnitine (C16:2)	-0.005	PC ae C44:5	-0.480	Hydroxyvalerylcarnitine (C5-OH (C3-DC-M))	-0.076

PC ae C42:0	-0.003	PC aa C40:6	-0.480	PC aa C32:3	-0.067
Dodecenoylcarnitine (C12:1)	-0.002	PC ae C42:0	-0.475	Ornithine	-0.062
lysoPC a C26:1	0.020	PC ae C42:4	-0.473	Decenoylcarnitine (C10:1)	-0.045
PC aa C34:2	0.022	PC ae C44:6	-0.470	PC aa C36:0	-0.043
Kynurenine	0.026	PC ae C40:6	-0.460	Aspartate	-0.043
PC aa C36:2	0.029	PC aa C42:0	-0.458	lysoPC a C18:2	-0.041
SM C24:0	0.030	PC ae C36:5	-0.456	Tiglylcarnitine (C5:1)	-0.040
Hexadecenoylcarnitine (C16:1)	0.032	PC aa C40:2	-0.443	Hydroxyhexanoylcarnitine (C5-DC (C6-OH))	-0.040
Glycine	0.036	PC ae C34:3	-0.442	Dodecenoylcarnitine (C12:1)	-0.035
PC ae C42:1	0.045	PC ae C36:4	-0.441	PC aa C32:1	-0.031
Octadecenoylcarnitine (C18:1)	0.046	PC ae C40:4	-0.438	Propenoylcarnitine (C3:1)	-0.026
Arginine	0.049	Tetradecenoylcarnitine (C14:1)	-0.432	Alpha-Aminoadipic acid (alpha-AAA)	-0.015
PC aa C40:6	0.053	PC aa C42:1	-0.431	Kynurenine	0.004
PC ae C30:1	0.056	PC aa C40:3	-0.430	lysoPC a C18:1	0.011
SM C26:0	0.058	PC aa C40:1	-0.423	Hexanoylcarnitine (C6 (C4:1-DC))	0.014
PC aa C42:2	0.059	PC aa C38:4	-0.409	Glutaconylcarnitine (C5:1-DC)	0.015
SM C24:1	0.059	Tryptophan	-0.408	Dodecanedioylcarnitine (C12-DC)	0.039
Carnitine (C0)	0.060	lysoPC a C16:0	-0.402	PC aa C34:2	0.048
Ornithine	0.062	PC aa C40:5	-0.401	PC aa C34:3	0.048
Leucine	0.062	PC aa C42:2	-0.400	PC ae C30:1	0.049
Creatinine	0.064	PC ae C38:4	-0.384	SM (OH) C24:1	0.050
PC aa C36:0	0.065	lysoPC a C18:0	-0.384	lysoPC a C16:1	0.052
PC aa C42:4	0.066	PC aa C40:4	-0.381	Isoleucine	0.070
lysoPC a C18:0	0.067	PC aa C38:5	-0.371	lysoPC a C20:3	0.073
Sarcosine	0.068	Tyrosine	-0.368	PC ae C40:1	0.082
PC aa C40:1	0.070	SM C16:0	-0.363	lysoPC a C20:4	0.085
Hydroxyoctadecenoylcarnitine (C18:1-OH)	0.075	PC aa C36:4	-0.358	Hydroxyoctadecenoylcarnitine (C18:1-OH)	0.086
PC ae C44:4	0.076	PC ae C44:4	-0.356	PC ae C36:0	0.088
Alpha-Aminoadipic acid (alpha-AAA)	0.079	Acetylcarnitine (C2)	-0.352	Serine	0.092
PC ae C44:3	0.083	PC ae C40:3	-0.349	PC aa C36:2	0.096

lysoPC a C28:0	0.084	SM C18:0	-0.344	PC aa C32:0	0.096
PC aa C38:3	0.084	PC ae C40:2	-0.337	Tryptophan	0.097
PC aa C38:0	0.087	PC aa C42:6	-0.334	PC aa C34:1	0.101
SM (OH) C24:1	0.088	Hydroxyhexadecenoylcarnitine (C16:1-OH)	-0.329	Acetylcarnitine (C2)	0.102
Alanine	0.090	Dodecenoylcarnitine (C12:1)	-0.328	Hexadecadienylcarnitine (C16:2)	0.105
Serine	0.090	Hydroxyhexadecanoylcarnitine (C16-OH)	-0.315	SM (OH) C22:1	0.107
Butyrylcarnitine (C4)	0.091	Octadecanoylcarnitine (C18)	-0.313	Tetradecenoylcarnitine (C14:1)	0.107
Hydroxyhexadecadienylcarnitine (C16:2-OH)	0.097	PC ae C36:3	-0.311	SM C24:0	0.107
lysoPC a C20:4	0.100	SM (OH) C16:1	-0.310	Sarcosine	0.115
PC aa C38:4	0.100	SM C16:1	-0.304	PC aa C36:1	0.115
Hydroxyhexanoylcarnitine (C5-DC (C6-OH))	0.101	PC aa C38:3	-0.303	Creatinine	0.124
SM C26:1	0.102	Glutamine	-0.294	lysoPC a C26:1	0.130
PC aa C40:5	0.103	Hydroxyhexadecadienylcarnitine (C16:2-OH)	-0.293	Lysine	0.132
PC aa C42:6	0.103	SM C24:1	-0.293	PC ae C34:1	0.136
Hydroxyhexadecanoylcarnitine (C16-OH)	0.106	PC ae C34:2	-0.290	lysoPC a C28:1	0.139
PC aa C34:1	0.107	PC ae C38:0	-0.286	SM (OH) C22:2	0.141
PC aa C38:6	0.108	SM C18:1	-0.284	Hexenoylcarnitine (C6:1)	0.149
PC ae C44:6	0.109	Symmetrical Dimethylarginine (SDMA)	-0.279	PC ae C36:2	0.154
PC aa C42:1	0.114	PC aa C32:0	-0.279	SM (OH) C16:1	0.156
Tiglylcarnitine (C5:1)	0.114	SM (OH) C14:1	-0.276	PC ae C36:1	0.156
PC ae C36:4	0.116	Serotonin	-0.273	SM (OH) C14:1	0.158
Asymmetric dimethylarginine (ADMA)	0.117	PC ae C32:2	-0.273	Hydroxyhexadecanoylcarnitine (C16-OH)	0.161
PC ae C38:2	0.119	SM (OH) C22:2	-0.272	SM C26:1	0.161
lysoPC a C28:1	0.120	Hydroxytetradecadienylcarnitine (C14:2-OH)	-0.271	SM C26:0	0.163
PC aa C40:3	0.123	Decenoylcarnitine (C10:1)	-0.269	PC aa C28:1	0.164
PC aa C42:5	0.123	Nonaylcarnitine (C9)	-0.267	PC aa C26:0	0.168
PC aa C42:0	0.124	PC ae C42:3	-0.266	PC ae C42:2	0.169
Hexenoylcarnitine (C6:1)	0.125	SM C20:2	-0.265	PC aa C36:6	0.169
lysoPC a C16:0	0.126	PC ae C32:1	-0.265	Decadienylcarnitine (C10:2)	0.174
Hexanoylcarnitine (C6 (C4:1-DC))	0.129	PC ae C42:2	-0.262	PC ae C38:2	0.177

PC ae C38:6	0.133	Dodecanedioylcarnitine (C12-DC)	-0.260	lysoPC a C28:0	0.179
PC ae C36:5	0.133	PC aa C36:3	-0.251	SM C18:1	0.182
Lysine	0.135	SM C26:1	-0.247	PC aa C36:5	0.183
Tetradecenoylcarnitine (C14:1)	0.136	PC ae C36:0	-0.247	PC ae C40:2	0.184
SM C16:1	0.136	PC ae C42:1	-0.247	PC ae C42:3	0.184
Glutaconylcarnitine (C5:1-DC)	0.136	PC aa C36:1	-0.245	Methylglutarylcarnitine (C5-M-DC)	0.187
Symmetrical Dimethylarginine (SDMA)	0.137	PC ae C44:3	-0.243	PC ae C34:2	0.194
SM (OH) C22:1	0.139	Hexadecadienylcarnitine (C16:2)	-0.237	PC aa C36:3	0.196
lysoPC a C20:3	0.139	Proline	-0.231	Octanoylcarnitine (C8)	0.198
Asparagine	0.142	PC aa C28:1	-0.228	SM C16:1	0.199
PC ae C44:5	0.151	PC aa C36:5	-0.223	Arginine	0.201
PC ae C30:2	0.152	lysoPC a C17:0	-0.220	PC ae C32:1	0.202
PC ae C36:3	0.154	Hexenoylcarnitine (C6:1)	-0.218	Hydroxytetradecenoylcarnitine C14:1-OH	0.203
Isoleucine	0.158	SM C26:0	-0.212	SM C20:2	0.203
PC aa C36:4	0.159	PC ae C34:1	-0.211	Histidine	0.204
lysoPC a C18:2	0.160	Hexanoylcarnitine (C6 (C4:1-DC))	-0.201	Symmetrical Dimethylarginine (SDMA)	0.204
PC aa C40:4	0.160	Glutaconylcarnitine (C5:1-DC)	-0.194	Methionine	0.206
PC ae C38:5	0.161	Decadienylcarnitine (C10:2)	-0.188	PC ae C38:3	0.207
Proline	0.163	Methionine	-0.187	SM C24:1	0.207
Hydroxyhexadecenoylcarnitine (C16:1-OH)	0.167	PC ae C40:1	-0.185	PC ae C42:1	0.207
PC ae C42:3	0.168	SM C24:0	-0.181	PC aa C36:4	0.210
PC aa C38:5	0.172	SM (OH) C22:1	-0.178	Asparagine	0.211
PC ae C42:4	0.176	PC ae C38:3	-0.176	Tetradecadienylcarnitine (C14:2)	0.212
PC ae C42:5	0.179	PC aa C36:6	-0.175	PC ae C30:2	0.214
Tetradecadienylcarnitine (C14:2)	0.179	Leucine	-0.166	Leucine	0.226
Octadecanoylcarnitine (C18)	0.180	Hydroxyoctadecenoylcarnitine (C18:1-OH)	-0.164	Octadecanoylcarnitine (C18)	0.234
PC ae C34:2	0.182	lysoPC a C20:4	-0.152	PC ae C44:3	0.234
Valine	0.183	Kynurenine	-0.151	SM C18:0	0.241
PC aa C36:3	0.187	lysoPC a C16:1	-0.151	PC ae C36:3	0.247
SM C18:1	0.187	PC ae C36:1	-0.149	PC ae C36:5	0.248

Pimelylcarnitine (C7-DC)	0.187	Hydroxyhexanoylcarnitine (C5-DC (C6-OH))	-0.149	Total Dimethylarginine (DMA)	0.250
PC aa C34:3	0.188	lysoPC a C18:1	-0.146	Pimelylcarnitine (C7-DC)	0.252
SM C16:0	0.191	SM (OH) C24:1	-0.144	SM C16:0	0.252
Acetylcarnitine (C2)	0.193	Arginine	-0.134	PC ae C38:0	0.254
Hexadecanoylcarnitine (C16)	0.196	Total Dimethylarginine (DMA)	-0.129	Hydroxytetradecadienylcarnitine (C14:2-OH)	0.260
PC ae C34:3	0.198	PC ae C30:2	-0.125	PC ae C32:2	0.264
PC ae C42:2	0.200	Sarcosine	-0.101	Dodecanoylcarnitine (C12)	0.272
SM C18:0	0.208	lysoPC a C20:3	-0.093	lysoPC a C26:0	0.278
Hydroxytetradecadienylcarnitine (C14:2-OH)	0.210	PC aa C34:1	-0.083	lysoPC a C24:0	0.280
Phenylalanine	0.211	PC ae C36:2	-0.081	Decanoylcarnitine (C10)	0.284
PC ae C40:1	0.213	Isoleucine	-0.069	Tyrosine	0.285
PC ae C38:0	0.219	PC aa C34:3	-0.068	PC ae C34:3	0.286
PC aa C32:1	0.225	PC aa C24:0	-0.065	Hydroxyhexadecadienylcarnitine (C16:2-OH)	0.289
SM (OH) C22:2	0.225	Asparagine	-0.057	lysoPC a C17:0	0.291
SM C20:2	0.233	Histidine	-0.054	PC ae C44:6	0.291
Methionine	0.235	Valerylcarnitine (C5)	-0.052	PC aa C42:6	0.298
lysoPC a C18:1	0.235	lysoPC a C24:0	-0.051	PC aa C24:0	0.299
PC aa C36:6	0.236	lysoPC a C28:1	-0.046	PC aa C40:4	0.301
PC aa C36:5	0.241	PC aa C32:3	-0.045	PC ae C44:5	0.301
Hydroxytetradecenoylcarnitine C14:1-OH	0.241	PC aa C32:1	-0.043	PC aa C42:0	0.306
PC ae C32:2	0.242	PC aa C36:0	-0.039	Hexadecenoylcarnitine (C16:1)	0.307
lysoPC a C16:1	0.243	PC aa C34:4	-0.029	PC aa C38:5	0.309
Threonine	0.250	lysoPC a C26:0	-0.023	PC ae C36:4	0.310
PC aa C32:3	0.253	lysoPC a C18:2	-0.018	PC ae C40:4	0.315
PC aa C34:4	0.255	lysoPC a C26:1	0.008	PC aa C38:3	0.316
PC aa C36:1	0.258	Propenoylcarnitine (C3:1)	0.022	PC ae C38:4	0.319
Tetradecanoylcarnitine (C14)	0.267	Lysine	0.039	Hydroxyhexadecenoylcarnitine (C16:1-OH)	0.326
PC ae C40:4	0.269	PC aa C36:2	0.039	Octadecenoylcarnitine (C18:1)	0.326
PC ae C40:6	0.274	Ornithine	0.047	Proline	0.328
Tyrosine	0.277	Methylglutarylcarnitine (C5-M-DC)	0.055	PC aa C38:4	0.336

Octanoylcarnitine (C8)	0.278	Asymmetric dimethylarginine (ADMA)	0.056	Glutamine	0.339
PC ae C40:3	0.285	Threonine	0.065	PC ae C40:3	0.346
Serotonin	0.292	PC aa C34:2	0.074	PC ae C38:6	0.351
Valerylcarnitine (C5)	0.293	lysoPC a C28:0	0.077	PC ae C42:5	0.355
lysoPC a C17:0	0.296	lysoPC a C14:0	0.080	PC aa C42:1	0.357
PC ae C36:0	0.298	PC ae C30:1	0.087	PC ae C38:5	0.360
SM (OH) C14:1	0.299	PC ae C34:0	0.087	PC aa C40:5	0.360
PC ae C38:3	0.301	Hydroxybutyrylcarnitine (C3-DC (C4-OH))	0.089	PC ae C42:4	0.366
PC ae C32:1	0.304	PC ae C38:2	0.092	PC ae C40:5	0.370
Decanoylcarnitine (C10)	0.306	Valine	0.093	PC ae C44:4	0.375
PC ae C38:4	0.308	Alpha-Aminoadipic acid (alpha-AAA)	0.116	PC aa C42:4	0.381
PC aa C28:1	0.312	PC aa C26:0	0.126	Tetradecanoylcarnitine (C14)	0.386
Dodecanoylcarnitine (C12)	0.314	Creatinine	0.130	PC aa C40:3	0.398
PC ae C36:2	0.320	Aspartate	0.131	lysoPC a C16:0	0.401
PC ae C40:5	0.321	Hydroxypropionylcarnitine (C3-OH)	0.141	PC aa C38:0	0.407
PC ae C40:2	0.324	Glycine	0.146	PC aa C42:2	0.408
PC aa C32:0	0.328	Serine	0.160	lysoPC a C18:0	0.420
lysoPC a C14:0	0.339	Tiglylcarnitine (C5:1)	0.165	PC ae C40:6	0.422
Propionylcarnitine (C3)	0.349	Butenylcarnitine (C4:1)	0.178	PC aa C40:2	0.424
PC aa C30:0	0.388	Citrulline	0.186	PC aa C40:1	0.434
SM (OH) C16:1	0.395	Hydroxyvalerylcarnitine (C5-OH (C3-DC-M))	0.216	Phenylalanine	0.437
PC ae C34:1	0.405	PC ae C30:0	0.255	PC aa C40:6	0.461
PC ae C36:1	0.415	Propionylcarnitine (C3)	0.264	PC ae C42:0	0.462
Tryptophan	0.450	Carnitine (C0)	0.279	PC aa C42:5	0.469
Nonaylcarnitine (C9)	0.468	PC aa C30:0	0.302	PC aa C38:6	0.473
Taurine	0.537	Hexose	0.331	Octadecadienylcarnitine (C18:2)	0.476
PC ae C34:0	0.540	Butyrylcarnitine (C4)	0.349	Hexadecanoylcarnitine (C16)	0.499
PC ae C30:0	0.542	Glutamate	0.678	Alanine	0.518

Table S3: OPLS-DA models (targeted analysis data) p(corr) values of the measured metabolites in models comparing day 1, 12:00 – 16:00 h with 00:00 – 04:00 h; 16:00 – 20:00 h with 04:00 – 08:00 h; 18:00 – 22:00 h with 06:00 – 10:00 h; 20:00 – 00:00 h with 08:00 – 12:00 h; and 22:00 – 02:00 h with 10:00 – 14:00 h.

12:00 - 16:00 h vs 00:00 - 04:00 h		16:00 - 20:00 h vs 04:00 - 08:00 h		18:00 - 22:00 h vs 06:00 - 10:00 h		20:00 - 00:00 h vs 08:00 - 12:00 h		22:00 - 02:00 h vs 10:00 - 14:00 h	
Metabolite	**p(corr)**	**Metabolite**	**p(corr)**	**Metabolite**	**p(corr)**	**Metabolite**	**p(corr)**	**Metabolite**	**p(corr)**
Glu	-0.676	Glu	-0.354	PC ae C30:0	-0.368	SM (OH) C16:1	-0.483	alpha-AAA	-0.591
C4	-0.503	PC ae C30:0	-0.329	lysoPC a C26:0	-0.328	PC ae C40:6	-0.424	Glu	-0.590
C3	-0.440	C0	-0.310	lysoPC a C28:1	-0.312	PC ae C30:0	-0.418	C4	-0.542
Hexose	-0.394	C4	-0.275	lysoPC a C28:0	-0.306	PC ae C34:1	-0.409	C3	-0.502
PC aa C30:0	-0.385	lysoPC a C28:0	-0.256	ADMA	-0.293	PC ae C40:3	-0.407	Val	-0.456
Asp	-0.327	PC aa C26:0	-0.239	total DMA	-0.290	C10:1	-0.400	C3-DC (C4-OH)	-0.451
Val	-0.301	C5-OH (C3-DC-M)	-0.224	C0	-0.266	SM (OH) C14:1	-0.398	Ile	-0.446
alpha-AAA	-0.298	lysoPC a C26:0	-0.211	PC ae C44:3	-0.249	PC ae C40:2	-0.391	Sarcosine	-0.439
Orn	-0.273	PC aa C30:0	-0.206	C5-OH (C3-DC-M)	-0.232	PC ae C40:5	-0.387	Orn	-0.431
Ile	-0.258	lysoPC a C28:1	-0.197	PC ae C30:2	-0.223	PC ae C36:1	-0.386	C5	-0.409
C3-OH	-0.238	Creatinine	-0.189	lysoPC a C24:0	-0.219	PC ae C34:0	-0.377	Pro	-0.384
PC ae C30:0	-0.213	ADMA	-0.180	PC aa C26:0	-0.217	SM C20:2	-0.373	Leu	-0.321
PC aa C34:2	-0.197	lysoPC a C24:0	-0.169	C4	-0.213	C14:1	-0.371	Met	-0.271
Ser	-0.189	Gly	-0.163	PC ae C34:0	-0.206	PC aa C28:1	-0.365	Hexose	-0.253
C0	-0.169	C3	-0.146	lysoPC a C26:1	-0.193	lysoPC a C26:0	-0.362	Asn	-0.241
C5-OH (C3-DC-M)	-0.164	lysoPC a C26:1	-0.141	PC aa C24:0	-0.185	PC ae C40:4	-0.361	Phe	-0.236
Pro	-0.163	C10:1	-0.134	SM (OH) C16:1	-0.157	PC ae C38:4	-0.356	Tyr	-0.214
PC ae C34:0	-0.161	PC ae C34:0	-0.132	SM (OH) C24:1	-0.136	C12:1	-0.349	lysoPC a C18:2	-0.212
C5:1	-0.161	PC ae C30:1	-0.132	PC ae C36:1	-0.133	C18:1	-0.348	Ser	-0.193
PC aa C36:2	-0.160	Hexose	-0.131	PC aa C34:4	-0.133	C10	-0.347	ADMA	-0.186
PC ae C38:2	-0.155	C4:1	-0.119	SM C20:2	-0.129	SM C16:0	-0.347	Cit	-0.172
C3-DC (C4-OH)	-0.145	Lys	-0.105	Kynurenine	-0.127	PC ae C44:3	-0.345	Arg	-0.161
lysoPC a C14:0	-0.144	PC ae C30:2	-0.101	C10:1	-0.125	PC ae C42:5	-0.339	Thr	-0.158

Sarcosine	-0.141	PC aa C24:0	-0.086	PC ae C34:1	-0.122	lysoPC a C28:1	-0.336	Lys	-0.153
Cit	-0.135	C3-OH	-0.053	Creatinine	-0.122	C18:2	-0.332	PC aa C30:0	-0.147
C5	-0.126	Thr	-0.051	PC aa C40:1	-0.121	PC ae C32:1	-0.330	C5:1	-0.142
Leu	-0.109	PC aa C34:4	-0.050	PC aa C28:1	-0.115	PC ae C36:0	-0.325	C3-OH	-0.116
Lys	-0.094	C12:1	-0.047	SM (OH) C14:1	-0.106	PC aa C42:5	-0.323	His	-0.108
Asn	-0.074	total DMA	-0.043	PC ae C30:1	-0.106	C8	-0.321	PC ae C30:1	-0.071
C4:1	-0.067	C5:1	-0.043	PC ae C40:2	-0.095	PC ae C32:2	-0.321	C9	-0.069
PC aa C34:1	-0.050	PC aa C32:3	-0.027	Gly	-0.094	total DMA	-0.318	C6:1	-0.063
Met	-0.042	Kynurenine	-0.022	C3	-0.094	lysoPC a C28:0	-0.314	lysoPC a C14:0	-0.058
PC aa C34:3	-0.036	PC aa C32:1	-0.013	SM (OH) C22:1	-0.090	PC aa C40:1	-0.311	Creatinine	-0.052
Arg	-0.034	SM (OH) C24:1	-0.003	PC ae C42:1	-0.086	PC aa C38:0	-0.310	C5:1-DC	-0.031
C3:1	-0.033	Cit	0.000	SDMA	-0.085	PC ae C38:3	-0.310	C12-DC	-0.030
C5-M-DC	-0.023	Ser	0.012	PC ae C40:3	-0.081	PC ae C30:2	-0.308	Trp	-0.027
Thr	-0.017	PC ae C38:2	0.012	PC ae C42:3	-0.080	PC ae C42:4	-0.307	C4:1	-0.021
His	-0.013	lysoPC a C14:0	0.015	PC aa C32:3	-0.075	PC ae C38:6	-0.306	Gly	-0.017
PC aa C32:1	-0.013	C5	0.017	PC ae C38:3	-0.072	SM C18:0	-0.306	Asp	-0.016
lysoPC a C18:2	-0.010	Val	0.028	PC aa C42:0	-0.071	SM (OH) C22:1	-0.306	PC aa C34:2	-0.010
Tyr	-0.002	PC ae C36:2	0.030	PC ae C42:2	-0.070	PC ae C38:5	-0.303	PC aa C26:0	0.014
Creatinine	0.005	PC aa C36:0	0.037	PC aa C42:2	-0.069	SM (OH) C22:2	-0.297	PC aa C36:2	0.035
ADMA	0.007	PC ae C34:1	0.048	PC ae C32:2	-0.069	PC aa C42:6	-0.294	lysoPC a C26:1	0.039
Gly	0.031	PC ae C36:1	0.055	PC aa C32:1	-0.067	SM (OH) C24:1	-0.294	C0	0.053
PC ae C30:1	0.032	His	0.055	SM C26:0	-0.065	PC aa C42:2	-0.293	C5-OH (C3-DC-M)	0.054
PC ae C36:2	0.036	C3:1	0.058	C5:1	-0.059	PC aa C42:0	-0.293	PC ae C38:2	0.084
PC aa C32:3	0.045	SM (OH) C22:1	0.059	PC ae C36:2	-0.059	PC ae C44:5	-0.287	C14:2-OH	0.085
PC aa C34:4	0.053	PC aa C28:1	0.063	PC aa C30:0	-0.055	PC aa C42:1	-0.286	PC aa C36:0	0.097
PC aa C36:0	0.058	Asp	0.071	PC ae C40:6	-0.052	PC aa C36:1	-0.266	C5-DC (C6-OH)	0.104
PC aa C26:0	0.073	SM C26:0	0.078	PC aa C36:0	-0.050	SM C16:1	-0.266	lysoPC a C24:0	0.122
lysoPC a C28:0	0.086	SM C20:2	0.078	SM C24:0	-0.048	PC ae C36:2	-0.264	PC aa C24:0	0.125
C5:1-DC	0.087	PC ae C44:3	0.079	PC aa C40:2	-0.042	C18	-0.263	lysoPC a C28:0	0.132
C9	0.098	SM C24:0	0.081	PC ae C32:1	-0.042	PC ae C44:6	-0.263	PC aa C34:3	0.142
lysoPC a C16:1	0.115	C5-DC (C6-OH)	0.081	PC ae C36:0	-0.042	PC aa C42:4	-0.260	C5-M-DC	0.144

PC aa C32:0	0.123	PC aa C34:3	0.085	C18	-0.038	C16:1	-0.258	lysoPC a C20:4	0.151
Phe	0.131	SM (OH) C16:1	0.091	PC aa C36:6	-0.037	PC aa C40:2	-0.257	C3:1	0.151
PC ae C36:1	0.133	PC ae C38:3	0.091	C3:1	-0.037	PC aa C40:3	-0.256	Serotonin	0.154
lysoPC a C20:3	0.145	PC aa C36:6	0.091	Cit	-0.035	C14:2	-0.256	C6 (C4:1-DC)	0.164
Kynurenine	0.161	SM (OH) C14:1	0.096	C12:1	-0.032	PC ae C44:4	-0.256	PC ae C40:1	0.172
PC aa C36:3	0.169	PC ae C42:3	0.096	Thr	-0.031	SDMA	-0.250	C10:2	0.174
PC ae C38:3	0.169	SM C26:1	0.103	SM C26:1	-0.029	C2	-0.250	C16-OH	0.184
PC aa C36:1	0.182	PC aa C34:2	0.104	PC aa C42:1	-0.027	PC ae C36:4	-0.248	lysoPC a C18:1	0.190
SM (OH) C24:1	0.184	C5-M-DC	0.113	Lys	-0.024	Kynurenine	-0.247	lysoPC a C20:3	0.198
PC ae C34:1	0.188	PC ae C42:1	0.119	PC ae C38:2	-0.024	PC aa C40:6	-0.246	C16:2	0.204
PC aa C36:5	0.194	alpha-AAA	0.125	PC aa C42:6	-0.023	PC ae C36:5	-0.246	Kynurenine	0.207
Trp	0.195	PC ae C32:2	0.128	PC ae C44:4	-0.019	PC ae C42:2	-0.243	Ala	0.209
lysoPC a C18:1	0.196	C2	0.130	C5	-0.010	SM C18:1	-0.243	PC aa C32:3	0.220
PC aa C36:6	0.198	PC ae C40:2	0.138	C3-OH	-0.008	PC aa C40:4	-0.236	lysoPC a C26:0	0.223
SM C24:0	0.201	PC aa C36:2	0.138	lysoPC a C17:0	-0.004	SM C24:1	-0.236	PC aa C34:1	0.231
PC ae C36:0	0.215	PC ae C36:0	0.140	PC ae C40:4	0.002	PC ae C42:3	-0.233	C16:1-OH	0.232
C12-DC	0.217	C16:1-OH	0.141	PC ae C42:4	0.004	PC aa C34:4	-0.227	lysoPC a C28:1	0.235
SM (OH) C22:1	0.218	PC ae C32:1	0.142	PC aa C36:1	0.006	lysoPC a C17:0	-0.225	Taurine	0.246
C6 (C4:1-DC)	0.220	C16:2	0.142	PC ae C38:4	0.006	PC aa C24:0	-0.224	lysoPC a C16:1	0.249
lysoPC a C17:0	0.223	SDMA	0.147	PC aa C42:4	0.007	SM C26:1	-0.222	Gln	0.254
PC aa C36:4	0.232	lysoPC a C17:0	0.150	PC ae C42:5	0.008	lysoPC a C24:0	-0.218	PC aa C36:4	0.256
lysoPC a C26:0	0.240	Arg	0.150	PC ae C44:6	0.011	C0	-0.215	PC aa C32:0	0.266
Serotonin	0.243	lysoPC a C16:1	0.154	SM C16:0	0.012	PC ae C42:1	-0.213	PC aa C40:2	0.267
SM C26:1	0.244	C14:1	0.154	Hexose	0.017	PC ae C36:3	-0.212	PC aa C36:5	0.267
C10:2	0.245	Met	0.154	SM C18:0	0.019	PC aa C38:6	-0.211	SM C24:0	0.271
PC ae C40:1	0.253	SM C18:1	0.158	PC ae C40:5	0.020	PC aa C40:5	-0.210	PC ae C42:1	0.272
SM C26:0	0.257	PC ae C42:2	0.160	SM (OH) C22:2	0.020	SM C26:0	-0.210	SDMA	0.281
lysoPC a C26:1	0.257	PC aa C36:1	0.161	Glu	0.022	PC aa C36:6	-0.208	C14	0.281
PC ae C34:2	0.257	PC ae C40:1	0.161	PC ae C40:1	0.025	PC ae C42:0	-0.207	SM C26:0	0.283
PC aa C24:0	0.273	Gln	0.166	C14:1	0.030	PC aa C32:1	-0.204	C10:1	0.290
SM C18:1	0.274	lysoPC a C18:1	0.167	PC ae C44:5	0.031	SM C24:0	-0.201	PC aa C34:4	0.293
lysoPC a C20:4	0.276	SM (OH) C22:2	0.167	Ala	0.031	PC aa C38:3	-0.197	PC ae C30:2	0.297

lysoPC a C24:0	0.276	lysoPC a C20:3	0.169	C5-DC (C6-OH)	0.033	C7-DC	-0.189	C18:1-OH	0.312
PC aa C38:3	0.276	PC aa C40:1	0.177	PC aa C38:0	0.034	PC ae C34:2	-0.182	PC aa C32:1	0.312
PC ae C38:0	0.279	SM C16:1	0.179	SM C16:1	0.035	PC aa C38:5	-0.179	PC aa C38:4	0.317
PC aa C28:1	0.279	PC aa C34:1	0.180	SM C24:1	0.036	PC aa C32:3	-0.171	PC ae C42:3	0.325
SM C24:1	0.283	SM C18:0	0.183	lysoPC a C14:0	0.036	C5-OH (C3-DC-M)	-0.170	PC aa C36:3	0.329
PC ae C36:3	0.289	Asn	0.185	SM C18:1	0.038	lysoPC a C26:1	-0.163	PC ae C42:2	0.329
C18:1-OH	0.289	Orn	0.187	PC ae C42:0	0.043	PC aa C26:0	-0.162	PC ae C38:0	0.330
C16:2	0.292	PC ae C40:3	0.190	PC aa C40:3	0.059	PC ae C34:3	-0.160	C12:1	0.330
C16:2-OH	0.304	C16-OH	0.190	C5-M-DC	0.063	PC ae C38:0	-0.133	PC ae C44:3	0.338
SM C16:1	0.304	SM C24:1	0.195	PC ae C36:3	0.067	lysoPC a C16:0	-0.131	PC ae C44:6	0.339
SM (OH) C22:2	0.305	C18:1-OH	0.197	PC aa C42:5	0.070	lysoPC a C18:0	-0.129	C14:1-OH	0.344
C6:1	0.309	PC ae C44:4	0.200	PC aa C38:3	0.071	C12	-0.112	SM C26:1	0.344
PC ae C32:1	0.309	PC aa C42:0	0.200	PC ae C38:6	0.072	C3:1	-0.110	PC aa C36:6	0.347
PC ae C40:2	0.310	C10:2	0.206	C4:1	0.076	PC aa C36:5	-0.105	SM (OH) C24:1	0.351
lysoPC a C28:1	0.320	Leu	0.206	Gln	0.077	PC aa C36:3	-0.100	PC aa C42:2	0.360
PC ae C30:2	0.325	C18:1	0.211	PC aa C40:4	0.081	PC aa C38:4	-0.095	PC ae C44:4	0.362
SM (OH) C14:1	0.326	PC aa C42:2	0.212	PC ae C36:5	0.085	PC aa C36:0	-0.082	PC aa C42:0	0.369
SM C20:2	0.326	SM C16:0	0.215	His	0.088	C18:1-OH	-0.077	PC aa C42:1	0.372
SM (OH) C16:1	0.333	PC ae C38:0	0.216	PC aa C40:6	0.089	PC ae C40:1	-0.075	PC aa C40:1	0.372
SM C18:0	0.335	PC ae C34:2	0.218	PC ae C38:0	0.090	PC aa C32:0	-0.066	total DMA	0.375
C14:2-OH	0.337	lysoPC a C20:4	0.223	C18:1	0.090	PC ae C30:1	-0.053	PC aa C38:5	0.375
C16-OH	0.342	PC aa C36:5	0.224	C2	0.093	PC ae C38:2	-0.043	lysoPC a C16:0	0.382
Gln	0.346	Ile	0.226	PC aa C34:3	0.095	lysoPC a C16:1	-0.037	lysoPC a C18:0	0.383
PC ae C32:2	0.350	lysoPC a C18:2	0.229	PC ae C34:2	0.095	Gly	-0.036	PC ae C42:0	0.388
PC ae C42:2	0.354	PC aa C38:3	0.229	PC ae C38:5	0.096	Gln	-0.035	PC ae C34:2	0.390
total DMA	0.365	PC ae C36:3	0.231	C16-OH	0.107	C16	-0.027	PC aa C42:6	0.391
PC ae C42:1	0.366	PC aa C36:3	0.234	PC ae C36:4	0.109	Creatinine	-0.024	PC ae C44:5	0.394
PC ae C40:3	0.367	Sarcosine	0.237	PC aa C40:5	0.113	C14:1-OH	-0.022	SM C24:1	0.397
SDMA	0.367	PC aa C42:1	0.238	C18:1-OH	0.127	PC aa C34:3	-0.021	C16:2-OH	0.401
PC ae C42:3	0.370	PC aa C42:6	0.240	lysoPC a C16:1	0.131	C3-OH	-0.017	PC ae C42:4	0.404
PC aa C40:4	0.370	PC ae C40:6	0.248	PC aa C38:5	0.131	PC aa C34:1	-0.017	PC aa C38:3	0.407

SM C16:0	0.372	PC ae C38:4	0.252	Serotonin	0.133	C4:1	-0.016	SM C18:1	0.412
PC aa C38:5	0.377	C18	0.258	PC aa C36:5	0.136	PC aa C36:4	-0.005	PC aa C36:1	0.413
lysoPC a C18:0	0.377	C16:1	0.262	PC aa C36:3	0.142	Serotonin	0.009	SM (OH) C22:1	0.418
lysoPC a C16:0	0.384	PC aa C40:2	0.264	Met	0.145	Asp	0.016	PC aa C40:6	0.419
PC aa C38:4	0.387	C6 (C4:1-DC)	0.266	PC aa C34:1	0.148	C10:2	0.018	PC ae C30:0	0.423
C18	0.397	Trp	0.276	Trp	0.157	C16:2	0.028	PC ae C36:2	0.425
PC aa C42:6	0.398	PC ae C44:6	0.276	lysoPC a C18:0	0.162	C16:2-OH	0.036	PC aa C40:3	0.429
PC aa C40:5	0.400	PC ae C42:4	0.282	PC aa C38:6	0.164	PC aa C30:0	0.057	PC aa C38:6	0.430
PC ae C44:3	0.403	PC aa C40:4	0.288	PC aa C38:4	0.165	ADMA	0.057	PC ae C34:3	0.430
PC ae C38:4	0.410	PC aa C32:0	0.288	lysoPC a C16:0	0.175	lysoPC a C18:1	0.063	PC aa C40:5	0.432
Ala	0.411	PC ae C44:5	0.291	C12-DC	0.179	C5-DC (C6-OH)	0.064	SM C16:1	0.434
PC aa C40:3	0.422	PC ae C40:4	0.299	PC aa C36:2	0.181	Taurine	0.066	PC aa C40:4	0.434
C5-DC (C6-OH)	0.424	C6:1	0.302	Arg	0.182	C4	0.075	PC aa C42:4	0.439
PC ae C34:3	0.434	PC aa C38:0	0.304	PC ae C34:3	0.192	C5:1	0.088	PC ae C36:5	0.443
PC ae C36:4	0.443	PC aa C40:5	0.308	Ser	0.196	lysoPC a C14:0	0.092	PC ae C36:3	0.445
C16:1-OH	0.458	C3-DC (C4-OH)	0.312	PC aa C34:2	0.201	lysoPC a C20:4	0.094	C18	0.449
PC aa C40:6	0.461	PC aa C38:5	0.316	C18:2	0.202	PC aa C36:2	0.105	C12	0.451
PC aa C42:2	0.462	PC aa C40:6	0.320	Asp	0.207	C16-OH	0.106	PC aa C28:1	0.458
PC aa C40:2	0.462	PC ae C42:5	0.320	C16:2	0.226	Ala	0.110	PC ae C32:2	0.459
PC aa C42:1	0.464	C7-DC	0.321	Taurine	0.229	C5-M-DC	0.114	PC ae C42:5	0.459
PC aa C40:1	0.466	PC ae C36:4	0.322	lysoPC a C18:1	0.232	Cit	0.127	lysoPC a C17:0	0.461
PC ae C44:4	0.467	PC aa C40:3	0.323	PC aa C32:0	0.235	lysoPC a C20:3	0.136	SM (OH) C22:2	0.475
PC aa C38:6	0.475	C14:1-OH	0.323	C16:1	0.236	C6 (C4:1-DC)	0.137	C14:2	0.476
PC ae C40:4	0.476	PC ae C42:0	0.325	PC aa C36:4	0.241	C5	0.138	PC aa C38:0	0.479
PC ae C40:6	0.486	lysoPC a C18:0	0.325	alpha-AAA	0.249	PC aa C34:2	0.143	C7-DC	0.480
PC ae C38:6	0.488	C14:2-OH	0.326	C10:2	0.261	C16:1-OH	0.144	PC ae C32:1	0.485
PC ae C36:5	0.494	C12-DC	0.326	Asn	0.268	C9	0.158	PC ae C34:0	0.492
PC ae C42:0	0.495	Ala	0.327	Val	0.275	C3	0.198	PC ae C36:4	0.494
PC aa C42:4	0.501	PC ae C38:6	0.329	Sarcosine	0.279	C6:1	0.218	PC ae C36:0	0.496
PC aa C38:0	0.505	PC aa C36:4	0.336	C8	0.282	Trp	0.219	SM C18:0	0.505
PC ae C38:5	0.513	PC ae C40:5	0.338	C7-DC	0.287	C14	0.221	PC ae C38:3	0.520
PC ae C42:4	0.528	PC aa C38:4	0.341	lysoPC a C20:3	0.294	Thr	0.227	PC ae C40:4	0.528

C10:1	0.529	PC ae C36:5	0.343	lysoPC a C20:4	0.302	Lys	0.229	C16	0.534
PC aa C42:5	0.543	lysoPC a C16:0	0.344	C14:2	0.308	His	0.243	PC ae C38:6	0.538
PC ae C40:5	0.543	PC aa C42:4	0.349	C16:1-OH	0.312	C14:2-OH	0.244	C14:1	0.543
Taurine	0.548	PC ae C38:5	0.361	C14:1-OH	0.327	C12-DC	0.251	PC ae C38:5	0.544
PC aa C42:0	0.549	PC aa C38:6	0.365	Orn	0.330	C5:1-DC	0.282	SM (OH) C14:1	0.549
PC ae C44:6	0.560	C16:2-OH	0.370	C10	0.334	Hexose	0.311	SM C20:2	0.550
PC ae C44:5	0.566	C8	0.375	C6:1	0.338	Arg	0.374	C8	0.552
C14:1	0.567	Serotonin	0.376	Tyr	0.370	Orn	0.378	PC aa C42:5	0.553
C2	0.569	PC aa C42:5	0.377	C14:2-OH	0.383	lysoPC a C18:2	0.421	PC ae C40:2	0.554
C12:1	0.571	C14:2	0.380	C6 (C4:1-DC)	0.385	Met	0.455	C2	0.558
C14	0.588	PC ae C34:3	0.387	Leu	0.388	alpha-AAA	0.468	SM C16:0	0.561
PC ae C42:5	0.597	C18:2	0.387	C16:2-OH	0.405	Tyr	0.471	PC ae C36:1	0.571
C8	0.615	Tyr	0.398	C9	0.405	Ser	0.494	PC ae C38:4	0.584
C16:1	0.625	C9	0.412	Pro	0.413	Sarcosine	0.521	PC ae C40:6	0.591
C14:1-OH	0.625	C5:1-DC	0.424	C16	0.441	Phe	0.536	C16:1	0.601
C14:2	0.627	Pro	0.455	Phe	0.445	Asn	0.578	C10	0.601
C7-DC	0.659	C10	0.467	lysoPC a C18:2	0.449	Pro	0.590	PC ae C40:5	0.601
C18:1	0.664	Phe	0.493	C5:1-DC	0.455	C3-DC (C4-OH)	0.600	C18:2	0.606
C16	0.670	C16	0.497	Ile	0.457	Val	0.603	PC ae C34:1	0.608
C10	0.684	Taurine	0.503	C3-DC (C4-OH)	0.458	Leu	0.628	PC ae C40:3	0.616
C12	0.687	C12	0.532	C12	0.494	Glu	0.631	SM (OH) C16:1	0.647
C18:2	0.773	C14	0.612	C14	0.627	Ile	0.723	C18:1	0.649

Table S4: Number of metabolites differing significantly ($p < 0.05$ and $q < 0.05$) between the different time periods, corresponding times of day (defined in Table S1), and the interaction term (time period*time of day).

Time period	Factor	Number of metabolites
1	Time period	7
	Time of day	159
	Interaction	45
2	Time period	0
	Time of day	145
	Interaction	96
3	Time period	41
	Time of day	59
	Interaction	4
4	Time period	21
	Time of day	121
	Interaction	3

Table S5: Percent increase in concentrations (mean ± SEM) during sleep deprivation of the 41 metabolites that had significantly higher levels ($p < 0.05$, $q < 0.05$) during sleep deprivation compared with during sleep (00:00 – 06:00 h).

Metabolite	**% increase mean ± SEM**
Serotonin	43.6 ± 20.1
PC ae C34:0	28.6 ± 7.3
Nonaylcarnitine (C9)	28.1 ± 8.7
PC ae C30:0	25.4 ± 5.8
PC aa C30:0	25.1 ± 7.2
PC aa C32:1	22.2 ± 6.4
lysoPC a C17:0	22.0 ± 6.4
PC ae C36:1	21.8 ± 6.0
Pimelylcarnitine (C7-DC)	21.5 ± 9.1
Taurine	21.0 ± 5.7
PC ae C38:3	20.8 ± 6.3
PC ae C34:1	20.7 ± 6.5
PC ae C38:2	20.2 ± 10.6
Tryptophan	19.6 ± 6.0
Decanoylcarnitine (C10)	19.2 ± 5.4
PC ae C36:2	18.5 ± 6.2
PC aa C34:4	17.1 ± 5.3
lysoPC a C16:1	17.1 ± 5.3
PC ae C40:2	16.6 ± 5.7
Dodecanoylcarnitine (C12)	16.6 ± 5.1
PC ae C36:0	16.2 ± 6.7
PC ae C32:1	15.9 ± 5.8
SM (OH) C16:1	15.9 ± 5.0
Valerylcarnitine (C5)	15.5 ± 5.1
PC aa C36:6	15.1 ± 5.8
Propionylcarnitine (C3)	14.9 ± 5.4
PC aa C32:0	14.6 ± 6.3
PC ae C38:4	13.5 ± 5.3
PC aa C28:1	13.0 ± 5.3
SM (OH) C14:1	12.3 ± 4.4
SM C20:2	12.3 ± 4.8
PC ae C40:6	12.2 ± 5.3
PC ae C40:3	12.2 ± 4.7
PC aa C32:3	12.1 ± 5.0
PC ae C40:5	12.0 ± 5.1
Octanoylcarnitine (C8)	11.9 ± 3.9
lysoPC a C18:1	11.0 ± 4.7
lysoPC a C14:0	10.9 ± 3.2
SM (OH) C22:2	10.7 ± 4.1
Tetradecanoylcarnitine (C14)	10.4 ± 4.1
Hydroxyhexadecanoylcarnitine (C16-OH)	6.7 ± 3.1

Table S6: Targeted metabolic profiling data: cosinor analysis (amplitude, acrophase) for each measured metabolite on day 1 and day 2 and the change in amplitude and phase between day 1 and day 2.

Metabolite	Day 1				Day 2				% change in amplitude	Phase shift △ϕ (dec. h)
	Amplitude z-score	Acrophase time (dec. h)	p-value		Amplitude z-score	Acrophase time (dec. h)	p-value			
Amino acids										
Alanine			0.342				0.241			
Arginine			0.262				0.082			
Asparagine			0.100				0.073			
Aspartate			0.100		0.38	23.51	0.009	**		
Citrulline			0.643				0.731			
Glutamate	1.00	01.45	<0.001	***	0.78	00.56	0.007	**	-22	0.89
Glutamine			0.169				0.117			
Glycine			0.347				0.579			
Histidine			0.360				0.127			
Isoleucine	0.71	22.75	0.042	*	0.91	22.48	0.021	*	29	0.27
Leucine			0.128				0.070			
Lysine			0.371				0.205			
Methionine			0.245				0.116			
Ornithine	0.49	22.91	0.013	*	0.75	22.62	0.012	*	53	0.29
Phenylalanine			0.061				0.069			
Proline	0.72	21.28	0.019	*	0.94	21.05	0.011	*	30	0.23
Serine			0.094				0.151			
Threonine			0.386				0.132			
Tryptophan			0.272				0.210			
Tyrosine			0.092		0.74	21.71	0.045	*		
Valine	0.62	23.55	0.037	*	0.85	23.06	0.016	*	38	0.49
Biogenic amines										
Alpha-Aminoadipic acid (alpha-AAA)	0.73	23.01	0.001	**	0.84	23.06	0.004	**	15	-0.05
Asymmetric dimethylarginine (ADMA)			0.337				0.094			
Symmetrical Dimethylarginine (SDMA)	0.54	12.72	0.001	***	0.52	13.46	<0.001	***	-3	-0.74
Total Dimethylarginine (DMA)	0.39	11.23	0.033	*			0.079			
Creatinine			0.530				0.427			
Kynurenine	0.55	11.25	0.015	*	0.61	09.29	0.002	**	10	1.96

Sarcosine	0.78	21.97	0.004	**	0.76	22.41	0.013	*	-2	-0.45
Serotonin			0.117				0.533			
Taurine	0.66	15.83	0.004	**			0.216			
Hexoses										
Hexose	0.36	01.45	0.027	*			0.431			
Acylcarnitines										
Carnitine (C0)	0.45	06.75	0.025	*	0.38	06.49	0.048	*	-16	0.26
Acetylcarnitine (C2)	0.84	13.14	0.007	**			0.196			
Propionylcarnitine (C3)			0.058		0.93	01.28	0.005	**		
Propenoylcarnitine (C3:1)	0.78	20.96	0.043	*	0.54	22.55	0.050	*	-31	-1.59
Hydroxybutyrylcarnitine (C3-DC (C4-OH))			0.892				0.685			
Hydroxypropionylcarnitine (C3-OH)			0.700				0.434			
Butyrylcarnitine (C4)	0.75	02.80	0.003	**	0.95	02.45	0.001	***	26	0.35
Butenylcarnitine (C4:1)			0.428				0.942			
Valerylcarnitine (C5)			0.318		0.70	01.04	0.004	**		
Tiglylcarnitine (C5:1)			0.253				0.898			
Glutaconylcarnitine (C5:1-DC)			0.674				0.293			
Hydroxyhexanoylcarnitine (C5-DC (C6-OH))			0.174				0.745			
Methylglutarylcarnitine (C5-M-DC)			0.387				0.697			
Hydroxyvalerylcarnitine (C5-OH (C3-DC-M))	0.47	18.17	0.003	**			0.259			
Hexanoylcarnitine (C6 (C4:1-DC))			0.052				0.294			
Hexenoylcarnitine (C6:1)			0.145				0.229			
Pimelylcarnitine (C7-DC)	1.05	14.38	0.005	**			0.059			
Octanoylcarnitine (C8)	1.00	13.99	0.002	**			0.073			
Nonaylcarnitine (C9)	0.56	18.02	0.012	*			0.068			
Decanoylcarnitine (C10)	1.06	14.21	0.001	***			0.063			
Decenoylcarnitine (C10:1)	0.72	12.16	0.019	*			0.479			
Decadienylcarnitine (C10:2)	0.41	14.81	0.030	*			0.066			
Dodecanoylcarnitine (C12)	1.08	15.24	0.001	***			0.076			
Dodecenoylcarnitine (C12:1)			0.171				0.456			
Dodecanedioylcarnitine (C12-DC)	0.74	12.49	0.023	*			0.511			
Tetradecanoylcarnitine (C14)	0.82	16.31	<0.001	***	0.71	17.91	0.016	*	-14	-1.60
Tetradecenoylcarnitine (C14:1)	0.91	13.24	0.001	***			0.054			
Hydroxytetradecenoylcarnitine C14:1-OH	0.70	14.66	0.007	**			0.120			
Tetradecadienylcarnitine (C14:2)	0.88	14.02	0.004	**	0.66	12.81	0.041	*	-25	1.21
Hydroxytetradecadienylcarnitine (C14:2-OH)	0.45	17.60	0.005	**			0.128			
Hexadecanoylcarnitine (C16)	0.93	15.24	<0.001	***	0.74	15.73	0.007	**	-21	-0.48
Hexadecenoylcarnitine (C16:1)			0.104				0.198			
Hydroxyhexadecenoylcarnitine (C16:1-OH)	0.83	13.68	0.008	**	0.71	13.26	0.021	*	-15	0.42

Hexadecadienylcarnitine (C16:2)			0.082				0.068			
Hydroxyhexadecadienylcarnitine (C16:2-OH)	0.51	13.65	0.017	*	0.53	12.97	0.023	*	4	0.68
Hydroxyhexadecanoylcarnitine (C16-OH)	0.51	16.19	0.035	*	0.53	14.41	0.036	*	4	1.78
Octadecanoylcarnitine (C18)	0.60	13.66	0.003	**			0.051			
Octadecenoylcarnitine (C18:1)	0.92	13.20	0.001	**	0.80	12.89	0.015	*	-13	0.31
Hydroxyoctadecenoylcarnitine (C18:1-OH)			0.073				0.292			
Octadecadienylcarnitine (C18:2)	1.01	13.83	<0.001	***	0.87	13.25	0.002	**	-14	0.58
Glycerophospholipids										
lysoPC a C14:0			0.333		0.54	00.28	0.005	**		
lysoPC a C16:0	0.79	15.08	0.001	***	0.67	16.20	0.001	**	-15	-1.12
lysoPC a C16:1			0.169				0.203			
lysoPC a C17:0	0.56	13.42	0.042	*	0.52	16.02	0.002	**	-6	-2.60
lysoPC a C18:0	0.79	14.94	<0.001	***	0.69	16.45	0.001	***	-13	-1.51
lysoPC a C18:1			0.148				0.098			
lysoPC a C18:2	0.55	20.81	0.022	*	0.68	21.82	0.001	**	23	-1.01
lysoPC a C20:3			0.150				0.098			
lysoPC a C20:4			0.051				0.217			
lysoPC a C24:0	0.44	11.22	0.040	*			0.085			
lysoPC a C26:0	0.50	10.43	0.018	*			0.179			
lysoPC a C26:1			0.059				0.445			
lysoPC a C28:0			0.062				0.301			
lysoPC a C28:1	0.55	10.91	0.014	*			0.344			
PC aa C24:0	0.36	11.42	0.049	*			0.050			
PC aa C26:0			0.122				0.186			
PC aa C28:1	0.50	12.56	0.047	*			0.165			
PC aa C30:0	0.42	02.41	0.023	*	0.86	00.17	<0.001	***	104	2.24
PC aa C32:0			0.086		0.50	20.28	0.007	**		
PC aa C32:1			0.300		0.23	22.97	0.046	*		
PC aa C32:3			0.612				0.122			
PC aa C34:1			0.570				0.065			
PC aa C34:2			0.689		0.37	18.21	0.045	*		
PC aa C34:3			0.832		0.60	21.69	<0.001	***		
PC aa C34:4			0.208				0.306			
PC aa C36:0			0.259		0.52	10.07	0.009	**		
PC aa C36:1			0.052				0.109			
PC aa C36:2			0.725		0.37	17.99	0.044	*		
PC aa C36:3			0.055		0.43	18.76	0.030	*		
PC aa C36:4	0.51	16.49	0.011	*	0.45	18.10	0.019	*	-13	-1.61
PC aa C36:5			0.056		0.43	18.95	0.011	*		

PC aa C36:6			0.071				0.060			
PC aa C38:0	0.84	14.15	<0.001	***	0.55	14.55	0.007	**	-34	-0.40
PC aa C38:3	0.75	14.66	0.001	***	0.58	16.19	0.003	**	-23	-1.53
PC aa C38:4	0.82	15.36	<0.001	***	0.60	16.44	0.005	**	-27	-1.08
PC aa C38:5	0.74	14.94	0.001	**	0.55	16.57	0.006	**	-26	-1.63
PC aa C38:6	0.77	15.02	0.001	***	0.67	16.54	0.002	**	-12	-1.52
PC aa C40:1	0.70	13.48	<0.001	***	0.59	15.61	0.012	*	-16	-2.13
PC aa C40:2	0.66	13.84	0.001	***	0.54	15.60	0.021	*	-18	-1.76
PC aa C40:3	0.77	14.22	<0.001	***	0.52	15.77	0.009	**	-33	-1.55
PC aa C40:4	0.85	14.57	<0.001	***	0.59	15.32	0.003	**	-31	-0.75
PC aa C40:5	0.85	14.70	<0.001	***	0.66	15.61	0.001	***	-23	-0.91
PC aa C40:6	0.84	14.72	<0.001	***	0.66	16.24	0.002	**	-20	-1.52
PC aa C42:0	0.79	13.96	<0.001	***	0.51	14.55	0.009	**	-36	-0.59
PC aa C42:1	0.73	14.07	0.001	***	0.54	15.32	0.021	*	-27	-1.24
PC aa C42:2	0.68	13.82	<0.001	***	0.55	15.83	0.015	*	-19	-2.01
PC aa C42:4	0.79	14.32	<0.001	***	0.55	15.16	0.018	*	-31	-0.83
PC aa C42:5	0.87	14.34	<0.001	***	0.60	15.42	0.007	**	-31	-1.08
PC aa C42:6	0.76	14.20	0.002	**	0.45	15.36	0.036	*	-40	-1.17
PC ae C30:0			0.195				0.078			
PC ae C30:1			0.440				0.756			
PC ae C30:2	0.51	11.55	0.021	*			0.331			
PC ae C32:1	0.67	13.19	0.002	**			0.075			
PC ae C32:2	0.71	13.34	0.003	**	0.45	15.53	0.028	*	-36	-2.19
PC ae C34:0			0.403				0.160			
PC ae C34:1			0.050				0.241			
PC ae C34:2	0.51	14.43	0.012	*	0.33	17.91	0.047	*	-36	-3.47
PC ae C34:3	0.98	15.07	<0.001	***	0.53	14.85	0.002	**	-46	0.22
PC ae C36:0	0.56	13.09	0.011	*			0.163			
PC ae C36:1			0.099				0.140			
PC ae C36:2			0.392		0.33	19.37	0.033	*		
PC ae C36:3	0.67	14.27	0.001	**	0.39	16.19	0.026	*	-42	-1.92
PC ae C36:4	0.89	14.33	<0.001	***	0.53	14.33	0.005	**	-40	-0.01
PC ae C36:5	0.94	14.37	<0.001	***	0.55	13.60	0.005	**	-42	0.76
PC ae C38:0	0.55	14.98	0.014	*	0.52	18.27	0.002	**	-5	-3.28
PC ae C38:2			0.918		0.54	20.55	0.003	**		
PC ae C38:3	0.49	12.72	0.045	*	0.36	15.54	0.027	*	-27	-2.82
PC ae C38:4	0.69	13.71	0.003	**	0.48	14.77	0.009	**	-31	-1.06
PC ae C38:5	0.85	14.22	<0.001	***	0.56	14.52	0.004	**	-34	-0.30
PC ae C38:6	0.85	14.14	<0.001	***	0.52	14.62	0.004	**	-39	-0.48
PC ae C40:1	0.41	14.56	0.049	*	0.45	20.44	0.041	*	10	-5.87

PC ae C40:2	0.54	13.07	0.018	*			0.067			
PC ae C40:3	0.64	13.38	0.009	**	0.45	14.86	0.015	*	-31	-1.48
PC ae C40:4	0.75	13.96	0.001	***	0.51	15.12	0.008	**	-32	-1.16
PC ae C40:5	0.76	13.91	<0.001	***	0.52	14.80	0.006	**	-32	-0.88
PC ae C40:6	0.71	13.48	0.002	**	0.56	14.83	0.002	**	-21	-1.35
PC ae C42:0	0.75	14.49	0.001	***	0.65	16.08	0.006	**	-14	-1.59
PC ae C42:1	0.58	13.54	0.005	**			0.156			
PC ae C42:2	0.59	13.73	0.009	**			0.211			
PC ae C42:3	0.57	13.56	0.009	**			0.204			
PC ae C42:4	0.78	14.28	0.000	***	0.50	15.46	0.010	*	-36	-1.18
PC ae C42:5	0.81	14.11	0.000	***	0.56	14.59	0.004	**	-31	-0.48
PC ae C44:3	0.59	12.45	0.003	**			0.160			
PC ae C44:4	0.70	14.11	<0.001	***	0.58	15.29	0.004	**	-18	-1.18
PC ae C44:5	0.80	14.29	<0.001	***	0.56	14.55	0.004	**	-30	-0.26
PC ae C44:6	0.83	14.27	<0.001	***	0.58	14.32	0.005	**	-31	-0.05
Sphingolipids										
SM (OH) C14:1	0.58	12.61	0.011	*			0.056			
SM C16:0	0.68	13.58	0.001	**	0.47	13.70	0.012	*	-30	-0.12
SM C16:1	0.72	13.48	0.001	***	0.51	13.73	0.007	**	-28	-0.25
SM (OH) C16:1	0.58	12.58	0.013	*	0.39	12.26	0.015	*	-32	0.32
SM C18:0	0.63	13.51	0.003	**	0.44	13.69	0.007	**	-30	-0.18
SM C18:1	0.67	13.34	0.003	**	0.51	13.24	0.003	**	-24	0.10
SM C20:2	0.49	12.57	0.012	*	0.35	14.54	0.021	*	-30	-1.97
SM (OH) C22:1	0.55	12.77	0.011	*	0.40	12.44	0.022	*	-27	0.33
SM (OH) C22:2	0.64	13.30	0.003	**	0.46	12.50	0.010	**	-27	0.81
SM C24:0	0.57	13.47	0.005	**	0.42	13.45	0.015	*	-27	0.02
SM C24:1	0.66	13.86	0.001	**	0.52	13.59	0.007	**	-21	0.27
SM (OH) C24:1	0.56	12.19	0.002	**	0.42	12.06	0.014	*	-25	0.14
SM C26:0	0.57	13.18	0.009	**	0.44	12.84	0.012	*	-22	0.34
SM C26:1	0.54	13.34	0.002	**	0.46	13.12	0.014	*	-15	0.23

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ cosine fit; $p > 0.05$, amplitude and acrophase cannot be calculated. Metabolites in bold (n = 41) were significantly increased during sleep deprivation compared with during the sleep period ($p < 0.05$, $q < 0.05$). Acrophase phase shift ($\triangle\phi$) (dec.h), postive values indicate a phase advance and negative values indicate a phase delay on day 2 compared to day 1.

Table S7: Targeted metabolic profiling data: mean (± SEM), minimum and maximum concentrations [µM] of 171 metabolites.

Metabolite	Day 1			Day 2		
	Concentration [µM]			Concentration [µM]		
	mean ± SEM	minimum (mean)	maximum (mean)	mean ± SEM	minimum (mean)	maximum (mean)
Amino acids						
Alanine	394.78 ± 12.71	182.75	579.87	393.17 ± 13.42	249.76	675.88
Arginine	103.08 ± 2.92	47.52	163.87	104.42 ± 3.94	52.74	176.60
Asparagine	56.91 ± 2.08	29.97	93.51	59.39 ± 2.57	32.33	109.71
Aspartate	6.35 ± 0.35	0.60	33.47	6.49 ± 0.27	0.10	33.47
Citrulline	35.91 ± 0.68	11.80	59.36	35.20 ± 0.65	18.52	54.87
Glutamate	48.78 ± 4.25	10.48	162.93	48.98 ± 3.92	8.68	128.98
Glutamine	758.43 ± 11.42	358.32	1043.57	742.45 ± 14.32	478.55	1292.59
Glycine	260.26 ± 3.92	121.15	394.80	255.61 ± 4.77	140.05	410.25
Histidine	109.54 ± 1.89	46.82	159.02	109.84 ± 2.55	72.44	155.48
Isoleucine	98.36 ± 6.29	39.95	172.22	102.92 ± 7.66	42.39	190.31
Leucine	182.04 ± 9.58	90.02	352.90	185.90 ± 11.74	81.29	358.40
Lysine	271.09 ± 9.05	139.04	494.51	280.22 ± 11.61	145.36	575.72
Methionine	32.42 ± 1.54	18.41	58.29	34.32 ± 1.78	17.73	65.47
Ornithine	69.64 ± 1.73	33.80	118.11	69.27 ± 2.50	32.35	118.41
Phenylalanine	79.61 ± 3.12	48.55	148.88	80.40 ± 3.22	36.24	143.56
Proline	351.68 ± 13.5	170.66	545.80	363.62 ± 16.94	175.31	606.51
Serine	115.28 ± 3.31	62.41	193.55	118.73 ± 4.22	56.91	210.25
Threonine	135.36 ± 3.35	81.03	226.05	139.17 ± 4.57	75.83	275.12
Tryptophan	72.24 ± 2.07	35.60	121.67	77.63 ± 2.29	35.60	139.35
Tyrosine	78.37 ± 2.99	44.07	133.01	80.89 ± 3.74	35.11	152.37
Valine	311.93 ± 10.00	185.73	470.47	318.70 ± 12.89	159.35	492.78
Biogenic amines						
Alpha-Aminoadipic acid (alpha-AAA)	0.95 ± 0.04	0.15	2.26	0.99 ± 0.05	0.05	2.34
Asymmetric dimethylarginine (ADMA)	0.55 ± 0.02	0.04	0.99	0.56 ± 0.02	0.04	1.11
Symmetrical Dimethylarginine (SDMA)	0.47 ± 0.01	0.19	0.71	0.48 ± 0.01	0.13	0.71
Total Dimethylarginine (DMA)	0.79 ± 0.01	0.18	1.32	0.79 ± 0.01	0.13	1.47
Creatinine	125.73 ± 4.25	27.05	303.90	133.97 ± 4.25	24.07	317.12
Kynurenine	3.73 ± 0.08	1.43	9.46	3.73 ± 0.08	1.51	9.07
Sarcosine	1.67 ± 0.06	0.67	3.01	1.71 ± 0.07	0.67	2.73
Serotonin	0.19 ± 0.01	0.04	0.74	0.20 ± 0.01	0.04	0.76
Taurine	49.39 ± 1.32	19.19	96.27	51.12 ± 0.56	26.47	92.54
Hexoses						
Hexose	8530.88 ± 221.63	3616.90	17177.82	9101.27 ± 227.29	3634.58	21141.69
Acylcarnitines						
Carnitine (C0)	37.04 ± 0.57	17.56	56.29	37.63 ± 0.50	21.26	58.23
Acetylcarnitine (C2)	3.66 ± 0.16	1.22	7.28	3.64 ± 0.15	1.46	7.85
Propionylcarnitine (C3)	0.34 ± 0.01	0.18	0.62	0.36 ± 0.02	0.18	0.68
Propenoylcarnitine (C3:1)	0.05 ± <0.01	0.02	0.11	0.04 ± <0.01	0.02	0.10
Hydroxybutyrylcarnitine (C3-DC (C4-OH))	0.01 ± <0.01	<0.01	0.01	0.01 ± <0.01	<0.01	0.02
Hydroxypropionylcarnitine (C3-OH)	0.01 ± <0.01	<0.01	0.01	0.01 ± <0.01	<0.01	0.01
Butyrylcarnitine (C4)	0.19 ± 0.01	0.10	0.34	0.19 ± 0.01	0.09	0.34
Butenylcarnitine (C4:1)	0.005 ± <0.01	<0.01	0.01	0.01 ± <0.01	<0.01	0.05
Valerylcarnitine (C5)	0.14 ± <0.01	0.06	0.33	0.14 ± 0.01	0.07	0.35
Tiglylcarnitine (C5:1)	0.01 ± <0.01	0.01	0.02	0.01 ± <0.01	0.01	0.02
Glutaconylcarnitine (C5:1-DC)	0.03 ± <0.01	0.02	0.07	0.03 ± <0.01	0.02	0.06
Hydroxyhexanoylcarnitine (C5-DC (C6-OH))	0.03 ± <0.01	0.02	0.07	0.03 ± <0.01	0.02	0.05
Methylglutarylcarnitine (C5-M-DC)	0.01 ± <0.01	0.01	0.02	0.01 ± <0.01	0.01	0.02
Hydroxyvalerylcarnitine (C5-OH (C3-DC-M))	0.01 ± <0.01	<0.01	0.02	0.01 ± <0.01	<0.01	0.02

Hexanoylcarnitine (C6 (C4:1-DC))	0.05 ± <0.01	0.02	0.09	0.05 ± <0.01	0.03	0.07
Hexenoylcarnitine (C6:1)	0.01 ± <0.01	<0.01	0.02	0.01 ± <0.01	0.00	0.02
Pimelylcarnitine (C7-DC)	0.01 ± <0.01	<0.01	0.06	0.01 ± <0.01	<0.01	0.05
Octanoylcarnitine (C8)	0.08 ± <0.01	0.03	0.20	0.08 ± <0.01	0.05	0.17
Nonaylcarnitine (C9)	0.03 ± <0.01	0.01	0.08	0.04 ± <0.01	0.01	0.07
Decanoylcarnitine (C10)	0.12 ± 0.01	0.04	0.42	0.12 ± 0.01	0.04	0.31
Decenoylcarnitine (C10:1)	0.13 ± <0.01	0.06	0.25	0.12 ± <0.01	0.05	0.25
Decadienylcarnitine (C10:2)	0.03 ± <0.01	0.02	0.06	0.03 ± <0.01	0.01	0.06
Dodecanoylcarnitine (C12)	0.06 ± <0.01	0.03	0.18	0.06 ± <0.01	0.03	0.18
Dodecenoylcarnitine (C12:1)	0.04 ± <0.01	0.03	0.05	0.04 ± <0.01	0.03	0.04
Dodecanedioylcarnitine (C12-DC)	0.09 ± <0.01	0.03	0.19	0.09 ± <0.01	0.03	0.19
Tetradecanoylcarnitine (C14)	0.03 ± <0.01	0.02	0.05	0.03 ± <0.01	0.02	0.05
Tetradecenoylcarnitine (C14:1)	0.05 ± <0.01	0.02	0.13	0.05 ± <0.01	0.01	0.13
Hydroxytetradecenoylcarnitine C14:1-OH	0.01 ± <0.01	<0.01	0.02	0.01 ± <0.01	<0.01	0.02
Tetradecadienylcarnitine (C14:2)	0.01 ± <0.01	<0.01	0.06	0.01 ± <0.01	<0.01	0.06
Hydroxytetradecadienylcarnitine (C14:2-OH)	0.01 ± <0.01	<0.01	0.01	0.01 ± <0.01	<0.01	0.01
Hexadecanoylcarnitine (C16)	0.08 ± <0.01	0.03	0.16	0.08 ± <0.01	0.04	0.14
Hexadecenoylcarnitine (C16:1)	0.004 ± <0.01	<0.01	0.01	0.004 ± <0.01	<0.01	0.01
Hydroxyhexadecenoylcarnitine (C16:1-OH)	0.01 ± <0.01	0.01	0.04	0.01 ± <0.01	0.01	0.04
Hexadecadienylcarnitine (C16:2)	0.01 ± <0.01	<0.01	0.01	0.01 ± <0.01	<0.01	0.01
Hydroxyhexadecadienylcarnitine (C16:2-OH)	0.004 ± <0.01	<0.01	0.02	0.004 ± <0.01	<0.01	0.01
Hydroxyhexadecanoylcarnitine (C16-OH)	0.01 ± <0.01	<0.01	0.01	0.01 ± <0.01	<0.01	0.01
Octadecanoylcarnitine (C18)	0.03 ± <0.01	0.01	0.09	0.04 ± <0.01	0.02	0.05
Octadecenoylcarnitine (C18:1)	0.07 ± <0.01	0.03	0.14	0.07 ± <0.01	0.03	0.14
Hydroxyoctadecenoylcarnitine (C18:1-OH)	0.003 ± <0.01	<0.01	0.01	0.004 ± <0.01	<0.01	0.01
Octadecadienylcarnitine (C18:2)	0.02 ± <0.01	0.01	0.04	0.02 ± <0.01	0.01	0.04
Glycerophospholipids						
lysoPC a C14:0	3.26 ± 0.03	2.05	4.25	3.49 ± 0.05	2.16	4.81
lysoPC a C16:0	78.73 ± 2.19	30.54	141.32	78.79 ± 2.00	24.85	141.90
lysoPC a C16:1	2.71 ± 0.06	1.07	6.04	2.92 ± 0.05	1.04	5.65
lysoPC a C17:0	1.65 ± 0.05	0.63	2.84	1.86 ± 0.04	0.53	2.93
lysoPC a C18:0	21.29 ± 0.58	7.18	40.93	20.81 ± 0.53	7.18	33.37
lysoPC a C18:1	20.72 ± 0.41	8.10	38.69	21.40 ± 0.38	6.58	36.43
lysoPC a C18:2	36.90 ± 1.07	16.03	62.47	37.95 ± 1.15	10.27	63.74
lysoPC a C20:3	2.83 ± 0.07	1.14	5.69	2.96 ± 0.07	1.08	4.80
lysoPC a C20:4	6.52 ± 0.15	2.97	13.17	6.48 ± 0.13	2.56	10.39
lysoPC a C24:0	0.26 ± 0.01	0.11	0.54	0.26 ± 0.01	0.11	0.52
lysoPC a C26:0	0.53 ± 0.03	0.17	1.70	0.57 ± 0.02	0.19	1.70
lysoPC a C26:1	0.24 ± 0.01	0.08	0.63	0.25 ± 0.01	0.10	0.60
lysoPC a C28:0	0.47 ± 0.02	0.21	1.10	0.52 ± 0.01	0.21	1.11
lysoPC a C28:1	0.50 ± 0.02	0.23	1.01	0.53 ± 0.01	0.24	1.01
PC aa C24:0	0.22 ± 0.01	0.08	0.49	0.22 ± 0.01	0.08	0.49
PC aa C26:0	1.40 ± 0.03	0.64	2.90	1.43 ± 0.04	0.65	2.66
PC aa C28:1	2.18 ± 0.05	1.15	3.21	2.33 ± 0.04	1.30	3.75
PC aa C30:0	4.09 ± 0.11	1.81	7.41	4.81 ± 0.17	1.75	7.79
PC aa C32:0	13.84 ± 0.27	5.92	19.39	14.69 ± 0.27	7.06	22.70
PC aa C32:1	16.29 ± 0.40	5.85	44.88	17.98 ± 0.28	7.58	36.08
PC aa C32:3	0.44 ± 0.01	0.22	0.71	0.46 ± 0.01	0.24	0.69
PC aa C34:1	192.03 ± 2.09	111.04	292.41	198.25 ± 2.73	116.45	326.02
PC aa C34:2	202.13 ± 3.88	132.96	499.08	213.95 ± 4.24	121.62	538.85
PC aa C34:3	16.57 ± 0.29	7.31	30.07	17.52 ± 0.36	8.20	29.04
PC aa C34:4	1.90 ± 0.04	0.80	2.95	2.08 ± 0.03	0.89	3.35
PC aa C36:0	2.03 ± 0.04	0.14	4.92	2.18 ± 0.05	0.55	4.99
PC aa C36:1	63.84 ± 1.45	18.00	124.93	65.86 ± 1.42	33.69	115.83
PC aa C36:2	174.97 ± 2.36	95.40	314.82	181.13 ± 2.79	113.33	345.74
PC aa C36:3	135.03 ± 2.43	53.26	191.12	137.97 ± 2.46	62.21	196.89
PC aa C36:4	178.33 ± 2.69	94.95	250.61	179.70 ± 2.77	86.25	269.66
PC aa C36:5	19.97 ± 0.45	9.80	34.44	21.02 ± 0.41	9.10	35.35
PC aa C36:6	0.70 ± 0.02	0.27	1.15	0.75 ± 0.01	0.33	1.20
PC aa C38:0	2.28 ± 0.06	0.85	3.31	2.24 ± 0.05	1.02	3.18

PC aa C38:3	49.89 ± 1.28	17.46	94.57	49.96 ± 1.24	20.01	78.81
PC aa C38:4	98.26 ± 2.47	41.20	171.18	95.63 ± 2.31	42.20	156.09
PC aa C38:5	50.54 ± 1.27	23.78	87.16	50.83 ± 1.16	22.41	86.37
PC aa C38:6	58.42 ± 1.49	23.99	94.56	57.99 ± 1.46	26.49	96.79
PC aa C40:1	0.32 ± 0.01	0.16	0.47	0.32 ± 0.01	0.16	0.47
PC aa C40:2	0.26 ± 0.01	0.07	0.47	0.25 ± 0.01	0.08	0.46
PC aa C40:3	0.52 ± 0.01	0.21	0.90	0.52 ± 0.01	0.24	0.83
PC aa C40:4	3.66 ± 0.10	1.50	7.49	3.67 ± 0.09	1.74	6.20
PC aa C40:5	9.93 ± 0.28	3.85	21.02	9.73 ± 0.26	4.13	17.22
PC aa C40:6	20.64 ± 0.55	7.67	34.95	20.11 ± 0.52	9.10	29.99
PC aa C42:0	0.50 ± 0.01	0.20	0.77	0.49 ± 0.01	0.19	0.75
PC aa C42:1	0.25 ± 0.01	0.10	0.37	0.25 ± 0.01	0.11	0.39
PC aa C42:2	0.22 ± 0.01	0.11	0.33	0.22 ± 0.01	0.11	0.35
PC aa C42:4	0.21 ± 0.01	0.09	0.35	0.20 ± 0.01	0.09	0.35
PC aa C42:5	0.32 ± 0.01	0.12	0.59	0.31 ± 0.01	0.15	0.53
PC aa C42:6	0.55 ± 0.01	0.25	1.03	0.54 ± 0.01	0.27	0.88
PC ae C30:0	0.49 ± 0.01	0.24	0.76	0.59 ± 0.01	0.27	0.89
PC ae C30:1	0.09 ± <0.01	<0.01	0.39	0.10 ± 0.01	<0.01	0.33
PC ae C30:2	0.11 ± <0.01	0.06	0.20	0.12 ± <0.01	0.06	0.20
PC ae C32:1	2.27 ± 0.06	0.90	3.55	2.43 ± 0.05	1.16	3.75
PC ae C32:2	0.59 ± 0.02	0.28	0.98	0.62 ± 0.01	0.32	0.99
PC ae C34:0	1.97 ± 0.05	0.79	3.22	2.35 ± 0.03	0.93	3.63
PC ae C34:1	11.11 ± 0.29	3.96	17.85	12.41 ± 0.18	6.41	18.21
PC ae C34:2	11.07 ± 0.23	3.95	18.79	11.23 ± 0.18	4.95	15.93
PC ae C34:3	6.38 ± 0.22	2.20	12.23	6.37 ± 0.15	2.66	9.73
PC ae C36:0	0.95 ± 0.02	0.33	1.57	1.01 ± 0.01	0.54	1.53
PC ae C36:1	15.10 ± 0.40	5.89	23.65	17.16 ± 0.23	8.27	24.23
PC ae C36:2	16.91 ± 0.40	6.43	28.10	18.86 ± 0.29	7.85	28.73
PC ae C36:3	7.22 ± 0.17	2.30	11.56	7.32 ± 0.14	3.62	10.63
PC ae C36:4	15.27 ± 0.43	5.66	28.87	14.86 ± 0.34	7.15	23.03
PC ae C36:5	9.68 ± 0.30	3.63	17.51	9.33 ± 0.23	4.01	15.58
PC ae C38:0	1.70 ± 0.04	0.76	2.58	1.79 ± 0.03	0.84	2.73
PC ae C38:2	1.27 ± 0.04	<0.01	2.70	1.46 ± 0.05	0.06	3.11
PC ae C38:3	6.47 ± 0.18	2.29	11.02	7.25 ± 0.12	3.50	11.62
PC ae C38:4	11.86 ± 0.31	4.63	21.95	12.49 ± 0.25	5.68	21.04
PC ae C38:5	14.60 ± 0.39	5.51	24.41	14.44 ± 0.32	7.17	21.68
PC ae C38:6	5.55 ± 0.15	1.95	8.73	5.45 ± 0.12	2.67	8.08
PC ae C40:1	1.14 ± 0.02	0.48	1.80	1.17 ± 0.02	0.52	2.08
PC ae C40:2	1.85 ± 0.05	0.70	2.84	2.01 ± 0.03	0.80	2.96
PC ae C40:3	1.49 ± 0.04	0.63	2.17	1.58 ± 0.03	0.79	2.15
PC ae C40:4	2.17 ± 0.06	0.90	4.30	2.22 ± 0.04	0.99	3.85
PC ae C40:5	3.66 ± 0.09	1.39	6.31	3.80 ± 0.07	1.76	6.03
PC ae C40:6	3.58 ± 0.09	1.33	5.81	3.74 ± 0.08	1.64	5.65
PC ae C42:0	0.60 ± 0.01	0.35	0.94	0.59 ± 0.01	0.37	0.81
PC ae C42:1	0.42 ± 0.01	0.20	0.77	0.41 ± 0.01	0.18	0.67
PC ae C42:2	0.58 ± 0.01	0.24	1.01	0.59 ± 0.01	0.26	0.93
PC ae C42:3	0.79 ± 0.02	0.31	1.26	0.79 ± 0.01	0.36	1.11
PC ae C42:4	0.79 ± 0.02	0.28	1.54	0.79 ± 0.02	0.29	1.31
PC ae C42:5	1.87 ± 0.04	0.80	3.37	1.86 ± 0.03	0.87	2.95
PC ae C44:3	0.16 ± <0.01	0.07	0.26	0.16 ± <0.01	0.08	0.28
PC ae C44:4	0.38 ± 0.01	0.15	0.69	0.38 ± 0.01	0.17	0.68
PC ae C44:5	1.66 ± 0.04	0.58	3.28	1.65 ± 0.03	0.62	2.79
PC ae C44:6	0.96 ± 0.03	0.41	1.74	0.94 ± 0.02	0.35	1.55
Sphingolipids						
SM (OH) C14:1	7.24 ± 0.16	3.68	10.92	7.68 ± 0.12	4.01	11.37
SM C16:0	164.93 ± 3.65	60.57	269.55	168.18 ± 3.14	101.67	254.46
SM C16:1	22.45 ± 0.52	9.02	41.84	22.66 ± 0.46	13.95	43.16
SM (OH) C16:1	4.77 ± 0.11	2.13	7.24	5.18 ± 0.08	2.63	7.27
SM C18:0	38.32 ± 0.84	16.49	57.68	39.81 ± 0.69	22.72	57.95
SM C18:1	16.37 ± 0.38	7.00	29.58	16.97 ± 0.33	9.02	30.58
SM C20:2	0.61 ± 0.01	0.20	1.15	0.65 ± 0.01	0.22	1.15
SM (OH) C22:1	40.14 ± 0.84	15.04	76.25	41.70 ± 0.70	21.80	69.74

SM (OH) C22:2	43.56 ± 1.00	19.92	74.55	45.42 ± 0.84	25.70	68.87
SM C24:0	43.05 ± 0.89	13.15	86.96	43.33 ± 0.80	20.57	79.09
SM C24:1	192.64 ± 4.12	62.24	331.10	194.20 ± 3.83	117.26	297.09
SM (OH) C24:1	2.06 ± 0.04	0.66	3.59	2.15 ± 0.03	1.07	3.56
SM C26:0	0.32 ± 0.01	0.10	0.53	0.32 ± 0.01	0.15	0.54
SM C26:1	0.62 ± 0.01	0.19	1.01	0.64 ± 0.01	0.35	1.01

Metabolites in bold (n = 41) were significantly increased during sleep deprivation compared with during the sleep period ($p < 0.05$, $q < 0.05$).

Table S8: Correlations between metabolites common to untargeted and targeted analysis.

Metabolite	Pearson r	R squared	P value	P value summary
Leucine	0.345	0.119	< 0.0001	****
Methionine	0.431	0.186	< 0.0001	****
Phenylalanine	0.474	0.225	< 0.0001	****
Proline	0.577	0.333	< 0.0001	****
Tryptophan	0.513	0.263	< 0.0001	****
Tyrosine	0.462	0.214	< 0.0001	****
Acetylcarnitine (C2)	0.596	0.355	< 0.0001	****
Propionylcarnitine (C3)	0.287	0.083	< 0.0001	****
Butyrylcarnitine (C4)	0.445	0.198	< 0.0001	****
Octanoylcarnitine (C8)	0.832	0.693	< 0.0001	****
Decanoylcarnitine (C10)	0.875	0.766	< 0.0001	****
Dodecanoylcarnitine (C12)	0.806	0.650	< 0.0001	****
Tetradecanoylcarnitine (C14)	0.573	0.329	< 0.0001	****
Tetradecenoylcarnitine (C14:1)	0.517	0.267	< 0.0001	****
Tetradecadienylcarnitine (C14:2)	0.764	0.583	< 0.0001	****
Hexadecanoylcarnitine (C16)	0.512	0.262	< 0.0001	****
Octadecanoylcarnitine (C18)	0.346	0.120	< 0.0001	****
Octadecenoylcarnitine (C18:1)	0.516	0.266	< 0.0001	****
LysoPC a C18:2	0.540	0.292	< 0.0001	****
LysoPC a C20:3	0.199	0.039	0.0005	***