Figure S1, related to Figure 1. Characteristics of the 6 animal cohorts used in this study.

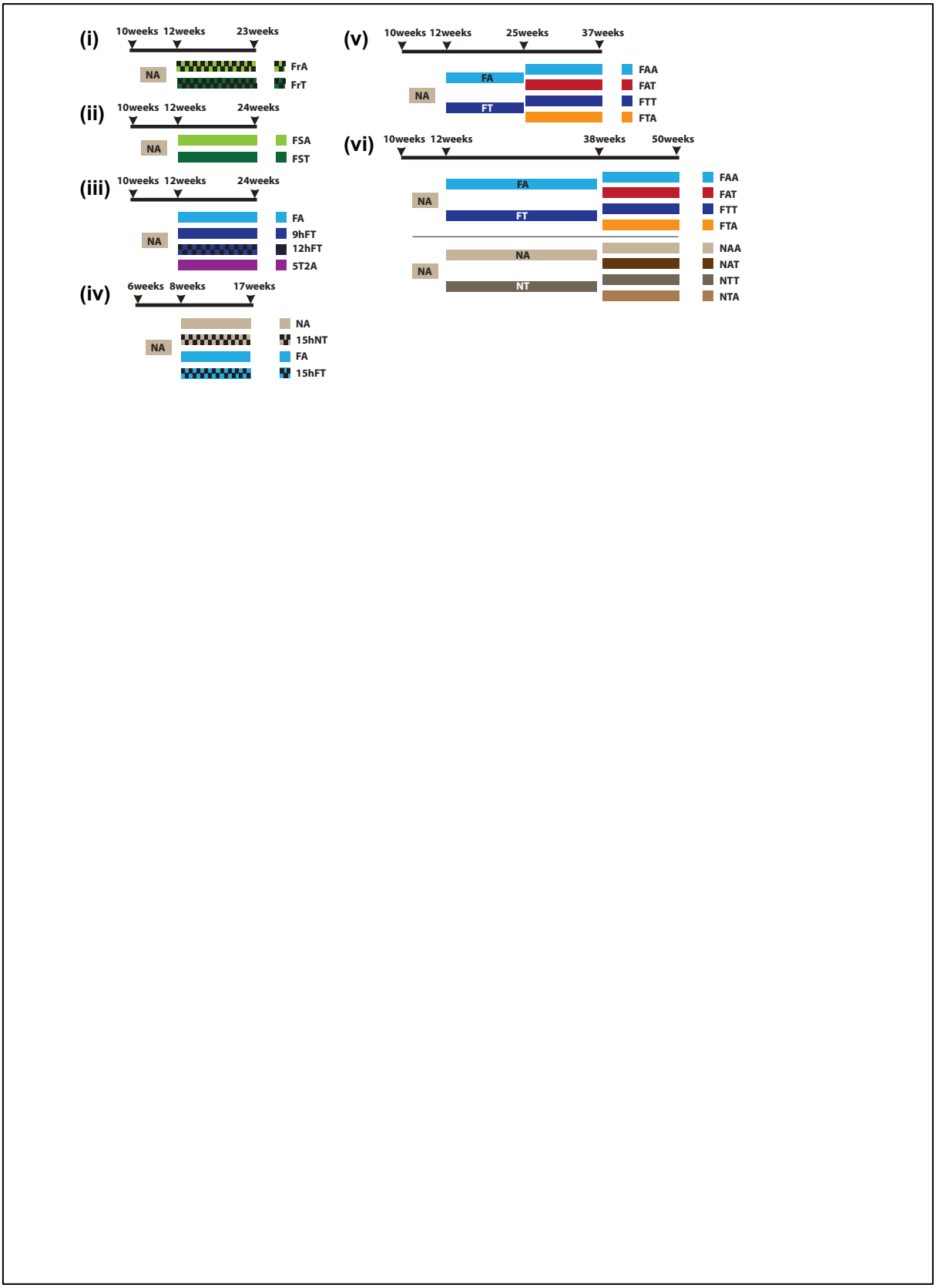


Figure S2, related to Figure 2. Additional details about food consumption, body weight and body composition.

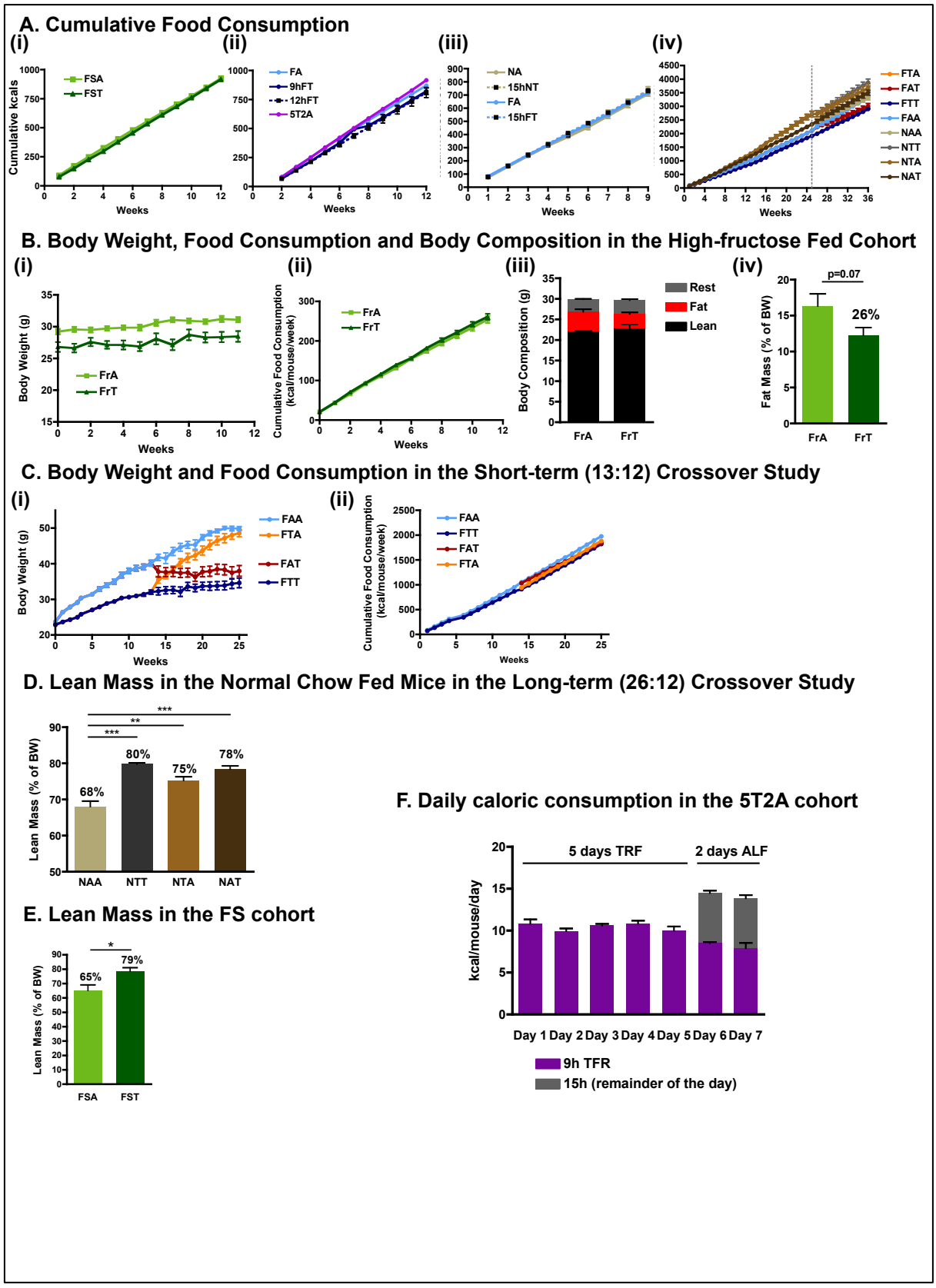


Figure S3, related to Figure 3. TRF modulates adipokines levels and counteracts liver steatosis.

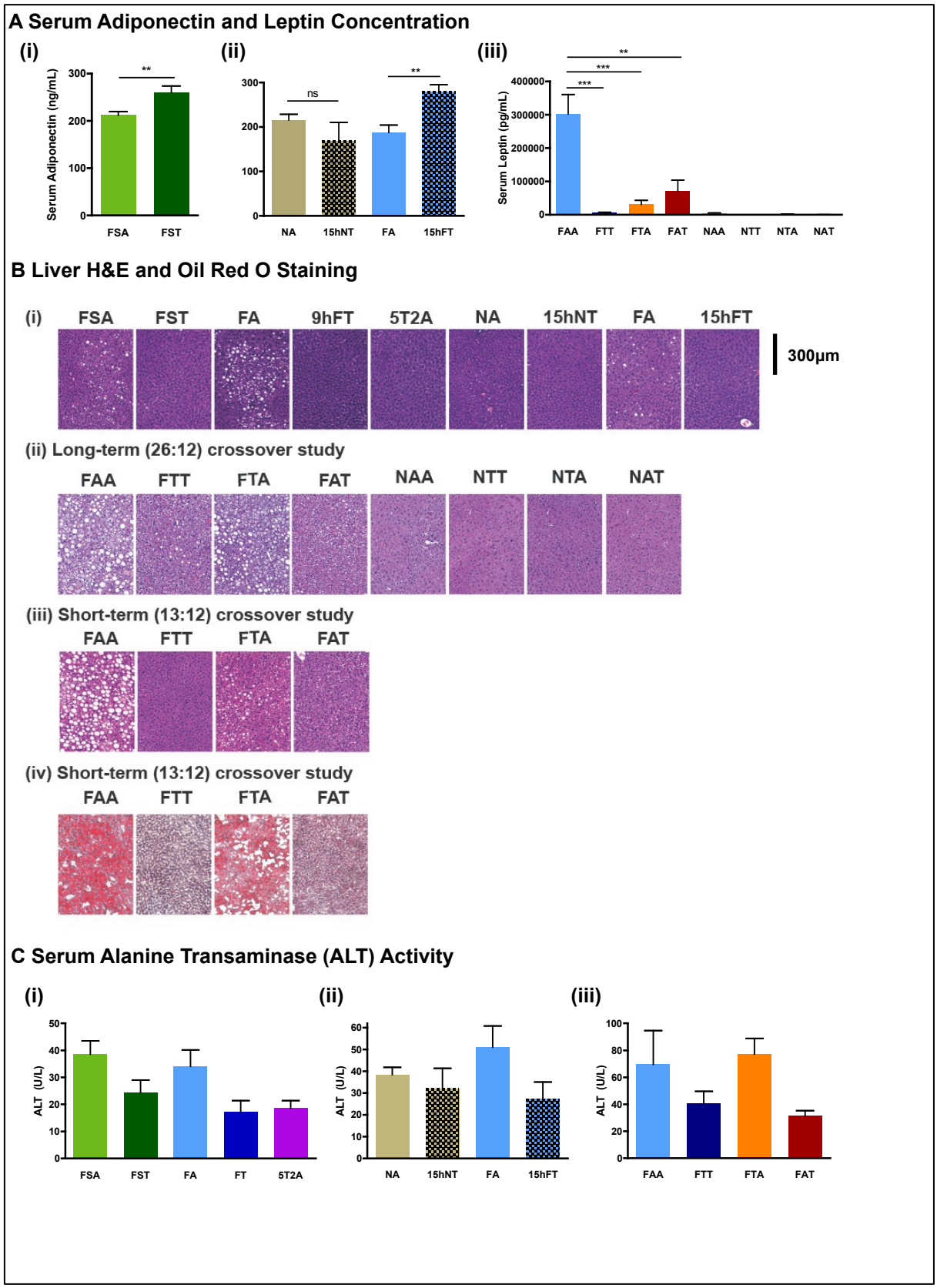


Figure S4, related to Figure 4. TRF improves glucose homeostasis.

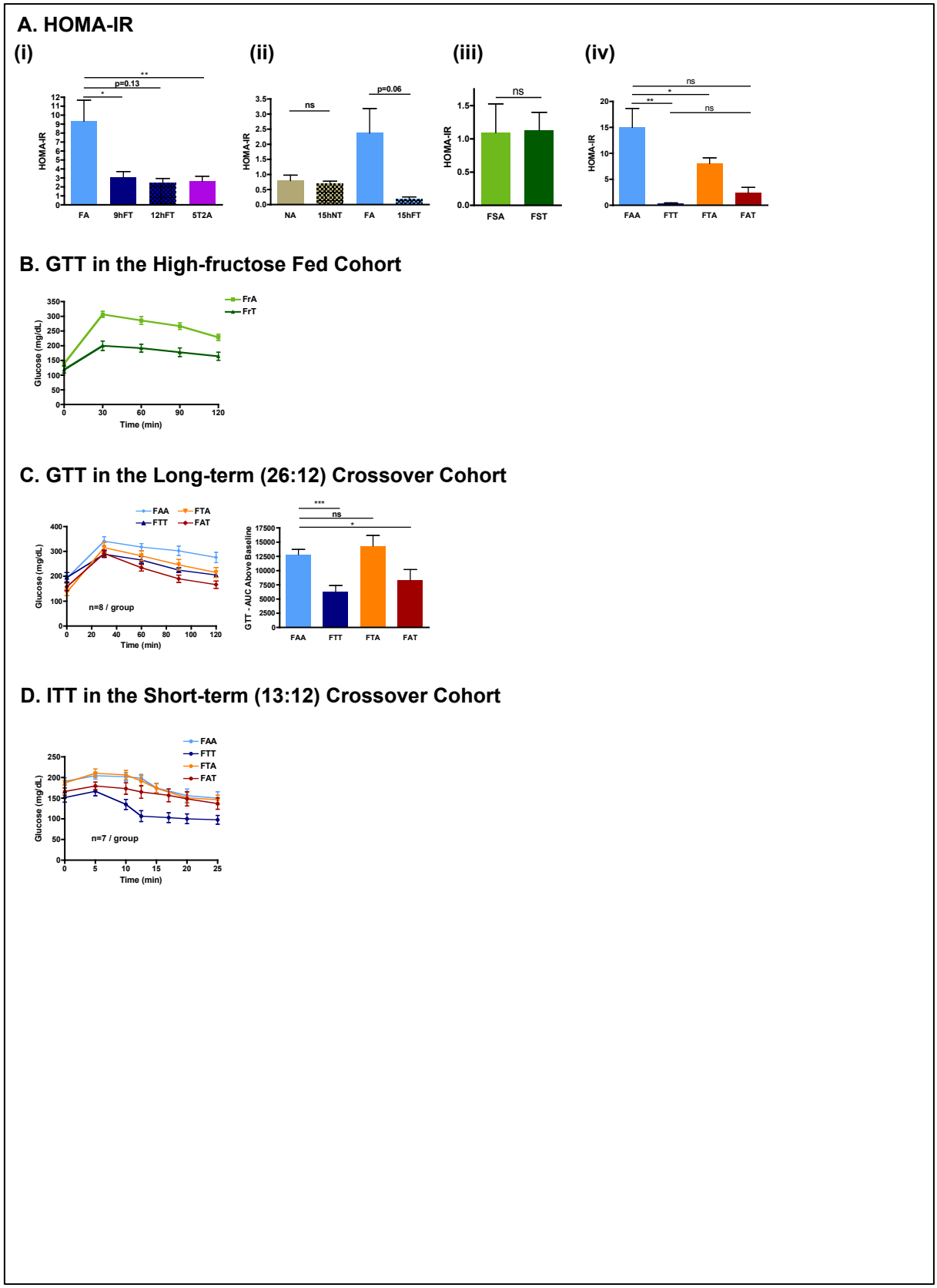


Figure S5, related to Figure 5. Activity, muscle physiology and glycogen storage upon time-restricted feeding.

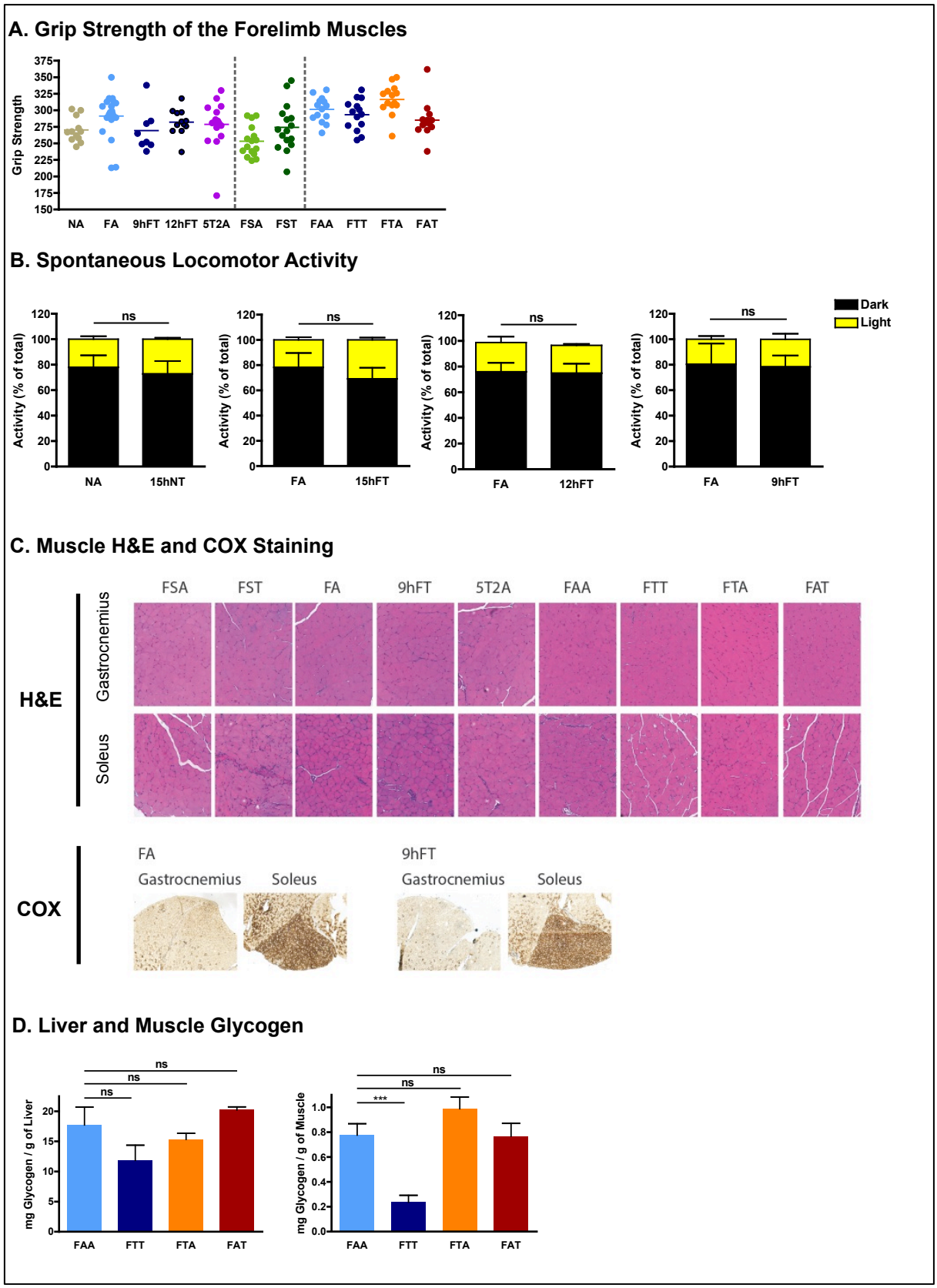


Figure S6, related to figure 6. Serum metabolomics.

## A. Statistical Comparisons of Serum Metabolites.

| Statistical Comparisons of Serum Metabolites | | | | | |
|---|---|---|---|---|---|
| Test | Factor Tested | Groups | | Significant Metabolites | |
| | | | | Number | Percent of total |
| Two-Factor ANOVA (p<0.05) | Time Effect | 6 time points: | ZT16;ZT20;ZT24;ZT4;ZT8;ZT12 | 68 | 24% |
| | Feeding Paradigm Effect | 4 feeding groups: | NTT FTT FAT FAA | 164 | 59% |
| t Test (p<0.05) | TRF effect | 6-plicates<br>2 groups: | TRF: NTT-FTT-FTA & ALF: FAA | 114 | 41% |
| Two-Factor ANOVA (p<0.05) on t Test significant metabolites | Time Effect | 6 time point<br>4 feeding groups | ZT16 ZT20 ZT24 ZT4 ZT8 ZT12<br>NTT FTT FAT FAA | 25 | 22% |

## B. Gamma-glutamyl Amino Acids and Sphingolipids.

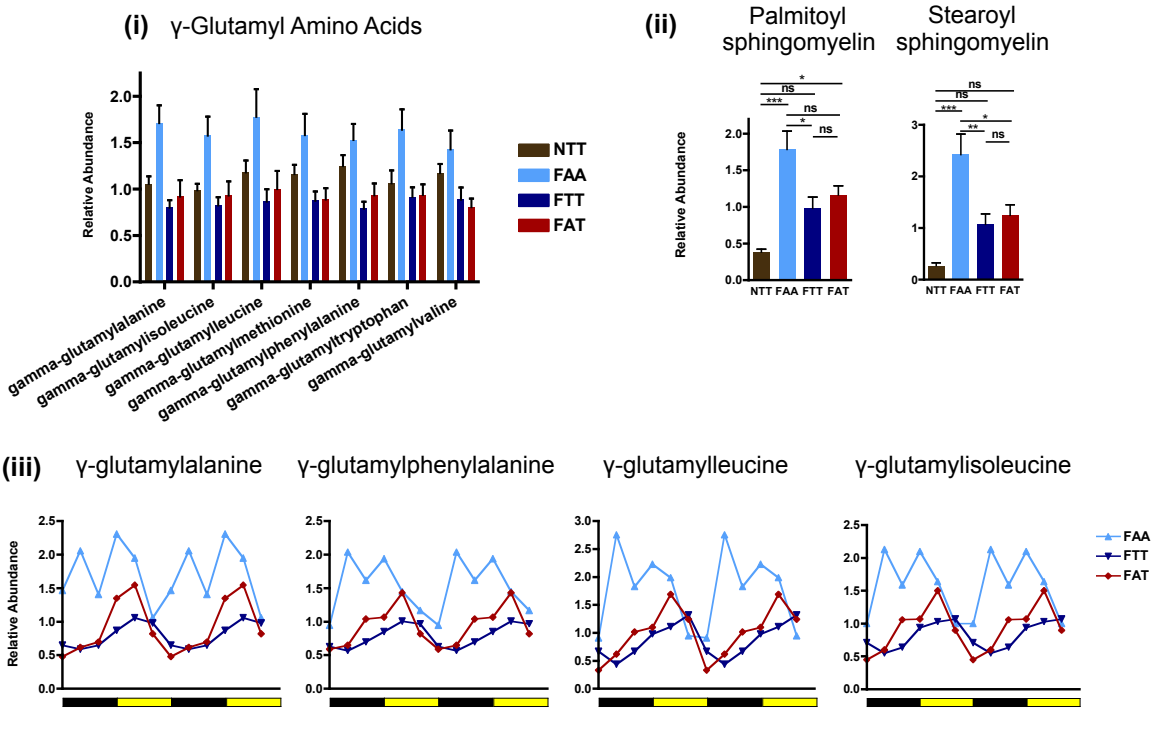
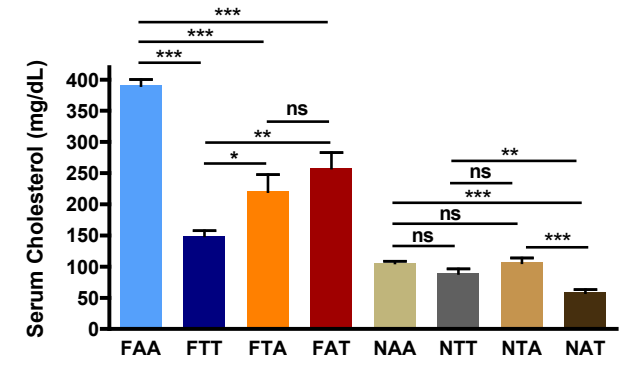
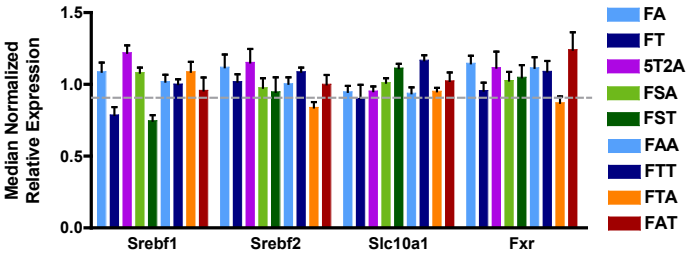


Figure S7, related to figure 7. Cholesterol levels in the long-term crossover study and hepatic expression of sterol pathway regulators.



**Supplemental Figures Legends:**

**Figure S1. Schematic of the animal cohorts used in this study.**

This study was undertaken to investigate four main questions: (1) the beneficial effect of TRF under different diets, (2) the impact of food availability duration on TRF benefits, (3) the legacy effect of TRF on different time-scale and (4) the therapeutic effect of TRF on pre-established DIO and associated metabolic disorders (see Fig 1). To address these questions, the following 6 animal cohorts were studied with variation in the age of the mice, the duration of the study and the lighting schedule. Because of these differences, results presented in Figs. 2 to 4 are grouped as these 6 mice cohorts which are as described:

(i) **high-fructose cohort**: one cohort of 12 weeks old mice fed a high-fructose diet (Fr) for 11 weeks on a 12:12 light:dark cycle (FrA and FrT feeding groups),

(ii) **high-fat high-sucrose cohort**: one cohort of 12 weeks old mice fed a high-fat high-sucrose diet (FS) for 12 weeks on a 12:12 light:dark cycle (FSA and FST feeding groups),

(iii) **high-fat TRF and 5T2A cohort**: one cohort of 12 weeks old mice fed a high-fat diet for 12 weeks on a 12:12 light:dark cycle (FA, 9hFT, 12hFT and 5T2A feeding groups),

(iv) **high-fat and normal chow cohort**: one cohort of 8 weeks old mice fed a high-fat diet (F) or a normal chow diet (N) for 9 weeks on a 12:12 dark:light cycle (FA, 15hFT, NA and 15hNT feeding groups),

(v) **short-term crossover cohort (13:12)**: one cohort of 12 weeks old mice fed a high-fat diet for 25 weeks on a 12:12 light:dark cycle during which the feeding regimen was switched for some mice midway through the experiment (FAA, FTT, FTA, FAT feeding groups),

(vi) **long-term crossover cohort (26:12)**: one cohort of 12 weeks old mice fed a high-fat diet or a normal chow diet on a 12:12 light:dark cycle for 48 weeks during which the feeding regimen was switched for some mice after 26 weeks and then maintained another 12 weeks (FAA, FTT, FTA, FAT feeding groups on HFD and NAA, NTT, NTA, NAT feeding groups on NC).

**Figure S2, related to Figure 2. Additional details about food consumption, body weight and body composition.**

**A.** Cumulative food consumption in each experimental cohort **(associated with Fig 2A)**. The number of mice (n) analyzed per group was (i-ii) n=16, (iii) n=12 and (iv) n=32 then 16.

**B.** (i) Body weight and corresponding (ii) cumulative food consumption (n=12 mice per group); (iii) body composition of the mice in each feeding group (n=7) at the end of the study and corresponding (iv) percent fat mass as a percent of total body weight in the **high-fructose diet**

fed cohort (The percentage reduction of fat mass compared to the ad libitum fed control group is indicated.).

**C.** (i) Body weight and corresponding (ii) cumulative food consumption in the **short-term (13:12) crossover study**. The number of mice (n) analyzed per group was n=16 then 8.

**D.** Lean mass as a percent of total body weight in the normal chow fed mice of the long-term (26:12) crossover study (calculated from fig 2C(iv)). The value of the percent lean mass in each group is indicated.

**E.** Lean mass as a percent of total body weight in FS cohort (calculated from fig 2C(i)). The value of the percent lean mass in each group is indicated.

**F.** Daily caloric consumption during the 5 days on 9hTRF and the 2 days ALF represented as calories ingested during the 9h TRF interval (ZT13-22; purple) and the remaining 15h (grey). Data represent the average over 3 weeks for 4 cages and 16 mice.

Data are presented as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus all other groups or versus the ad libitum fed control group as indicated.

**Figure S3, related to Figure 3. TRF modulates adipokine levels and counteracts liver steatosis.**

**A.** Serum adiponectin and leptin and concentration. The number of mice (n) analyzed per group was (i) n=10, (ii) n=6 and (iii) n=8.

**B.** Representative H&E (i-iii) and Oil Red O (iv) stained histological sections of the liver in the different groups as shown. Scale bar shown is for B(i)-(iv).

**C.** Serum alanine transaminase (ALT) activity. (i) n=4, (ii) n=7 and (iii) n=4. Intergroup differences were not significant.

**Figure S4, related to Figure 4. Time-restricted feeding improves glucose homeostasis.**

**A.** HOMA-IR for the different experimental groups. The number of mice (n) analyzed per group was (i) n=4-12, (ii) n=4-6, (iii) n=5 and (iv) n=4.

**B.** GTT in the high-fructose fed cohort. 8 mice per group were analyzed.

**C.** GTT in the long-term (26:12) crossover cohort. 8 mice per group were analyzed.

**D.** ITT in the short-term (13:12) crossover cohort. 7 mice per group were analyzed.

**Figure S5, related to Figure 5. Activity, muscle physiology and glycogen storage upon time-restricted feeding.**

**A.** Grip strength of the forelimb muscles.

**B.** Spontaneous locomotor activity.

**C.** Representative H&E and cytochrome oxidase (Cox) stained histological transverse sections of the muscle.

**D.** Glycogen tissue content of the liver (left) and the muscle (right) in the short-term (13:12) crossover study. 12 mice per group were analyzed. Data are presented as mean ± SEM. t-test, *p < 0.05, **p < 0.01, ***p < 0.001 as indicated.

**Figure S6, related to Figure 6. Serum metabolomics.**

**A.** Statistical comparisons of 278 serum metabolites.

**B.** Relative serum abundance (median normalized) of (i) γ-glutamyl amino acids, (ii) pro-inflammatory sphingolipids palmitoyl- and stearoyl-sphingomyelin, and (iii) temporal profile of γ-glutamyl amino acids.

**Figure S7, related to Figure 7. Cholesterol levels in the long-term crossover study and hepatic expression of sterol pathway regulators.**

**A.** Serum cholesterol levels in the long-term (26:12) crossover study. 8 mice per group were analyzed. Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 versus the indicated group.

**B.** qPCR analysis of cholesterol and bile acids metabolism transcriptional regulators *srebf1*, *srebf2* and *fxr*, and bile acid transporter *scl10a1*. Data are shown as average expression ± SEM. (n=12 mice per group).

**Supplemental Table 1, related to Figure 1.**

Formula of the different diets used in the study.

**Supplemental Table 2, related to Figure 1.**

Detailed characteristics of the animal cohorts used in this study.

**Supplemental Table 3, related to Figures 2, 3, 4, 5 and 7.**

Results summary. Grey cells signify that no measurements were recorded. N/M: Not Measured.

**Supplemental Table 4, related to Figure 6.**

Metabolomics results showing the raw data and the outputs values form JTK_Cycle (Hughes, 2010).

**Table S1, related to Figure 1. Formula of the different diets used in the study.**

| | | High Fructose | High-Fat High-Fructose | High Fat Diet | Normal Chow |
|---|---|---|---|---|---|
| | | HFr | FS | HFD | NC |
| | | Harlan TD.89247 | Research Diet D12266B | TestDiet 58Y1 | LabDiet 5010 |
| Energy from: (% kcal) | **Fat** | 13% | 32% | 62% | 13% |
| | **Carbohydrates** | 67% (88% being fructose) | 51% (50% being sucrose) | 20% | 59% |
| | **Proteins** | 20% | 17% | 18% | 28% |

**Table S2, related to Figure 1. Detailed characteristics of the animal cohorts used in this study.**

| COHORT | Serial # | Acronym | Acclimatation | Food Availability | Variable of Interest Goal | Diet | Lighting cycle (ZT0= light off) | Age Started | Duration of the feeding paradigm | Number of Mice per Group | Diet catalog number | Diet description |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| i | 1 | FrA | 2 weeks *ad libitum* normal chow | Wk 0-11: *ad libitum* | Diet | High-Fructose (Fr) | 12:12 Light:Dark "natural" cycle | 12 weeks | 11 weeks | 12 | Harlan TD 89247 | 60% Fructose 13% Fat |
| | 2 | FrT | 2 weeks *ad libitum* normal chow | Wk 0-11: 9h food access (ZT13-ZT22) | Diet and Time | High-Fructose (Fr) | 12:12 Light:Dark | 12 weeks | 11 weeks | 12 | Harlan TD 89247 | 60% Fructose 13% Fat |
| ii | 3 | FSA | 2 weeks *ad libitum* normal chow | Wk 0-12: *ad libitum* | Diet | High-Fat High-Sucrose (FS) | 12:12 Light:Dark | 12 weeks | 12 weeks | 16 | Research Diets D12266B | 25% Sucrose 32% fat |
| | 4 | FST | 2 weeks *ad libitum* normal chow | Wk 0-12: 9h food access (ZT13-ZT22) | Diet and Time | High-Fat High-Sucrose (FS) | 12:12 Light:Dark | 12 weeks | 12 weeks | 16 | Research Diets D12266B | 25% Sucrose 32% fat |
| iii | 5 | FA | 2 weeks *ad libitum* normal chow | Wk 0-12: *ad libitum* | Diet | 60% High-Fat (F) | 12:12 Light:Dark | 12 weeks | 12 weeks | 16 | TestDiets 58Y1 | 62% Fat |
| | 6 | 9hFT | 2 weeks *ad libitum* normal chow | Wk 0-12: 9h food access (ZT13-ZT22) | Diet and Time | 60% High-Fat (F) | 12:12 Light:Dark | 12 weeks | 12 weeks | 16 | TestDiets 58Y1 | 62% Fat |
| | 7 | 12hFT | 2 weeks *ad libitum* normal chow | Wk 0-12: 12h food access (ZT12-ZT24) | Diet and Time | 60% High-Fat (F) | 12:12 Light:Dark | 12 weeks | 12 weeks | 16 | TestDiets 58Y1 | 62% Fat |
| | 8 | 5T2A | 2 weeks *ad libitum* normal chow | Wk 0-12:<br>5 days: 9h food access (ZT13-ZT22)<br>2 days: *ad libitum* | Legacy Effect | 60% High-Fat (F) | 12:12 Light:Dark | 12 weeks | 12 weeks | 16 | TestDiets 58Y1 | 62% Fat |
| iv | 9 | NA | 2 weeks *ad libitum* normal chow | Wk 0-9: *ad libitum* | Diet | Nornal Chow (N) | 12:12 Dark:Light "reverse" cycle | 8 weeks | 9 weeks | 12 | LabDiets 5010 | 14% Fat 59% Carbohydrates |
| | 10 | 15hNT | 2 weeks *ad libitum* normal chow | Wk 0-9: 15h food access (ZT10-ZT25) | Diet and Time | Nornal Chow (N) | 12:12 Dark:Light | 8 weeks | 9 weeks | 12 | LabDiets 5010 | 14% Fat 59% Carbohydrates |
| | 11 | FA | 2 weeks *ad libitum* normal chow | Wk 0-9: *ad libitum* | Diet | 60% High-Fat (F) | 12:12 Dark:Light | 8 weeks | 9 weeks | 12 | TestDiets 58Y1 | 62% Fat |
| | 12 | 15hFT | 2 weeks *ad libitum* normal chow | Wk 0-9: 15h food access (ZT10-ZT25) | Diet and Time | 60% High-Fat (F) | 12:12 Dark:Light | 8 weeks | 9 weeks | 12 | TestDiets 58Y1 | 62% Fat |
| v | 13 | FAA | 2 weeks *ad libitum* normal chow | Wk 0-25: *ad libitum* | Therapeutic Effect Control | 60% High-Fat (F) | 12:12 Light:Dark | 12 weeks | 25 weeks | 8 | TestDiets 58Y1 | 62% Fat |
| | 14 | FAT | 2 weeks *ad libitum* normal chow | Wk 0-13: *ad libitum*<br>Wk 14-25: 9h food access (ZT13-ZT22) | Therapeutic effect | 60% High-Fat (F) | 12:12 Light:Dark | 12 weeks | 25 weeks | 8 | TestDiets 58Y1 | 62% Fat |
| | 15 | FTT | 2 weeks *ad libitum* normal chow | Wk 0-25: 9h food access (ZT13-ZT22) | Legacy Effect Control | 60% High-Fat (F) | 12:12 Light:Dark | 12 weeks | 25 weeks | 8 | TestDiets 58Y1 | 62% Fat |
| | 16 | FTA | 2 weeks *ad libitum* normal chow | Wk 0-13: 9h food access (ZT13-ZT22)<br>Wk 14-25: *ad libitum* | Legacy Effect | 60% High-Fat (F) | 12:12 Light:Dark | 12 weeks | 25 weeks | 8 | TestDiets 58Y1 | 62% Fat |
| vi | 17 | FAA | 2 weeks *ad libitum* normal chow | Wk 0-38: *ad libitum* | Therapeutic Effect Control | 60% High-Fat (F) | 12:12 Light:Dark | 12 weeks | 38 weeks | 16 | TestDiets 58Y1 | 62% Fat |
| | 18 | FAT | 2 weeks *ad libitum* normal chow | Wk 0-26: *ad libitum*<br>Wk 27-38: 9h food access (ZT13-ZT22) | Therapeutic effect | 60% High-Fat (F) | 12:12 Light:Dark | 12 weeks | 38 weeks | 16 | TestDiets 58Y1 | 62% Fat |
| | 19 | FTT | 2 weeks *ad libitum* normal chow | Wk 0-38: 9h food access (ZT13-ZT22) | Legacy Effect Control | 60% High-Fat (F) | 12:12 Light:Dark | 12 weeks | 38 weeks | 16 | TestDiets 58Y1 | 62% Fat |
| | 20 | FTA | 2 weeks *ad libitum* normal chow | Wk 0-26: 9h food access (ZT13-ZT22)<br>Wk 27-38: *ad libitum* | Legacy Effect | 60% High-Fat (F) | 12:12 Light:Dark | 12 weeks | 38 weeks | 16 | TestDiets 58Y1 | 62% Fat |
| | 21 | NAA | 2 weeks *ad libitum* normal chow | Wk 0-38: *ad libitum* | Therapeutic Effect Control | Nornal Chow (N) | 12:12 Light:Dark | 12 weeks | 38 weeks | 16 | LabDiets 5010 | 13.5% fat |
| | 22 | NAT | 2 weeks *ad libitum* normal chow | Wk 0-26: *ad libitum*<br>Wk 27-38: 9h food access (ZT13-ZT22) | Therapeutic effect | Nornal Chow (N) | 12:12 Light:Dark | 12 weeks | 38 weeks | 16 | LabDiets 5010 | 13.5% fat |
| | 23 | NTT | 2 weeks *ad libitum* normal chow | Wk 0-38: 9h food access (ZT13-ZT22) | Legacy Effect Control | Nornal Chow (N) | 12:12 Light:Dark | 12 weeks | 38 weeks | 16 | LabDiets 5010 | 13.5% fat |
| | 24 | NTA | 2 weeks *ad libitum* normal chow | Wk 0-26: 9h food access (ZT13-ZT22)<br>Wk 27-38: *ad libitum* | Legacy Effect | Nornal Chow (N) | 12:12 Light:Dark | 12 weeks | 38 weeks | 16 | LabDiets 5010 | 13.5% fat |

**Table S3, related to Figures 2, 3, 4, 5 and 7. Results summary.**

| | | Cohort: | i | | | | ii | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Diet: | Fr | | | | FS | | | |
| | | Duration (weeks): | 2 + 11 | | | | 2 + 12 | | | |
| | | | ALF | | TRF | | ALF | | TRF | |
| | | | FrA | | FrT | | FSA | | FST | |
| | | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| Body Weight | Initial (g) | | 29.2 | 0.5 | 26.8 | 0.8 | 25.2 | 0.4 | 26.3 | 0.5 |
| | Final (g) | | 31.1 | 0.4 | 28.5 | 0.8 | 35.8 | 1.4 | 31.9 | 0.9 |
| | % BW gain over the study | | 6.7% | 2.6 | 7.0% | 4.1 | 42.9% | 6.2 | 21.5% | 3.5 |
| Cumulative Food Consumption | Final (kcals/mouse) | | 254.4 | 9.3 | 261.2 | 7.2 | 924.5 | 19.1 | 915.9 | 9.9 |
| Body Composition | Lean (g) | | 21.91 | 0.3 | 22.67 | 1.1 | 22.45 | 0.6 | 23.63 | 1.0 |
| | Fat (g) | | 4.94 | 0.6 | 3.65 | 0.4 | 9.00 | 1.8 | 3.38 | 0.9 |
| | Rest (g) | | 3.05 | 0.1 | 3.35 | 0.3 | 3.23 | 0.2 | 3.06 | 0.3 |
| | Fat (% of BW) | | 16.32% | 1.7 | 12.25% | 1.1 | 25.47% | 4.3 | 11.15% | 2.7 |
| | % Fat reduction compared to ALF | | | | 26.1% | | | | 62.4% | |
| Serum Biochemestry | Leptin (pg/mL) | | N/M | N/M | N/M | N/M | 6312 | 2605 | 1322 | 675.3 |
| | Adiponectin (ng/mL) | | N/M | N/M | N/M | N/M | 212 | 7.8 | 260.5 | 13.3 |
| | Triglyceride (mg/dL) | | N/M | N/M | N/M | N/M | 74.6 | 6.8 | 76.4 | 4.2 |
| | Fasted Glucose (mg/dL) | | N/M | N/M | N/M | N/M | 62.4 | 7.0 | 66.8 | 2.0 |
| | Refed Glucose (mg/dL) | | N/M | N/M | N/M | N/M | 223.5 | 37.0 | 138.6 | 19.3 |
| | Fasted Insulin (pg/mL) | | N/M | N/M | N/M | N/M | 211 | 58.1 | 244.6 | 60.4 |
| | Refed Insulin (pg/mL) | | N/M | N/M | N/M | N/M | 494.9 | 155.7 | 260.6 | 126.5 |
| | ALT activity (U/L) | | N/M | N/M | N/M | N/M | 38.6 | 4.9 | 24.3 | 4.7 |
| | Cholesterol (mg/dL) | | N/M | N/M | N/M | N/M | 167.6 | 17.4 | 120.5 | 4 |
| Liver Physiology | Triglycerides (mg/g of liver) | | N/M | N/M | N/M | N/M | 56.9 | 10.7 | 26.7 | 3.2 |
| | % TG reduction compared to ALF | | N/M | N/M | N/M | N/M | | | 53.1% | |
| Performances | Grip Strength | | N/M | N/M | N/M | N/M | 253 | 5.8 | 274 | 8.9 |
| | Treadmill (min) | | N/M | N/M | N/M | N/M | 58.3 | 7.9 | 104.5 | 11.6 |

| | | Cohort: | iii | | | | | | | | iv | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Diet: | HFD | | | | | | | | HDF | | NC | | HDF | | NC | |
| | | Duration (weeks): | 2 + 12 | | | | | | | | 2 + 9 | | | | | | | |
| | | | ALF | | 9hTRF | | 12hTRF | | 5d TRF: 2d ALF | | ALF | | | | 15hTRF | | | |
| | | | FA | | 9hFT | | 12hFT | | 5T2A | | FA | | NA | | 15hFT | | 15hNT | |
| | | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| Body Weight | Initial (g) | | 26.3 | 0.5 | 27.5 | 0.6 | 25.5 | 0.6 | 28.5 | 0.5 | 21.2 | 0.5 | 21.9 | 0.4 | 22.8 | 0.3 | 21.3 | 0.3 |
| | Final (g) | | 42.4 | 1.5 | 34.7 | 0.9 | 33.7 | 0.5 | 36.8 | 1.0 | 36.2 | 1.4 | 28.5 | 0.6 | 32.7 | 0.5 | 29.2 | 0.4 |
| | % BW gain over the study | | 61.6 | 7.2 | 26.4 | 2.7 | 33.3 | 4.4 | 29.8 | 4.6 | 72.2 | 7.9 | 30.3 | 3.0 | 43.9 | 2.8 | 36.6 | 2.1 |
| Cumulative Food Consumption | Final (kcals/mouse) | | 871.2 | 13.7 | 820.9 | 27.7 | 811.2 | 43.7 | 916.7 | 9.8 | 716.4 | 13 | 708.5 | 17.2 | 730.4 | 6.7 | 735.6 | 28.0 |
| Body Composition | Lean (g) | | 24.72 | 0.5 | 25.2 | 0.6 | 24.76 | 0.6 | 25.12 | 0.4 | 22.05 | 0.6 | 21.58 | 0.6 | 23.78 | 0.4 | 21.85 | 0.6 |
| | Fat (g) | | 15.24 | 1.4 | 6.55 | 1.9 | 7.72 | 1.2 | 7.96 | 2.3 | 7.5 | 1.4 | 2.37 | 0.2 | 4.25 | 0.4 | 2.33 | 0.3 |
| | Rest (g) | | 4.78 | 0.2 | 3.48 | 0.1 | 4.51 | 0.1 | 3.37 | 0.3 | 3.97 | 0.1 | 3.87 | 0.2 | 3.99 | 0.1 | 3.79 | 0.1 |
| | Fat (% of BW) | | 32.99% | 2.1 | 17.69% | 4 | 20.34% | 2.8 | 20.86% | 4.9 | 27.47% | 2.6 | 8.56% | 0.8 | 13.15% | 1.1 | 8.40% | 1 |
| | % Fat reduction compared to ALF | | | | 57.0% | | 49.3% | | 47.8% | | | | | | 43.3% | | 1.7% | |
| Serum Biochemestry | Leptin (pg/mL) | | 18579.0 | 3981.0 | 2291.0 | 1334.0 | 2049.0 | 1183.0 | 4016.0 | 1898.0 | 4288.0 | 1774.0 | 151.6 | 37.7 | 543.8 | 117.1 | 98.5 | 30.6 |
| | Adiponectin (ng/mL) | | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | 187.6 | 16.9 | 215.7 | 12.8 | 280.2 | 16.9 | 280.2 | 15.1 |
| | Triglyceride (mg/dL) | | 179.1 | 18.3 | 73.1 | 0.7 | 120.6 | 9.3 | 95.3 | 4.9 | 107.8 | 8.0 | 91.0 | 9.0 | 87.0 | 7.3 | 75.7 | 3.5 |
| | Fasted Glucose (mg/dL) | | 137.8 | 10.2 | 110.5 | 14.0 | 61.9 | 13.5 | 118.6 | 10.6 | 62.9 | 8.7 | 42.2 | 3.7 | 57.1 | 3.7 | 51.7 | 3.8 |
| | Refed Glucose (mg/dL) | | 257.3 | 15.1 | 195.3 | 17.3 | 134.8 | 21.3 | 176.3 | 19.8 | 155.7 | 14.1 | 123.7 | 17.3 | 118.5 | 7.1 | 117.9 | 10.4 |
| | Fasted Insulin (pg/mL) | | 749.3 | 182.9 | 504.2 | 129.2 | 572.7 | 150.8 | 318.6 | 45.5 | 752.7 | 205.0 | 258.2 | 54.5 | 59.4 | 15.0 | 165.1 | 41.6 |
| | Refed Insulin (pg/mL) | | 840.0 | 94.9 | 361.4 | 187.7 | 376.6 | 78.7 | 353.5 | 83.8 | 704.9 | 157.0 | 205.7 | 39.9 | 136.0 | 19.9 | 201.7 | 52.2 |
| | ALT activity (U/L) | | 34.1 | 6.2 | 17.4 | 4.0 | N/M | N/M | 18.6 | 2.8 | 51.1 | 9.8 | 38.4 | 3.5 | 27.5 | 7.6 | 32.2 | 9.2 |
| | Cholesterol (mg/dL) | | 215.7 | 13.2 | 117.7 | 28.2 | 120.6 | 9.3 | 124.6 | 5.9 | 225.1 | 9.1 | 110.0 | 1.7 | 130.2 | 7.0 | 112.5 | 4.3 |
| Liver Physiology | Triglycerides (mg/g of liver) | | 110.6 | 15.6 | 31.2 | 5.4 | N/M | N/M | 22.1 | 2.5 | 25.4 | 3.3 | 8.0 | 0.6 | 12.6 | 1.9 | 6.0 | 1.0 |
| | % TG reduction compared to ALF | | | | 71.8% | | N/M | N/M | 80.0% | | | | | | 50.4% | | 25.0% | |
| Performances | Grip Strength | | 291 | 7.4 | 269 | 11.4 | 282 | 5.8 | 279 | 9.0 | N/M | N/M | 270 | 5.4 | N/M | N/M | N/M | N/M |
| | Treadmill (min) | | 50.2 | 4.7 | 141.3 | 4.6 | 73.3 | 3.3 | 122.7 | 9.2 | N/M | N/M | 77.0 | 8.0 | N/M | N/M | N/M | N/M |

| | | Cohort: | v | | | | | | | | vi | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Diet: | HFD | | | | | | | | HFD | | | | | | NC | | | | | | | |
| | | Duration (weeks): | 2 + 13:12 | | | | | | | | 2 + 26:12 | | | | | | | | | | | | | |
| | | | 13w TRF: 12w ALF | | ALF | | 9hTRF | | 13w ALF: 12w TRF | | 26w TRF: 12w ALF | | ALF | | 9hTRF | | 26w ALF: 12w TRF | | 26w TRF: 12w ALF | | ALF | | 9hTRF | | 26w ALF: 12w TRF | |
| | | | FTA | | FAA | | FTT | | FAT | | FTA | | FAA | | FTT | | FAT | | NTA | | NAA | | NTT | | NAT | |
| | | | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM |
| Body Weight | Initial (g) | | 22.9 | 0.3 | 23.7 | 0.2 | 22.9 | 0.3 | 23.7 | 0.2 | 23.5 | 0.3 | 23.2 | 0.3 | 23.5 | 0.3 | 23.2 | 0.3 | 23.5 | 0.3 | 23.3 | 0.3 | 23.5 | 0.3 | 23.3 | 0.3 |
| | Crossover week (g) | | 32 | 0.6 | 40 | 0.8 | 32 | 0.6 | 40 | 0.8 | 36.0 | 0.9 | 54.0 | 0.7 | 36.0 | 0.9 | 54.0 | 0.7 | 33.0 | 0.4 | 32.4 | 0.4 | 33.0 | 0.4 | 32.0 | 0.4 |
| | Final (g) | | 48.6 | 1 | 49.9 | 0.6 | 34.6 | 1.4 | 38 | 1.5 | 48.5 | 1.7 | 59.7 | 1.1 | 40.3 | 1.8 | 47.5 | 1.9 | 32.7 | 0.5 | 34.4 | 0.6 | 32.7 | 0.4 | 31.4 | 0.4 |
| | % BW gain over the study (final/initial) | | 115.8 | 6.5 | 107.7 | 2.4 | 53.5 | 5.9 | 59.8 | 6.1 | 106.3 | 8.0 | 158.2 | 6.2 | 71.9 | 9.6 | 106.2 | 9.5 | 40.4 | 3.2 | 51.4 | 3.9 | 40.7 | 3.9 | 38.1 | 3.4 |
| | % BW gain after crossover (final/crossover) | | 50.3 | 5.4 | 24.2 | 3.1 | 9.3 | 5.2 | -6.2 | 3.8 | 39.1 | 5.9 | 13.4 | 3.6 | 15.2 | 5.7 | -11.3 | 3.9 | -0.2 | 1.8 | 11.3 | 2.6 | -0.7 | 1.6 | 1.7 | 2.8 |
| Cumulative Food Consumption | Final (kcals/mouse) | | 1883.2 | 43.9 | 1975.8 | 29.1 | 1822.2 | 80.8 | 1846.4 | 27.2 | 2945 | 41.9 | 3317 | 65.8 | 2903.5 | 52.8 | 3010.3 | 94.9 | 3775.4 | 154.4 | 3317.3 | 82.9 | 3878.2 | 128 | 3504.7 | 89.3 |
| Body Composition | Lean (g) | | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | 26.01 | 0.5 | 26.85 | 0.4 | 25.36 | 0.6 | 25.16 | 0.8 | 24.72 | 0.4 | 23.79 | 0.3 | 26.7 | 0.6 | 25.7 | 0.9 |
| | Fat (g) | | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | 15.81 | 2.1 | 27.8 | 1.0 | 7.6 | 1.7 | 15.49 | 2.6 | 4 | 0.4 | 7.37 | 0.7 | 2.77 | 0.1 | 3.33 | 0.2 |
| | Rest (g) | | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | 5.47 | 0.2 | 5.9 | 0.1 | 5.27 | 0.2 | 5.49 | 0.1 | 4.18 | 0.1 | 3.87 | 0.1 | 3.98 | 0.1 | 3.72 | 0.1 |
| | Fat (% of BW) | | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | 32.31% | 3.1 | 45.82% | 0.9 | 18.95% | 3.5 | 32.12% | 3.4 | 12.08% | 1.1 | 20.91% | 1.7 | 8.30% | 0.3 | 10.20% | 0.7 |
| | % Fat reduction compared to ALF | | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | 43.1% | | | | 72.7% | | 44.3% | | 45.7% | | | | 62.4% | | 54.8% | |
| Serum Biochemestry | Leptin (pg/mL) | | 3957.0 | 658.7 | 3299.0 | 418.9 | 43.8 | 11.2 | 1180.0 | 631.4 | 31219.0 | 11939.0 | 302391.0 | 58477.0 | 5134.0 | 2367.0 | 71572.0 | 32213.0 | 1388.0 | 683.6 | 4460.0 | 1180.0 | 175.1 | 21.2 | 458.1 | 163.3 |
| | Adiponectin (ng/mL) | | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M |
| | Triglyceride (mg/dL) | | 252.4 | 38.0 | 288.9 | 54.0 | 196.9 | 19.0 | 213.7 | 22.0 | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M |
| | Fasted Glucose (mg/dL) | | 109.8 | 6.0 | 125.3 | 7.6 | 78.3 | 5.6 | 92.4 | 10.6 | 182.3 | 44.4 | 130.2 | 18.8 | 130.0 | 9.0 | 148.1 | 19.0 | 73.9 | 12.6 | 87.8 | 2.7 | 74.5 | 6.1 | 65.8 | 0.9 |
| | Refed Glucose (mg/dL) | | 227.2 | 9.4 | 259.9 | 17.6 | 101.0 | 16.9 | 157.6 | 20.8 | 302.5 | 34.7 | 291.1 | 31.9 | 198.2 | 16.2 | 224.8 | 24.9 | 186.1 | 12.3 | 188.3 | 32.7 | 147.3 | 14.5 | 178.5 | 15.7 |
| | Fasted Insulin (pg/mL) | | 1208.2 | 218.1 | 1578.1 | 290.9 | 77.0 | 15.7 | 326.1 | 123.5 | 1370.4 | 594.3 | 4647.7 | 1263.7 | 821.7 | 248.2 | 2553.4 | 395.0 | 305.9 | 151.0 | 313.8 | 42.1 | 379.0 | 142.0 | 215.1 | 73.9 |
| | Refed Insulin (pg/mL) | | 1385.9 | 148.7 | 1745.5 | 27.6 | 165.8 | 46.4 | 844.2 | 333.0 | 2051.2 | 460.2 | 3775.3 | 836.9 | 622.2 | 91.9 | 2264.3 | 791.2 | 532.4 | 190.6 | 641.7 | 374.8 | 198.7 | 113.9 | 395.6 | 217.6 |
| | ALT activity (U/L) | | 77.3 | 11.5 | 69.6 | 25.1 | 40.6 | 9.1 | 31.4 | 3.9 | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M |
| | Cholesterol (mg/dL) | | 200.8 | 21.6 | 173.9 | 10.8 | 94.4 | 5.2 | 115.3 | 16.0 | 220.4 | 27.1 | 390.4 | 9.8 | 149.1 | 8.7 | 258 | 24.9 | 106.1 | 7.7 | 105.7 | 3.1 | 89.1 | 7.1 | 58.3 | 4.9 |
| Liver Physiology | Triglycerides (mg/g of liver) | | 95.8 | 5.5 | 92.1 | 7.0 | 23.6 | 5.5 | 52.9 | 13.3 | 91.5 | 14.8 | 126.1 | 6.9 | 33.2 | 5.5 | 79.0 | 13.2 | 20.3 | 1.4 | 16.4 | 2.2 | 11.0 | 0.8 | 9.6 | 1.0 |
| | % TG reduction compared to ALF | | -4.0% | | | | 74.4% | | 42.6% | | 27.4% | | | | 73.7% | | 37.4% | | -23.8% | | | | 32.9% | | 41.5% | |
| | Glycogen (mg/g of liver) | | 15.3 | 1.0 | 17.7 | 3.0 | 11.8 | 2.5 | 20.2 | 0.5 | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M |
| Performances | Grip Strength | | 316 | 6.0 | 301 | 5.1 | 293 | 6.2 | 285 | 7.1 | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M |
| | Treadmill (min) | | 43.5 | 7.7 | 20.3 | 5.3 | 96.8 | 3.3 | 79.3 | 3.8 | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M |
| | Rotarod (sec) | | N/M | N/M | N/M | N/M | N/M | N/M | N/M | N/M | 61.6 | 6.7 | 30.5 | 4.4 | 63.8 | 6.1 | 67.6 | 9.1 | N/M | N/M | 59.2 | 5.0 | 53.6 | 5.4 | N/M | N/M |

**Supplemental Methods**

**Animal cohorts and feeding regimens** (see also supplemental table 1)

**(i) high fructose cohort**: after 2 weeks of acclimation with ad libitum access to normal chow diet (LabDiet-5010), 12 weeks old mice were fed a high fructose diet (Harlan-TD.89247) (**Fr**) for 11 weeks on a 12:12 light:dark cycle. The **FrA** group (n=12 mice) had ad libitum access to food and the **FrT** group (n=12 mice) had access to food from ZT13 to ZT 22 (9h during the dark phase) where ZT0 denotes the time of light off.

**(ii) high fat high sucrose cohort**: after 2 weeks of acclimation with ad libitum access to normal chow diet (LabDiet-5010), 12 weeks old mice fed a high fat high sucrose diet (ResearchDiets-D12266B) (**FS**) for 12 weeks on a 12:12 light:dark cycle. The **FSA** group (n=16 mice) had ad libitum access to food and the **FST** group (n=16 mice) had access to food from ZT13 to ZT 22 (9h during the dark phase) where ZT0 denotes the time of light off.

**(iii) high fat TRF and 5T2A cohort**: after 2 weeks of acclimation with ad libitum access to normal chow diet (LabDiet-5010), 12 weeks old mice were fed a 60% high fat diet (TestDiet-58Y1) (**F**) for 12 weeks on a 12:12 light:dark cycle. The **FA** group (n=16 mice) had ad libitum access to food. The **9hFT** group (n=16 mice) had access to food from ZT13 to ZT 22 (9h during the dark phase) and the **12hFT** group (n=16 mice) had access to food from ZT12 to ZT 24 (12h of the dark phase) where ZT0 denotes the time of light off. The **5T2A** group (n=16 mice) alternates between 5 days of 9h time-restricted feeding (ZT13-ZT22) and 2 days of ad libitum feeding.

**(iv) high fat and normal chow cohort**: after 2 weeks of acclimation with ad libitum access to normal chow diet (LabDiet-5010), 8 weeks old mice were fed a 60% high fat diet (TestDiet-58Y1) (**F**) or a normal chow diet (**N**) for 9 weeks on a 12:12 dark:light cycle. The **FA** and **NA** groups (n=12 mice each) had ad libitum access to their respective food. The **15hFT** and **15hNT** groups (n=12 mice each) had access to food from ZT10 to ZT 25 (12h during the dark phase and 3h during the light phase) where ZT0 denotes the time of light off.

**(iv) short-term crossover cohort (13:12)**: after 2 weeks of acclimation with ad libitum access to normal chow diet (LabDiet-5010), 12 weeks old mice were fed a 60% high fat diet (TestDiet-58Y1) (**F**). The **FA** group (n=16 mice) had ad libitum access to food. The **FT** group (n=16 mice) had access to food from ZT13 to ZT 22 (9h during the dark phase). After 13 weeks of this feeding regimen, 8 mice from the FA group where switched to 9h TRF (**FAT**, n=8 mice) and 8 maintained on FA regimen (**FAA**, n=8 mice). Reciprocally, 8 mice from the FT group where switched to ALF (**FTA**, n=8 mice) and 8 maintained on 9h TRF regimen (**FTT**, n=8 mice). The

new feeding regimens were maintained for another 12 weeks. The week of the crossover, the food access duration was progressively reduced to maintain isocaloric consumption.

**(vi) long-term crossover cohort (26:12)**: after 2 weeks of acclimation with ad libitum access to normal chow diet (LabDiet-5010), 12 weeks old mice were fed a 60% high fat diet (TestDiet-58Y1) (**F**) or a normal chow diet (**N**) on a 12:12 light:dark cycle. The **FA** and **NA** groups (n=32 mice each) had ad libitum access to their respective food. The **FT** and **NT** groups (n=32mice) had access to food from ZT13 to ZT 22 (9h during the dark phase). After 26 weeks of this feeding regimen, 16 mice from the ALF groups where switched to 9h TRF (**FAT** and **NAT**, n=16 mice each) and 16 maintained on ALF regimen (**FAA** and **NAA**, n=8 mice). Reciprocally, 16 mice from the TRF groups where switched to ALF (**FTA** and **NAT**, n=16 mice) and 16 maintained on 9h TRF regimen (**FTT** and **NTT**, n=8 mice). The new feeding regimens were maintained for another 12 weeks. The week of the crossover, the food access duration was progressively reduced to maintain isocaloric consumption.

**Body composition**

Body composition was analyzed in live mice using a body composition analyzer (EchoMRI™-100H).

**Organ collection**

Within each feeding group, 2-4 mice were sacrificed every 3 or 4 hours (depending on the cohort) over a 24-hour period. Liver, muscle, WAT (epididymal), and BAT from individual mice were flash frozen or stored in formalin for histological examination. Samples were ground to fine powder in liquid nitrogen and aliquots were used for RNA and protein analyses.

**Histology**

Sections (6 µm) of formalin-fixed liver, muscle, epididimal WAT, and BAT were stained with H&E. Frozen liver sections were stained with Oil Red O. For COX staining, 8-µm thick cross-sections of frozen muscle were incubated in 5.5 mM 3,3′-diaminobenzidine tetra hydrocholoride (DAB) (Sigma), 163.5 µM reduced cytochrome c (Sigma), 8 µM catalase (Sigma) in 0.05 M phosphate buffer (pH 7.4) for 15 min at 37ºC.

**Performance assays**

The rotarod performance test was performed using the Rotamex rota rod (Columbus Instruments). Each day at ZT 15, mice were placed on an accelerating rotating rod and the time

to fall was recorded. Mice were trained for 2 consecutive days and performance recorded on day 3. Data represent the time spent on the rod for the last 2 trials on day 3. Grip strength was determined using a digital grip strength meter (Columbus Instruments). Each animal was tested thrice on 2 consecutive days at ZT16. Data represents the average of the 3 measurements on day 2. The treadmill exhaustion test was performed using the Exer 6M first generation treadmill (Columbus Instruments) at ZT 15. On day 1, mice were trained at low speed for 15 minutes with speed ramping up every 5 minutes. On day 2, mice were trained for 20 minutes at intermediate speed with 5 minutes of ramping. On day 3, maximal speed was reached by ramping up every 5 minutes and, when applicable, a 5º angle incline was applied to the treadmill when running time exceeded 1 hour. Mice were run until exhaustion, which was defined as the inability to continue running despite repeated stimulation by compressed air.

**Metabolic Cages**

Activity was monitored using the activity module of PhenoMaster/LabMaster equipment following the manufacturer's instructions (TSE Systems). Single-housed mice were habituated for 3 days prior to 2 days of recording. Lighting and feeding conditions were maintained identical to that of the home cages.

**Western blotting**

Total lysates were prepared in RIPA buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 1 mM $Na_3VO_4$) supplemented with protease and phosphatase inhibitor cocktails (cOmplete and PhosSTOP tablets, Roche). Western blots were conducted as described (Vollmers et al., 2009). Membranes were cut and probed simultaneously with antibodies directed against phospho-S6 (S235/236) (CST 4858) and SREBP (Sc-13551), then stripped and reprobed for loading control with antibodies against S6 (CST 2217) and Actin (ab1801) or ERK (Sc-94). Signals were quantified using ImageJ.

**qPCR Primers**

| Official Symbol | Official Full Name | mRNA | Forward Primer | Reverse Primer |
|---|---|---|---|---|
| **Acaca** | Acetyl-Coenzyme A carboxylase alpha | NM_133360.2 | CCGATTCATAATTGGGTCTGTGT | CCATCCTGTAAGCCAGAGATCC |
| **Ccl8** | Chemokine (C-C motif) ligand 8 | NM_021443.3 | GACGCTAGCCTTCACTCCAA | GAGCCTTATCTGGCCCAGTC |
| **Cyp7a1** | Cytochrome P450, family 7, subfamily a, polypeptide 1 | NM_007824.2 | CTTCTGCGAAGGCATTTGGAC | ATTTAGGAAGGCCCGGAGGT |
| **Cyp7b1** | Cytochrome P450, family 7, subfamily b, polypeptide 1 | NM_007825.4 | GGTCTGCCTGGAAAGCACTA | AGCATCGAAGATTTCCGGGT |
| **Dhcr7** | 7-dehydrocholesterol reductase | NM_007856.2 | CAGTGCCGAAGACTGCAAAT | CAGGCTACAATCCCTGGTCG |

| | | | | |
|---|---|---|---|---|
| **Elovl3** | Elongation of very long chain fatty acids | NM_007703.2 | ACTTCGAGACGTTTCAGGACTTA | GACGACCACTATGAGAAATGAGC |
| **Elovl5** | ELOVL family member 5, elongation of long chain fatty acids | NM_134255.3 | GAACATTTCGATGCGTCACTCA | GGAGGAAC ATCCTTTGACTCTT |
| **Fasn** | Fatty acid synthase | NM_007988.3 | AGGTGGTGATAGCCGGTATGT | TGGGTAATCCATAG GCCCAG |
| **G6pc** | Glucose-6-phosphatase | NM_008061.3 | CAGTGGTCGGAGACTGGTTC | GTCCAGGACCCACCAATACG |
| **Gck** | Glucokinase | NM_010292.4 | AACGACCCCTGCTTATCCTC | CTGCCAGGATCTGCTCTACC |
| **Il1b** | Interleukin 1 beta | NM_008361.3 | AGTTGACGGACCCCAAAAG | AGCTGGATGCTCATCAGG |
| **Nr1h4/ FXR** | Nuclear receptor subfamily 1, group H, member 4 | NM_001163700.1 | GGGATGTTGGCTGAATGTATG | GAGTTCCGTTTTCTCCCTGC |
| **Pcx** | Pyruvate carboxylase | NM_001162946.1 | GTTCCGTGTCCGAGGTGTAA | AACTGGGTGTCCACTGTGC |
| **Pparg** | Peroxisome Proliferator Activated Receptor gamma | NM_011146.3 | TGCTGTTATGGGTGAAACTCTG | CTGTGTCAACCATGGTAATTTCTT |
| **Ppargc1a/ Pgc1a** | Peroxisome Proliferative Activated Receptor, Gamma, Coactivator 1 alpha | NM_008904.2 | GAAAGGGCCAAACAGAGAGA | GTAAATCACACGGCGCTCTT |
| **Rn18S** | Mus musculus 18S ribosomal RNA (Rn18s) | NR_003278.3 | GCAATTATTCCCCATGAACG | GGGACTTAATCAACGCAAGC |
| **Rpl10** | Ribosomal Protein L10 | NM_052835.3 | GTGGGAGCGTCTCTTTTCCT | GCAGAAACGAGACTTTGGGTA |
| **Slc10a1** | Solute carrier family 10 (sodium/bile acid cotransporter family), member 1 | NM_011387.2 | GGACATGAACCTCAGCATTGTG | GCCTTTGTAGGGCACCTTGT |
| **Sqle** | Squalene epoxidase | NM_009270.3 | TCGGGGAGAATTGCCAAGAA | AGGACGCCTCGTTTGTTCAC |
| **Srebf1** | Sterol Regulatory Element Binding Transcription Factor 1 | NM_011480.3 | CTTTTCCTTAACGTGGGCCT | GAGCTGGAGCATGTCTTCGAT |
| **Srebf2** | Sterol regulatory element binding factor 2 | NM_033218.1 | CCAAAGAAGGAGAGAGGCGG | CGCCAGACTTGTGCATCTTG |
| **Tnf** | Tumor Necrosis Factor | NM_013693.3 | AAGTTCCCAAATGGCCTCCC | CACTTGGTGGTTTGCTACGAC |
| **Ucp1** | Uncoupling protein 1 | NM_009463.3 | GGCCTCTACGACTCAGTCCA | TAAGCCGGCTGAGATCTTGT |