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RESEARCH ARTICLE

Physical Activity, Mitochondria, and Disease

Social jet lag impairs exercise volume and attenuates physiological and metabolic adaptations to voluntary exercise training

Michael B. Dial,[1] Elias M. Malek,[1] Austin R. Cooper,[1] Greco A. Neblina,[1] Nikoleta I. Vasileva,[1] Dustin J. Hines,[2] and Graham R. McGinnis[1]

[1]*Department of Kinesiology and Nutrition Sciences, School of Integrated Health Sciences, University of Nevada, Las Vegas, Nevada, United States and* [2]*Department of Psychology, Psychological and Brain Sciences and Interdisciplinary Neuroscience Programs, University of Nevada, Las Vegas, Nevada, United States*

Abstract

Social jet lag (SJL) is a misalignment between sleep and wake times on workdays and free days. SJL leads to chronic circadian rhythm disruption and may affect nearly 70% of the general population, leading to increased risk for cardiometabolic diseases. This study investigated the effects of SJL on metabolic health, exercise performance, and exercise-induced skeletal muscle adaptations in mice. Ten-week-old C57BL/6J mice ($n = 40$) were allocated to four groups: control sedentary (CON-SED), control exercise (CON-EX), social jet lag sedentary (SJL-SED), and social jet lag exercise (SJL-EX). CON mice were housed under a 12:12-h light-dark cycle. SJL was simulated by implementing a 4-h phase delay for 3 days to simulate "weekends," followed by a 4-h phase advance back to "weekdays," for 6 wk. EX mice had free access to a running wheel. Graded exercise tests (GXTs) and glucose tolerance tests (GTTs) were performed at baseline and after intervention to monitor the effects of exercise and social jet lag on cardiorespiratory and metabolic health, respectively. SJL led to alterations in activity and running patterns and clock gene expression in skeletal muscle and decreased average running distance ($P < 0.05$). SJL-SED mice gained significantly more weight compared with CON-SED and SJL-EX mice ($P < 0.01$). SJL impaired fasting blood glucose and glucose tolerance compared with CON mice ($P < 0.05$), which was partially restored by exercise in SJL-EX mice. SJL also blunted improvements in exercise performance and mitochondrial content in the quadriceps. These data suggest that SJL blunted some cardiometabolic adaptations to exercise and that proper circadian hygiene is necessary for maintaining health and performance.

NEW & NOTEWORTHY In mice, disrupting circadian rhythms with social jet lag for 6 wk caused significant weight gain, higher fasting blood glucose, and impaired glucose tolerance compared with control. Voluntary exercise in mice experiencing social jet lag prevented weight gain, though the mice still experienced increased fasting blood glucose and impaired exercise performance compared with trained mice not experiencing social jet lag. Social jet lag seems to be a potent circadian rhythm disruptor that impacts exercise-induced training adaptations.

cardiometabolic adaptations; circadian rhythm disruption; exercise performance; glucose tolerance; social jet lag

INTRODUCTION

Circadian rhythms are natural daily cycles that exist in almost all living organisms and function at all biological levels including cellular, tissue, and systemic. These rhythms are regulated by an intrinsic mechanism known as the circadian clock, which orchestrates biological processes in a time of day-dependent fashion (1, 2). Maintaining health relies significantly on circadian rhythms, which play a crucial role in regulating vital physiological processes including body temperature, hormone production, and metabolism and lead to disease states when disrupted (3). The suprachiasmatic nucleus (SCN) located in the hypothalamus serves as the "central clock" for these circadian rhythms, which coordinates the rhythmicity of neighboring brain regions as well as peripheral tissues through neural humoral pathways. Peripheral tissues harbor their own intrinsic circadian clocks, which are ubiquitously present in extracerebral tissues including skeletal muscle. Synchronization of circadian clocks is achieved through three primary extrinsic cues or zeitgebers: light, food/nutrition, and activity/exercise. The central clock integrates photic cues to synchronize the central clock in the SCN and is thus predominantly influenced by light. Conversely, peripheral clocks exhibit greater responsiveness to bioenergetic cues such as nutrient intake and physical exercise (4–6). This synchronization ensures

Correspondence: G. R. McGinnis (graham.mcginnis@unlv.edu).
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coordinated circadian activity throughout the body, allowing for anticipation of physiological and metabolic demands and preparing the body for patterns of activity, stress, and subsequent recovery (1, 4).

Within modern societies, the natural diurnal patterns of sunlight and darkness have been supplanted by artificial light sources. Consequently, our light exposure is increasingly dictated by our behavioral and societal needs, often leading to misalignment of light exposure leading to desynchronization of the circadian rhythm (7). In the most extreme cases, individuals engaged in night shift work experience pronounced disturbances in their circadian rhythms (8). Extensive research has established an association between this disruption and heightened inflammation, metabolic, and cardiovascular diseases among night shift workers (9–14).

Whereas the population of chronic night shift workers is relatively small, a large portion of the population undergoes a far more pervasive form of circadian rhythm disruption called "social jet lag" (SJL) (15). SJL occurs as the result of misalignment between behavioral schedules on free days and workdays. It is determined by calculating the absolute difference in the midsleep phase between free days and work days (15). Weekday behaviors, such as sleep/wake times and meals, are typically regulated by school or work schedules. In contrast, on weekends people often adjust their behaviors to align with their social preferences. This commonly involves delaying the sleep/wake schedule and staying up and waking up later than usual. This shift in behavior, often by several hours each weekend, disrupts the circadian rhythm in a manner similar to conventional jet lag experienced when traveling across different time zones (16, 17). Epidemiological data demonstrate a positive association with SJL and diabetes risk factors including excess body weight (18), dyslipidemia (19), and insulin resistance (20). Animal model studies have demonstrated that a single weekend of SJL (6-h shift) delayed locomotor activity and circadian clock gene expression in several peripheral tissues, which persisted throughout the entire subsequent "workweek" (21). In effect, this suggests that, in those with SJL, clock gene expression is chronically out of synchrony throughout the week. This is similar to what is seen in chronic shift workers, with higher rates of hyperglycemia and type 2 diabetes mellitus (22, 23), as well as genetic and environmental rodent models of circadian rhythm disruption (24–26).

Exercise is a frontline intervention to prevent metabolic diseases including obesity, diabetes, and metabolic syndrome (among others) (27), but it is unclear how SJL impacts the benefits of exercise. SJL has been associated with impaired cardiorespiratory fitness (28, 29), though these findings are based on cross-sectional data, making it difficult to determine whether SJL impedes exercise training-induced adaptations. Furthermore, exercise has also been demonstrated to facilitate faster synchronization of activity rhythms in mice (30) as well as the circadian clock in other peripheral tissues (6), though how exercise interacts with the metabolic effects of SJL is largely uninvestigated. Therefore, the purpose of this study was to investigate the effects of concurrent exercise and SJL on metabolic health (i.e., body weight, glucose tolerance, fasting blood glucose) as well as exercise-induced skeletal muscle adaptations in mice.

MATERIALS AND METHODS

Mice and Housing

Forty adult male C57BL/6J mice (aged 10 wk) were randomly assigned to four experimental groups and were housed and maintained according to University of Nevada, Las Vegas (UNLV) Institutional Animal Care and Use Committee (IACUC) protocols: *1*) sedentary mice housed on a control light-dark (LD) cycle (CON-SED), *2*) sedentary mice housed on a SJL schedule (SJL-SED), *3*) exercised mice housed on a control LD cycle (CON-EX), and *4*) exercised mice housed on a SJL schedule (SJL-EX). The mice were housed individually in microisolator cages maintained at controlled temperature and humidity (~23°C, 20% humidity). The control LD cycle involved a strict 12:12-h flipped cycle, with the light phase (ZT0) starting at 3:00 AM and the dark phase (ZT12) starting at 3:00 PM. This schedule ensured that all handling of the mice was conducted during the dark/active period under dim red light. The intervention period was 6 wk, after which running wheels were locked for at least 24 h and mice were euthanized under isoflurane anesthesia (Covetrus North America, Portland, ME) (3% vaporized in 100% O_2). All mice were euthanized at ZT5 to minimize circadian variation. The skeletal muscles, including quadriceps, gastrocnemius, and soleus, were collected for gravimetric and molecular analysis of exercise-induced adaptations. Tissues were snap-frozen in liquid nitrogen and stored at −80°C for subsequent analysis. All aspects of this study were approved by the UNLV IACUC.

Simulated Social Jet Lag

To simulate a social jet lag (SJL) schedule, mice were transferred between two vivarium rooms with different light-dark (LD) schedules. The first room followed the control LD schedule, as described above, representing the "weekday" schedule. The second room had a 4-h delay, with ZT0 starting at 7:00 AM and ZT12 at 7:00 PM, mimicking a delayed sleep onset. To simulate the delayed sleep onset on weekends, a 4-h phase delay was implemented on Fridays, extending the dark period by 4 h, and this schedule persisted throughout the weekend. After 3 days, the mice were returned to the original LD schedule, undergoing a 4-h phase advance. This simulated the pattern of delayed activity onset followed by a shift back to the regular schedule.

Voluntary Wheel Running Exercise

In the exercise groups (CON-EX and SJL-EX), mice were given access to a wireless running disk (Med Associates, Fairfax, VT) and underwent a 6-wk period of voluntary wheel running exercise. The running disk was available for the mice to use ad libitum on both weekdays and weekends. The exercise volume was recorded with Wheel Manager software (Med Associates). The running distance was continuously monitored in 10-min intervals throughout the 6-wk duration and aggregated to calculate the daily distance covered. For the sedentary mice, a running disk without the revolving base was provided to account for cage enrichment without exercise.

Passive Infrared Activity Monitoring

Sedentary mice were housed individually in cages equipped with a passive infrared (PIR) sensor attached to each cage. This

sensor enabled the monitoring of circadian rhythms in activity without the presence of the exercise stimulus provided by the running disk. The PIR sensor used in this study has been developed and validated with open-source software, demonstrating its accuracy and reliability in tracking locomotive behavior in mice (31). The PIR sensor recorded activity data, which were digitally stored on a flash disk with a 1-min bin resolution. Subsequently, the activity data were processed and aggregated into 10-min bins to analyze both total activity levels and the circadian rhythm in activity.

Glucose Tolerance Tests

To measure glucose tolerance, an intraperitoneal glucose tolerance test (GTT) was administered to mice during the baseline period, before any shifts in LD, and after the 6-wk study period. GTTs were performed consistently at the beginning of the active phase (~ZT12–ZT14) to reduce any time of day-dependent variance in glucose tolerance. Mice were fasted for 12 h with ad libitum access to water during the inactive phase before the GTT. After mouse weight was obtained, fasting blood glucose was measured via tail prick with a glucometer (Contour Next One; Contour, Smyrna TN). An intraperitoneal glucose injection (2 g/kg, 20% glucose solution in sterile 0.9% NaCl) was administered, and blood was sampled at 30, 60, 90, and 120 min from the same tail prick. For SJL mice, GTTs were performed 2 days after the last shift (Fig. 1).

Graded Exercise Tests

To measure exercise capacity and the adaptation to exercise training, mice performed graded exercise tests (GXTs) on a motorized treadmill (Panlab Harvard Apparatus, Barcelona, Spain) at baseline and at the conclusion of the study. Before exercise tests, mice were habituated to the treadmill by exposing them to at least two brief (~5 min) sessions at low speed (~10 m/min). After habituation, mice performed a GXT to determine their baseline level of fitness. The GXT protocol started at 10 m/min and an incline of 10% for 10 min, followed by sequential increases of 2 m/min every 2 min throughout the protocol until mice reached volitional fatigue (32). GXTs were repeated at the conclusion of the study period to evaluate training adaptations. Both GXTs were performed at the same time of day (~ZT12) to minimize time of day-dependent variances in exercise performance (33), 4 days after changes in LD cycle for SJL mice (Fig. 1).

Western Blotting

Frozen skeletal muscle was powdered with a mortar and pestle in liquid nitrogen. Approximately 20 mg of the powdered tissue was mixed with lysis buffer containing protease and phosphatase inhibitors (the soleus muscle was homogenized whole). The resulting lysates were electrophoretically separated on polyacrylamide gels (4–20% gradient) and transferred to polyvinylidene fluoride (PVDF) membranes. After blocking with 5% nonfat dry milk (NFDM) diluted in Tris-buffered saline with Tween (TBS-T), membranes were treated with a cocktail of primary antibodies targeting specific constituent proteins within the five oxidative phosphorylation (OXPHOS) complexes [C-I: NDUFB8, C-II: succinate dehydrogenase B (SDHB), C-III: ubiquinol-cytochrome *c* reductase core protein 2 (UQCRC2), C-IV: mitochondrially encoded cytochrome *c*-oxidase I (MT-CO1), and C-V: ATP5A; Abcam, ab110413]. This cocktail antibody was diluted 1:1,000 in 5% bovine serum albumin (BSA)-based blocking reagent and applied overnight. A horseradish peroxidase (HRP)-conjugated anti-mouse secondary antibody was used to visualize protein expression with enhanced chemiluminescence (ECL) substrate (Bio-Rad ChemiDoc, Hercules, CA), and the resulting images were analyzed with ImageJ software (34).

RNA Isolation and PCR

RNA was isolated with conventional TRIzol methods. Approximately 20 mg of powdered quadriceps tissue was homogenized in TRIzol by shaking in a BeadBeater for 2 min at maximum speed (BioSPEQ, Irvine, CA). The RNA concentration and quality were checked with a NanoDrop 2000 (NanoDrop Technologies, Wilmington, DE), and 1,000 ng of RNA was used for cDNA library preparation. RT-PCR was performed with 25 ng of cDNA in a master mix containing gene-specific primers and SYBR Green (PerfeCTa no. 95072-250), and the reactions were tracked through 40 PCR cycles.

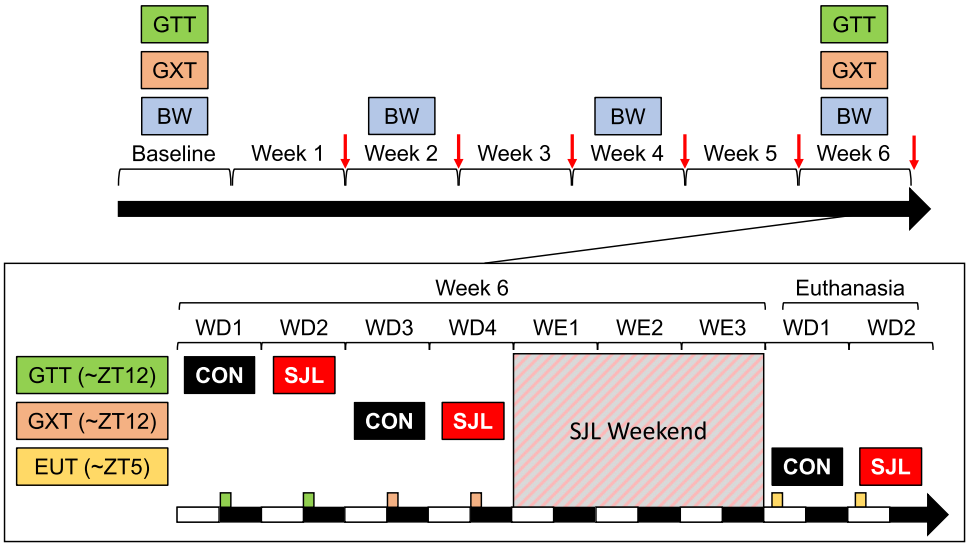

Figure 1. Graphical representation of study design. Mice underwent intraperitoneal glucose tolerance tests (GTTs) and graded exercise tests (GXTs) at baseline and after the 6-wk social jet lag (SJL) intervention. Body weight (BW) was recorded biweekly. All GTTs, GXTs, and BW measurements were taken at ZT12 to minimize circadian variation. During *week 6*, GTTs were performed on control groups (CON) on *weekday 1* (WD1) and on social jet lag groups (SJL) on WD2, ~2 days after the last light-dark (LD) shift. GXTs were performed on CON groups on WD3 and on SJL groups on WD4, ~4 days after the last LD shift. CON mice were euthanized (EUT) on WD1, and SJL mice were euthanized on WD2, ~2 days after the last LD shift. Red arrows indicate LD shifts simulating SJL.

Canonical circadian clock genes were evaluated with specific primer sequences listed below: *Bmal1* F′–CACCAACCCATACACAGAAG, *Bmal1* R′–GGTCACATCCTACGACAAAC, *Per1* F′–CAGACCAGGTGTCGTGATTAAA, *Per1* R′–CGAAACAGGGAAGGTGAAGAA, *Per2* F′–ATGAGTCTGGAGGACAGAAG, *Per2* R′–CCTGAGCTGTCCCTTTCTA, *Clock* F′–ACTCAGGACAGACAGATAAGA, *Clock* R′–TCACCACCTGACCCATAA, *Rev-erb-α* F′–TGGCCTCAGGCTTCCACTATG, *Rev-erb-α* R′–CCGTTGCTTCTCTCTCTTGGG, *Cry1* F′–AGAGGGCTAGGTCTTCTCGC, *Cry1* R′–CTACAGCTCGGGACGTTCTC, *Cry2* F′–GTCTGTGGGCATCAACCGA, *Cry2* R′–TGCATCCCGTTCTTTCCCAA. Gene expression was quantified with the $2^{\Delta\Delta C_t}$ method (where C_t is threshold cycle) and reported as fold change from CON-SED for each gene.

Calculations and Statistical Analysis

Incremental (AUC_i) and total (AUC_t) area under the curve for glucose were calculated with GraphPad Prism 9 (GraphPad Software Inc., La Jolla, CA). Glucose AUC_i, AUC_t, fasting blood glucose, GXT performance, and body mass were analyzed with repeated-measures two-way ANOVA (condition × time). Relative body mass (percent change) was analyzed with a one-way ANOVA. When interaction effects were significant, Tukey's honestly significant difference (HSD) post hoc tests were used to identify where significant differences occurred. All data were analyzed with GraphPad Prism 9 (GraphPad Software Inc.). The level of statistical significance was set at $P < 0.05$. All data are expressed as means ± standard error of the mean.

RESULTS

SJL alters activity rhythms and clock gene expression in sedentary and exercise mice. To assess the effect of SJL and exercise on circadian rhythms in activity, we housed mice with passive infrared activity monitors for sedentary mice (recording activity counts) or wireless running wheels for exercised mice (recording activity in distance). A 3-wk period of activity is shown in Fig. 2*A*, where each bar represents the average activity count for sedentary mice or distance in exercised mice per 10-min time period. To examine the impact of SJL with or without exercise on the skeletal muscle circadian clock, the expressions of canonical circadian clock genes were measured in the quadriceps muscle by PCR after the intervention period (Fig. 2*B*; note that mice were only euthanized at 1 time of day, ~ZT5). We found significant effects of exercise and/or SJL on *Per1*, *Per2*, and *Cry2*. Exercise decreased the expression of *Per2* [SED = 0.618 ± 0.19 vs. EX = 0.293 ± 0.04; main effect (ME) EX: $P = 0.032$] and *Cry2* (SED = 0.613 ± 0.19 vs. EX = 0.276 ± 0.04; ME EX: $P = 0.030$). SJL resulted in increased expression of *Per1* (CON = 1.01 ± 0.20 vs. SJL = 2.04 ± 0.39; ME SJL: $P = 0.041$) and decreased expression of both *Per2* (CON = 0.624 ± 0.19 vs. SJL = 0.288 ± 0.05; ME SJL: $P = 0.029$) and *Cry2* (CON = 0.626 ± 0.19 vs. SJL = 0.265 ± 0.06; ME SJL: $P = 0.023$). No other effects or interactions were observed for other circadian clock genes.

Mice Undergoing SJL Perform Less Voluntary Wheel Running

To evaluate the effects of SJL on voluntary exercise performance, running distance was compared between CON-EX and SJL-EX and was analyzed as the average daily distance covered in each week of the study (*weeks 1–6*; Fig. 3*A*). A significant time × group interaction was found ($P < 0.001$). Post hoc pairwise comparisons revealed that whereas running distance was not different between groups during *week 1* (before the first SJL shift; $P > 0.999$), there were significantly different average weekly distances at *week 2* (CON-EX = 14.3 ± 0.84 km vs. SJL-EX = 8.56 ± 0.92 km, $P = 0.002$) and *week 3* (CON-EX = 16.2 ± 0.86 km vs. SJL-EX = 10.5 ± 0.85 km, $P = 0.001$). Differences in distance approached significance at *week 4* (CON-EX = 14.8 ± 1.31 km vs. SJL-EX = 9.96 ± 1.15 km, $P = 0.08$; Fig. 3*A*) and were not different at *weeks 5* or *6*. These results suggest that SJL has a negative effect on voluntary wheel running activity. To determine whether wheel running was different during the phase shifts inducing SJL, the average daily distance for weekends and weekdays was also assessed (Fig. 3*B*). Whereas there was a significant main effect of SJL (CON-EX weekday = 13.7 ± 1.29 km vs. CON-EX weekend 14.0 ± 1.07 km, $P = 0.89$; SJL-EX weekday = 10.1 ± 0.97 km vs. SJL-EX weekend = 10.1 ± 0.77, $P < 0.001$), indicating lower running distance compared with CON-EX, there was no difference between weekdays and weekends in running distance ($P = 0.89$).

Exercise Prevents SJL-Induced Body Weight Gain

As one index of metabolic health, we assessed body mass (reported as absolute body mass and relative change from baseline body mass) throughout 6-wk SJL. At baseline, there were no significant differences in body mass between groups. We observed a significant group × time interaction in body mass during the study period ($P < 0.001$; Fig. 4*A*). Post hoc comparisons revealed significant increases in absolute body mass for all groups except CON-EX mice (CON-SED baseline: 24.35 ± 0.41 g vs. 6 wk: 25.66 ± 0.45 g, $P < 0.001$; SJL-SED baseline: 23.68 ± 0.53 g vs. 6 wk: 26.43 ± 0.45 g, $P < 0.001$; SJL-EX baseline: 25.04 ± 0.43 g vs. 6 wk: 26.07 ± 0.37 g, $P = 0.003$). However, there were no significant differences between groups at any time point throughout the study. We subsequently compared the change in body mass from mice at baseline to 6 wk with a one-way ANOVA (Fig. 4*B*). We found a significant difference in the change in body mass between groups ($P < 0.001$). Sedentary mice with SJL gained a significantly larger amount of weight during the study compared with sedentary mice on a control schedule (CON-SED: 5.4 ± 1.1% vs. SJL-SED: 11.8 ± 1.1%, $P = 0.002$). Exercise was able to prevent the weight gain due to SJL (SJL-SED: 11.8 ± 1.1% vs. SJL-EX: 4.2 ± 1.4%, $P < 0.001$), with SJL-EX mice gaining a similar amount of weight as CON-EX mice. These results suggest that exercise is protective against increased body mass caused by SJL.

SJL Impairs Glucose Tolerance and Fasting Blood Glucose

Fasting blood glucose and glucose tolerance were assessed at baseline (Fig. 5*A*) and after 6 wk of SJL (Fig. 5*B*). Glycemic responses are reported as AUC_t (Fig. 5*C*) and as AUC_i (Fig. 5*D*). A significant group × time interaction was found for fasting blood glucose ($P < 0.001$; Fig. 5*E*).

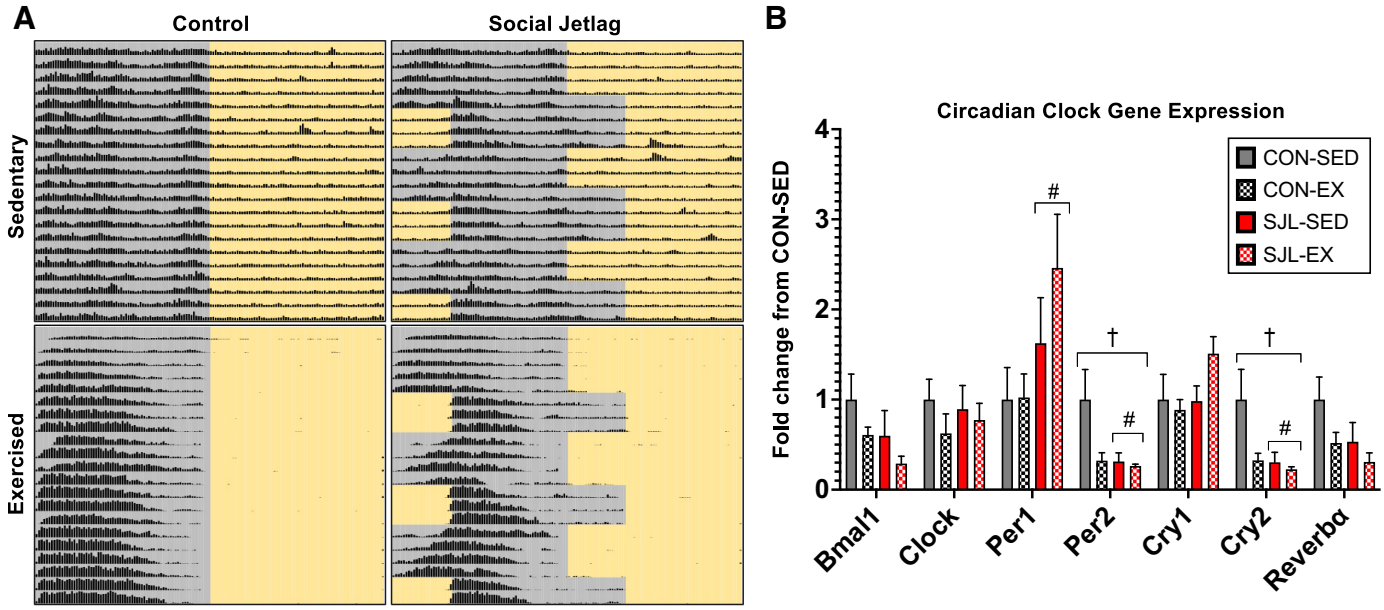

Figure 2. Social jet lag (SJL) induces alterations in activity patterns and circadian clock gene expression in sedentary (SED) and exercised (EX) mice. Example actogram from a period of control (CON) and SJL light-dark (LD) conditions with sedentary mice [activity recorded through passive infrared monitors (PIR)] and exercised mice (activity recorded with running wheels) *A*: data represent activity from $n = 10$ mice/group. Actograms show disruption in activity following SJL shifts. Gray background indicates dark/active phase (ZT12–ZT24), and yellow background indicates light/rest phase (ZT0–ZT12). Amplitude of activity plots between sedentary (PIR) and exercised (wheel) are not directly comparable. *B*: expression of canonical circadian clock genes (*Bmal1*, *Clock*, *Per1*, *Per2*, *Cry1*, *Cry2*, and *Reverbα*) was measured in quadriceps muscles. Mice were euthanized at ~ZT5. Data represent $n = 4$ or 5 mice/group. #Main effect SJL ($P < 0.05$), †main effect exercise ($P < 0.05$).

Post hoc analysis revealed that SJL led to a significant increase in fasting blood glucose in sedentary mice (SJL-SED Pre: 149.5 ± 5.1 mg·dL^{-1} vs. Post: 169.0 ± 5.2 mg·dL^{-1}, $P = 0.018$), which was not prevented by exercise training (SJL-EX Pre: 157.2 ± 8.7 mg·dL^{-1} vs. Post: 170.2 ± 8.6 mg·dL^{-1}, $P = 0.008$). Furthermore, SJL-EX mice had a significantly higher fasting blood glucose than CON-EX mice (CON-EX: 155.1 ± 3.6 mg·dL^{-1} vs. SJL-EX: 176.0 ± 5.6 mg·dL^{-1}, $P = 0.044$). Analysis of glycemic responses during GTT demonstrated that SJL impaired glucose tolerance, as we found a significant increase in AUC_t in sedentary mice with SJL [SJL-SED Pre: 842.9 ± 46.0 arbitrary units (AU) vs. Post: 1,072.0 ± 43.7 AU, $P = 0.017$]. There were no significant changes in other groups during the study period. Furthermore, there was not a significant group × time interaction for AUC_i ($P = 0.635$; Fig. 5*D*). These data reveal that SJL increases fasting blood glucose and impairs the glycemic response. Exercise was capable of preventing increases in glucose AUC during GTT but did not prevent the increase in fasting blood glucose caused by SJL.

SJL Impedes Exercise-Induced Improvement in GXT

Time to exhaustion (TTE) during GXTs was assessed as an index of cardiorespiratory fitness at baseline and after 6 wk of SJL or CON conditions (Fig. 6). A significant group × time interaction was found for time to exhaustion ($P < 0.001$; Fig. 6*A*). Post hoc analysis revealed that neither CON-SED nor SJL-SED improved from their baseline GXT performance (CON-SED Pre: 19.64 ± 0.83 min vs. Post: 17.30 ± 1.67 min, $P = 0.182$; SJL-SED Pre: 17.44 ± 0.62 min vs. Post: 15.55 ± 0.70 min, $P = 0.410$). However, both CON-EX (Pre: 17.48 ± 0.71 min; Post: 29.72 ± 0.99 min, $P <$ 0.001) and SJL-EX (Pre: 20.62 ± 1.15; Post: 25.71 ± 1.01 min,

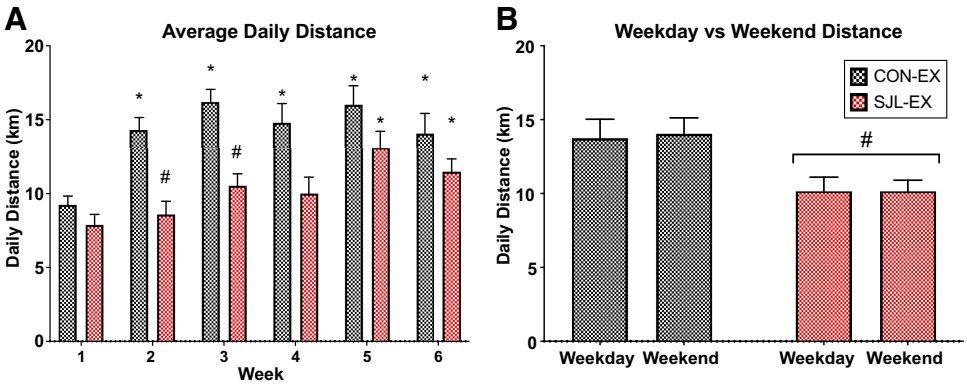

Figure 3. Mice undergoing social jet lag (SJL) perform less voluntary wheel running. *A*: daily running distances were averaged during each week of the study. *B*: daily running distances were averaged for weekdays and weekend days. Data represent $n = 10$ mice/group and are presented as means ± SE. CON, control; EX, exercise; SED, sedentary. *Significantly different from *week 1* ($P < 0.05$), #significantly different from CON ($P < 0.05$) or main effect SJL ($P < 0.05$).

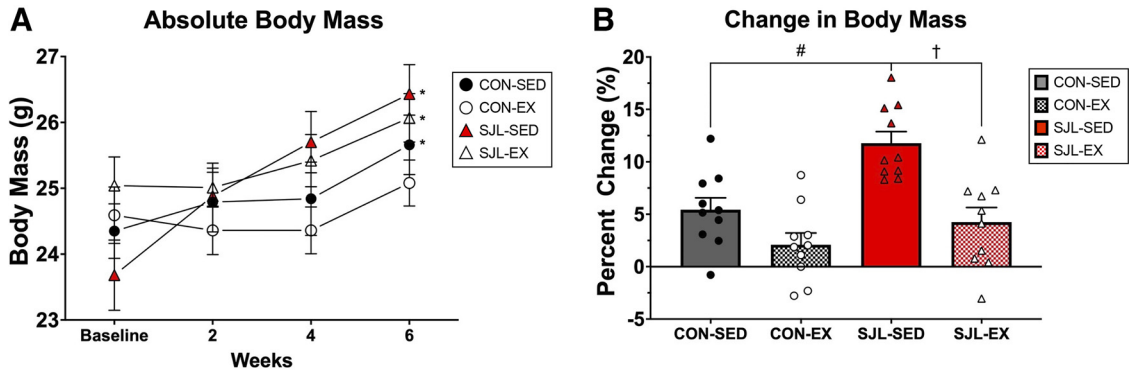

Figure 4. Exercise protected mice from social jet lag (SJL)-induced weight gain. *A*: body mass of mice at baseline and biweekly during 6-wk SJL protocol. Mice were weighed at the same time of day throughout the study. *B*: change in body mass at 6 wk from baseline, expressed as percent change. Data represent $n = 10$ mice/group and are presented as means ± SE. CON, control; EX, exercise; SED, sedentary. *Significantly different from baseline ($P < 0.05$), #significantly different from CON ($P < 0.05$), †significantly different from SED ($P < 0.05$).

$P < 0.001$) significantly increased GXT time to exhaustion compared with baseline. SJL did, however, blunt some of the improvements in GXT performance. At 6 wk, CON-EX mice had a significantly longer TTE compared with SJL-EX (CON-EX Post = 29.72 ± 0.99 min vs. SJL-EX Post 25.71 ± 1.01 min, $P = 0.032$). GXT performance is also depicted as percent change from baseline (Fig. 6*B*). Over the 6-wk training period, CON-EX mice improved their TTE by ~66% compared with baseline, whereas SJL-EX mice improved by only ~27% ($P < 0.05$).

Exercise Increased Mitochondrial Content in Skeletal Muscle, Which Is Dampened by SJL

To evaluate underpinning mechanisms of the reduced exercise capacity in mice with SJL, we assessed quadriceps muscles for the expression of component proteins in mitochondrial

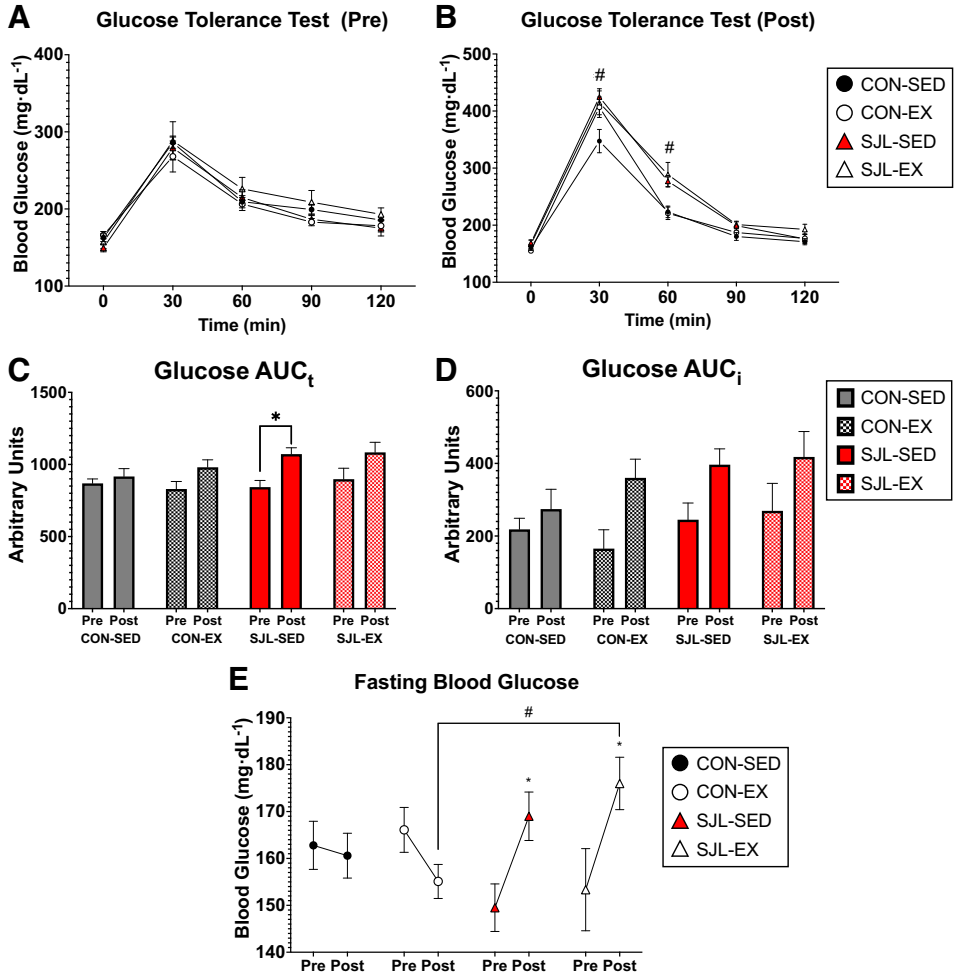

Figure 5. Social jet lag (SJL) disrupts fasting blood glucose and glucose tolerance in mice. *A–D*: glucose tolerance tests were performed at baseline (Pre; *A*) and after 6-wk SJL intervention (Post; *B*) and presented as total area under the curve (AUC_t; *C*) and incremental area under the curve (AUC_i; *D*). *E*: fasting blood glucose was measured at baseline and after 6-wk SJL. Data represent $n = 10$ mice/group and are presented as means ± SE. CON, control; EX, exercise; SED, sedentary. *Significantly different from baseline ($P < 0.05$), #significantly different from CON ($P < 0.05$).

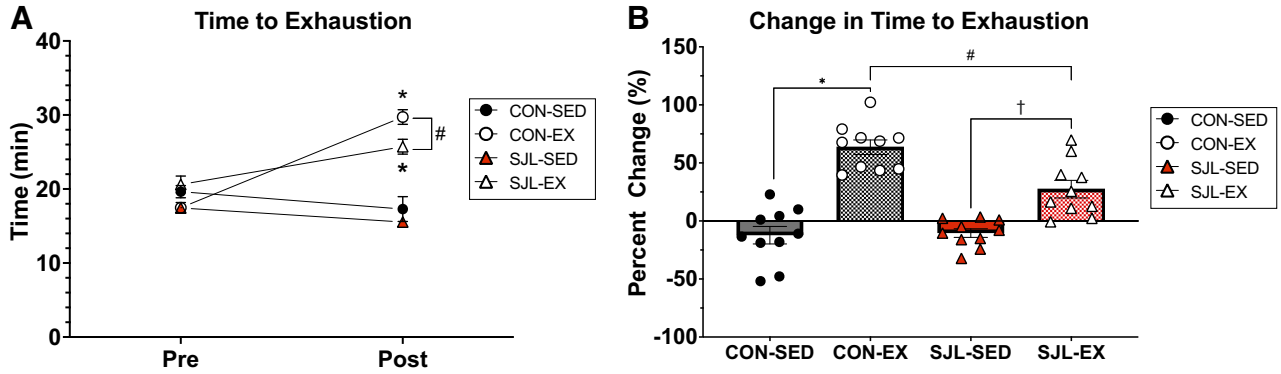

Figure 6. Social jet lag (SJL) prevents exercise training-induced improvements in time to exhaustion (TTE) during graded exercise tests (GXTs). GXTs were performed at baseline and after the 6-wk SJL intervention. *A*: during the exercise tests, TTE was recorded to evaluate cardiorespiratory fitness. *B*: TTE is also presented as a percent change from baseline. Data represent $n = 10$ mice/group and are presented as means ± SE. CON, control; EX, exercise; SED, sedentary. *Significantly different from baseline ($P < 0.05$), #significantly different from CON ($P < 0.05$), †significantly different from SED ($P < 0.05$).

OXPHOS complexes via Western blot (Fig. 7). Exercise increased the expression of several OXPHOS complexes (main effects of EX: C-V, $P = 0.0256$; C-III, $P < 0.001$; and C-I, $P < 0.001$). SJL also decreased expression of C-II (ME SJL: $P = 0.0025$) and C-I (ME SJL: $P = 0.0071$). Analysis of C-IV expression revealed a significant exercise × SJL interaction ($P = 0.0257$). Post hoc analysis revealed that C-IV expression increased in response to exercise training in CON mice (CON-SED: 1.00 ± 0.16 vs. CON-EX: 2.75 ± 0.33, $P < 0.001$) as well as SJL mice (SJL-SED: 0.802 ± 0.070 vs. SJL-EX: 1.587 ± 0.212, $P = 0.0470$). However, C-IV levels were significantly lower in SJL-EX compared with CON-EX mice (CON-EX: 2.749 ± 0.327 vs. SJL-EX: 1.587 ± 0.212, $P = 0.0021$). These findings suggest that SJL inhibits some exercised-induced increases in mitochondrial OXPHOS complex abundance (specifically Complex IV), which may relate to their impaired exercise performance. Similar effects were observed in the gastrocnemius (data not shown).

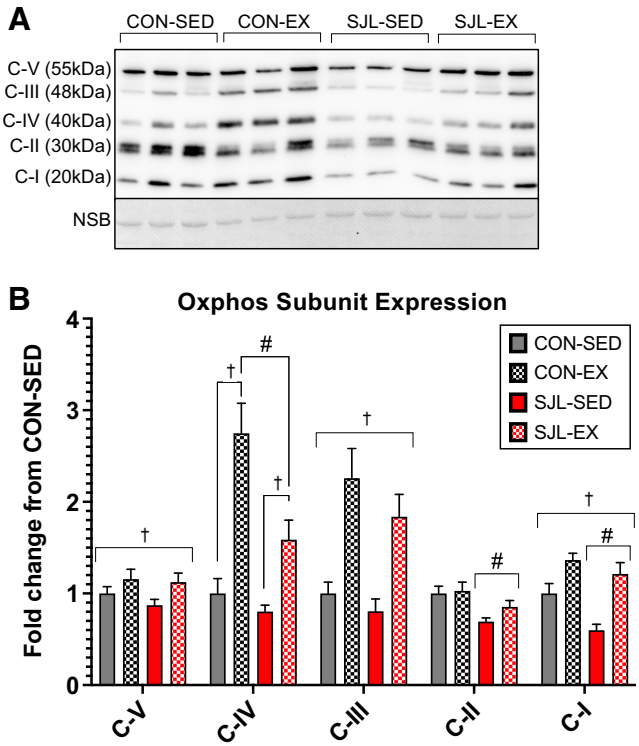

Figure 7. Exercise-induced increases in mitochondrial oxidative phosphorylation proteins are impeded by social jet lag (SJL): representative Western blot image (*A*) and quantification for CV alpha subunit (C-V), CIV subunit I (C-IV), CIII-Core protein 2 (C-III), CII-30 kDa (C-II), and CI subunit NDUFB8 (C-1) (*B*). Data represent $n = 10$ mice/group and are presented as means ± SE. CON, control; EX, exercise; SED, sedentary. #Significantly different from CON ($P < 0.05$) or main effect SJL ($P < 0.05$), †significantly different from SED ($P < 0.05$) or main effect exercise ($P < 0.05$). NSB, nonspecific band.

DISCUSSION

The main purpose of this study was to investigate the interplay of both exercise and SJL on metabolic health, incorporating measures of exercise performance, glucose tolerance, and exercise-induced adaptations in the skeletal muscle. A critical finding of this study was that SJL caused sedentary mice to gain significantly more weight throughout the study, whereas exercise largely protected SJL mice from dramatic increases in body mass. These changes in weight occurred despite all groups having similar weights at the beginning of the study ($P = 0.2176$). This finding in sedentary mice is in agreement with several epidemiological studies associating SJL with obesity in humans (18, 20, 35, 36). Parsons and colleagues (35) investigated the effects of SJL in 815 nonshift workers and found positive associations between the magnitude of SJL and body mass index (BMI), fat mass, obesity, and metabolic syndrome. Other large-scale investigations have found similar associations between SJL and waist circumference and adiposity (18, 20, 36). Similar studies have revealed links to not only obesity phenotypes but also unhealthy lifestyle behaviors that contribute to obesity. A recent study found that higher levels of SJL were associated with lower likelihood of eating breakfast, fruits, and vegetables and higher likelihood of consuming sugar-sweetened drinks (37). In young male rats, a high-calorie diet in combination with SJL resulted in severe increases in risk factors for metabolic syndrome, including high insulin and dyslipidemia, whereas SJL or the high-calorie diet alone resulted in mild changes (38), suggesting a synergistic effect between unhealthy eating practices and SJL. Other studies

have demonstrated how exercise affects the metabolic effects of circadian rhythm disruption. In a "chronic jet lag" model (6-h phase advances every 2 days), sedentary mice experienced a significant weight gain (~15%), whereas exercised mice did not (39). These findings are echoed in the present study, which, to the authors' knowledge, is the first study to demonstrate that exercise may have a protective effect against some of the detrimental metabolic effects specifically associated with SJL.

The present study also found that SJL decreased voluntary wheel running and GXT performance. SJL-EX mice averaged significantly less running distance than CON-EX mice over the course of the 6-wk intervention (average daily distance: CON-EX: 13.38 ± 0.51 km; SJL-EX: 10.12 ± 0.35 km) and had worse GXT performance during after 6-wk SJL. This may have occurred because of SJL blunting the ability to adapt to the exercise training. Although SJL resulted in lower running volume for the first 3 wk of the intervention, other studies have found significant mitochondrial adaptation at much lower running volumes. Running an average distance of 5.3 km/day for 6 wk resulted in significant increases in time to exhaustion, citrate synthase, PGC-1α, and other markers of mitochondrial abundance in male mice (40). Another 6-wk intervention in female mice running 4.6 km/day also found increased mitochondrial abundance and activity (41). It is also possible that GXT outcomes and mitochondrial abundance may have been decreased because of lower training volumes, and subsequent experiments with clamped exercise volumes are needed to determine this possibility.

Although no studies to date have monitored exercise behavior and capacity during prolonged SJL, other models of circadian rhythm disruption have been used. Using *Clock* mutant mice ($CLOCK^{\Delta 19}$), Pastore and Hood (42) found similar voluntary wheel running behavior between $CLOCK^{\Delta 19}$ and wild-type (WT) male mice, though the mutant-type mice decreased running distance by 8 wk of training. They also found COX1 expression to be similarly elevated by exercise in WT and $CLOCK^{\Delta 19}$ mice. This is somewhat in contrast to our findings, where SJL inhibited the exercise-induced increased expression of Complex IV. Complex IV is cytochrome *c*-oxidase 1 (COX1), the main subunit of the cytochrome *c*-oxidase complex, and is the ultimate complex of mitochondrial oxidative phosphorylation in the electron transport chain. COX1 protein content in the skeletal muscle is very sensitive to endurance-type exercise, increasing ~50% after 5 wk of training for 60 min five times per week (43). An in vitro investigation of murine skeletal muscle demonstrated that just 4 days (3 h per day) of muscle contraction elicited a 2.5-fold increase of COX protein (44).

Other clock genes have also been implicated in exercise performance and mitochondrial regulation, including *Per1/2*, *Bmal1*, *Cry1/2*, and *REV-ERBα* (45–47). *Bmal1* inactivation in vitro and in vivo attenuates skeletal muscle mitochondrial function (48). When *REV-ERBα* is inactivated, total mitochondrial count and endurance capacity are decreased (49). Genetic models of circadian clock disruption clearly negatively impact mitochondrial function and may impede exercise adaptations. However, the present findings offer a novel perspective on environmental circadian rhythm disruption via SJL.

The present study demonstrated that exercise affected clock gene expression in the quadriceps muscle, decreasing *Per2* and *Cry2* gene expression. All mice were euthanized at the same time (~ZT5), allowing for a single-time point analysis of gene expression. A growing body of literature suggests that acute exercise can shift skeletal muscle-specific circadian clock genes without shifting the central circadian clock within the SCN (6, 50–53). Four weeks of regular, low-intensity exercise in mice during the light phase significantly shifted *Per2* rhythms in the soleus muscle but not in the SCN (6). In mice, as little as 60 min of moderate endurance exercise during the inactive phase (ZT5 and ZT11) shifted *Per2* rhythms in the soleus muscle, whereas exercise in the active phase (ZT17) did not (53). The mice in the present study had ad libitum wheel access, and both exercise groups (CON-EX and SJL-EX) showed decreases in *Per2* and *Cry2* expression despite performing nearly all exercise activity during the active phase (Fig. 2*A*).

The findings in the present study are congruent with recent studies investigating SJL in human populations, both corroborating previous datasets and showing the significance and validity of our murine model. Previous observational work showed that adolescents with higher levels of SJL have lower cardiorespiratory fitness (28). Another investigation from our laboratory explored the effects of SJL in ROTC cadets. Cadets who reported <2 h of SJL scored significantly higher on the Army Physical Fitness Test than those reporting >2 h of SJL (29). Although these observations were observational/associative in nature, no previous studies have evaluated the consequences of SJL on adaptation to exercise training in a prescriptive way. The present study reveals a novel relationship between circadian rhythm disruption, exercise adaptation, and exercise performance, where disrupting the circadian rhythm with SJL impairs exercise-induced adaptation and performance.

An additional novel finding of this study was the effects of SJL and exercise on glucose tolerance. SJL resulted in impaired glucose tolerance in sedentary mice (AUC_t), whereas the sedentary control mice had no change in glucose tolerance. SJL with exercise did not cause a significant increase in glucose tolerance (AUC_t), suggesting that exercise may provide protection against some of the detrimental effects of SJL on glucose tolerance.

Circadian clock gene knockout models have demonstrated the important role of the circadian clock in glucose metabolism. In muscle-specific *Bmal1*-knockout mice, Dyar and colleagues (24) found that knockout mice had impaired insulin-dependent glucose uptake, lower GLUT-4 protein levels, and impaired ability to oxidize glucose. Another study found that skeletal muscle-specific *Bmal1*-knockout mice lacked sufficient intermediates in the tricarboxylic acid cycle to oxidize glucose efficiently and thus relied predominantly on fat as a substrate (54). In the present study, effects of SJL were observed on *Per1*, *Per2*, and *Cry2* in the skeletal muscle. *Per2* has been shown to play a role in promoting liver glycogen storage and fasting glucose (55). Additionally, *Cry1* and *Cry2*-knockout mice showed impaired insulin secretion and glucose tolerance in male mice (56). The alterations in clock genes via SJL may have contributed to the altered glucose metabolism in the present study.

SJL also led to an elevation of fasting blood glucose levels in sedentary and exercised mice. This is in agreement with human studies investigating circadian rhythm disruption in

shift workers (23). Scheer and colleagues (23) found that when participants shifted sleep 12 h outside of their normal time, blood glucose increased despite increased insulin, suggesting that circadian rhythm disruption affects insulin sensitivity and glucose tolerance. Other investigations into sleep restriction have found similar impairments of glucose tolerance (57). SJL specifically has been associated with elevated fasting insulin but not fasting glucose in humans (20). However, after monitoring subjects for a year, the same group found a positive correlation between SJL and fasting blood glucose values (58). It is possible that changes in body composition (e.g., fat vs. lean mass) that are not detected by changes in body weight contribute to differences in glycemia. Skeletal muscle plays a primary role in glucose disposal, so increases in body weight attributed to lean mass should improve glucose handling. However, in the present study we found no significant differences in tissue weight (adipose tissue or skeletal muscle) between exercise or SJL groups. Thus, it is possible that tissue insulin sensitivity could be responsible for differences in glycemia, though additional experiments are needed to support this.

It is important to note that the present study was conducted in young healthy male mice, and SJL had a negative effect on markers of glucose tolerance. This is similar to findings from several large-scale epidemiological studies investigating the effect of SJL on metabolic health. In a cohort of >1,500 individuals, 2 h of SJL was associated with a twofold increase of risk for type 2 diabetes and prediabetes (59). SJL was also associated with elevated HbA1c in >150 type 1 diabetes patients (60, 61)

There are limitations to this study that should be considered. Notably, only male mice were included. Sex as a biological variable is important to include in subsequent studies to allow more general extrapolation of these findings. Additionally, although body weight was monitored, we did not assess body composition, which could have revealed changes in lean or fat mass in these mice. Finally, food intake was not monitored in this study. These additional parameters could lend important insight into behavioral and physiological consequences of SJL and should be considered for future studies.

Social jet lag is a prevalent and potent disruptor of circadian rhythms. To the best of our knowledge, the present study is the first to directly measure the effects of SJL on metabolic health (body weight, glucose tolerance) and exercise capacity after a voluntary training program in a murine model. Our data reveal that SJL negatively affects markers of metabolic health, whereas exercise counteracted some of these negative effects. Furthermore, this is the first study to show that SJL impairs the functional and molecular adaptations to exercise training. These findings extend previous epidemiological findings, strengthening the causal relationship between SJL and metabolic disease risk. Subsequent interventional studies are needed to more clearly understand the direct impacts of SJL on metabolic health, including the prevention of obesity, diabetes, and comorbid cardiovascular conditions.

DATA AVAILABILITY

Authors will provide all raw data, as well as access to biological samples, produced within the described experiments upon reasonable request submitted to the corresponding author.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

D.J.H. and G.R.M. conceived and designed research; M.B.D., E.M.M., A.R.C., G.A.N., N.I.V., and G.R.M. performed experiments; M.B.D., E.M.M., A.R.C., G.A.N., N.I.V., and G.R.M. analyzed data; M.B.D., E.M.M., and G.R.M. interpreted results of experiments; M.B.D. and G.R.M. prepared figures; M.B.D. drafted manuscript; M.B.D., D.J.H., and G.R.M. edited and revised manuscript; M.B.D., E.M.M., A.R.C., G.A.N., N.I.V., D.J.H., and G.R.M. approved final version of manuscript.

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