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RESEARCH ARTICLE

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Effect of Aerobic Exercise and Time-Restricted Feeding on Metabolic Markers and Circadian Rhythm in Mice Fed with the High-Fat Diet

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Scope: Diet and exercise are significant players in obesity and metabolic diseases. Time-restricted feeding (tRF) has been shown to improve metabolic responses by regulating circadian clocks but whether it acts synergically with exercise remains unknown. It is hypothesized that forced exercise alone or combined with tRF alleviates obesity and its metabolic complications.

Methods and results: Male C57bl6 mice are fed with high-fat or a control diet for 12 weeks either ad libitum or tRF for 10 h during their active period. High-fat diet (HFD)-fed mice are divided into exercise (treadmill for 1 h at 12 m min^{-1} alternate days for 9 weeks and 16 m min^{-1} daily for the following 3 weeks) and non-exercise groups. tRF and tRF-Ex significantly decreased body weight, food intake, and plasma lipids, and improved glucose tolerance. However, exercise reduced only body weight and plasma lipids. tRF and tRF-Ex significantly downregulated *Fasn, Hmgcr*, and *Srebp1c*, while exercise only *Hmgcr*. HFD feeding disrupted clock genes, but exercise, tRF, and tRF-Ex coordinated the circadian clock genes *Bmal1, Per2*, and *Rev-Erbα* in the liver, adipose tissue, and skeletal muscles.

Conclusion: HFD feeding disrupted clock genes in the peripheral organs while exercise, tRF, and their combination restored clock genes and improved metabolic consequences induced by high-fat diet feeding.

1. Introduction

Metabolic diseases increase dramatically and are serious global threats.[1] Diets rich in saturated fats have detrimental effects on metabolic health, inducing lipid and inflammatory profile alterations and impaired insulin sensitivity.[2,3] Energy metabolism is strongly linked with daily rhythms of sleep-wake cycles. Shift workers have shown a higher risk for metabolic syndrome,[4] and human cohort studies ("28-h days" or shiftwork) have reported acute effects on circadian desynchronization with changes in glucose, insulin, and leptin levels and reduced resting metabolic rate.[5,6] Circadian disruption in mice, either by genetic or environmental means also causes metabolic disorders.[7] ClockΔ19/Δ19-mutant mice are hyperglycemic with increased food intake during the rest period and are susceptible to an increased body weight gain on a high-fat diet.[8]

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Circadian oscillations are entertained daily through environmental cyclic variables called Zeitgeber ("time giver") and light is the major Zeitgeber via the suprachiasmatic nucleus (SCN), which in turn synchronizes circadian rhythms in the peripheral tissues.[9] In mice, light exposure during nighttime led to increased food intake during the rest period with weight gain and impaired glucose homeostasis.[10]

Intermittent fasting (IF) is a prevalent dietary approach for losing weight and to improve metabolic health in humans. IF reduces blood lipids, improves glucose control and insulin sensitivity, and reduces visceral fat.[11,12] Time-restricted feeding (tRF) is a modified IF that limits the schedule and availability of meals without lowering calorie consumption. Obese mice benefited from tRFs by reduction of weight and serum cholesterol levels and improved insulin sensitivity.[13] The tRF regimen allows subjects to consume their normal diet ad libitum whenever they choose within a set time frame (e.g., 3–4 h, 7–9 h, or 10–12 h), resulting in prolonged daily fasting periods.[14] Fasting protocols are part of religious and cultural practices. During religious fasting, body weight, low-density lipoproteins cholesterol (LDL-C), and total cholesterol improve, and high-density lipoproteins cholesterol (HDL-C) increase.[15,16] Importantly, human trials have demonstrated that tRF improved insulin sensitivity and blood pressure in males with prediabetes.[17]

The circadian clock drives the expression of a substantial number of target genes known as "clock-controlled genes" which are highly tissue-specific, and display different distributions of circadian phases.[18,19] In mice, 43% of genes oscillate with circadian rhythm in at least one organ;[20] however, in male baboons, more than 80% of the genes showed daily rhythm in their expressions.[21] The tissue-specific rhythmic gene expression is controlled by both central and peripheral circadian clocks via nuclear receptors, generating numerous metabolic enzymes in a highly time-of-day-specific manner. The circadian clock in mammals consists of cell-autonomous transcription-translation feedback loops (TTFL) that function together to produce a robust 24-h rhythm.[22] TTFL is driven by four integral clock proteins, two activators—brain and muscle ARNT like-1 (BMAL1) and circadian locomotor output cycles kaput (CLOCK), and the two repressors (PER (Period circadian protein homolog) and CRY (cryptochrome circadian regulator)). CLOCK and BMAL1 form heterodimers that bind to E-boxes and activate transcription of their repressors *Cry*1 and *Cry*2, and *Per1* and *Per2* in addition to other clock-controlled genes (CCGs). PER and CRY proteins heterodimerize in the cytoplasm, translocate to the nucleus, and limit CLOCK/BMAL1 transcriptional activities.[23,24] In addition, a second TTFL is generated by orphan nuclear receptors *Rev-Erbα*/*Rev-Erbβ* (repressed) and retinoid-related orphan receptor (activate) transcription.[25] In mammals, the SCN coordinates the peripheral individual clocks in the liver, muscles, kidneys, and lungs. Rhythmic expression of clock genes in the periphery is opposite or phase-delayed to the rhythm of the same clock genes in the SCN.[26,27] Furthermore, peripheral clocks are different from SCN clocks and can regulate synchronization independent of SCN.[26]

In addition to tRF, exercise is a well-established option for improving skeletal muscle functions.[28] Exercise has been widely utilized as an effective intervention to reduce obesity and associated metabolic diseases,[29] to improve body composition and function, and insulin sensitivity.[30] Previously, it has been shown that exercise enhanced insulin sensitivity and reduced body fat mass and lipids in obese mice under high-fat diet.[31,32] Exercise also improves metabolism, but it has not been investigated whether the improvement is mediated via changes in the circadian clock. In addition, the combination of exercise and time-restricted feeding on the peripheral circadian clock is not known. We hypothesized that forced exercise alone or combined with time-restricted feeding coordinates the clock machinery in peripheral organs reduces obesity and improves metabolism.

2. Experimental Section

2.1. Animals

The animal experiment was conducted in accordance with the guidelines set by the European Community Council Directives 2010/63/EU and the 3Rs principles. Animal study was approved by the National Animal Experiment Board of Finland (license no: ESAVI/708/2015 and amendment license ESAVI/609/2017).

Male C57BL/6NCrl mice (22–25 g), aged 6–7 weeks were purchased from the Laboratory Animal Center, University of Oulu, Finland. The mice were housed individually in plexiglass cages and maintained at 22 ± 1 °C with a relative humidity of 45 ± 5% with a 12 h light-dark cycle. Mice were acclimatized to the experimental condition on normal chow for 1 week with free access to water and pelleted food. Mice were fed with the purified high-fat diet D12492 (60% kcal fat) and control diet D12450J (10% kcal fat) purchased from Research Diets, Inc (New Brunswick, NJ, USA). After acclimatization, mice were weighed and randomized into six groups (n = 8) based on body weight and fed with their respective diet and schedules for 12 weeks (**Figure 1**). The dietary groups were (I) CD-Ad, (II) CD-tRF, (III) HFD-Ad, (IV) HFD-tRF, (V) HFD-Ad-Ex, and (VI) HFD-tRF-Ex. Zeitgeber time 0 (ZT0) was designated as lights-on time and ZT12 as lights-off time (lights on between 7:00–19:00). Under tRF, mice were allowed to eat for 10 hours (ZT15-ZT24) during their active period. Only HFD (ad libitum and tRF) fed mice were forced exercise on a treadmill for 1 h with the speed of 12 m min^{-1} on alternate days

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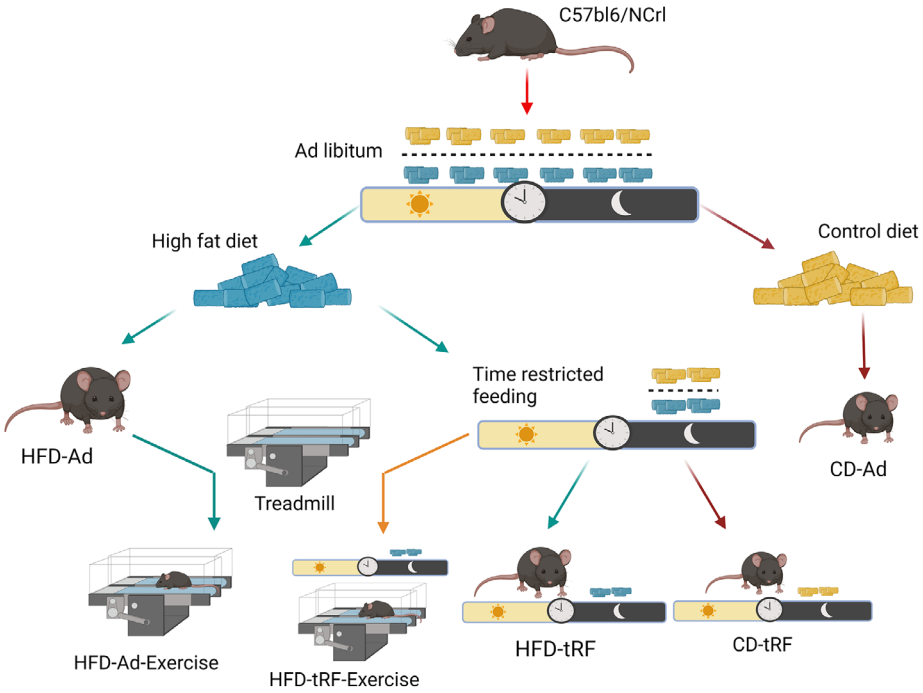

Figure 1. Study design: C57BL6/NCrl mice fed either time-restricted (tRF:10 h food) or ad libitum with HFD or control (CD) diet for 12 weeks. Only HFD mice fed either tRF or ad libitum were exercised on a treadmill 12 m min^{-1} alternate day for 1 h week 0-9 and daily from week 10 to 12 speed 16 m min^{-1}.

for 9 weeks. From week 10, the exercise regimen was increased to 1 h daily with a speed 16 m min^{-1} for an additional 3 weeks. Body weight of the animals was recorded twice weekly, and food intake was measured on alternate days throughout the feeding period.

2.2. Insulin Tolerance Test (ITT), Glucose Tolerance Test (GTT) and Blood Samples

ITT was performed after 8 weeks and GTT after week 8 and week 12 of feeding with their respective diet and schedules. For ITT, animals were fasted for 4 h and Actrapid insulin (0.35 IU kg^{-1} of body weight) was injected intraperitoneally. Blood glucose was measured at 0, 15, 30, and 60 min with glucometer using glucose strips (FreeStyle lite, Abbott laboratories, Green Oaks, IL, USA). Food was provided to ad libitum fed mice immediately after ITT. For GTT, animals were fasted for 6 h, and basal blood glucose was measured. After basal samples, all mice were injected intraperitoneally with 1.5 g kg^{-1} body weight glucose solution. Blood glucose was measured at 15, 30, 60, and 120 min after glucose injection using same glucometer and glucose strips as indicated above.

Terminal blood samples were collected after 12 weeks of feeding. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes from all animals under isoflurane anesthesia, and plasma was separated by centrifuging samples at 8000 rpm for 7 min at 4 °C. Immediately after blood collection, animals were euthanized at ZT0.5, and tissues were collected for metabolic and circadian gene expression analysis. Plasma LDL-C, HDL-C, total cholesterol, and triglycerides were measured using DiaSys kits (Diagnostic Systems GmbH, Holzheim, Germany).

2.3. Gene Expression Analysis from Liver, Muscle, and Adipose Tissue

The gene expression analysis was performed by real-time PCR. RNA was extracted from livers with Total RNA NucleoSpin kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany), muscle tissues with RNeasy fibrous tissue kit from Qiagen (Venlo, Netherlands), and adipose tissue with Qiazol reagent from Qiagen. Genomic DNA was removed from liver tissues by gDNA columns, while muscle and adipose tissues by DNase digestion using rDNase set (Macherey-Nagel GmbH & Co. KG, Düren, Germany). Concentration and quality of RNA were estimated by NanoDrop ND-1000 ultraviolet–visible (UV–vis) spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). cDNAs were synthesized from 1 µg of RNA using SensiFast cDNA synthesis kit (Meridian Biosciences Inc., Cincinnati, OH, USA) as per manufacturer instructions. Mouse specific primers for glucose, fatty acid, and cholesterol metabolism such as 3-hydroxy-3-methylglutaryl-CoA reductase (*Hmgcr*), cholesterol 7α hydroxylase (*Cyp7a1*), glucose 6 phosphatase (*G6Pase*), phosphoenolpyruvate carboxykinase (*Pepck*), fatty acid synthase (*Fasn*), sterol regulatory element-binding protein 1c (*Srebp1c*) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (*Pgc-1α*) were designed and supplied by TAG Copenhagen, Copenhagen, Denmark. In addition, circadian clock genes *Bmal1*, *Clock*, *Per1*, *Per2*, *Cry1*, *Cry2*, *Rev-Erbα*, and albumin D-site binding protein (*Dbp*) were analyzed. Samples were normalized

to glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*) and ribosomal protein large P0 (*Rplp0*) as endogenous control. Primer sequences are presented in supplementary information (Table S1, supporting information). PCR reactions were performed in an ABI-PRISM 7300 sequence detection system (Applied Biosystems, Foster City, CA, USA) and QuantStudio 5 (ThermoFisher Scientific Waltham, MA, USA) in a total volume of 20–30 µL. All samples were measured in duplicates using the following conditions: 2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Each assay included a standard curve of three serial dilutions of cDNA from the fasted mouse and no template controls. Results were calculated according to the instructions of the manufacturer (ABI PRISM 7300 sequence detection system, Applied Biosystems).

2.4. Immunoblotting for Protein Analysis

Liver tissues from mice were homogenized in RIPA buffer (50 mM Tris, 150 mM NaCl, 0.1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS), containing protease inhibitor cocktail (Sigma, St.Luis, MO. USA) using a ball mill (Brinkmann Instruments, Westbury, NY, USA). Homogenates were centrifuged at 13 000 rpm for 20 min at +4 °C, and supernatants were collected. Total protein concentrations were determined by the Bradford reagent (Bio-Rad Laboratories Inc., Hercules, CA, USA). Equal amounts of protein (30 µg) were separated electrophoretically on 8% SDS-PAGE gels and transferred to PVDF membrane (Millipore). Ponceau staining of the membrane was performed and photographed. Membranes were blocked by incubating 1 h with 5% non-fat dry milk in TBST and incubated overnight +4 °C with primary anti-PGC-1α antibody and loading control protein GAPDH antibody (1:500 polyclonal antibody of rabbit PGC-1α sc-13067 - Santa Cruz Biotechnology Inc, Texas, USA, 1:10 000 GAPDH). After overnight incubation, membranes were washed with TBST and incubated with secondary antibody (1:10 000, Goat anti-rabbit IgG horseradish peroxidase-conjugated anti-rabbit IgG) for 1 h at room temperature. Chemiluminescence for PGC-1α were detected with SuperSignal West Femto Maximum Sensitivity Substrate (cat. no. 34095, Thermo Fisher Scientific, Rockford, USA) according to the manufacturer's instructions. Blots were visualized with Odyssey Fc imaging system (LI-COR Biosciences, Ltd, Cambridge, UK), and results normalized to loading controls for GAPDH.

2.5. Statistical Analysis

One-way analysis of variance (ANOVA) was used to analyze for statistical significance between the groups using GraphPad Prism, version 7 (GraphPad Software, Inc., La Jolla, CA, USA) and SPSS, version 21 (SPSS, Inc., Chicago, IL, U.S.A.). The two-way ANOVA was used to analyze statistical difference in GTT and ITT between the groups at different time intervals. Dunnett's multiple comparison was applied to analyze the difference between treatment groups. The different treatment groups were compared with each other except CD-tRF, which was compared with CD-Ad and HFD-tRF. The data are expressed as the mean ± standard error of mean (SEM). Differences were considered to be statistically significant when $p < 0.05$.

3. Results

3.1. Body Weight and Organ Weight

HFD-Ad feeding significantly increased ($p < 0.0001$) body weight gain compared to control (CD-Ad) fed mice. HFD-tRF ($p < 0.0001$), HFD-Ad-Ex ($p < 0.006$) and their combination HFD-tRF-Ex ($p < 0.0001$) significantly reduced body weight gain compared to HFD-Ad (**Figure** 2A). Moderate exercise (treadmill 12 m min^{-1} for 1 h on alternate days) did not cause significant reduction in body weights during 8 weeks of trial, but 16 m min^{-1} daily from week 9 to 12 significantly reduced body weights in HFD ad libitum fed mice (Figure 2A). However, there was no significant change in total area under curve (AUC) with HFD-Ad-Ex compared to HFD-Ad (Figure 2B). The weight reductions under HFD-tRF and HFD-tRF-Ex were significantly ($p < 0.05$) higher compared to HFD-Ad-Ex and no significant differences were observed between HFD-tRF and HFD-tRF-Ex as shown in total AUC (Figure 2A,B). No significant differences in body weight gain were found between CD-tRF and controls (CD-Ad) fed mice (Figure 2A,B). Liver weight/body weights of the mice were significantly increased ($p < 0.01$) with CD-tRF compared to controls (CD-Ad) and HFD-tRF fed mice (Figure 2C). HFD-Ad significantly ($p < 0.004$) increased epididymal fat weight/body weights of the mice compared to control CD-Ad (Figure 2D). HFD-tRF, HFD-Ad-Ex, and HFD-tRF-Ex did not reduce fat weight/body weight of mice compared to HFD-Ad fed mice. However, CD-tRF significantly ($p < 0.0001$) reduced epididymal fat weights/body weight of the mice compared to HFD-tRF (Figure 2D). No significant differences in brown adipose tissue (BAT) weight/body weight of the mice were found between the different groups (Figure 2E).

3.2. Food and Energy Intake

HFD-tRF ($p < 0.005$) and HFD-tRF-Ex ($p < 0.003$) significantly decreased food intake/48 hours during first 2-week feeding (**Figure** 3A,B). During week 3–9, mice on HFD-tRF and HFD-tRF-Ex ate the same as HFD-Ad, but food intake was reduced from week 10 of the experiment in both tRF groups. Total food intake throughout the study period was significantly reduced in HFD-Ad fed mice compared to control (CD-Ad) fed mice (Figure 3B). HFD-tRF ($p < 0.007$) and HFD-tRF-Ex ($p < 0.0007$) reduced total food intake compared to HFD-Ad fed mice while HFD-Ad-Ex did not change food intake (Figure 3B). In addition, HFD-tRF significantly reduced total food intake compared to CD-tRF fed mice (Figure 3B). No significant changes in food intake /gram body weight of the mice and cumulative food intake/gram body weight were observed between HFD-fed groups (Figure 3C,D). However, CD-fed mice consumed significantly ($p < 0.001$) more food/gram body weight and cumulative food intake/gram body weight than HFD-fed mice (Figure 3C,D). Energy intake of mice was calculated based on the food intake data and results were included in the supplementary information (Figure S1, supporting information). HFD-tRF, and HFD-tRF-Ex mice reduced energy intake per 48 h after two weeks of feeding compared to HFD-Ad mice (Figure S1A, supporting information). Total energy intake throughout the study period was significantly increased in HFD-Ad compared to CD-Ad

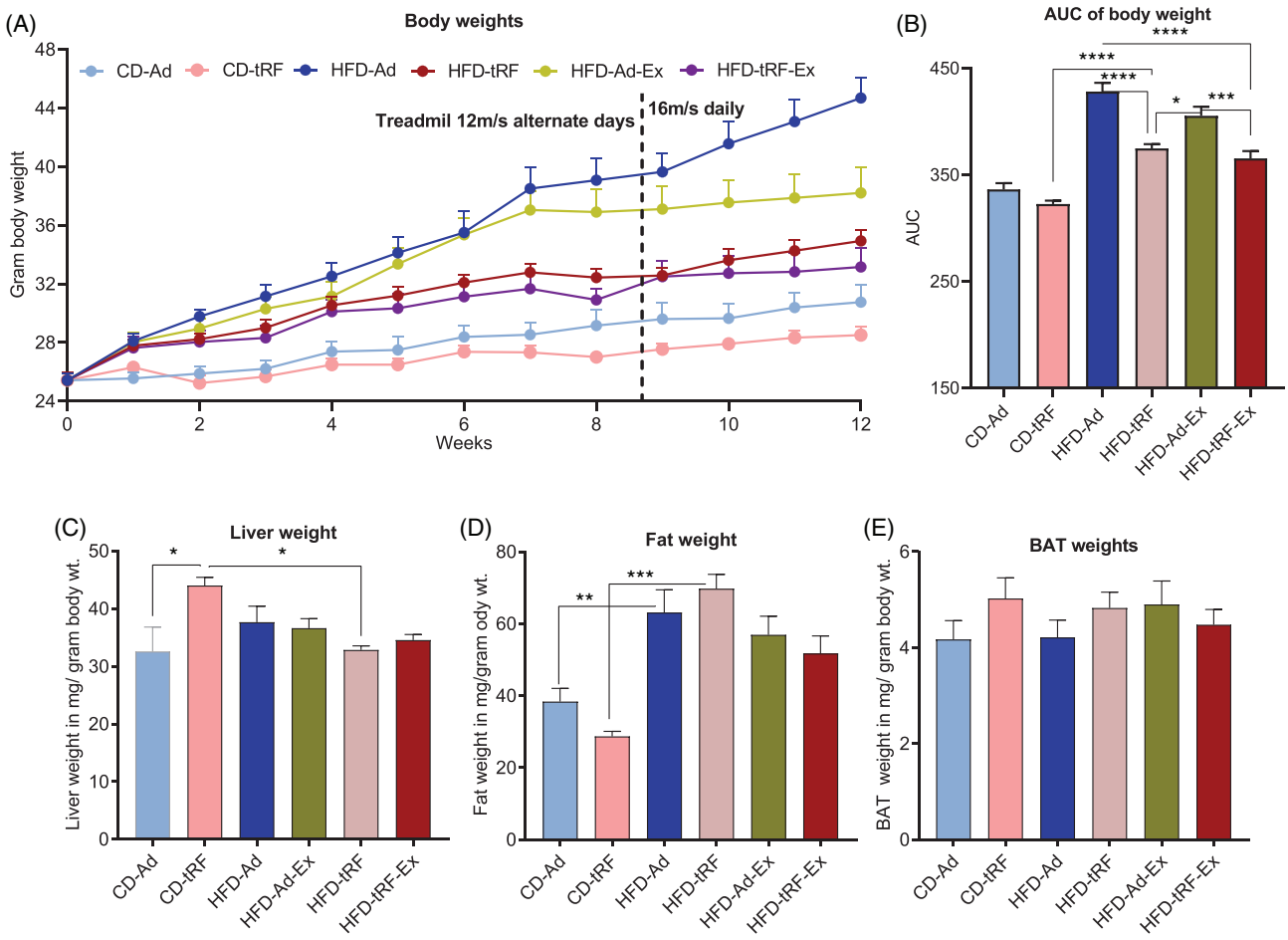

Figure 2. HFD-Ad increased body weight compared to control CD-Ad. HFD-tRF, HFD-Ad-Ex and HFD-tRF-Ex reduced body weight compared to HFD-Ad (A). Weight reduction under HFD-tRF and HFD-tRF-Ex was greater than HFD-Ad-Ex (B). There was no difference in body weight between CD-tRF and CD-Ad fed mice (A). Liver weight per gram body weight was increased with CD-tRF compared to CD-Ad and HFD-tRF (C). HFD-Ad increased epididymal fat weight per gram body weight compared to CD-Ad (D). In addition, CD-tRF reduced fat weight compared to HFD-tRF and CD-Ad. No difference in BAT weights were found between the groups (E). The values represent the mean ± standard error of mean (SEM), and differences were considered statistically significant when $p < 0.05$ and $n = 8$ (*$p < 0.05$, **$p < 0.01$ ***$p < 0.001$).

(control) fed mice (Figure S1B, supporting information). HFD-tRF and HFD-tRF-Ex significantly reduced total energy intake compared to HFD-Ad fed mice (Figure S1B, supporting information). CD-tRF mice had the same total energy intake compared to CD-Ad (control) mice, but their total energy intake was significantly reduced compared to HFD-tRF mice (Figure S1B, supporting information). No significant changes in energy intake/gram body weight of the mice or cumulative energy intake/gram body weight of the mice were observed between the groups (Figure S1C,D, supporting information).

3.3. Insulin and Glucose Tolerance

HFD-Ad fed mice had significantly impaired insulin ($p < 0.01$), but not glucose tolerance compared to control (CD-Ad) fed mice after 8 weeks of feeding as shown by total AUC (**Figure** 4A,B). However, no significant changes in ITT incremental area under curve (iAUC) were observed between HFD-Ad and control (CD-Ad) fed mice (Figure 4C). HFD-tRF, HFD-Ad-Ex, and their combination (HFD-tRF-Ex) did not improve insulin resistance, however only CD-tRF fed mice significantly improved insulin resistance compared to HFD-tRF after 8 weeks of feeding (Figure 4A–F). At 12 weeks, HFD-Ad fed mice displayed significantly impaired glucose tolerance while HFD-tRF ($p < 0.002$) and HFD-tRF-Ex ($p < 0.0001$) significantly improved glucose tolerance, total AUC, and iAUC of GTT (Figure 4G–I). CD-tRF significantly improved glucose tolerance and total AUC, but not iAUC at 12 weeks compared to HFD-tRF fed mice (Figure 4I). HFD-Ad-Ex did not improve glucose tolerance and no significant difference in insulin sensitivity was found between HFD-tRF, HFD-Ad-Ex, and HFD-tRF-Ex during 12 weeks of feeding trial (Figure 4G–I).

3.4. Plasma Lipids

Mice fed HFD-Ad significantly increased plasma LDL-C and total cholesterol ($p < 0.0001$) compared to control (CD-Ad) fed mice. HFD-tRF ($p < 0.0001$) and HFD-tRF-Ex ($p < 0.0004$) significantly

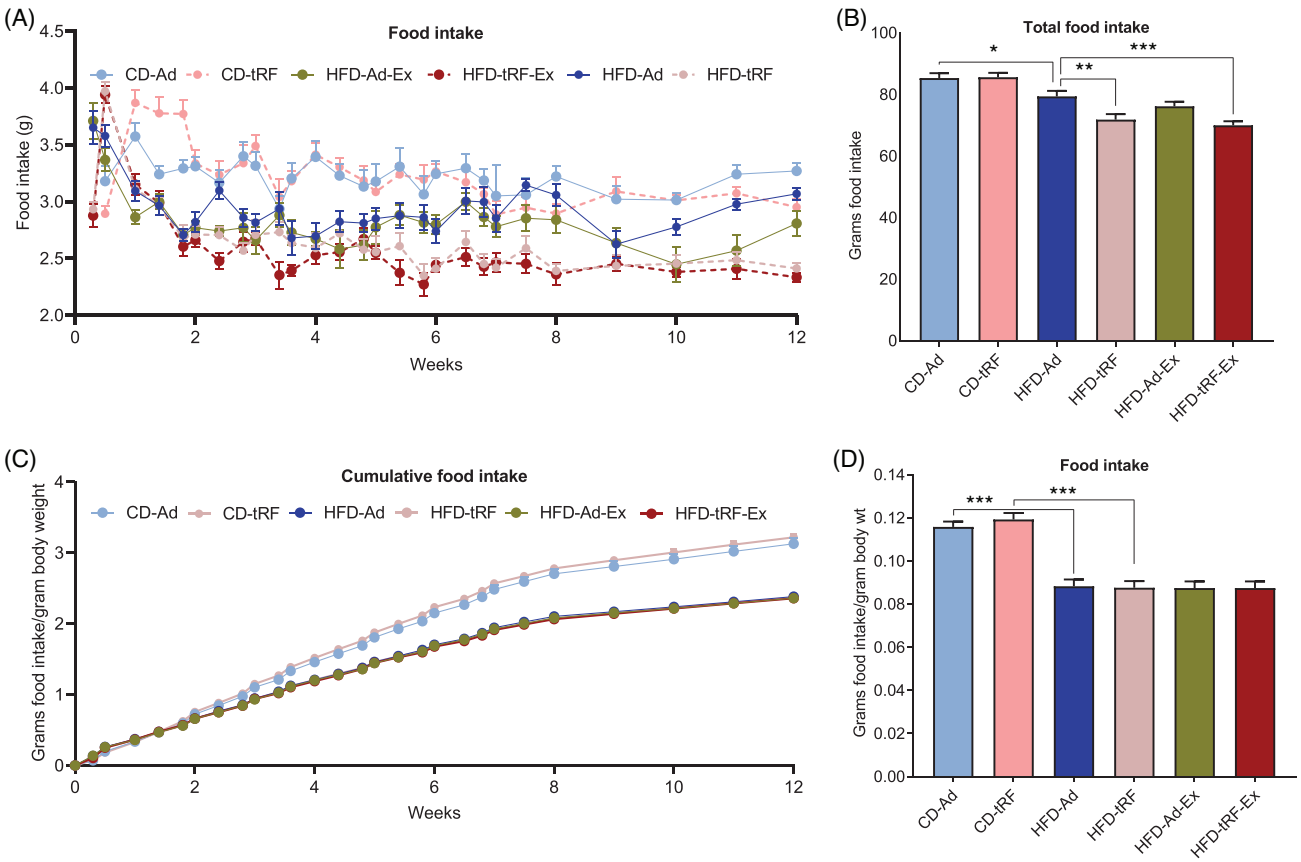

Figure 3. Mice fed CD ate more food in grams per 48 h and total food intake for 12 weeks was more compared to HFD (A,B). HFD-Ad-Ex did not inhibit food intake per 48 h and HFD-tRF and HFD-tRF-Ex significantly reduced food intake compared to HFD-Ad. The food intake per gram body weight (D) and cumulative food intake per gram body weight (C) was significantly higher in CD fed mice compared to HFD, however there was no difference between HFD fed mice. The values represent the mean ± standard error of mean (SEM), and differences were considered statistically significant when $p < 0.05$ and $n = 8$ (*$p < 0.05$, **$p < 0.01$ ***$p < 0.001$).

reduced plasma LDL-C and total cholesterol compared to HFD-Ad fed mice (**Figure** 5A,B). HFD-Ad-Ex did not change plasma LDL-C and total cholesterol, but significantly ($p < 0.01$) increased plasma HDL-C compared to HFD-Ad fed mice (Figure 5C). In addition, HFD-Ad-Ex significantly ($p < 0.02$) decreased plasma triglyceride compared to HFD-Ad fed mice (Figure 5D). No significant changes in plasma triglyceride and HDL-C were observed with HFD-tRF and HFD-tRF-Ex.

3.5. Glucose, Fatty Acid, and Cholesterol Metabolism Genes

HFD-Ad feeding upregulated the liver expressions ($p < 0.01$) of *Hmgcr* and *Fasn* genes compared to livers of control (CD-Ad) fed mice (**Figure** 6A,B). HFD-Ad-Ex and HFD-tRF-Ex significantly ($p < 0.05$) downregulated the liver expression of *Hmgcr*, HFD-tRF and HFD-tRF-Ex downregulated ($p < 0.05$) *Fasn* expressions compared to livers of HFD-Ad fed mice (Figure 6A,B). No significant differences in *Hmgcr* expressions were observed with HFD-Ad-Ex and HFD-tRF-Ex and *Fasn* expression with HFD-tRF and HFD-tRF-Ex compared to controls (Figure 6A,B). In addition, CD-tRF feeding significantly downregulated *Hmgcr* expression in livers compared to HFD-tRF fed mice (Figure 6A,B). No significant changes in *Cyp7a1* or *G6pase* genes were found between the groups, however HFD-tRF-Ex feeding significantly inhibited liver *Pepck* expression compared to livers of HFD-Ad fed mice (Figure 6C–E). *Srebp1c* expression was significantly increased in HFD-Ad fed mice compared to control (CD-Ad) fed mice (Figure 6F). HFD-tRF-Ex significantly inhibited *Srebp1c* expressions in livers while HFD-tRF and HFD-Ad-Ex feeding did not compared to HFD-Ad fed mice (Figure 6F). Liver *Pgc-1α* gene expressions were significantly downregulated by HFD-Ad feeding compared to control CD-Ad fed mice and HFD-tRF, HFD-Ad-Ex and HFD-tRF-Ex increased *PGC-1α* expression in livers, which was not statistically significant (Figure 6G).

3.6. Clock Gene Expression in Liver, Adipose and Skeletal Muscle Tissue

Liver *Bmal1*, *Clock* and *Per1* gene expressions were reduced but not significantly by HFD-Ad feeding compared to control (CD-Ad) (**Figure** 7 and **Table** 1). HFD-tRF, HFD-Ad-Ex, and HFD-tRF-Ex increased *Bmal1*, and *Clock* expressions compared to HFD-Ad feeding in liver. HFD-Ad feeding significantly upregulated *Per2* and *Dbp* liver expressions compared to control

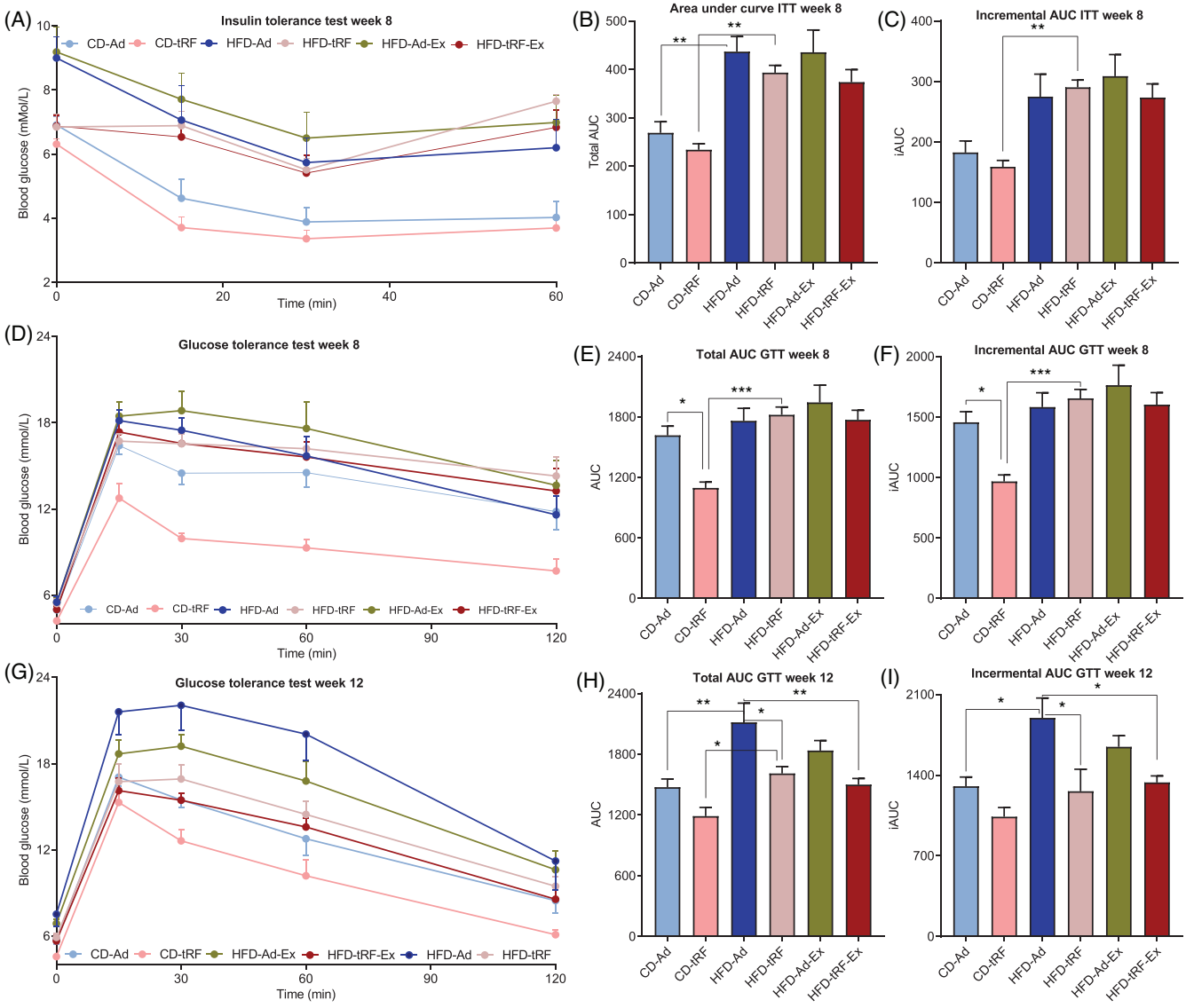

Figure 4. HFD-Ad fed mice showed impaired insulin tolerance and glucose tolerance compared to CD fed mice and CD-tRF improved insulin resistance compared to HFD-tRF at 8 weeks of feeding (A,B). At week 12, HFD-tRF and HFD-tRF-Ex improved glucose tolerance while HFD-Ad-Ex did not change compared to HFD-Ad fed mice (G–I). There was no change in glucose tolerance between HFD-tRF and HFD-tRF-Ex. The values represent the mean ± standard error of mean (SEM), and differences were considered statistically significant when $p < 0.05$ and $n = 8$ (*$p < 0.05$, **$p < 0.01$ ***$p < 0.001$).

CD-Ad fed mice (Figure 7 and Table 1). HFD-tRF, HFD-Ad-Ex, and HFD-tRF-Ex significantly downregulated *Per2* and *Dbp* expressions in livers. HFD-tRF and HFD-tRF-Ex significantly downregulated *Rev-Erbα* expression while HFD-Ad-Ex did not change *Rev-Erbα* liver expressions (Figure 7 and Table 1). HFD-Ad feeding downregulated *Bmal1* and *Clock* genes and upregulated *Dbp* expressions in adipose tissues compared to control (CD-Ad) fed mice (**Figure 8**). HFD-tRF, HFD-Ad-Ex and HFD-tRF-Ex significantly upregulated *Bmal1* expression compared to HFD-Ad fed mice in adipose tissues. No significant difference in adipose tissue *Bmal1* expressions with HFD-tRF, HFD-Ad-Ex and HFD-tRF-Ex compared to control (Figure 8 and Table 1). HFD-tRF significantly induced adipose tissue *Cry1* expression compared to HFD-Ad fed mice and CD-tRF reduced *Cry1* expressions compared to HFD-tRF. HFD-Ad-Ex reduced *Cry2* and *Dbp* expressions compared to HFD-Ad fed mice. HFD-tRF and HFD-tRF-Ex feeding significantly downregulated *Rev-Erbα*, but HFD-Ad-Ex did not change *Rev-Erbα* expressions in adipose tissue compared to HFD-Ad fed mice (Figure 8 and Table 1). CD-tRF feeding significantly reduced *Per2, Cry2,* and *Rev-Erbα* expressions compared to CD-Ad fed mice. In addition, CD-tRF feeding significantly downregulated *Per2, Cry1,* and *Cry2* expressions in adipose tissue compared to HFD-tRF (Figure 8 and Table 1). HFD-Ad feeding downregulated *Bmal1* and *Clock* gene and upregulated *Per1, Per2, Cry2,* and *Dbp* expressions in skeletal muscle compared to control CD-Ad fed mice (**Figure 9** and Table 1). HFD-tRF, HFD-Ad-Ex and HFD-tRF-Ex significantly upregulated *Bmal1* and *Cry1* and downregulated *Per2, Cry2 and Dbp* expressions compared to HFD-Ad fed mice in skeletal muscles (Figure 9 and Table 1). Muscle *Per1* expression was

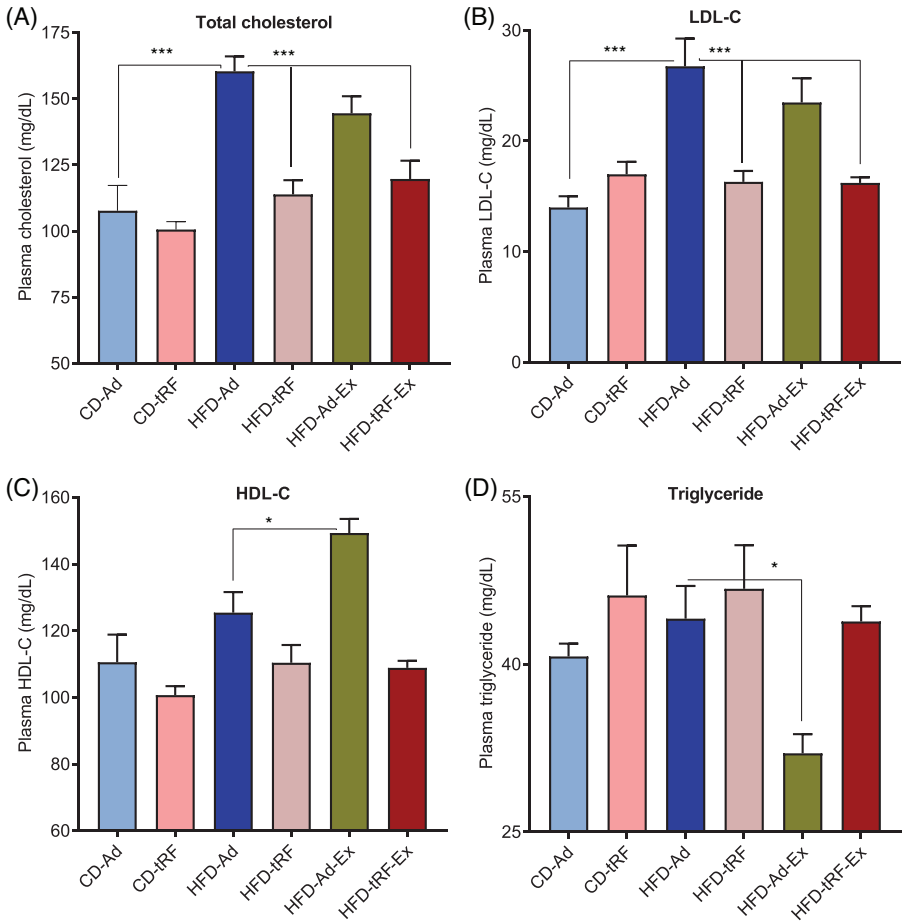

Figure 5. HFD-tRF and HFD-tRF-Ex decreased total plasma cholesterol and LDL-C compared to HFD-Ad (A,B). HFD-Ad-Ex increased plasma HDL-C and decreased plasma triglyceride levels compared to HFD-Ad (C,D). The values represent the mean ± standard error of mean (SEM), and differences were considered statistically significant when $p < 0.05$ and $n = 8$ (*$p < 0.05$, **$p < 0.01$ ***$p < 0.001$).

significantly downregulated in HFD-tRF, and HFD-tRF-Ex fed mice compared to HFD-Ad fed mice. HFD-Ad-Ex significantly upregulated *Clock* expressions, while HFD-tRF and HFD-tRF-Ex did not affect the expressions in skeletal muscles compared to HFD-Ad (Figure 9 and Table 1). Table 1 summarizes the results of clock gene expressions in the liver, adipose tissue and skeletal muscle.

3.7. Immunoblotting for PGC-1α Expressions

Immunoreactivity of PGC-1α (≈90 kDa molecular weight) was found in total protein extractions in all liver samples (**Figure 10A**). PGC-1α protein expressions were increased (but not significantly) in the CD-Ad fed mice compared to HFD-Ad fed mice (Figure 10B). HFD-tRF and HFD-tRF-Ex showed an increase in PGC-1α expressions, but the increase was not statistically significant (Figure 10B).

4. Discussion

Time-restricted feeding significantly inhibited body weight gain, reduced plasma cholesterol, and improved insulin sensitivity in mice under a high-fat diet. However, time-restrictions under control diet improved only insulin sensitivity without affecting body weight and plasma lipids. These results are in accordance with previous findings in animals with different feeding schedules: tRF in mice for 6–8 h day^{-1} reduced body weight, body fat, plasma triglyceride and cholesterol, and improved glucose tolerance.[13,33,34] Ten hours tRF/day in whole body *Cryl:Cry2* and liver specific *Bmal1* and *Rev-Erbα/β* knockout mice prevented HFD-induced obesity, dyslipidemia, and glucose intolerance by protecting aberrant activation of genes involved in lipid metabolism.[22] Chaix and colleagues reported that tRF increased mammalian target of rapamycin (mTORC1) activation and integrated stress response pathways, reducing stress of HFD feeding and thereby preserving cellular homeostasis and metabolic functions.[22] Similar results on body weight and insulin resistance were reported in humans with 6–10 h tRF but in contrast to animal data a reduction in energy intake of about 20% was reported.[35–38] Recently, Tsitsou et al. summarized that tRF produced a moderate weight loss in overweight and obese subjects, but a weight loss >5% was observed when tRF combined with caloric restrictions.[39] We found a decrease in total food and energy intake with tRF and Ex-tRF in HFD fed mice compared to

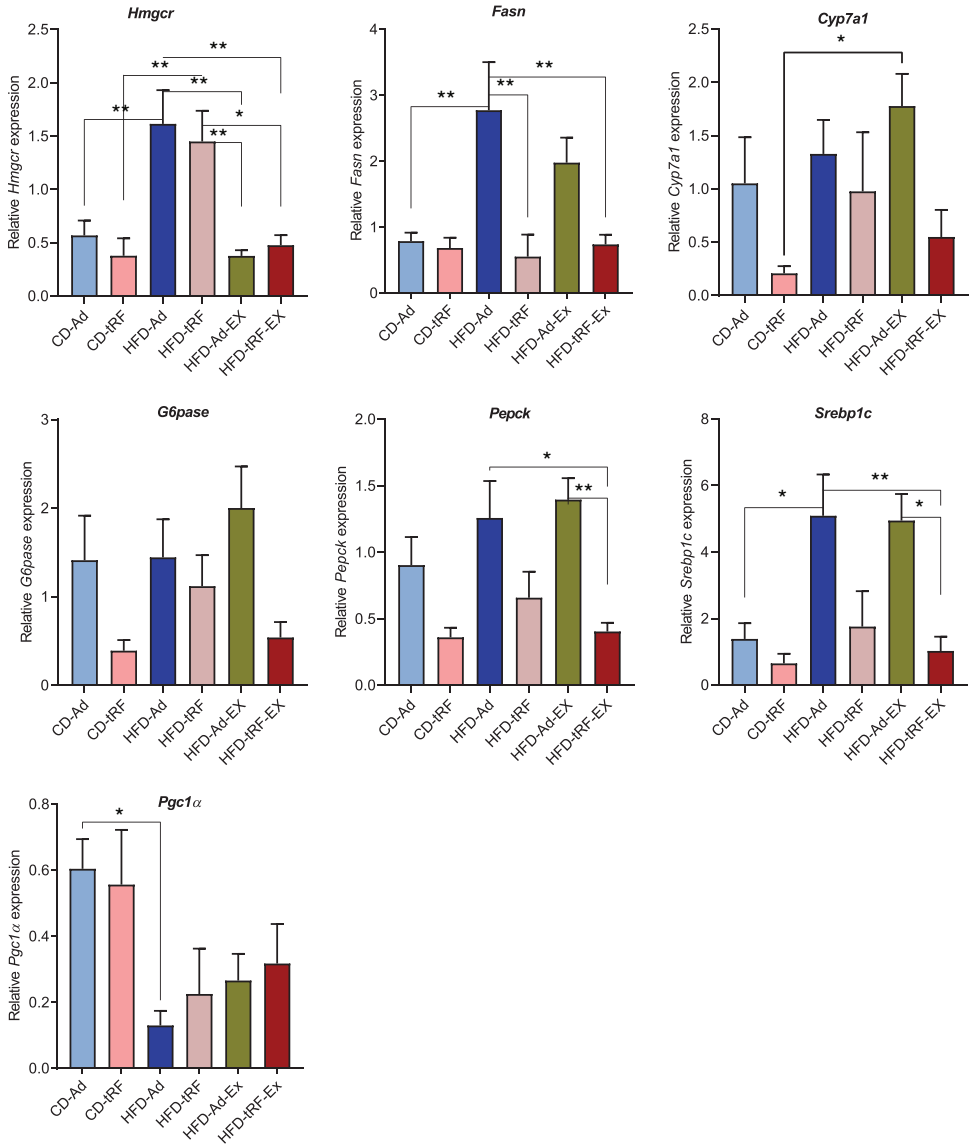

Figure 6. HFD-Ad upregulated the expressions of *Hmgcr* and *Fasn* genes in liver compared to CD-Ad fed mice. *Hmgcr* was downregulated with HFD-Ad-Ex and HFD-tRF-Ex and *Fasn* was downregulated with HFD-tRF and HFD-tRF-Ex in liver compared to HFD-Ad. In addition, CD-tRF significantly downregulated *Hmgcr* expression compared to HFD-tRF. No significant changes in *Cyp7a1*, *G6pase* gene were found. However, HFD-tRF-Ex inhibited *Pepck* expression in liver compared to HFD-Ad fed mice. HFD-tRF-Ex inhibited *Srebp1c* expressions compared to HFD-Ad fed mice. *Pgc-1α* gene expression was downregulated by HFD-Ad compared to CD-Ad fed mice. HFD-tRF, HFD-Ad-Ex, and HFD-tRF-Ex increased the expressions of PGC-1α. The values presented as mean ± standard error of the mean (SEM) and differences were considered statistically significant when $p < 0.05$ and $n = 8$ (*$p < 0.05$, **$p < 0.01$ ***$p < 0.001$).

ad libitum fed but no significant changes in food intake per gram body weight of the mice (Figure 3 and S1, supporting information). In contrast, Hatori et al., reported that tRF did not reduce energy intake but improved diurnal rhythm of food intake and respiratory exchange ratio in HFD fed mice,[34] yet the authors calculated energy intake per body weight of the mice. When we calculated energy intake in the similar way, we also found no difference between the feeding groups.

The moderate exercise intensity used in our study (1 h treadmill 12 m min^{-1} on alternate days) for 8 weeks did not result in observable changes nor seemed to have improved metabolic consequences in HFD fed mice. However, 1 h treadmill 16 m min^{-1} daily reduced body weight gain (Figure 2A) and plasma triglycerides (Figure 5D) in HFD fed obese mice but did not reduce overall food intake (Figure 3). Similar results with exercise on body weight gain and plasma lipids were reported in humans and animals.[40,41] Rodent studies demonstrated that different modes of exercise such as treadmill, wheel running, and swimming reduced diet-induced obesity and insulin resistance.[42–44] Our exercise regime did not improve glucose tolerance in HFD-induced obese mice. Similar findings were observed in type-2 diabetic mice (*db*/*db*) during 6 weeks of daily treadmill exercise 12–18 m min^{-1} for 30 min.[45] In contrast, treadmill exercise with varying speeds (6 m min^{-1}-exhaustion

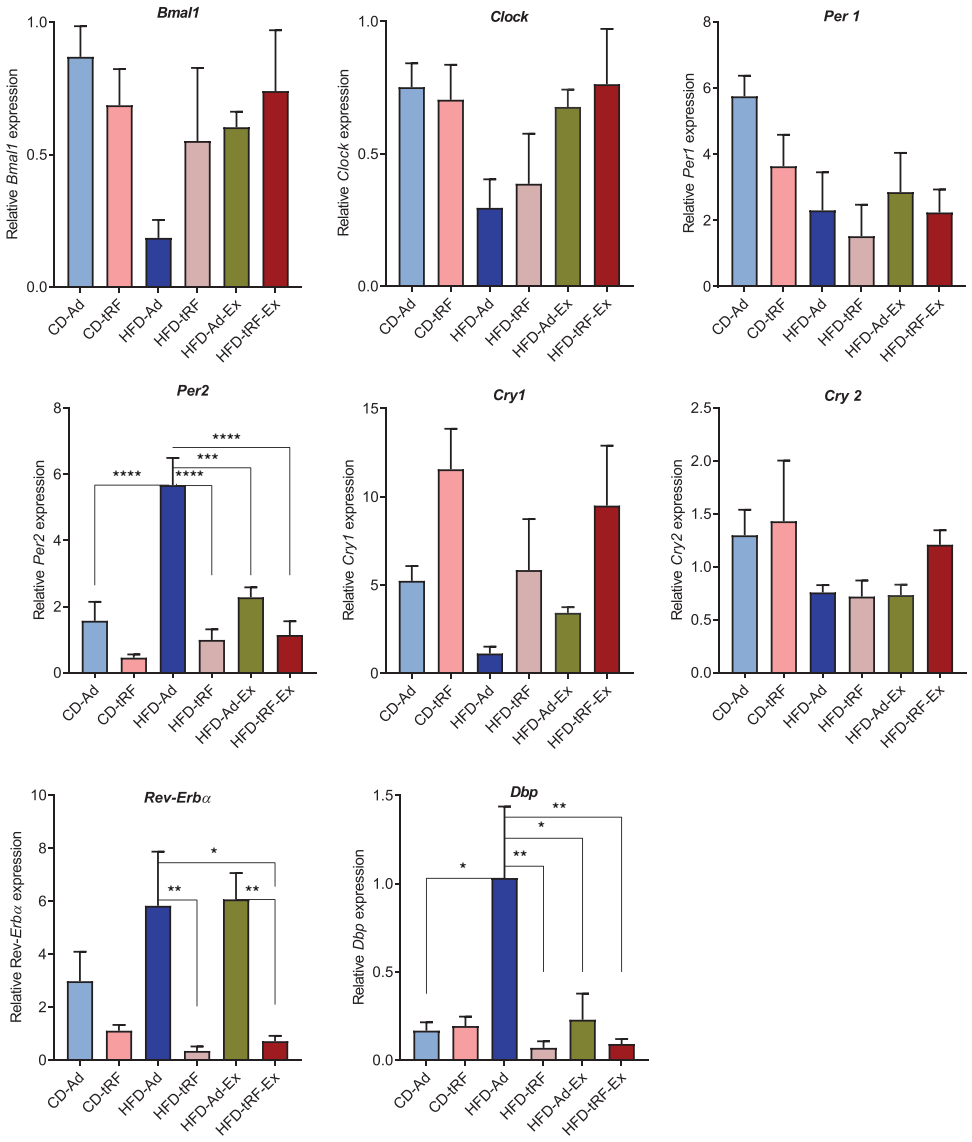

Figure 7. HFD-tRF, HFD-Ad-Ex and HFD-tRF-Ex increased *Bmal1*, and *Clock* expression compared to HFD-Ad fed mice in the liver. HFD-Ad significantly upregulated *Per2* and *Dbp* expressions in the liver compared to CD-Ad fed mice. HFD-tRF, HFD-Ad-Ex and HFD-tRF-Ex significantly downregulated *Per2* and *Dbp* expressions in liver. In addition, HFD-tRF and HFD-tRF-Ex significantly downregulated *Rev-Erbα* expression in liver. HFD-Ad-Ex did not change *Rev-Erbα* expressions. The values presented as mean ± standard error of the mean (SEM) and differences were considered statistically significant when $p < 0.05$ and $n = 8$ (*$p < 0.05$, **$p < 0.01$ ***$p < 0.001$).

80 min per day 5 times per week) for 8 weeks in *db/db* mice improved glucose tolerance and exercise capacity.[46] In type-2 diabetic subjects exercise reduced obesity and improved glucose homeostasis.[47] The failure to improve glucose tolerance in our study could be due to the exercise intensity and timing, which was in the morning (ZT1-ZT2) 1 h after lights on. Low and moderate intensity exercise clearly showed better performance late in their active phase (2 h before morning) compared to early active phase (2 h after evening) in mice, however no such differences in performance were observed with high intensity exercise.[48] Similar observations (better at 6:00pm than early hours 8:00 am of the day) with exercise efficiency were reported in humans, indicating that effects of exercise depend on the circadian system.[48,49] The authors reported that oxygen consumption were lower in evening than the morning hours of the day.[48] Several studies in humans demonstrated that exercise in the morning (6:00–11:00) was associated with greater weight loss, BMI, waist circumference, and abdominal fat compared to evening times (15:00–20:00) in overweight and obese subjects.[50–52] Some studies showed that late afternoon exercise is more effective than morning exercise.[53,54] In contrast, others reported no significant difference in weight loss between morning and evening exercise schedules.[55] A recent systematic review of 35 studies with 17 259 participants on physical activity found no consistent evidence which time of the day to perform exercise provided the most favorable health benefits.[56] However, in a controlled environment with mice wheel running altered the expression of clock genes *Per1/2* in SCN indicating that exercise shifts the circadian rhythm.[57]

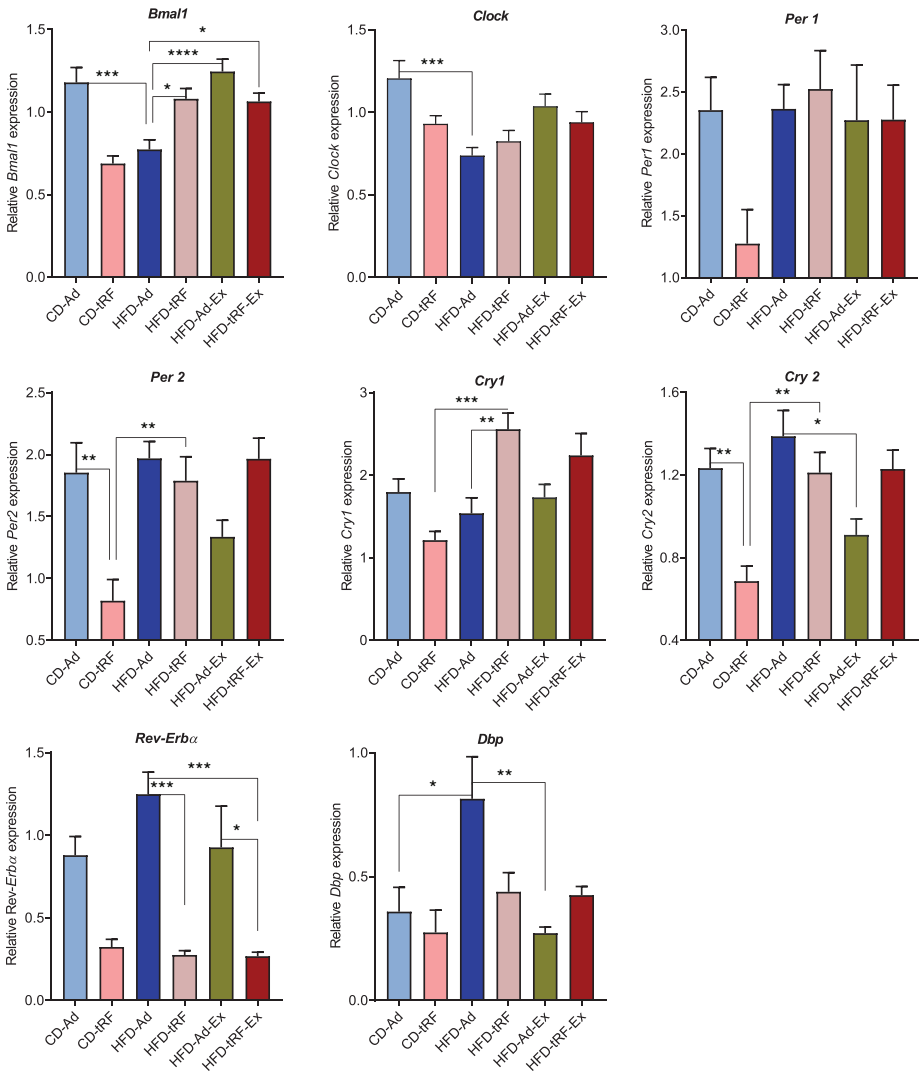

Figure 8. HFD-Ad feeding downregulated *Bmal1* and *Clock* gene and upregulated *Dbp* expressions and HFD-tRF, HFD-Ad-Ex and HFD-tRF-Ex upregulated *Bmal1* expression in adipose tissue. HFD-tRF increased *Cry1* expression and reduced *Rev-Erbα* expressions in adipose tissue. HFD-tRF, and HFD-tRF-Ex HFD-Ad-Ex reduced *Cry2* and *Dbp* expressions compared to HFD-Ad fed mice. CD-tRF significantly reduced *Per2*, *Cry2*, and *Rev-Erbα* expressions compared to CD-Ad fed mice. *Per2*, *Cry1* and *Cry2* were significantly downregulated in mice adipose tissue fed with CD-tRF compared to HFD-tRF. The values presented as mean ± standard error of the mean (SEM) and differences were considered statistically significant when $p < 0.05$ and $n = 8$ ($*p < 0.05$, $**p < 0.01$ $***p < 0.001$).

Time-dependent variations in exercise performance is depended on clock proteins PER1/2, regulating glycolytic and fatty acid oxidation.[48] Early tRF increased the expression of circadian genes *BMAL1*, *CRY1/2* in the morning, and *REV-ERBα*, *CRY1/2* in the evening in human blood cells, indicating a bidirectional feedback loop between meal timing and the circadian clock.[58] We found that exercise during an early inactive period altered circadian clock genes in periphery including liver, adipose, and skeletal muscles in mice (Figures 7–9 and Table 1).

Our results show that tRF combined with aerobic exercise (tRF-Ex) reduced body weight, fat mass, plasma lipids and improved insulin sensitivity in HFD fed mice. Recently, it has been shown that tRF-Ex reduced fat mass and improved lipid metabolism, and glycemic control in mice fed with HFD.[59] Our results are consistent with these previous findings in terms of insulin sensitivity and fat mass with tRF-Ex.[59] Haganes and colleagues demonstrated in overweight and obese women that tRF combined exercise reduced Hb1Ac and improved body compositions.[60] The author showed that reduction in fat mass was more pronounced with tRF-Ex compared to tRF or exercise alone.[60] Our results are consistent with those on body weight and fat mass and therapeutically superior to tRF or exercise alone in preventing obesity and metabolic diseases in animal model.

The central and peripheral circadian clocks regulate nutrient intake and energy metabolism. Short-term (6 weeks) HFD feeding in mice altered diurnal rhythms of clock genes, such as *Bmal1*, *Clock*, and *Per2* in adipose tissue.[61] Exercise, tRF, and their combinations (Ex-tRF) prevented *Bmal1* downregulation induced by HFD in muscles, adipose tissue, and liver. We found an upregulation in *Bmal1* and *Cry1*, downregulation of *Cry2*,

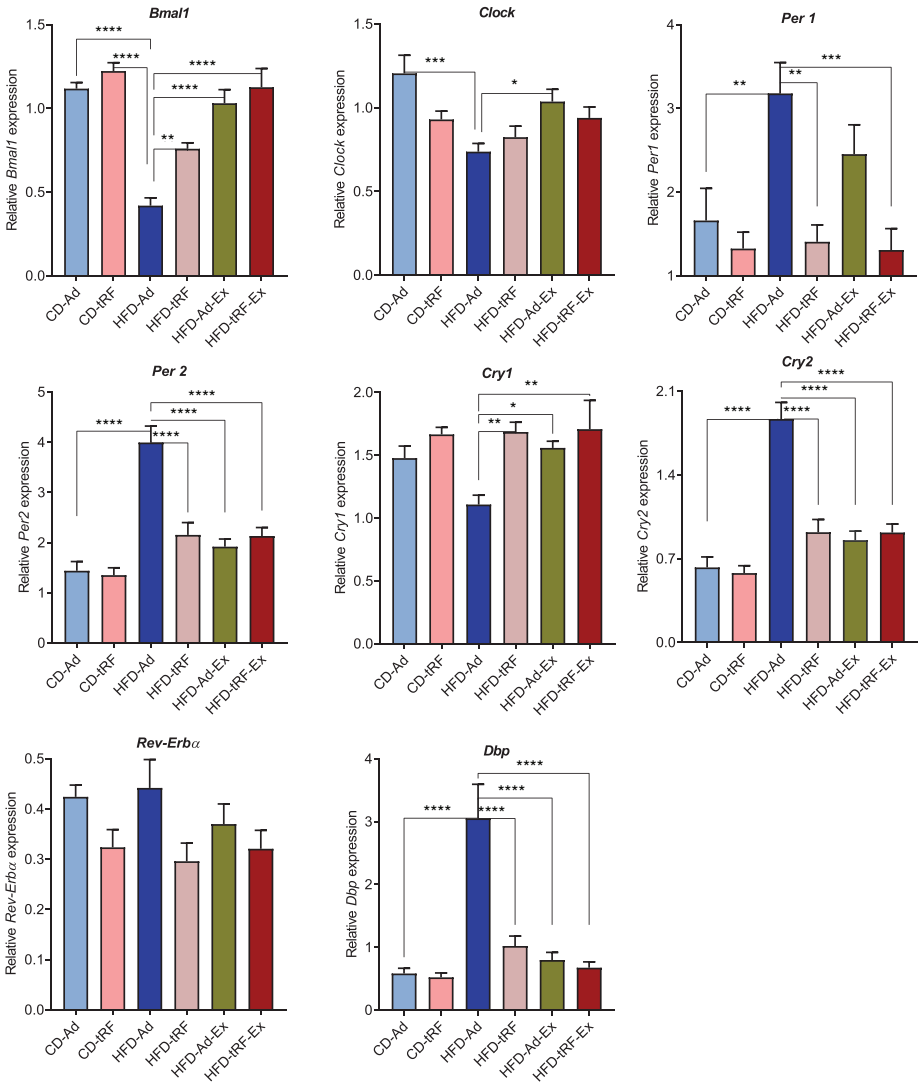

Figure 9. HFD-Ad downregulated *Bmal1* and clock gene and upregulated *Per1*, *Per2*, *Cry2*, and Dbp expressions in skeletal muscles compared to CD-Ad. HFD-tRF, HFD-Ad-Ex and HFD-tRF-Ex upregulated *Bmal1* and *Cry1* and downregulated *Per2*, *Cry2* and *Dbp* expression compared to HFD-Ad. *Per1* was downregulated by HFD-tRF, and *Clock* expressions were upregulated with HFD-tRF-Ex and HFD-Ad-Ex in skeletal muscles. The values presented as mean ± standard error of the mean (SEM) and differences were considered statistically significant when $p < 0.05$ and $n = 8$ (*$p < 0.05$, **$p < 0.01$ ***$p < 0.001$).

Per2, and *Rev-Erbα* with tRF, Ex and tRF-Ex in peripheral tissues (Figures 7–9 and Table 1). *Bmal1*-deficient mice displayed glucose intolerance and higher total fat content and similar metabolic defects were reported in *Per1*$^{-/-}$ and *Per2*$^{-/-}$ double mutant mice.[62] In contrast, liver specific *Bmal1*-knockout exhibited hypoglycemia that is limited to fasting, as well as increased glucose clearance due to increase expressions of *Glut2* and genes involved in hepatic glucose regulations glucokinase and pyruvate kinase.[62] Whole-body *Bmal1* knockout resulted in muscle atrophy and decreased total activity.[63,64] In contrast, muscle-specific *Bmal1* knockout showed normal muscle ultrastructure with a slight decrease in muscle force.[65] These findings suggest that the dramatic muscle atrophy in whole-body *Bmal1* knockout mice is unlikely to be the consequence of a disrupted muscle clock.[66] *Bmal1* expression spikes during the transit from the active to the rest phase, whereas *Per1/2*, *Cry1/2* are antiphase to *Bmal1* and their expressions peak during the transition from rest to activity phase in human skeletal muscles.[67] The genes of key enzymes involved in cholesterol and bile acid metabolism such as *Hmgcr* and *Cyp7a1* showed diurnal oscillation in mice liver.[68] The *Bmal1* is found in the promoter region of genes involved in lipid metabolism and coincides with the increased liver expression of *Fasn*, sterol regulatory element binding transcription factor 1 (*Srebf1*) and *Hmgcr* in mice.[69,70] Liver expression of *Srebp1c* and *Fasn* increased during day-time feeding compared to nighttime feeding.[71] We measured the expressions of lipid metabolizing genes in livers during the transition from active to the rest phase at ZT0.5. tRF and Ex-tRF downregulated *Fasn* and *Srebp1c* while *Hmgcr* was downregulated by exercise and Ex-tRF (Figure 6). These findings indicate that *Bmal1* upregulation

Table 1. Summary table of the expression levels of circadian genes in liver, adipose tissue, and skeletal muscle. CD-tRF and HFD-Ad were compared with control (CD-Ad). HFD-Ad HFD-tRF, HFD-Ad-Ex and HFD-tRF-Ex were compared with HFD-Ad. (↓ = Downregulation) (↑ = upregulation) and (– = no differences).

Genes	Target organs	CD-tRF	HFD-Ad	HFD-tRF	HFD-Ad-Ex	HFD-tRF-Ex	Comments
Bmal1	Liver	–	↓	–	–	–	HFD-tRF, HFD-Ad-Ex, and HFD-tRF-Ex upregulated *Bmal1* in adipose tissues and skeletal muscle
	Adipose tissue	–	↓	↑	↑	↑	
	Skeletal muscle	–	↓	↑	↑	↑	
Clock	Liver	–	↓	–	–	–	HFD-Ad-Ex upregulated *Clock* in skeletal Muscle
	Adipose tissue	–	↓	–	–	–	
	Skeletal muscle	–	↓	–	↑	–	
Per1	Liver	–	↓	–	–	–	*Per1* was downregulated in skeletal muscle of HFD-tRF, and HFD-tRF-Ex fed mice
	Adipose tissue	–	↓	–	–	–	
	Skeletal muscle	–	↑	↓	–	↓	
Per2	Liver	–	↑	↓	↓	↓	*Per2* downregulation in all the tissues except HFD-tRF-Ex in adipose tissue
	Adipose tissue	↓	↑	↓	↓	–	
	Skeletal muscle	–	↑	↓	↓	↓	
Cry1	Liver	–	↓	–	–	–	HFD-tRF upregulated *Per2* in adipose tissue and HFD-tRF, HFD-Ad-Ex and HFD-tRF-Ex in skeletal Muscles
	Adipose tissue	–	↓	↑	–	–	
	Skeletal muscle	–	↓	↑	↑	↑	
Cry2	Liver	–	↓	–	–	–	*Cry2* downregulated in adipose tissue with HFD-Ad-Ex. HFD-tRF, HFD-Ad-Ex and HFD-tRF-Ex reduced *Cry*2 in skeletal muscle
	Adipose tissue	↓	↑	–	↓	–	
	Skeletal muscle	–	↑	↓	↓	↓	
Rev-Erbα	Liver	–	↓	↓	–	↓	HFD-tRF and HFD-tRF-Ex reduced *Rev-Erbα* in liver. HFD-tRF, HFD-Ad-Ex, HFD-tRF-Ex reduced *Rev-Erbα* in adipose tissue
	Adipose tissue	–	↑	↓	↓	↓	
	Skeletal muscle	–	↑	–	–	–	
Dbp	Liver	–	↑	↓	↓	↓	HFD-tRF, HFD-Ad-Ex, HFD-tRF-Ex reduced *Dbp* in liver and skeletal muscle. HFD-Ad-Ex reduced *Dbp* in adipose tissue.
	Adipose tissue	–	↑	–	↓	–	
	Skeletal muscle	–	↑	↓	↓	↓	

is most likely not the cause for the reduction in plasma lipids under tRF and Ex-tRF since *Fasn* and *Hmgcr* are in antiphase to *Bmal1* expression. It has been demonstrated that *Bmal1* repressed adipogenesis via the Wnt signaling pathway,[72] and *Bmal1*$^{-/-}$ mice displayed reduced capacity for fat storage. These observations indicate that *Bmal1* plays a role in adipogenesis. In addition, exercise upregulated *Bmal1* in periphery and did not reduce plasma lipids. Our results are consistent with the previous studies in mice with *Fasn* downregulations under tRF.[34] tRF induced rhythmicity in *Fasn* expression in both the light and dark-fed animals which is due to food intake.[73]

Aerobic exercise affects lipid metabolism by reducing mRNA levels of *Hmgcr* and acetyl Co-A acetyltransferase 1 (*Acat1*) in liver of high-fat and high cholesterol-fed mice.[74] Our results with exercise are consistent with previous studies on *Hmgcr* in the liver.[74] tRF inhibited *Hmgcr* and *Cyp7a1* during dark phase in the liver of mice fed with high-fat and high-cholesterol diet.[75] Hua et al. demonstrated that tRF suppressed lipogenesis, but did not find significant changes in *Hmgcr* and *Fasn* expression under tRF in HFD fed mice liver, which might be due to the timing of the sacrifice of the mice.[76] In addition, *Rev-Erbα* acts as a repressor of genes involved in lipid synthesis.[77] We found reduced expression of *Rev-Erbα* under tRF and Ex-tRF in the liver and adipose tissues. The different results could be due to time of animal sacrifice at ZT0.5 (within 30 min of lights on) since *Rev-Erbα* expressions is lowest in the morning and peaks during start of the dark phase in peripheral tissues.[78] PGC-1*α* regulates many metabolic processes including mitochondrial biogenesis, hepatic gluconeogenesis, and thermogenesis.[79,80] In our study, *PGC-1α* increased

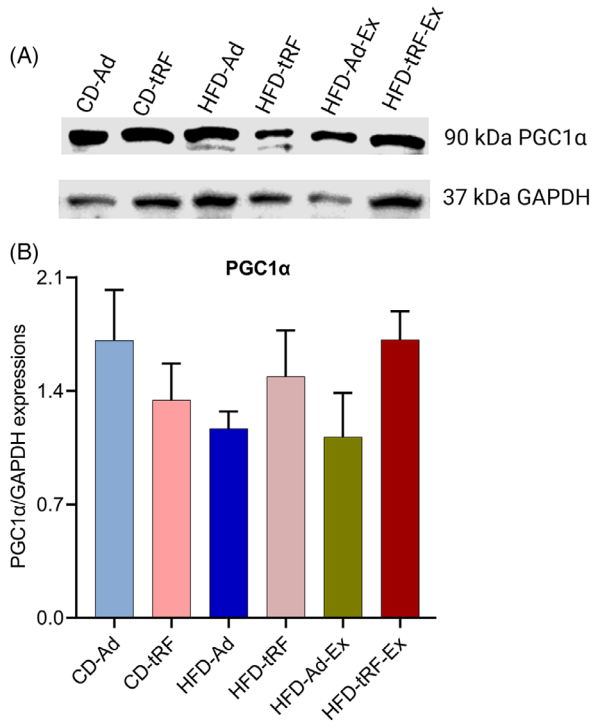

Figure 10. PGC-1*α* protein expression was downregulated by HFD-Ad compared to control CD-Ad in the liver. HFD-tRF and HFD-tRF-Ex increased the PGC-1*α* protein expressions compared to HFD-Ad. The values presented as mean ± standard error of the mean (SEM) and differences were considered statistically significant when $p < 0.05$ and $n = 8$ ($*p < 0.05$, $**p < 0.01$ $***p < 0.001$).

with tRF, exercise, and tRF-Ex in the liver. Similar findings on *PGC-1α* were reported under tRF in adipose tissues and BAT of mice.[78]

Clock mRNA expression can highly vary with either extremely low or no circadian fluctuation.[81,82] We found that *Clock* gene expressions did not change between the groups in liver and adipose tissues but exercise upregulated *Clock* expressions in skeletal muscles (Figure 9 and Table 1). Exercise provides a time cue for clock genes in peripheral tissues.[83,84] Wheel access for 6 h in the early night (ZT12-18) and late night (ZT18-24) amplified circadian rhythms, improved ambulatory activity and physiological rhythms in wild type and vasoactive intestinal peptide (VIP) deficient mice.[85] The authors reported an increased amplitude of *Per2* rhythms in the SCN and in peripheral organs such as the liver and adrenals.[85] In contrast, our results showed reduced expressions of *Per2* in the liver and adipose tissues of mice fed with HFD, which could be due to the time of exercise (Figures 7,8, and Table 1). Resistance training in humans upregulated *BMAL1*, PER2, and *CRY1*, but PER1, *CRY2*, and *Rev-Erb* remained constant in the skeletal muscles even 6 h after exercise.[86] Treadmill running increased *Per1* and *Per2* expression in plantaris muscle 1 h after exercise in mice.[87] In humans, acute exercise (80% Vo2) for 15 min in the morning enhanced *PER1* and *PER2* and reduced *Rev-Erbα* expressions in skeletal muscles.[88] Our results demonstrated that exercise increased *Bmal1* and reduced *Per2* expression in skeletal muscles and adipose tissues and tRF downregulated *Per2* expression in skeletal muscles. Similar results on *BMAL1* gene expressions with exercise were reported in human skeletal muscles 4–8 h after exercise.[89] *Rev-Erbα* expression was downregulated by tRF and tRF-Ex in the liver and adipose tissues of mice fed with HFD (Figures 7,8, and Table 1). In contrast, Hatori et al. reported that tRF increased *Rev-Erbα* expressions in the mice liver.[34] This could be due to the different time of sacrifice as *Rev-Erb* rhythms are low in the morning and peak during the evening. Global homozygous *Rev-Erbα* ablation inhibited glycolysis and fatty acid-oxidation in white adipose tissue (WAT), whereas heterozygous ablation stimulated these metabolic processes.[90] Whole-body *Rev-Erbα/β* knockout resulted in increased food intake, body weight, blood glucose, and altered activity pattern with reduced nighttime running,[91,92] whereas SCN-specific knockdown resulted in increased weight gain, food intake, blood glucose, and liver triglyceride levels.[92] Adipocyte-specific *Rev-Erbα* knockout mice developed obesity under HFD feeding, indicating that Rev-Erbα regulates WAT metabolism in a state-dependent manner.[93] Liver specific *Rev-Erbα* knockdown in mice revealed limited impact no difference in lipid accumulation in the liver nor on lipogenic genes.[93] These observations suggest that the favorable effect with tRF and Ex-tRF in our study is not due to the down-regulation of liver *Rev-Erbα*.

Our study has some limitations: We had only HFD fed (ad libitum and tRF) mice exercised while CD fed mice did not. Exercise was performed only once per day for 1 h after lights on. Mice were sacrificed at only one time point within 0.5 h of lights on.

5. Conclusion

Forced exercise at the start of inactive phase alleviated obesity in mice fed with HFD. Time-restricted feeding restored the circadian clocks in peripheral tissue,[34] and prevent detrimental metabolic effects of HFD. Forced exercise affected clock genes *Bmal1* and *Rev-Erbα* in adipose and skeletal muscle tissues, suggesting that forced exercise functions as Zeitgeber in these tissues. Time-restricted feeding seems to be a stronger Zeitgeber compared to forced exercise in peripheral tissues. The combination of exercise and time-restricted feeding resulted in the greatest reduction in body weight, plasma lipids and improved insulin sensitivity compared to either exercise or time-restricted feeding alone. Additional investigations are needed for the optimal combinations of forced exercise and time-restricted feeding to maintain and improve metabolic health and their translation to humans.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

G.S.R., K.H.H. and K.A.M. conceptualized the study, K.H.H. provided the funds, G.S.R. and Y.K. performed the experiments and analysis. R.S. and S.J.M. helped with the analyses of western blots and V.S. performed the statistical analysis. G.S.R. wrote the first version of the manuscript. All authors contributed to the final version of the manuscript, proofread the manuscript, and agreed with the publication.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Keywords

circadian clocks, exercise, metabolic regulations, obesity, time-restricted feeding

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