PNAS
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**Supplementary Information for**

# "Reshaping Circadian Metabolism in the Suprachiasmatic Nucleus and Prefrontal Cortex by Nutritional Challenge"

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www.pnas.org/cgi/doi/10.1073/pnas.2016589117

## Supplementary Information

## Material and Methods

### Platform for Metabolomic Profile

The metabolome analysis was performed by Metabolon Inc. (617 Davis Drive, Suite 400, Durham, NC 27713) using the samples reported in (1), as explained below:

**Sample Preparation:** Samples were prepared using the automated MicroLab STAR® system from Hamilton Company. Several recovery standards were added prior to the first step in the extraction process for QC purposes. To remove protein, dissociate small molecules bound to protein or trapped in the precipitated protein matrix, and to recover chemically diverse metabolites, proteins were precipitated with methanol under vigorous shaking for 2 min (Glen Mills GenoGrinder 2000) followed by centrifugation. The resulting extract was divided into five fractions: two for analysis by two separate reverse phase (RP)/UPLC-MS/MS methods with positive ion mode electrospray ionization (ESI), one for analysis by RP/UPLC-MS/MS with negative ion mode ESI, one for analysis by HILIC/UPLC-MS/MS with negative ion mode ESI, and one sample was reserved for backup. Samples were placed briefly on a TurboVap® (Zymark) to remove the organic solvent. The sample extracts were stored overnight under nitrogen before preparation for analysis.

**UPLC-MS/MS:** All methods utilized a Waters ACQUITY ultra-performance liquid chromatography (UPLC) and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. The sample extract was dried then reconstituted in solvents compatible to each of the four methods. Each reconstitution solvent contained a series of standards at fixed concentrations to ensure injection and chromatographic consistency. One aliquot was analyzed using acidic positive ion conditions, chromatographically optimized for more hydrophilic compounds. In this method, the extract was gradient eluted from a C18 column (Waters UPLC BEH C18-2.1x100 mm, 1.7 µm) using water and methanol, containing 0.05% perfluoropentanoic acid (PFPA) and 0.1% formic acid (FA). Another aliquot was also analyzed using acidic positive ion conditions, however it was chromatographically optimized for more hydrophobic compounds. In this method, the extract was gradient eluted from the same afore mentioned C18 column using methanol, acetonitrile, water, 0.05% PFPA and 0.01% FA and was operated at an overall higher organic content. Another aliquot was analyzed using basic negative ion optimized conditions using a separate dedicated C18 column. The basic extracts were gradient eluted from the column using methanol and water, however with 6.5mM Ammonium Bicarbonate at pH 8. The fourth aliquot was analyzed via negative ionization following elution from a HILIC column (Waters UPLC BEH Amide 2.1x150 mm,

1.7 µm) using a gradient consisting of water and acetonitrile with 10mM Ammonium Formate, pH 10.8. The MS analysis alternated between MS and data-dependent MSn scans using dynamic exclusion. The scan range varied slighted between methods but covered 70-1000 m/z.

**Statistical Analysis of the Metabolome experiment**

The data are represented as mean ± Standard deviation. For the analysis of circadian rhythmicity the nonparametric test JTK_CYCLE (2) was used and results were corroborated with BIO_CYCLE, an improved circadian oscillation detection method based on a neural network approach (3). A threshold of $p < 0.05$ was used to identify significantly circadian metabolites. Amplitude, phase and period predicted by JTK_CYCLE were further analyzed. Distributions of phase and amplitude of circadian metabolites were compared done using 'sci-kit learn' and 'pandas' in python. Amplitude distribution of oscillating metabolites was analyzed by Mann-Whitney test. Evaluation of phase lag between conditions was carried out by Wilcoxon Signed Rank Test. Heat maps of the rhythmic expressions were generated by R package 'gplots'.

To check differences among the different levels of metabolites between the two diets at specific time points a two-way Student's t-test with Bayesian regularization was performed with CyberT (4, 5).

Phase lag analysis was performed based on the "LAG" (predicted phase) obtained by JTK-CYCLE for every single metabolite. The metabolites with the same "LAG" were grouped together and the data reported in the radar plot as percentage. Anderson-Darling tests were performed using the R package 'kSamples' to determine whether or not the phase distributions of NC and HFD circadian metabolites were statistically different ($p<0.0001$).

**RNA extraction and library preparation**

SCN total RNA was extracted from SCN-enriched region of the brain using RNeasy Micro Kit (QIAGEN). Pre-frontal cortex total RNA from one brain side was extracted with TRIzol reagent, according to the manufacturer's protocol (Invitrogen). RNA quality and concentration were evaluated with Nanodrop spectrophotometer (Fisher scientific). One microgram of total RNA was reverse-transcribed with iScript (Biorad) and random hexamers, according to the manufacturer's protocol. PCR reactions were carried out using Biorad qPCR system, from 2.5 µl of cDNA diluted 1:100, 5 µl 2x BIORAD SYBR-GREEN master mix solution (Biorad) and 0.6 µl of primer mix at 300 nM in a total volume of 10 µl.

***Primer sequences used for qPCR***

| Genes | Forward Primers 5'-3' | Reverse Primers 5'-3' |
|---|---|---|
| *Nr3c1* | GTGGAAGGACAGCACAATTACCT | GCGGCATGCTGGACAGTT |
| *Nr3c2* | GCCGTGGAAGGACAACACA | CCTAAGTTCATGCCGGCTTG |
| *Arntl* | GCAGTGCCACTGACTACCAAGA | TCCTGGACATTGCATTGCAT |
| *Per2* | CGCCTAGAATCCCTCCTGAGA | CCACCGGCCTGTAGGATCT |
| *Sgk1* | CGTCAAAGCCGAGGCTGCTCGAAGC | GGTTTGGCGTGAGGGTTGGAGGAC |
| *Bdnf* | CACAATGTTCCACCAGGTGAGA | GCCTTCATGCAACCGAAGT |
| *Fkbp1a* | TCCTCTCGGGACAGAAACAAGC | AGTTTGGCTCTCTGACCCACAC |
| *Per1* | ACCAGCGTGTCATGATGACATA | GTGCACAGCACCCAGTTCCC |
| *18s* | CGCCGCTAGAGGTGAAATTC | CGAACCTCCGACTTTCGTTCT |

For RNA-sequencing, total RNA was monitored for quality control using the Agilent Bioanalyzer Nano RNA chip and Nanodrop absorbance ratios for 260/280 nm and 260/230 nm. Library construction was performed according to the Illumina TruSeq mRNA stranded protocol. The resulting libraries were validated by qPCR and sized by Agilent Bioanalyzer DNA high sensitivity chip. The samples were sequenced on an Illumina HiSeq 2500 instrument during two single-end 100 cycles sequencing runs by the Genomics High-Throughput Facility at the University of California, Irvine. For details see (6).

**Transcriptome oscillation analysis**

The transcriptomic dataset, generated from the above analysis, containing genes and their expression levels was then run through JTK_CYCLE to determine oscillation period, amplitude, phase, and p-value of each gene in both the high-fat and normal chow conditions (2). This analysis for the transcriptomic data is publicly available on the CircadiOmics web portal. The output from JTK_CYCLE was then analyzed using pipelines implemented for the CircadiOmics database and web portal (7-9). Running these pipelines produces Venn-diagrams of oscillating transcripts, heatmaps, phase graphs, and radar plots.

**Functional TFBS Enrichment Analysis**

The functional enrichment analysis was performed by querying Pathway Commons (10) and ConsensusPathDB (11). From these sources, the pathway hits were counted and compared to a background hit counts and a Fisher's exact test was performed to establish enrichment. To determine binding sites, experimental CHiP Seq data and MotifMap results, for the mouse genome build mm9 (BBLS > 1, FDR < 0.25), were used. Transcription factors binding sites were analyzed in the promoter regions (−10000, +2000 base pairs of the

transcription start site) of circadian genes. A Fisher's exact test was also performed between the circadian genes and the whole genome to establish enrichment.

**Correlation Analysis between Circadian SCN Transcriptome and Metabolome**

The correlation analysis between transcriptome and metabolome was done by performing a pairwise analysis of enzymes and metabolites oscillating with a p-value < 0.05 to determine interactions. For each enzyme-metabolite pair, a score representative of possible interaction was calculated. The calculation of this score takes into consideration data from databases such as KEGG (10-12), the number of occurrences in literature of that enzyme-metabolite pair, and evidence of interaction from mass spectrometry experiments. The outputted interaction score is proportional to the likelihood of interaction between the enzyme and metabolite. Upon completion of the pairwise analysis, the output was filtered to only include scores from the 50th percentile and above. The pairs of enzymes and metabolites with interactions scores in the 50th percentile and above were then analysis to determine what pathways they had in common. In addition to this analysis, the list of enzymes was considered alone and analyzed using the Gene Ontology Consortium (http://geneontology.org/) to obtain functional enrichment pathway analysis.

The above analysis was also performed on a subset of this data where instead of considering all enzymes and metabolites oscillating with p-value < 0.05, enzyme metabolite pairs were only considered if the enzymes and metabolites oscillating with p-value < 0.05 and the lag time between the enzyme and metabolite was within +/-2 hours of each other.

*Fig. S1*

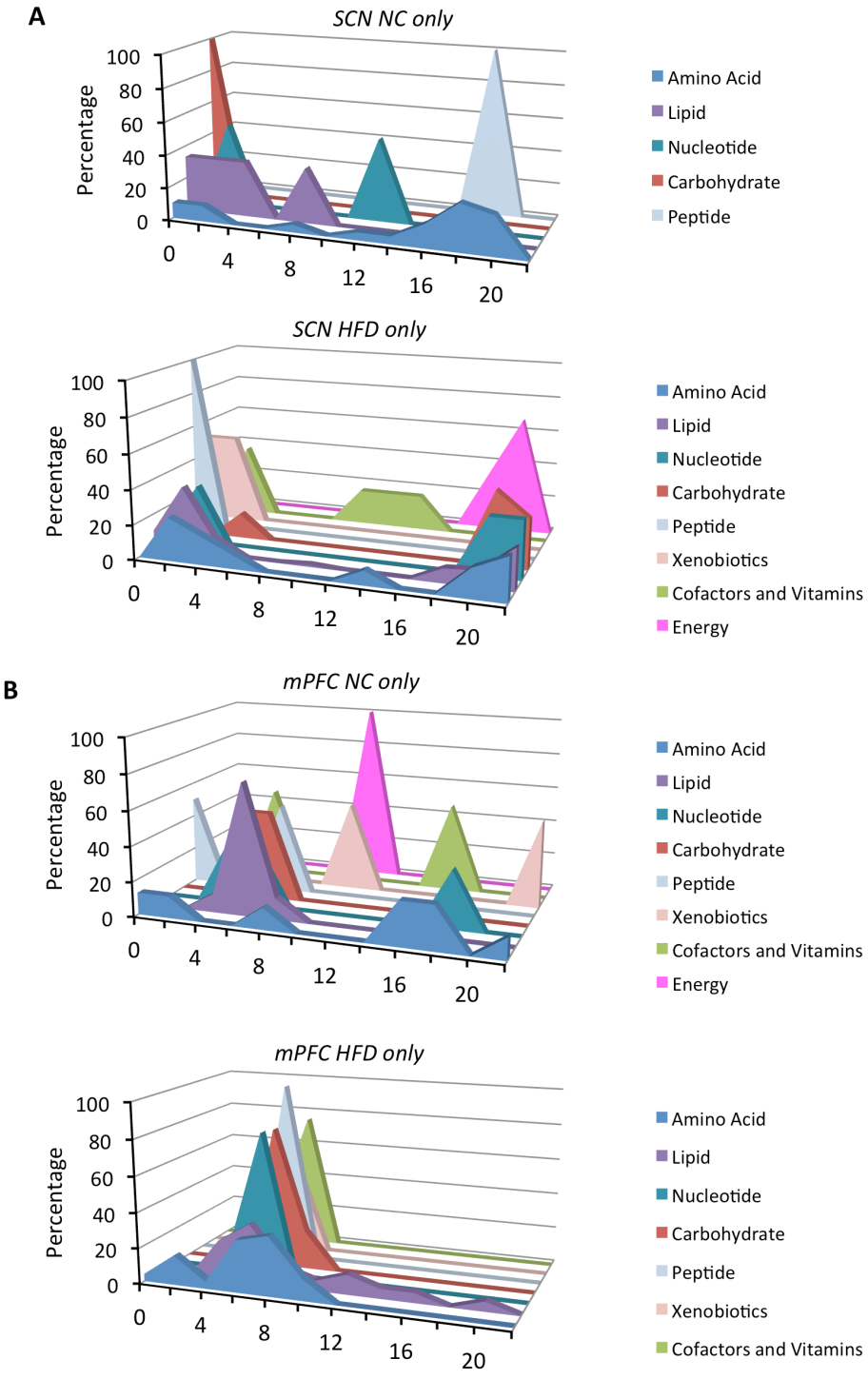


**Fig. S1. Metabolic super-pathway phase distribution in SCN and mPFC.**

Metabolic landscape showing the percentage of oscillatory metabolites peaking at specific ZTs in "NC or HFD condition only" compared to the total number of oscillatory metabolites in that metabolic pathway. The SCN is displayed in (A) and the mPFC in (B).

*Fig. S2*

## *SCN Circadian metabolites: "Sub-Pathway" analysis*

**A**

| Subpathway SCN NC_only | # |
|---|---|
| Leucine, Isoleucine and Valine Metabolism | 5 |
| Methionine, Cysteine, SAM and Taurine Metabolism | 4 |
| Glutamate Metabolism | 3 |
| Glycine, Serine and Threonine Metabolism | 3 |
| Urea cycle; Arginine and Proline Metabolism | 3 |
| Phospholipid Metabolism | 2 |
| Pyrimidine Metabolism, Cytidine containing | 2 |
| Alanine and Aspartate Metabolism | 1 |
| Endocannabinoid | 1 |
| Gamma-glutamyl Amino Acid | 1 |
| Glutathione Metabolism | 1 |
| Pentose Phosphate Pathway | 1 |
| Phenylalanine and Tyrosine Metabolism | 1 |
| Sphingolipid Metabolism | 1 |

**B**

| Subpathway SCN_Both | # |
|---|---|
| Diacylglycerol | 3 |
| Histidine Metabolism | 3 |
| Lysine Metabolism | 2 |
| Methionine, Cysteine, SAM and Taurine Metabolism | 2 |
| Creatine Metabolism | 1 |
| Fatty Acid Metabolism(Acyl Carnitine) | 1 |
| Fatty Acid, Dicarboxylate | 1 |
| Glutamate Metabolism | 1 |
| Glutathione Metabolism | 1 |
| Nicotinate and Nicotinamide Metabolism | 1 |
| Phenylalanine and Tyrosine Metabolism | 1 |
| Pyrimidine Metabolism, Uracil containing | 1 |
| Sphingolipid Metabolism | 1 |
| Urea cycle; Arginine and Proline Metabolism | 1 |

**C**

| Subpathway SCN HFD_only | # |
|---|---|
| Sphingolipid Metabolism | 21 |
| Phospholipid Metabolism | 20 |
| Diacylglycerol | 14 |
| Fatty Acid Metabolism(Acyl Carnitine) | 13 |
| Lysolipid | 7 |
| Plasmalogen | 6 |
| Lysine Metabolism | 4 |
| Lysoplasmalogen | 4 |
| Glycolysis, Gluconeogenesis, and Pyruvate Metabolism | 3 |
| Polyamine Metabolism | 3 |
| TCA Cycle | 3 |
| Aminosugar Metabolism | 2 |
| Leucine, Isoleucine and Valine Metabolism | 2 |
| Nicotinate and Nicotinamide Metabolism | 2 |
| Nucleotide Sugar | 2 |
| Phosphatidylserine (PS) | 2 |
| Polyunsaturated Fatty Acid (n3 and n6) | 2 |
| Vitamin B6 Metabolism | 2 |
| Alanine and Aspartate Metabolism | 1 |
| Carnitine Metabolism | 1 |
| Ceramides | 1 |
| Chemical | 1 |
| Endocannabinoid | 1 |
| Food Component/Plant | 1 |
| Gamma-glutamyl Amino Acid | 1 |
| Glycerolipid Metabolism | 1 |
| Long Chain Fatty Acid | 1 |
| Methionine, Cysteine, SAM and Taurine Metabolism | 1 |
| Mevalonate Metabolism | 1 |
| Purine and Pyrimidine Metabolism | 1 |
| Purine Metabolism, Guanine containing | 1 |
| Pyrimidine Metabolism, Orotate containing | 1 |
| Sterol | 1 |
| Tocopherol Metabolism | 1 |
| Urea cycle; Arginine and Proline Metabolism | 1 |

**Fig. S2. Metabolic sub-pathway analysis of metabolites oscillating in the SCN.**

(A) Table of the metabolic pathways oscillating in "NC only" in the SCN. The number of metabolites with a circadian profile for each specific pathway is represented on the right column (#).

(B) Table of the metabolic pathways oscillating in “both conditions” in the SCN. The number of metabolites with a circadian profile for each specific pathway is represented on the right column (#).
(C) Table of the metabolic pathways oscillating in “HFD only” in the SCN. The number of metabolites with a circadian profile for each specific pathway is represented on the right column (#).

*Fig. S3*

## *mPFC Circadian metabolites: "Sub-Pathway" analysis*

**A**

| Subpathway mPFC NC_only | # |
|---|---|
| Phospholipid Metabolism | 25 |
| Sphingolipid Metabolism | 13 |
| Diacylglycerol | 8 |
| Lysolipid | 6 |
| Fatty Acid Metabolism(Acyl Carnitine) | 3 |
| Plasmalogen | 3 |
| Ceramides | 2 |
| Gamma-glutamyl Amino Acid | 2 |
| Glycolysis, Gluconeogenesis, and Pyruvate Metabolism | 2 |
| Methionine, Cysteine, SAM and Taurine Metabolism | 2 |
| Urea cycle; Arginine and Proline Metabolism | 2 |
| Chemical | 1 |
| Food Component/Plant | 1 |
| Glutamate Metabolism | 1 |
| Glutathione Metabolism | 1 |
| Hemoglobin and Porphyrin Metabolism | 1 |
| Leucine, Isoleucine and Valine Metabolism | 1 |
| Long Chain Fatty Acid | 1 |
| Lysoplasmalogen | 1 |
| Monoacylglycerol | 1 |
| Pentose Phosphate Pathway | 1 |
| Phenylalanine and Tyrosine Metabolism | 1 |
| Phosphatidylserine (PS) | 1 |
| Purine Metabolism, Adenine containing | 1 |
| Purine Metabolism, Guanine containing | 1 |
| Pyrimidine Metabolism, Uracil containing | 1 |
| Sterol | 1 |
| TCA Cycle | 1 |
| Vitamin B6 Metabolism | 1 |

**B**

| Subpathway mPFC_Both | # |
|---|---|
| Aminosugar Metabolism | 1 |
| Diacylglycerol | 4 |
| Fatty Acid, Dicarboxylate | 1 |
| Glycolysis, Gluconeogenesis, and Pyruvate Metabolism | 1 |
| Hemoglobin and Porphyrin Metabolism | 1 |
| Histidine Metabolism | 4 |
| Lysine Metabolism | 2 |
| Lysolipid | 1 |
| Nicotinate and Nicotinamide Metabolism | 2 |
| Pentose Phosphate Pathway | 1 |
| Polyamine Metabolism | 1 |
| Purine Metabolism, (Hypo)Xanthine/Inosine containing | 1 |
| Pyrimidine Metabolism, Thymine containing | 1 |
| Pyrimidine Metabolism, Uracil containing | 1 |
| Sphingolipid Metabolism | 1 |
| Urea cycle; Arginine and Proline Metabolism | 1 |

**C**

| Subpathway mPFC HFD_only | # |
|---|---|
| Phospholipid Metabolism | 6 |
| Lysine Metabolism | 4 |
| Nucleotide Sugar | 4 |
| Fructose, Mannose and Galactose Metabolism | 3 |
| Glycine, Serine and Threonine Metabolism | 3 |
| Glycolysis, Gluconeogenesis, and Pyruvate Metabolism | 3 |
| Histidine Metabolism | 3 |
| Methionine, Cysteine, SAM and Taurine Metabolism | 3 |
| Urea cycle; Arginine and Proline Metabolism | 3 |
| Aminosugar Metabolism | 2 |
| Ascorbate and Aldarate Metabolism | 2 |
| Lysolipid | 2 |
| Pantothenate and CoA Metabolism | 2 |
| Pentose Metabolism | 2 |
| Polyamine Metabolism | 2 |
| Pyrimidine Metabolism, Orotate containing | 2 |
| Tryptophan Metabolism | 2 |
| Alanine and Aspartate Metabolism | 1 |
| Chemical | 1 |
| Dipeptide | 1 |
| Endocannabinoid | 1 |
| Fatty Acid Synthesis | 1 |
| Fatty Acid, Dicarboxylate | 1 |
| Fatty Acid, Monohydroxy | 1 |
| Food Component/Plant | 1 |
| Gamma-glutamyl Amino Acid | 1 |
| Glutamate Metabolism | 1 |
| Glutathione Metabolism | 1 |
| Glycerolipid Metabolism | 1 |
| Inositol Metabolism | 1 |
| Mevalonate Metabolism | 1 |
| Monoacylglycerol | 1 |
| Phenylalanine and Tyrosine Metabolism | 1 |
| Plasmalogen | 1 |
| Polyunsaturated Fatty Acid (n3 and n6) | 1 |
| Purine Metabolism, (Hypo)Xanthine/ Inosine containing | 1 |
| Purine Metabolism, Adenine containing | 1 |
| Pyrimidine Metabolism, Cytidine containing | 1 |

**Fig. S3. Metabolic sub-pathway analysis of metabolites oscillating in the mPFC.**

(A) Table of the metabolic pathways oscillating in "NC only" in the mPFC. The number of

metabolites with a circadian profile for each specific pathway is represented on the right column (#).

(B) Table of the metabolic pathways oscillating in “both conditions” in the mPFC. The number of metabolites with a circadian profile for each specific pathway is represented on the right column (#).

(C) Table of the metabolic pathways oscillating in “HFD only” in the mPFC. The number of metabolites with a circadian profile for each specific pathway is represented on the right column (#).

In the main text the most represented sub-pathways (based on the number of metabolites) are reported as example of specific pathway enrichment in the oscillatory metabolome of the SCN and mPFC.

*Fig. S4*

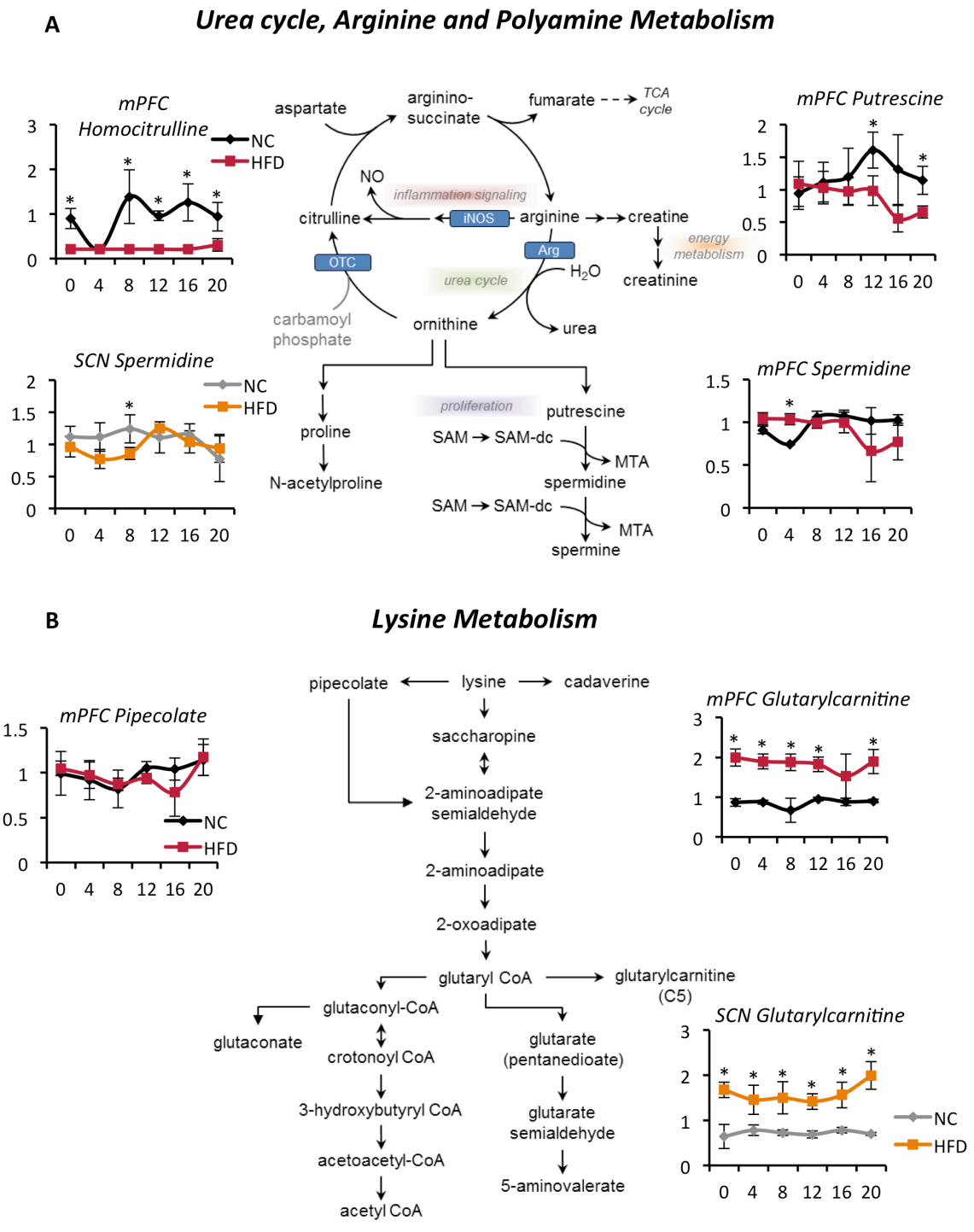


**Fig. S4. Daily profile of metabolites belonging to pathways impacted in SCN and mPFC in a similar manner upon nutritional challenge.**

(A) "Urea Cycle, Arginine and Polyamines Metabolism" chart. mPFC Homocitrulline, Sperimidine and Putresceine and SCN Spermidine are shown (n=3-5 per time-point, per group, CyberT test *p<0.05).

(B) “Lysine Metabolism” chart. mPFC Glutarate, Glutarylcarnitine and SCN Glutarylcarnitine are shown (n=3-5 per time-point, per group, CyberT test *p<0.05).
Error bars represent Standard Deviation.

*Fig. S5*

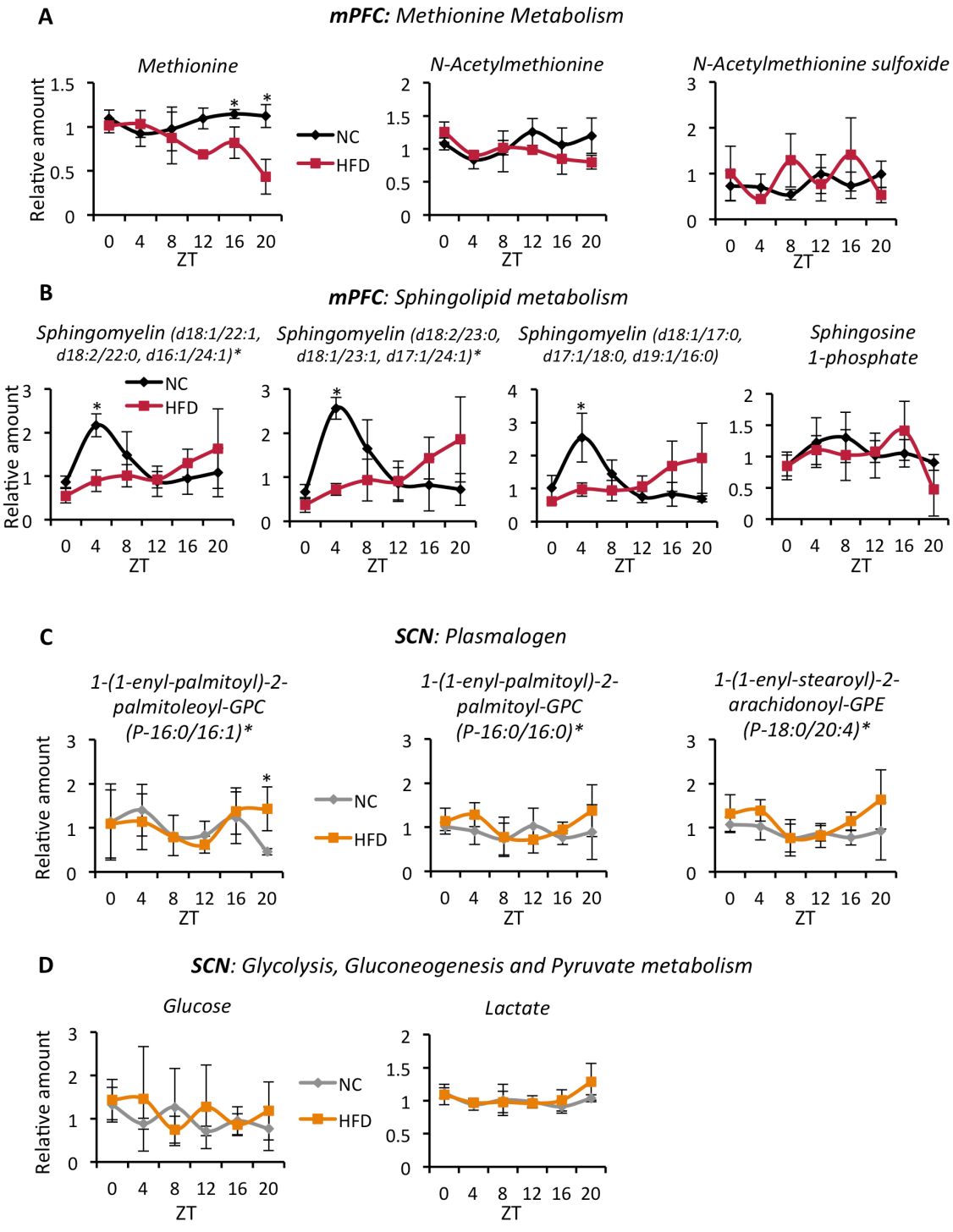


**Fig. S5. Daily profile of metabolites belonging to metabolic pathways oscillating exclusively in SCN or mPFC upon HFD.**

(A) Metabolites belonging to the “Methionine Metabolic Pathway” in the mPFC are displayed. These metabolites were circadian exclusively in NC SCN and were not rhythmic in the mPFC (n=3-5 per time-point, per group, CyberT test *p<0.05).
(B) mPFC “Sphingolipids” are reported in the graph. These metabolites gained a daily oscillation in the SCN upon HFD but not in the mPFC (n=3-5 per time-point, per group, CyberT test *p<0.05).
(C) “Plasmalogen” profile in the SCN. These specific types of lipid showed rhythmicity exclusively in NC mPFC but not in the SCN (n=5 per time-point, per group, CyberT test *p<0.05).
(D) Glucose and Lactate levels throughout the circadian cycle in the SCN did not show any rhythmicity (n=5 per time-point, per group).
Error bars represent Standard Deviation.

*Fig. S6*

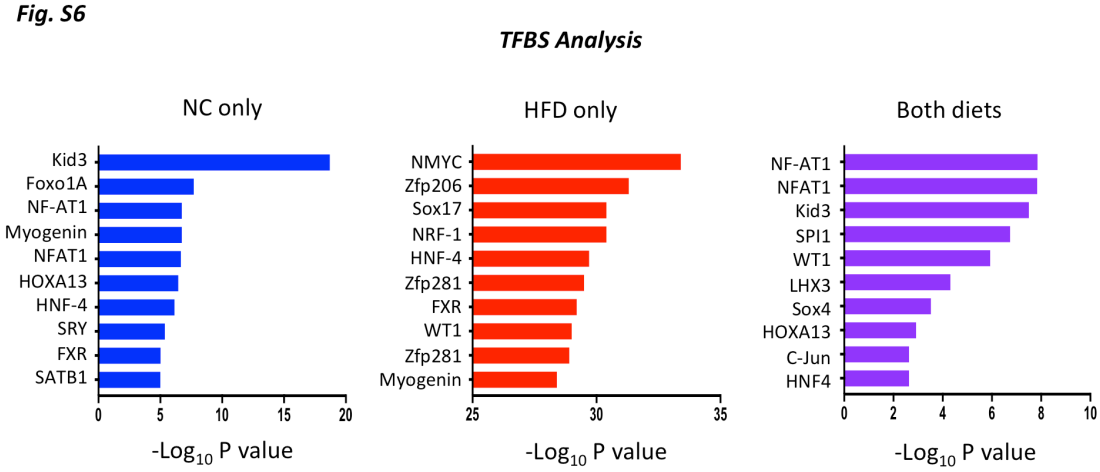


**Fig. S6. Transcription factor binding site analysis (TFBS) of the SCN circadian transcriptome.**
The analysis showed specific differences in the putative transcription factor binding sites present in the promoter region of the oscillatory transcripts upon the diverse nutritional conditions.

*Fig. S7*

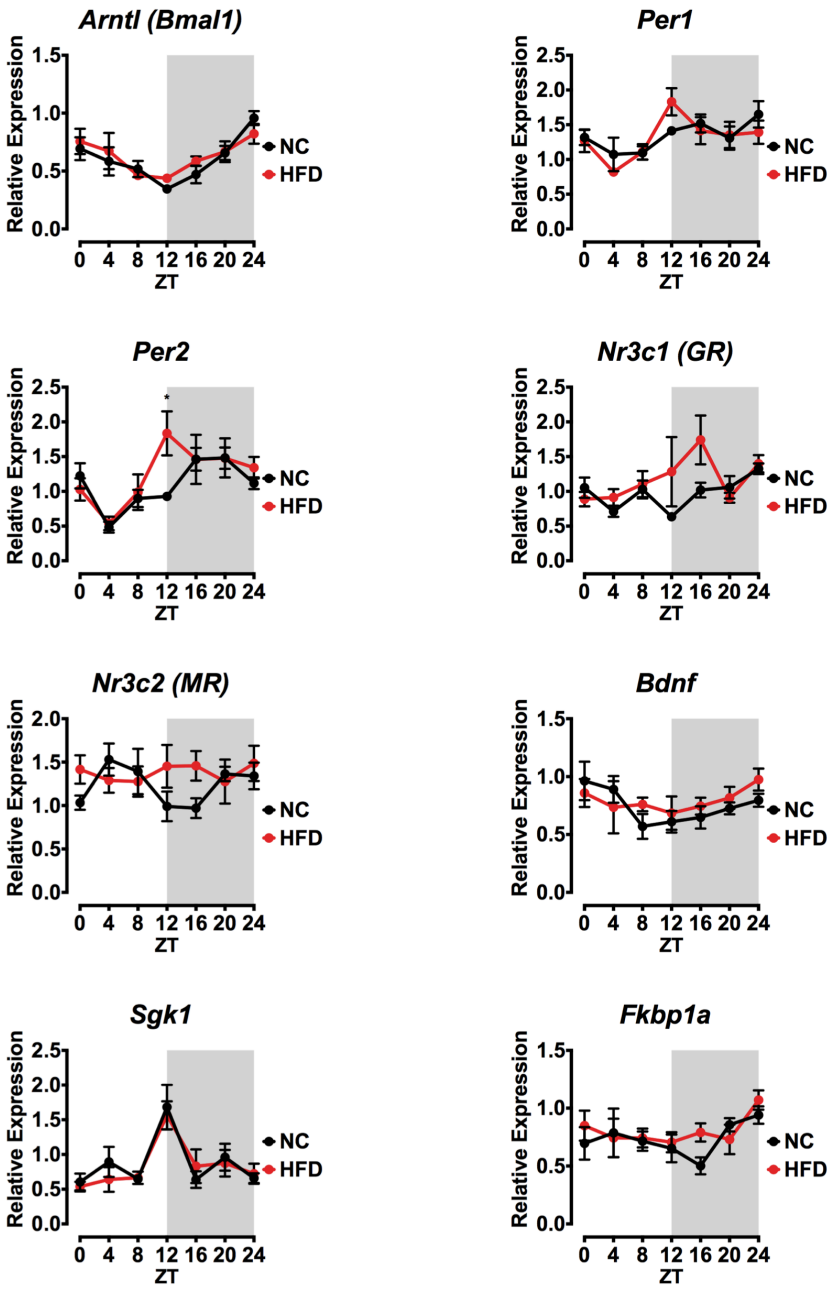


**Fig. S7. Gene expression analysis in the mPFC.**
Diurnal qPCR analysis of specific genes in the mPFC of mice fed to NC or HFD for ten weeks (N=5 animals per time point per group).
The results showed a limited effect of HFD on core-clock genes oscillation. No significant impact on the oscillation of genes implicated in brain stress reactions was observed upon HFD feeding. (Two-way ANOVA, *Per2:* interaction $p<0.05$, Post-hoc Holm-Sidak NC vs HFD, ZT12 $P<0.05$; all the other genes: no significant differences).
Error bars represent SEM.

**Table S1.**
Crossing of SCN and mPFC metabolites oscillating in "NC only" (JTK_CYCLE p<0.05)

| Metabolite Name | SCN | mPFC |
|---|---|---|
| 6-phosphogluconate | x | x |
| glutamate | x | x |
| methionine sulfoxide | x | x |
| urea | x | x |
| 1,2-dioleoyl-GPE (18:1/18:1) | | x |
| 1,2-dioleoyl-GPS (18:1/18:1) | | x |
| 1,2-dipalmitoyl-GPG (16:0/16:0) | | x |
| 1-(1-enyl-palmitoyl)-2-palmitoleoyl-GPC (P-16:0/16:1)* | | x |
| 1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/16:0)* | | x |
| 1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4)* | | x |
| 1-(1-enyl-stearoyl)-GPE (P-18:0)* | | x |
| 1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4n6)* | | x |
| 1-linoleoyl-2-arachidonoyl-GPE (18:2/20:4)* | | x |
| 1-linoleoyl-GPC (18:2) | | x |
| 1-oleoyl-2-arachidonoyl-GPE (18:1/20:4)* | | x |
| 1-oleoyl-2-arachidonoyl-GPI (18:1/20:4) * | | x |
| 1-oleoyl-2-linoleoyl-GPC (18:1/18:2)* | | x |
| 1-oleoyl-GPE (18:1) | | x |
| 1-palmitoyl-2-arachidonoyl-GPC (16:0/20:4n6) | | x |
| 1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)* | | x |
| 1-palmitoyl-2-arachidonoyl-GPI (16:0/20:4)* | | x |
| 1-palmitoyl-2-gamma-linolenoyl-GPC (16:0/18:3n6)* | | x |
| 1-palmitoyl-2-linoleoyl-GPC (16:0/18:2) | | x |

| | | |
|---|---|---|
| 1-palmitoyl-2-linoleoyl-GPE (16:0/18:2) | | **x** |
| 1-palmitoyl-2-oleoyl-GPC (16:0/18:1) | | **x** |
| 1-palmitoyl-2-oleoyl-GPE (16:0/18:1) | | **x** |
| 1-palmitoyl-2-oleoyl-GPG (16:0/18:1) | | **x** |
| 1-palmitoyl-2-oleoyl-GPS (16:0/18:1) | | **x** |
| 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1)* | | **x** |
| 1-palmitoyl-2-stearoyl-GPC (16:0/18:0) | | **x** |
| 1-palmitoyl-GPC (16:0) | | **x** |
| 1-palmitoyl-GPE (16:0) | | **x** |
| 1-stearoyl-2-arachidonoyl-GPC (18:0/20:4) | | **x** |
| 1-stearoyl-2-arachidonoyl-GPE (18:0/20:4) | | **x** |
| 1-stearoyl-2-arachidonoyl-GPI (18:0/20:4) | | **x** |
| 1-stearoyl-2-arachidonoyl-GPS (18:0/20:4) | | **x** |
| 1-stearoyl-2-oleoyl-GPC (18:0/18:1) | | **x** |
| 1-stearoyl-2-oleoyl-GPE (18:0/18:1) | | **x** |
| 1-stearoyl-GPE (18:0) | | **x** |
| 1-stearoyl-GPS (18:0)* | | **x** |
| 2-hydroxybutyrate/2-hydroxyisobutyrate | | **x** |
| 2-linoleoylglycerol (18:2) | | **x** |
| 3-hydroxyisobutyrate | | **x** |
| 3-phosphoglycerate | | **x** |
| 3-ureidopropionate | | **x** |
| 4-cholesten-3-one | | **x** |
| N-acetyltyrosine | | **x** |
| N-stearoyl-sphingosine (d18:1/18:0)* | | **x** |
| S-(3-hydroxypropyl)mercapturic acid (HPMA) | | **x** |
| acetylcarnitine (C2) | | **x** |
| adenosine 3',5'-cyclic | | **x** |

| | | |
|---|---|---|
| monophosphate (cAMP) | | |
| behenoyl sphingomyelin (d18:1/22:0)* | | **x** |
| biliverdin | | **x** |
| ceramide (d18:2/24:1, d18:1/24:2)* | | **x** |
| citrate | | **x** |
| dihomo-linoleoylcarnitine (C20:2)* | | **x** |
| gamma-glutamylglutamine | | **x** |
| gamma-glutamylvaline | | **x** |
| gluconate | | **x** |
| glycosyl ceramide (d18:1/20:0, d16:1/22:0)* | | **x** |
| glycosyl-N-stearoyl-sphingosine (d18:1/18:0) | | **x** |
| guanosine 5'- diphosphate (GDP) | | **x** |
| linoleoylcarnitine (C18:2)* | | **x** |
| oleoyl-arachidonoyl-glycerol (18:1/20:4) [2]* | | **x** |
| oleoyl-linoleoyl-glycerol (18:1/18:2) [2] | | **x** |
| ophthalmate | | **x** |
| ornithine | | **x** |
| palmitoyl sphingomyelin (d18:1/16:0) | | **x** |
| palmitoyl-arachidonoyl-glycerol (16:0/20:4) [1]* | | **x** |
| palmitoyl-arachidonoyl-glycerol (16:0/20:4) [2]* | | **x** |
| palmitoyl-docosahexaenoyl-glycerol (16:0/22:6) [1]* | | **x** |
| palmitoyl-docosahexaenoyl-glycerol (16:0/22:6) [2]* | | **x** |
| palmitoyl-linoleoyl-glycerol (16:0/18:2) [2]* | | **x** |
| phosphoenolpyruvate (PEP) | | **x** |
| pyridoxamine | | **x** |
| sphingomyelin (d18:0/18:0, d19:0/17:0)* | | **x** |
| sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0) | | **x** |
| sphingomyelin (d18:1/18:1, d18:2/18:0) | | **x** |

| | | |
|---|---|---|
| sphingomyelin (d18:1/19:0, d19:1/18:0)* | | **x** |
| sphingomyelin (d18:1/20:0, d16:1/22:0)* | | **x** |
| sphingomyelin (d18:1/24:1, d18:2/24:0)* | | **x** |
| sphingomyelin (d18:2/16:0, d18:1/16:1)* | | **x** |
| sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)* | | **x** |
| sphingomyelin (d18:2/24:1, d18:1/24:2)* | | **x** |
| stearate (18:0) | | **x** |
| stearoyl-arachidonoyl-glycerol (18:0/20:4) [1]* | | **x** |
| 5-oxoproline | **x** | |
| N-acetylarginine | **x** | |
| N-acetylglutamate | **x** | |
| N-acetylmethionine | **x** | |
| N-acetylmethionine sulfoxide | **x** | |
| N-stearoyltaurine | **x** | |
| O-methyltyrosine | **x** | |
| alanine | **x** | |
| argininosuccinate | **x** | |
| betaine | **x** | |
| choline phosphate | **x** | |
| cytidine | **x** | |
| dimethylglycine | **x** | |
| gamma-glutamylthreonine | **x** | |
| glutamine | **x** | |
| isobutyrylcarnitine (C4) | **x** | |
| isoleucine | **x** | |
| leucine | **x** | |
| methionine | **x** | |
| threonine | **x** | |
| thymidine | **x** | |
| tiglylcarnitine (C5:1-DC) | **x** | |
| tricosanoyl sphingomyelin (d18:1/23:0)* | **x** | |
| trimethylamine N-oxide | **x** | |
| valine | **x** | |

**Table S2.**
Crossing of SCN and mPFC metabolites oscillating in "both diets" (JTK_CYCLE p<0.05)

| Metabolite Name | SCN | mPFC |
|---|---|---|
| 1-methylhistamine | x | x |
| 1-methylhistidine | x | x |
| 1-methylimidazoleacetate | x | x |
| 2'-deoxyuridine | x | x |
| 2-hydroxyadipate | x | x |
| linoleoyl-docosahexaenoyl-glycerol (18:2/22:6) [2]* | x | x |
| sphinganine | x | x |
| 1-oleoyl-GPC (18:1) | | |
| 3-methylhistidine | | x |
| Isobar: fructose 1,6-diphosphate, glucose 1,6-diphosphate, myo-inositol 1,4 or 1,3-diphosphate | | x |
| N1-Methyl-2-pyridone-5-carboxamide | | x |
| bilirubin (Z,Z) | | x |
| erythronate* | | x |
| homoarginine | | x |
| inosine 5'-monophosphate (IMP) | | x |
| lysine | | x |
| nicotinamide adenine dinucleotide reduced (NADH) | | x |
| pipecolate | | x |
| putrescine | | x |
| sedoheptulose-7-phosphate | | x |
| stearoyl-arachidonoyl-glycerol (18:0/20:4) [2]* | | x |
| stearoyl-docosahexaenoyl-glycerol (18:0/22:6) [1]* | | x |
| stearoyl-docosahexaenoyl-glycerol (18:0/22:6) [2]* | | x |
| thymidine | | x |
| 2-aminoadipate | x | |
| 2-hydroxybutyrate/2-hydroxyisobutyrate | x | |
| 3-(4-hydroxyphenyl)lactate | x | |
| 3-hydroxybutyrylcarnitine (2) | x | |

| | | |
|---|---|---|
| N1-Methyl-4-pyridone-3-carboxamide | **x** | |
| S-methylcysteine | **x** | |
| arginine | **x** | |
| glutamate, gamma-methyl ester | **x** | |
| glutarate (pentanedioate) | **x** | |
| guanidinoacetate | **x** | |
| ophthalmate | **x** | |
| palmitoleoyl-oleoyl-glycerol (16:1/18:1) [2]* | **x** | |
| palmitoyl-linoleoyl-glycerol (16:0/18:2) [2]* | **x** | |

**Table S3.**
Crossing of SCN and mPFC metabolites oscillating in “HFD only” (JTK_CYCLE p<0.05)

| Metabolite Name | SCN | mPFC |
|---|---|---|
| 3-hydroxy-3-methylglutarate | x | x |
| UDP-N-acetylglucosamine | x | x |
| UDP-galactose | x | x |
| glycerophosphoglycerol | x | x |
| glycerophosphoinositol* | x | x |
| orotate | x | x |
| saccharopine | x | x |
| spermidine | x | x |
| thioproline | x | x |
| 1,2-distearoyl-GPC (18:0/18:0) | | x |
| 1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (P-16:0/20:4)* | | x |
| 1-linoleoylglycerol (18:2) | | x |
| 1-palmitoleoyl-GPC (16:1)* | | x |
| 2'-deoxycytidine | | x |
| 2'-deoxyinosine | | x |
| 2-aminoadipate | | x |
| 2-hydroxyglutarate | | x |
| 2-hydroxypalmitate | | x |
| 2-methylmalonylcarnitine (C4-DC) | | x |
| 2-piperidinone | | x |
| 2-stearoyl-GPE (18:0)* | | x |
| 3'-dephosphocoenzyme A | | x |
| 5-oxoproline | | x |
| N-acetyl-1-methylhistidine* | | x |
| N-acetyl-glucosamine 1-phosphate | | x |
| N-acetylalanine | | x |
| N-acetylarginine | | x |
| N-acetylglucosamine 6-phosphate | | x |
| N-acetylhistidine | | x |
| N-acetylputrescine | | x |
| N-acetylserine | | x |
| N6-acetyllysine | | x |
| O-sulfo-L-tyrosine | | x |
| S-methylcysteine | | x |
| UDP-glucose | | x |
| adenosine 2'- | | x |

| | | |
|---|---|---|
| monophosphate (2'-AMP) | | |
| arabinose | | **x** |
| arachidonoyl ethanolamide | | **x** |
| arginine | | **x** |
| betaine | | **x** |
| choline | | **x** |
| cystathionine | | **x** |
| cytidine 5'-monophospho-N-acetylneuraminic acid | | **x** |
| cytidine-5'-diphosphoethanolamine | | **x** |
| dehydroascorbate | | **x** |
| dimethylarginine (SDMA + ADMA) | | **x** |
| dimethylglycine | | **x** |
| eicosapentaenoate (EPA; 20:5n3) | | **x** |
| galactitol (dulcitol) | | **x** |
| galactonate | | **x** |
| gamma-glutamylhistidine | | **x** |
| glucose | | **x** |
| glucose 6-phosphate | | **x** |
| glutamate, gamma-methyl ester | | **x** |
| glutarate (pentanedioate) | | **x** |
| glycerophosphoethanolamine | | **x** |
| gulonate* | | **x** |
| histidine | | **x** |
| lactate | | **x** |
| mannitol/sorbitol | | **x** |
| methionine | | **x** |
| myo-inositol | | **x** |
| orotidine | | **x** |
| p-cresol sulfate | | **x** |
| phosphopantetheine | | **x** |
| prolylglycine | | **x** |
| ribose | | **x** |
| serotonin | | **x** |
| trimethylamine N-oxide | | **x** |
| 1,2-dioleoyl-GPE (18:1/18:1) | **x** | |
| 1-(1-enyl-oleoyl)-2-oleoyl-GPE (P-18:1/18:1)* | **x** | |
| 1-(1-enyl-oleoyl)-GPE (P-18:1)* | **x** | |

| | | |
|---|---|---|
| 1-(1-enyl-palmitoyl)-2-linoleoyl-GPE (P-16:0/18:2)* | **x** | |
| 1-(1-enyl-palmitoyl)-2-oleoyl-GPC (P-16:0/18:1)* | **x** | |
| 1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1)* | **x** | |
| 1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/16:0)* | **x** | |
| 1-(1-enyl-palmitoyl)-GPE (P-16:0)* | **x** | |
| 1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4)* | **x** | |
| 1-(1-enyl-stearoyl)-2-oleoyl-GPE (P-18:0/18:1) | **x** | |
| 1-(1-enyl-stearoyl)-GPE (P-18:0)* | **x** | |
| 1-arachidonoyl-GPE (20:4n6)* | **x** | |
| 1-oleoyl-2-arachidonoyl-GPE (18:1/20:4)* | **x** | |
| 1-oleoyl-2-linoleoyl-GPE (18:1/18:2)* | **x** | |
| 1-oleoyl-GPC (18:1) | **x** | |
| 1-oleoyl-GPE (18:1) | **x** | |
| 1-palmitoyl-2-arachidonoyl-GPC (16:0/20:4n6) | **x** | |
| 1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)* | **x** | |
| 1-palmitoyl-2-arachidonoyl-GPI (16:0/20:4)* | **x** | |
| 1-palmitoyl-2-linoleoyl-GPC (16:0/18:2) | **x** | |
| 1-palmitoyl-2-linoleoyl-GPE (16:0/18:2) | **x** | |
| 1-palmitoyl-2-oleoyl-GPE (16:0/18:1) | **x** | |
| 1-palmitoyl-2-oleoyl-GPI (16:0/18:1)* | **x** | |
| 1-palmitoyl-2-oleoyl-GPS (16:0/18:1) | **x** | |
| 1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1)* | **x** | |
| 1-palmitoyl-2-stearoyl-GPC (16:0/18:0) | **x** | |

| | | |
|---|---|---|
| 1-palmitoyl-GPC (16:0) | **x** | |
| 1-stearoyl-2-arachidonoyl-GPC (18:0/20:4) | **x** | |
| 1-stearoyl-2-arachidonoyl-GPE (18:0/20:4) | **x** | |
| 1-stearoyl-2-arachidonoyl-GPI (18:0/20:4) | **x** | |
| 1-stearoyl-2-arachidonoyl-GPS (18:0/20:4) | **x** | |
| 1-stearoyl-2-oleoyl-GPC (18:0/18:1) | **x** | |
| 1-stearoyl-2-oleoyl-GPE (18:0/18:1) | **x** | |
| 1-stearoyl-2-oleoyl-GPS (18:0/18:1) | **x** | |
| 1-stearoyl-GPC (18:0) | **x** | |
| 1-stearoyl-GPE (18:0) | **x** | |
| 1-stearoyl-GPS (18:0)* | **x** | |
| 2-methylbutyrylcarnitine (C5) | **x** | |
| 3-hydroxyisobutyrate | **x** | |
| 3-sulfo-nervonoyl-galactosylceramide (d18:1/24:1) | **x** | |
| 4-acetamidobutanoate | **x** | |
| 5-(galactosylhydroxy)-L-lysine | **x** | |
| 5-methylthioadenosine (MTA) | **x** | |
| Isobar: fructose 1,6-diphosphate, glucose 1,6-diphosphate, myo-inositol 1,4 or 1,3-diphosphate | **x** | |
| N-acetylasparagine | **x** | |
| N-acetylneuraminate | **x** | |
| N-formylmethionine | **x** | |
| N-glycolylneuraminate | **x** | |
| N-stearoyl-sphingadienine (d18:2/18:0)* | **x** | |
| N-stearoyl-sphingosine (d18:1/18:0)* | **x** | |
| N1-Methyl-2-pyridone-5-carboxamide | **x** | |
| adrenoylcarnitine (C22:4)* | **x** | |
| alpha-tocopherol | **x** | |
| arachidonoylcarnitine (C20:4) | **x** | |
| carnitine | **x** | |

| | | |
|---|---|---|
| ceramide (d18:2/24:1, d18:1/24:2)* | **x** | |
| cholesterol | **x** | |
| dihomo-linoleate (20:2n6) | **x** | |
| dihomo-linolenoylcarnitine (20:3n3 or 6)* | **x** | |
| dihomo-linoleoylcarnitine (C20:2)* | **x** | |
| docosapentaenoate (n6 DPA; 22:5n6) | **x** | |
| eicosenoylcarnitine (C20:1)* | **x** | |
| erythronate* | **x** | |
| fumarate | **x** | |
| gamma-glutamylvaline | **x** | |
| glycerophosphoserine* | **x** | |
| glycosyl-N-(2-hydroxynervonoyl)-sphingosine (d18:1/24:1(2OH))* | **x** | |
| glycosyl-N-arachidoyl-sphingosine (d18:1/20:0)* | **x** | |
| glycosyl-N-erucoyl-sphingosine (d18:1/22:1)* | **x** | |
| glycosyl-N-nervonoyl-sphingadienine (d18:2/24:1)* | **x** | |
| glycosyl-N-stearoyl-sphingosine (d18:1/18:0) | **x** | |
| glycosyl-N-tricosenoyl-sphingosine (d18:1/23:1)* | **x** | |
| guanosine 5'- diphosphate (GDP) | **x** | |
| laurylcarnitine (C12) | **x** | |
| lysine | **x** | |
| malate | **x** | |
| margaroylcarnitine* | **x** | |
| methylphosphate | **x** | |
| myristoleoylcarnitine (C14:1)* | **x** | |
| myristoylcarnitine (C14) | **x** | |
| nicotinamide riboside | **x** | |
| oleoyl-arachidonoyl-glycerol (18:1/20:4) [2]* | **x** | |
| oleoyl-linoleoyl-glycerol (18:1/18:2) [2] | **x** | |
| oleoyl-oleoyl-glycerol (18:1/18:1) [2]* | **x** | |

| | | |
|---|---|---|
| oleoylcarnitine (C18:1) | **x** | |
| palmitate (16:0) | **x** | |
| palmitoleoylcarnitine (C16:1)* | **x** | |
| palmitoyl ethanolamide | **x** | |
| palmitoyl sphingomyelin (d18:1/16:0) | **x** | |
| palmitoyl-arachidonoyl-glycerol (16:0/20:4) [1]* | **x** | |
| palmitoyl-arachidonoyl-glycerol (16:0/20:4) [2]* | **x** | |
| palmitoyl-dihomo-linolenoyl-glycerol (16:0/20:3n3 or 6) [2]* | **x** | |
| palmitoyl-docosahexaenoyl-glycerol (16:0/22:6) [1]* | **x** | |
| palmitoyl-docosahexaenoyl-glycerol (16:0/22:6) [2]* | **x** | |
| palmitoyl-oleoyl-glycerol (16:0/18:1) [2]* | **x** | |
| palmitoylcarnitine (C16) | **x** | |
| phosphoenolpyruvate (PEP) | **x** | |
| pipecolate | **x** | |
| pyridoxal | **x** | |
| pyridoxamine | **x** | |
| pyruvate | **x** | |
| sphingadienine* | **x** | |
| sphingomyelin (d18:0/18:0, d19:0/17:0)* | **x** | |
| sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0) | **x** | |
| sphingomyelin (d18:1/19:0, d19:1/18:0)* | **x** | |
| sphingomyelin (d18:1/20:0, d16:1/22:0)* | **x** | |
| sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)* | **x** | |
| sphingomyelin (d18:1/24:1, d18:2/24:0)* | **x** | |
| sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)* | **x** | |
| sphingosine | **x** | |
| sphingosine 1-phosphate | **x** | |
| stearoyl sphingomyelin (d18:1/18:0) | **x** | |
| stearoyl-arachidonoyl- | **x** | |

| | | |
|---|---|---|
| glycerol (18:0/20:4) [1]* | | |
| stearoyl-arachidonoyl-glycerol (18:0/20:4) [2]* | **x** | |
| stearoyl-docosahexaenoyl-glycerol (18:0/22:6) [1]* | **x** | |
| stearoyl-docosahexaenoyl-glycerol (18:0/22:6) [2]* | **x** | |
| stearoyl-linoleoyl-glycerol (18:0/18:2) [2]* | **x** | |
| stearoylcarnitine (C18) | **x** | |
| succinate | **x** | |
| trans-4-hydroxyproline | **x** | |

**Dataset S1 (separate file).** SCN matabolome Two-way ANOVA analysis.

**Dataset S2 (separate file).** mPFC matabolome Two-way ANOVA analysis.

**Dataset S3 (separate file).** SCN matabolome JTK_CYCLE analysis.

**Dataset S4 (separate file).** mPFC matabolome JTK_CYCLE analysis.

**Dataset S5 (separate file).** SCN transcriptome Normal Chow JTK_CYCLE analysis.

**Dataset S6 (separate file).** SCN transcriptome High Fat Diet JTK_CYCLE analysis.

**Dataset S7 (separate file).** SCN transcriptome KEGG Pathway analysis.

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