Circadian disruption impairs glucose homeostasis in male but not in female mice and is dependent on gonadal sex hormones

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RESEARCH ARTICLE

Circadian disruption impairs glucose homeostasis in male but not in female mice and is dependent on gonadal sex hormones

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Abstract

Circadian disruption (CD) is the consequence of a mismatch between endogenous circadian rhythms and behavior, and frequently occurs in shift workers. CD has often been linked to impairment of glucose and lipid homeostasis. It is, however, unknown if these effects are sex dependent. Here, we subjected male and female C57BL/6J mice to 6-h light phase advancements every 3 days to induce CD and assessed glucose and lipid homeostasis. Within this model, we studied the involvement of gonadal sex hormones by injecting mice with gonadotropin-releasing hormone-antagonist degarelix. We demonstrate that CD has sex-specific effects on glucose homeostasis, as CD elevated fasting insulin levels in male mice while increasing fasting glucose levels in female mice, which appeared to be independent of behavior, food intake, and energy expenditure. Absence of gonadal sex hormones lowered plasma insulin levels in male mice subjected to CD while it delayed glucose clearance in female mice subjected to CD. CD elevated plasma triglyceride (TG) levels and delayed plasma clearance of TG-rich lipoproteins in both sexes, coinciding with reduced TG-derived FA uptake by adipose tissues. Absence of gonadal sex hormones did not notably alter the effects of CD on lipid metabolism. We conclude that CD causes sex-dependent effects on glucose metabolism, as aggravated by male gonadal sex hormones and partly rescued by female gonadal sex hormones. Future studies on CD should consider the inclusion of both sexes, which may eventually contribute to personalized advice for shift workers.

KEYWORDS

circadian disruption, glucose homeostasis, gonadal hormones, lipid homeostasis, sex

1 | INTRODUCTION

Nearly every process in the body follows a circadian rhythm of about 24 h. These rhythms are synchronized by the master clock located in the suprachiasmatic nucleus (SCN) of the brain. The SCN is particularly responsive to light, and this so-called *Zeitgeber* facilitates finetuning of rhythm and adaptation to changes in environmental light–dark cycles. Other *Zeitgebers* such as environmental temperature, food intake, and physical activity are able to directly shift circadian rhythms in the SCN as well as in peripheral organs. However, in present-day society nocturnal eating and light exposure, and the need for shift work causes profound circadian disruption (CD), which has been associated with increased risk for various diseases, including cancer and cardiometabolic diseases.[1–7] A plethora of mechanisms have been described that could contribute to this elevated risk, among which disturbances in lipid and glucose homeostasis.

Compared to men, women tend to have an earlier chronotype (i.e., the preference to be awake in the morning versus the evening).[8,9] In addition, sleep problems in women significantly increase during menopause, suggesting involvement of sex hormones in sleep regulation.[8] Studies in rodents have shown that depletion of sex hormones before menopause or hormonal replacement therapy after menopause affects voluntary physical activity and sleep rhythms.[10,11] Whether CD also has sex-dependent effects on energy metabolism and cardiometabolic disease risk remains largely unknown. Observational studies addressing disease risk associated with shift work are inconclusive, possibly due to the differences in occupation between men and women.[12–14] Furthermore, most experimental studies in rodents have been conducted using a single sex only.

The identification of sex-specific risks might present an important consideration when designing interventions aimed to minimize the health risks associated with for example shift work. Here, we identified sex-dependent impairments in glucose homeostasis in response to CD that were partly modulated through gonadal sex hormones. Notably, impairments in lipid homeostasis following CD appeared to be independent of sex.

2 | MATERIALS AND METHODS

2.1 | Animals

Experiments were performed in accordance with the Institute for Laboratory Animal Research Guide for the Care and Use of Laboratory Animals and were approved by the National Committee for Animal experiments of the Netherlands.

Male and female C57BL/6J mice of 9 weeks of age were housed in groups of 3–4 mice per cage with ad libitum access to water and standard chow diet (Rat and Mouse No. 3 Breeding, SDS, Horley, United Kingdom). Diffuse white fluorescent light (550–600 lux; Bailey True-Light fluorescent tubes, 139985, Technische Unie, Alphen aan den Rijn, The Netherlands) was used to acclimatize the mice to a 12:12 h light–dark cycle. While mice of the control groups were maintained under these conditions throughout the experiment, CD mice were subjected to a phase advancement of 6 h (i.e., shortening of the dark phase) every 3 days.

In experiment 1, mice were exposed to CD or control light–dark cycles for a total duration of 10 weeks ($n = 8$ mice per group per sex). CD was confirmed by monitoring voluntary physical activity during the first 12 days of the study. Body weight, body composition (EchoMRI 100-Analyzer; EchoMRI, Houston, Texas), and food intake were monitored at regular intervals. One day after the last shift of the light–dark cycle for the CD group, all mice were fasted for 4 h after which blood was collected from the tail vein at *Zeitgeber* Time (ZT)12 to assess plasma glucose and triglyceride (TG) levels. Mice were subsequently killed by CO_2 inhalation and perfused transcardially with ice-cold phosphate-buffered saline (PBS) for 5 min before organs were collected for further analyses.

In experiment 2, mice were exposed to CD or control light–dark cycles for a total duration of 6 weeks. In addition, the mice received subcutaneous injections at days 1 and 29 with either vehicle ($n = 8$ per group per sex) or gonadotropin-releasing hormone (GnRH)-antagonist degarelix (25 mg/kg; MedChemExpress, Monmouth Junction, New Jersey, USA) ($n = 8$ per group per sex) to suppress the production and secretion of gonadal sex hormones as a less-invasive alternative to orchiectomy and ovariectomy.[15] Voluntary physical activity was assessed between days 1–12 and days 23–34. Body weight, body composition, and food intake were monitored at regular intervals. In week 5, one day after the shift in the light–dark cycle for the CD group, all mice were fasted for 4 h and subjected to a glucose tolerance test at ZT12. In week 6, mice were individually housed in metabolic home cages to assess energy expenditure by means of indirect calorimetry. On the day after the very last shift in the light–dark cycle for the CD group, mice were again fasted for 4 h and injected at ZT12 with radiolabeled TG-rich lipoprotein-like particles to assess organ uptake of TG-derived fatty acids.

2.2 | Voluntary physical activity and rhythm strength

Passive infrared sensors were used to monitor beam breaks, from which representative actograms were

generated and from which F-periodograms were constructed using Clocklab software version 6.1.05 (Actimetrics Software, Wilmette, Illinois, USA). Rhythm strength was defined by assessing the amplitude of corresponding periodograms.[16,17]

2.3 | Plasma measurements

Plasma glucose was measured by Accu-Chek Aviva (Roche Diagnostics GmbH, Mannheim, Germany) and plasma insulin by Ultra-Sensitive Mouse Insulin ELISA Kit (90080, Crystal Chem, Downers Grove, Illinois, USA), according to the manufacturers' protocols. The insulin stimulation index was calculated by dividing insulin levels measured 15 min after glucose injection by insulin levels measured prior to injection. Plasma TG was measured using Cobas Triglycerides enzymatic kit (106571, Roche Diagnostics, Mannheim, Germany), by adding 200 µl reagent to 2.5 µl sample and incubating at room temperature for 30 min prior to measuring absorption at 492 nm versus 650 nm.

2.4 | Indirect calorimetry and sleep

Mice were individually housed in automated metabolic home cages (Promethion System; Sable Systems, Las Vegas, Nevada, USA). Data on voluntary locomotor activity (by infrared beam breaks), food intake, O_2 consumption, and CO_2 production were continuously collected in 5 min bins, and energy expenditure was calculated and shown without normalization to body composition. Sleep was estimated from voluntary locomotor activity and defined as inactivity of 40 or more consecutive seconds. Data of two consecutive 12:12 h light–dark cycles were analyzed, starting directly after the phase advancement in the CD group.

2.5 | Clearance of radiolabeled lipoprotein-like emulsion particles

Fasted mice received an intravenous injection with an emulsion of TG-rich lipoprotein-like particles (80 nm) containing glycerol tri[^{3}H]oleate (1 mg TG in 200 µl saline per mouse), prepared as described previously.[18] Blood was collected to determine plasma decay of ^{3}H. After 15 min, mice were killed by CO_2 inhalation and perfused transcardially with ice-cold PBS for 5 min. Various organs were collected and tissue pieces of 50–200 mg were dissolved in 0.5 ml Solvable (6NE9100, PerkinElmer, Waltham, Massachusetts, USA) at 56°C overnight, after which 5.0 ml Ultima Gold (6013329, PerkinElmer, Waltham, Massachusetts, USA) was added. Plasma was directly added to 2.5 ml Ultima Gold. ^{3}H-activity was measured with a liquid scintillation counter (Tri-Carb 2910 TR, PerkinElmer, Waltham, Massachusetts, USA) and expressed as a percentage of the injected dose. A total of six mice had to be excluded from the analysis due to technical problems. Mice were excluded for calculating the AUC (between 2 and 15 min after injection) of plasma decay if one or more plasma samples were missing.

2.6 | Glucose tolerance test

Mice were fasted for 4 h and baseline plasma glucose was measured in tail vein blood before glucose was intraperitoneally injected (2 g/kg body weight). Plasma glucose was monitored over 2 h and plasma insulin was measured prior to and 15 min after the glucose injection.

2.7 | Statistical analyses

For practical reasons, male and female mice were investigated in separate cohorts, and the data were analyzed separately. Significant outliers were identified with a Grubbs outlier test and removed from the analysis. Statistical analyses between groups were performed with unpaired t-tests or two-way analysis of variance (ANOVA) and following Šídák's or Tukey's multiple-comparison post-hoc test, where applicable. Differences in energy expenditure between groups were analyzed by analysis of covariance (ANCOVA), listing a regression-based component score of body weight, fat mass, and lean mass as covariate. $p < .05$ was considered statistically significant. Statistical analyses were performed with GraphPad Prism software, version 9.0.1 (GraphPad, La Jolla, California) or SPSS Statistics 25 (IBM, Armonk, New York, USA). Data are presented as means ± SEM.

3 | RESULTS

3.1 | CD has sex-specific effects on glucose and sex-independent effects on lipid metabolism

To explore potential sexual dimorphism in the response to CD, male and female C57BL/6J mice were subjected to 6-h light phase advancements every 3 days for a total duration of 10 weeks. CD was confirmed through monitoring of voluntary physical activity during four consecutive phase shifts, of which the actogram data of the first two shifts are shown (Figure 1A). From these, the rhythm strength was

calculated, revealing a significant reduction in both male and female mice after CD (Figure 1B). Total food intake throughout the experiment was unaffected by CD for both sexes (Figure 1C). While body weight and lean mass were also unaffected, CD caused a transient increase in fat mass in male mice, which was normalized after week 10 (Figure 1D–F). In line with these data, CD did not affect weights of gonadal white adipose tissue (gWAT), subcutaneous WAT (sWAT), interscapular brown adipose tissue (iBAT) and subscapular BAT (sBAT) after 10 weeks (Figure 1G). Interestingly, CD elevated fasting plasma glucose levels in female mice (Figure 1H), while increasing plasma insulin levels and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) in male mice (Figure 1I,J). CD elevated plasma TG levels in both sexes (Figure 1K). These data suggest that CD has sex-specific effects on glucose, and sex-independent effects on lipid metabolism.

3.2 | Depletion of gonadal sex hormones impairs adaptation to CD

To obtain further insight in sex-specific effects of CD and the role of sex hormones therein, we evaluated the effect of CD in combination with suppression of endogenous gonadal sex hormones via GnRH antagonist degarelix. The total duration for this experiment was 6 weeks after which seminal vesicles of male mice and ovaries of female mice were weighed. Decreased seminal vesicle and ovary weight in the degarelix-treated groups confirmed the suppression of gonadal sex hormone production (Figure 2A).[15] In line with the results of the first experiment, CD caused an acute reduction in rhythm strength in both sexes, which was transient in the presence but not in the absence of gonadal sex hormones (Figure 2B). Also in accordance with the first study, CD did not affect total food intake (Figure 2C) or body composition after 6 weeks (Figure 2D–F) regardless of depletion of sex hormones. Depletion of gonadal sex hormones by itself, however, reduced total food intake (Figure 2C) and lean body mass (Figure 2D,E) exclusively in male mice, while it increased fat mass (Figure 2F) and white adipose tissue weight (Figure 2G) exclusively in female mice.

3.3 | CD shifts rhythms of energy intake and energy expenditure independent of gonadal sex hormones

We next investigated the effects of CD on diurnal behavior and whole-body energy homeostasis through indirect calorimetric measurements in metabolic home-cages during week 6 of the intervention. Two consecutive 12:12 h light–dark cycles were analyzed, starting directly after the phase advancement in the CD group. In both sexes, CD caused a shift in voluntary activity, food intake, and energy expenditure from the dark to the light phase, without affecting total daily levels (Figure 3A–F).

In the absence of gonadal sex hormones, the effects of CD were unaltered. Depletion of sex hormones by itself attenuated voluntary activity and energy expenditure in both sexes, while food intake was reduced in male mice only. The reduction in energy expenditure was explained by lower fat oxidation in female mice, while fat and carbohydrate oxidation were both lower in male mice (Figure 3G–J). The ANCOVA analysis suggested that the effects in males were mainly caused by differences in body weight and body composition (Tables 1 and 2).

In both sexes, CD caused a relative shift in inactivity-defined sleep time from the light phase to the dark phase without affecting total sleep duration (Figure 3K,L). Notably, depletion of sex hormones in female mice also caused a pronounced shift in inactivity-defined sleep from light to dark phase, and CD did not further promote this effect, but actually counteracted this shift to the dark phase.

3.4 | CD attenuates lipid metabolism independent of gonadal sex hormones

To investigate whether sex hormones have a role in the CD-induced elevation in plasma TG levels, we injected the mice with TG-rich lipoprotein-like particles containing glycerol tri[^{3}H]oleate to investigate the rate of clearance from the circulation. In accordance with the first experiment, CD elevated plasma TG levels in both sexes (CD effect by two-way ANOVA: $p = .023$ and $p < .001$ for males and females, respectively) (Figure 4A). Accordingly, plasma decay of ^{3}H-derived activity was delayed by CD in both sexes (CD effect by two-way ANOVA: $p = .010$ and $p < .001$ for males and females, respectively), although in males it did not reach statistical significance in the post-hoc analysis (Figure 4B,C). The delay in plasma TG clearance was explained by reduced uptake of [^{3}H]oleate by WAT and BAT in both sexes following CD (Figure 4D). Removal of gonadal sex hormones did not notably alter the effects of CD on these parameters, indicating that the effects of CD on lipid metabolism are independent of gonadal sex hormones.

3.5 | CD alters glucose homeostasis which is dependent on gonadal sex hormones

We further assessed glucose tolerance in the mice subjected to CD with or without depletion of gonadal sex hormones.

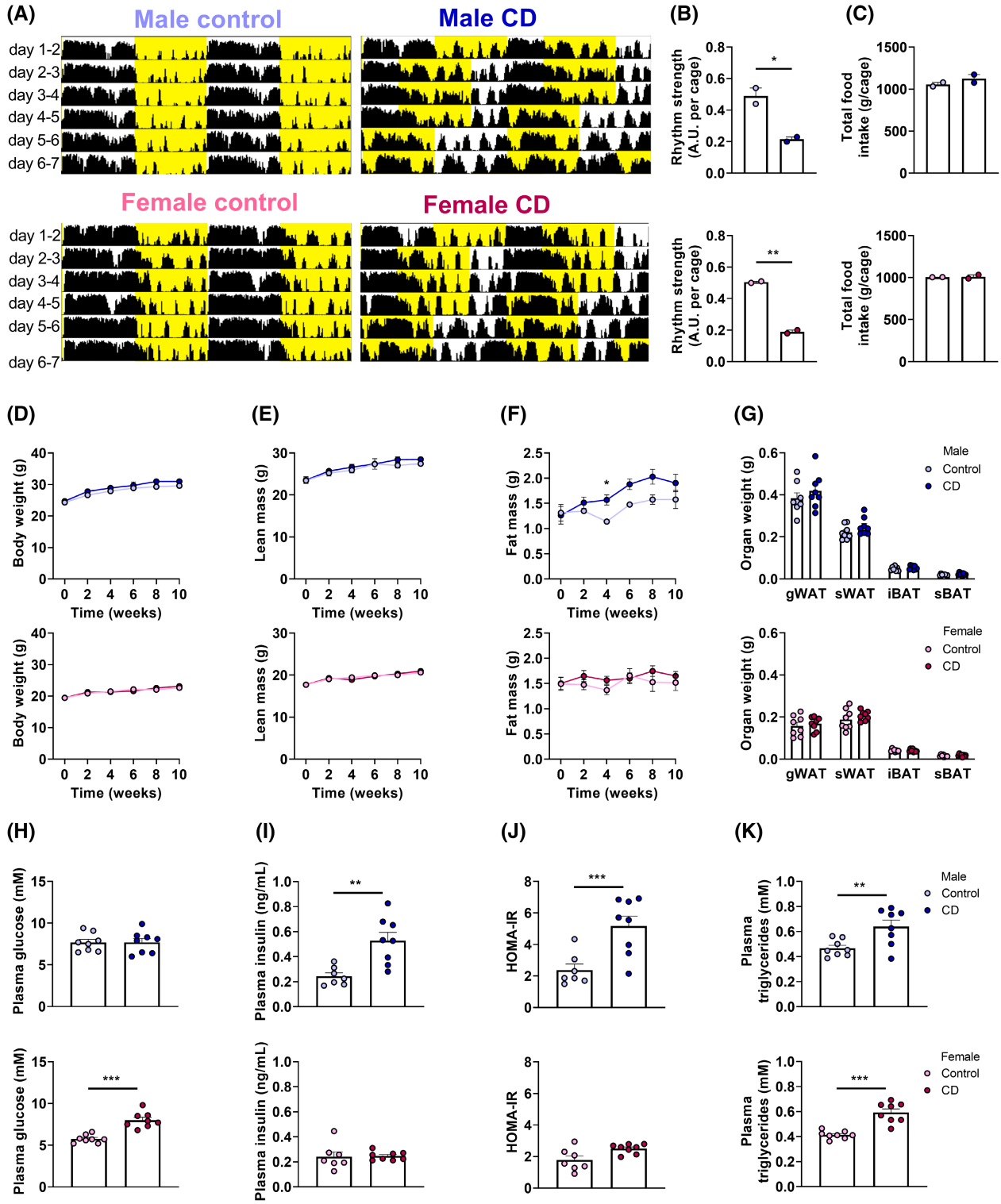

FIGURE 1 Voluntary physical activity rhythms, food intake, body composition, tissue weights, and glucose and lipid metabolism during circadian disruption. Male and female C57BL/6J mice were exposed to a 6-h phase advancement every 3 days (circadian disruption; CD) or regular 12:12 light–dark cycle (control) for a total duration of 10 weeks. (A) Actograms of the first week of the study were constructed ($n = 2$ cages of 4 mice per group) and (B) rhythm strength was calculated from periodogram analysis. (C) Total food intake per cage ($n = 2$ cages of 4 mice per group), (D) body weight, (E) lean mass, and (F) fat mass were monitored during the study. (G) Weight of gonadal white adipose tissue (gWAT), subcutaneous WAT (sWAT), interscapular brown adipose tissue (iBAT) and subscapular BAT (sBAT) was assessed at the endpoint ($n = 8$ mice per group), as well as (H) plasma glucose, (I) insulin, (K) triglycerides, and (J) Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) ($n = 7$–8 mice per group). Data are presented as means ± SEM. *Control vs. CD. $^*p < .05$; $^{**}p < .01$; $^{***}p < .001$, according to unpaired t-test (B, C, G–K) or two-way ANOVA and following Šídák's multiple-comparison test (D–F).

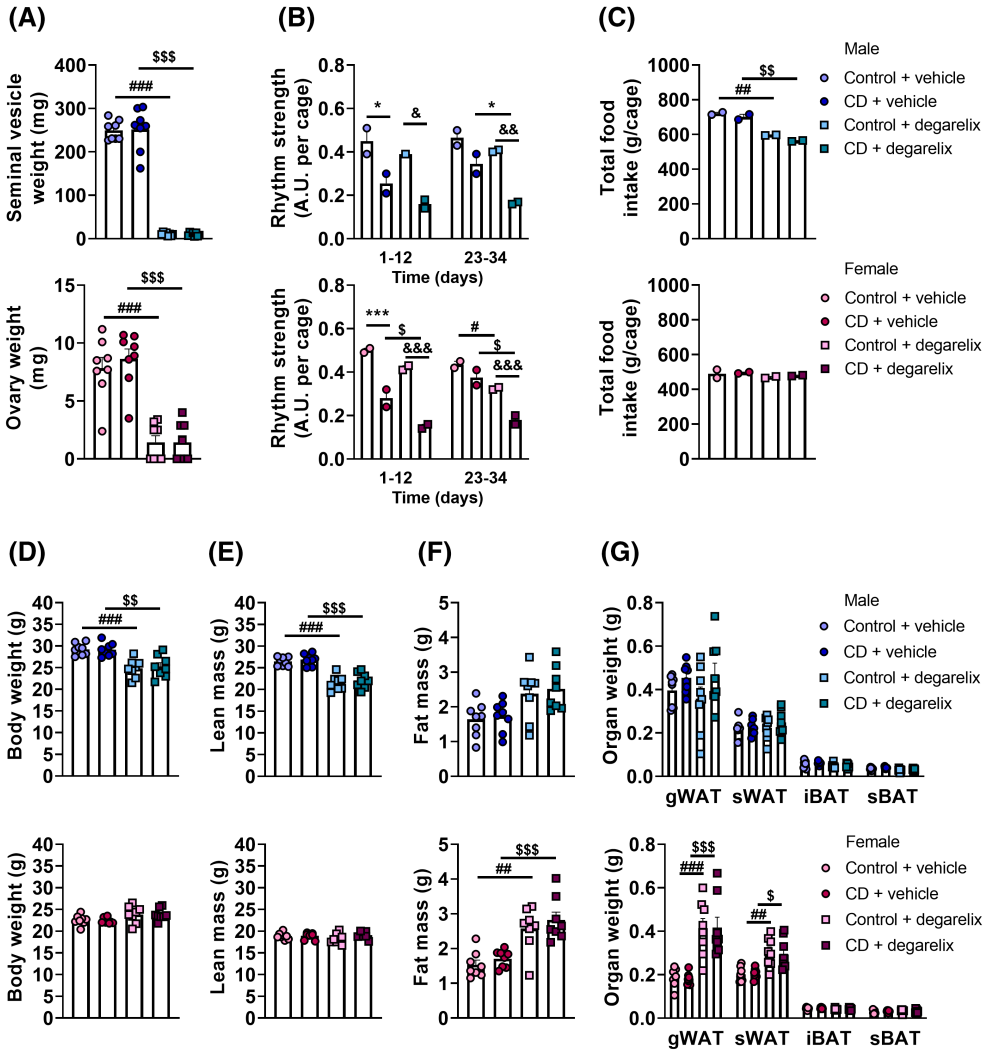

FIGURE 2 Gonadal weight, voluntary physical activity rhythms, and food intake during circadian disruption with chemical castration. Male and female C57BL/6J mice were exposed to a 6-h phase advancement every 3 days (circadian disruption; CD) or regular 12:12 light–dark cycle (control) for a total duration of 6 weeks. Mice were additionally treated with vehicle or degarelix to suppress endogenous production of gonadal sex hormones. (A) Weight of seminal vesicles from males and ovaries from females was assessed at the endpoint ($n = 8$ mice per group). (B) Rhythm strength between days 1–12 and 23–34 was calculated from periodogram analysis ($n = 2$ cages of 4 mice per group). (C) Total food intake was measured per cage ($n = 2$ cages of 4 mice per group). (D) Body weight, (E) lean mass, and (F) fat mass, and (G) weights of gonadal white adipose tissue (gWAT), subcutaneous WAT (sWAT), interscapular brown adipose tissue (iBAT) and subscapular BAT (sBAT) were measured at the end of the study ($n = 8$ mice per group). Data are presented as means ± SEM. $^{\#}$Control + vehicle vs. Control + degarelix; $^{\$}$CD + vehicle vs. CD + degarelix; *Control + vehicle vs. CD + vehicle; $^{\&}$Control + degarelix vs. CD + degarelix. $^{\$,*,\&}p < .05$; $^{\#\#,\$\$,\&\&}p < .01$; $^{\#\#\#,\$\$\$,***,\&\&\&}p < .001$, according to two-way ANOVA and following Tukey's multiple-comparison test.

In contrast to the first experiment, in which fasting glucose and insulin were measured after 10 weeks of CD, we observed no significant differences in fasting glucose or insulin levels between mice subjected to 6 weeks of CD or regular light–dark cycles in the two sexes (Figure 5A–D). Postprandial glucose and insulin levels also remained largely unaffected upon CD (Figure 5B–D). Interestingly, CD in the absence of gonadal sex hormones lowered plasma insulin levels in male mice without altering the insulin stimulation index (Figure 5D,E). In contrast, CD in the absence of gonadal sex hormones delayed glucose clearance without altering insulin levels, while the insulin stimulation index tended to be lowered in female mice (Figure 5B–D), indicative of reduced glucose-stimulated insulin release by β cells. These data suggest that gonadal sex hormones are responsible for the differential effects of CD on glucose homeostasis in male and female mice, in which the absence of male gonadal sex hormones ameliorates impaired glucose homeostasis and the absence of female gonadal sex hormones aggravates glucose homeostasis.

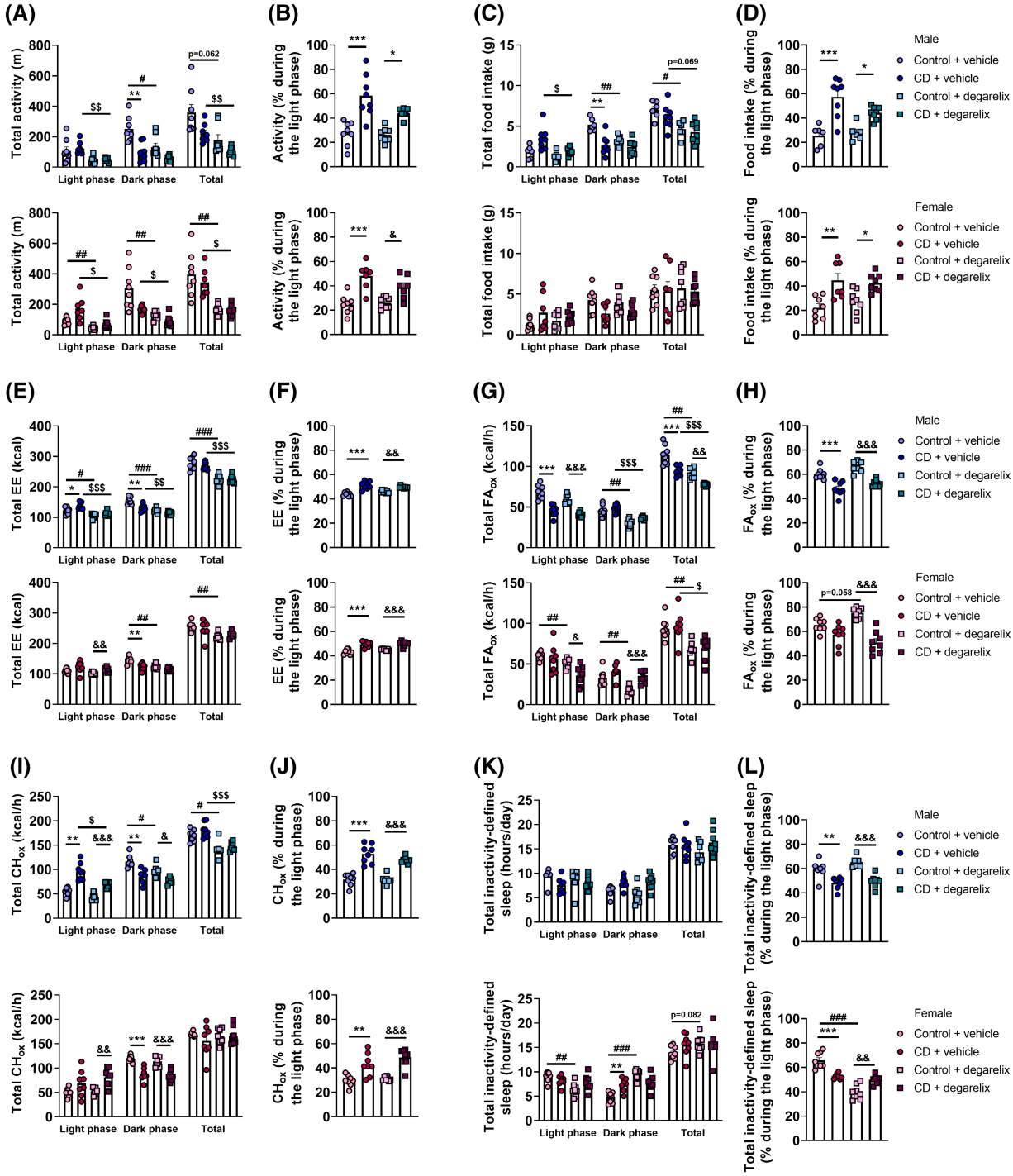

FIGURE 3 Behavior and energy expenditure during circadian disruption with chemical castration. Male and female C57BL/6J mice were exposed to a 6-h phase advancement every 3 days (circadian disruption; CD) or regular 12:12 light–dark cycle (control) for a total duration of 6 weeks. Mice were additionally treated with vehicle or degarelix to suppress endogenous production of gonadal sex hormones. Mice were single-housed in metabolic home-cages for continuous measurement of (A, B) voluntary locomotor activity, (C, D) food intake, (E, F) energy expenditure (EE), (G, H) fat oxidation (FA_{ox}), (I, J) carbohydrate oxidation (CH_{ox}), (K, L) and inactivity-defined sleep, after which the percentage of each parameter during the light phase was calculated ($n = 6$–8 mice per group). Data are presented as means ± SEM. $^{\#}$Control + vehicle vs. Control + degarelix; $^{\$}$CD + vehicle vs. CD + degarelix; *Control + vehicle vs. CD + vehicle; $^{\&}$Control + degarelix vs. CD + degarelix. $^{\#,\$,*,\&}p < .05$; $^{\#\#,\$\$,**,\&\&}p < .01$; $^{\#\#\#,\$\$\$,***,\&\&\&}p < .001$, according to two-way ANOVA and following Tukey's multiple-comparison test.

TABLE 1 Energy expenditure of male mice during circadian disruption with depletion of gonadal sex hormones

Tests of between-subjects effects						
Dependent variable: Energy expenditure						
Source	**Type III sum of squares**	***df***	**Mean square**	***F***	***p*-Value**	**Partial eta squared**
Corrected model	230 517.679[a]	4	57 629.420	44.478	.000	0.872
Intercept	23 828 840.940	1	23 828 840.940	18 390.986	.000	0.999
BW_FM_LM	48 676.812	1	48 676.812	37.569	.000	0.591
CD	4717.789	1	4717.789	3.641	.067	0.123
degarelix	167.108	1	167.108	0.129	.722	0.005
CD * degarelix	1713.429	1	1713.429	1.322	.261	0.048
Error	33 687.692	26	1295.680			
Total	24 494 627.980	31				
Corrected total	264 205.371	30				

Note: Male C57BL/6J mice were exposed to a 6-h phase advancement every 3 days (circadian disruption; CD) or regular 12:12 light–dark cycle (control) for a total duration of 6 weeks. Mice were additionally treated with vehicle or degarelix to suppress endogenous production of gonadal sex hormones. Analysis of covariance was conducted using a univariate general linear model (GLM) with energy expenditure as dependent variable, and group as independent variable. Regression-based factor component scores were created combining body weight (BW), fat mass (FM), and lean mass (LM), referred to as BW_FM_LM, which was used as covariate in the GLM.

[a]*R* squared = 0.872 (Adjusted *R* squared = 0.853).

TABLE 2 Energy expenditure of female mice during circadian disruption with depletion of gonadal sex hormones

Tests of between-subjects effects						
Dependent variable: Energy expenditure						
Source	**Type III sum of squares**	**df**	**Mean square**	**F**	***p*-Value**	**Partial eta squared**
Corrected model	5474.285[a]	4	1368.571	4.572	.006	0.413
Intercept	1 761 705.045	1	1 761 705.045	5884.980	.000	0.996
BW_FM_LM	604.146	1	604.146	2.018	.167	0.072
CD	117.153	1	117.153	0.391	.537	0.015
degarelix	5125.642	1	5125.642	17.122	.000	0.397
CD * degarelix	103.242	1	103.242	0.345	.562	0.013
Error	7783.260	26	299.356			
Total	1 796 597.460	31				
Corrected total	13 257.544	30				

Note: Female C57BL/6J mice were exposed to a 6-h phase advancement every 3 days (circadian disruption; CD) or regular 12:12 light–dark cycle (control) for a total duration of 6 weeks. Mice were additionally treated with vehicle or degarelix to suppress endogenous production of gonadal sex hormones. Analysis of covariance was conducted using a univariate general linear model (GLM) with energy expenditure as dependent variable, and group as independent variable. Regression-based factor component scores were created combining body weight (BW), fat mass (FM), and lean mass (LM), referred to as BW_FM_LM, which was used as covariate in the GLM.

[a]*R* squared = 0.413 (Adjusted *R* squared = 0.323).

4 | DISCUSSION

Circadian rhythms are pivotal for maintenance of lipid and glucose homeostasis. Previous studies have shown that CD causes disturbances in lipid and glucose homeostasis in both animal models and humans.[1–6] However, it was unknown if CD affects lipid and glucose homeostasis differently in males and females. Here we demonstrated that CD impairs glucose and lipid homeostasis and that only the effects of CD on glucose homeostasis are different for both sexes, which is at least partly explained by gonadal sex hormones where male hormones seem to aggravate the metabolic phenotype while female hormones are protective.

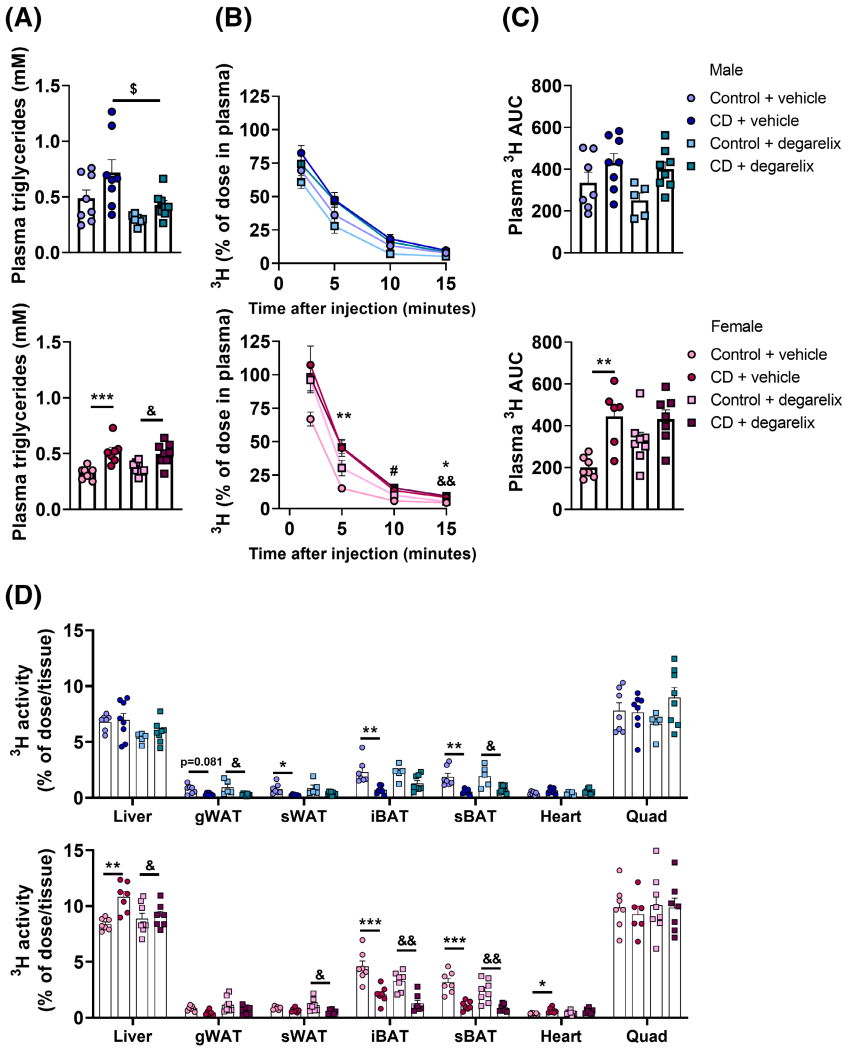

FIGURE 4 Lipid metabolism during circadian disruption with chemical castration. Male and female C57BL/6J mice were exposed to a 6-h phase advancement every 3 days (circadian disruption; CD) or regular 12:12 light–dark cycle (control) for a total duration of 6 weeks. Mice were additionally treated with vehicle or degarelix to suppress endogenous production of gonadal sex hormones. At the end of the study, (A) plasma triglycerides and (B) plasma decay of [^{3}H]oleate were measured from which (C) area under the curve (AUC) was calculated. Simultaneously, (D) uptake of [^{3}H]oleate by the liver, gonadal white adipose tissue (gWAT), subcutaneous WAT (sWAT), interscapular brown adipose tissue (iBAT), subscapular brown adipose tissue (sBAT), heart, and quadriceps was assessed (n = 5–8 mice per group). Data are presented as means ± SEM. $^{\#}$Control + vehicle vs. Control + degarelix; $^{\$}$CD + vehicle vs. CD + degarelix; *Control + vehicle vs. CD + vehicle; $^{\&}$Control + degarelix vs. CD + degarelix. $^{\#,\$,*,\&}p < .05$; $^{**,\&\&}p < .01$; $^{***}p < .001$, according to two-way ANOVA and following Tukey's multiple-comparison test.

We observed that CD causes a transient increase in fat mass only in males, suggesting a sex-dependent change in energy balance that is normalized over time. In contrast, numerous studies investigating CD have shown variable outcomes ranging from no effect of CD to a consistent acceleration in body weight or fat mass gain.[6,19–21] We next observed sex-specific effects of CD on glucose homeostasis, which, similar to body weight gain, has shown variable outcomes in literature.[6,19–21] In one study, male and female C57BL/6 mice were exposed to 12-h phase-advancements, which is considered a more severe CD protocol than what we used in our current study,[22,23] resulting in body weight gain, glucose intolerance, and insulin resistance in both sexes.[24] It is possible that the threshold at which CD induces metabolic disruption is higher for female mice than for male mice, but once exceeded, metabolic disruption is similar between sexes. Future studies should therefore include both sexes when studying effects of CD on energy metabolism to study sexual dimorphisms using various protocols with ranging severities of CD.

We subsequently assessed the involvement of gonadal sex hormones in effects of CD on lipid and glucose metabolism by injecting mice with GnRH-antagonist degarelix. With the exception of the reduction in plasma TG in males

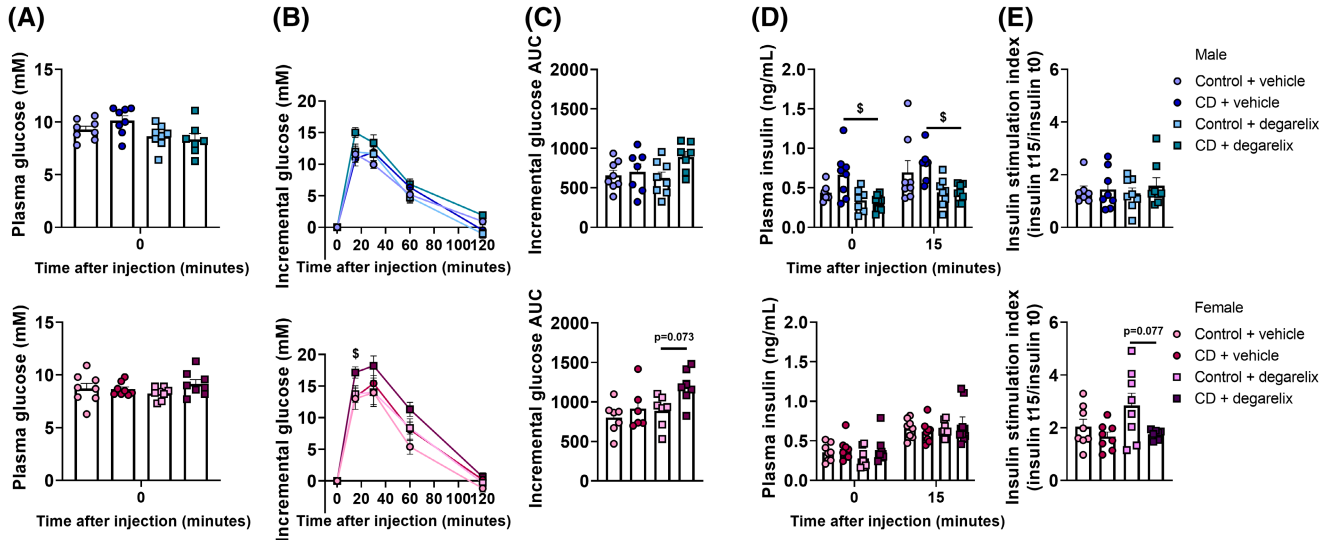

FIGURE 5 Glucose homeostasis during circadian disruption with chemical castration. Male and female C57BL/6J mice were exposed to a 6-h phase advancement every 3 days (circadian disruption; CD) or regular 12:12 light–dark cycle (control) for a total duration of 6 weeks. Mice were additionally treated with vehicle or degarelix to suppress endogenous production of gonadal sex hormones. (A) Fasted plasma glucose and (B) incremental plasma glucose levels were measured during a glucose tolerance test, and (C) area under the curve (AUC) was calculated. (D) Plasma insulin levels were measured at baseline and after 15 min, from which (E) the insulin stimulation index was calculated by dividing insulin levels after 15 min by insulin levels at baseline ($n = 6$–8 mice per group). Data are presented as means ± SEM. $^{\$}$CD + vehicle vs. CD + degarelix. $^{\$}p < .05$, according to two-way ANOVA and following Tukey's multiple-comparison test.

caused by removal of gonadal sex hormones, we observed no sexual dimorphism or involvement of gonadal sex hormones on markers of lipid metabolism. Nonetheless, we showed that CD caused pronounced elevations in plasma TG levels in males and females. Elevated TG levels are linked to increased cardiovascular disease risk in humans.[25] In line with previous findings,[26] we observed that CD delayed the clearance of circulating lipids by reducing the uptake of TG-derived FA by BAT and WAT. Both BAT and WAT display pronounced diurnal variation in metabolic functioning[27–29] that is disturbed upon CD.[26,30] Interestingly, while both testosterone and estradiol have been linked to metabolic BAT activity,[31,32] depletion of gonadal sex hormones did not affect TG-derived FA uptake by BAT in our study. This suggests that gonadal sex hormones might influence BAT activity more strongly during development of BAT in early life, rather than acutely.

Although the primary aim of this study was to elucidate the role of gonadal sex hormones in the effects of CD on lipid and glucose metabolism, we also observed noteworthy effects of gonadal sex hormone depletion alone. In females, removal of gonadal sex hormones under regular light–dark cycles induced adiposity and a reduction in energy expenditure that was explained by a reduction in fat oxidation, independent of changes in body composition. It also caused a pronounced shift in inactivity-defined sleep from light to dark phase under regular light–dark cycles. In line with these findings, estradiol has previously been shown to promote sleep during the resting phase[10] and has been identified as a protective factor against the development of type 2 diabetes,[33,34] as well as other cardiometabolic diseases in female mice and women.[35,36]

In the context of CD, removal of female gonadal sex hormones further impaired glucose homeostasis. This is in line with a previous study, which showed that removal of estradiol in female mice during CD impairs glucose homeostasis that is rescued by estradiol repletion.[37] We now subjected both sexes to the same CD protocol to show differential impairments in glucose homeostasis and demonstrated involvement of both male and female gonadal sex hormones. During CD, male gonadal sex hormones appear to elevate insulin levels, while female sex hormones appear to promote glucose-stimulated insulin secretion. This latter is in line with previous studies in mice and humans showing that CD impairs circadian β-cell function,[38–40] as well as the previously described protective effects of estradiol against β-cell failure.[33] In contrast to the consensus that estradiol protects against cardiometabolic disease risk, effects of testosterone on cardiometabolic health are inconclusive in the literature.[41] While low testosterone levels in men and testosterone removal in mice are related to impaired cardiometabolic health,[42–44] exogenous administration of testosterone to men with low endogenous levels actually elevates cardiovascular disease risk.[45] The contradictive observations of the effects of testosterone on cardiometabolic health may be a matter of dosage,[46] but could alternatively raise the question of whether other

hormonal factors are involved in the effects of testosterone on cardiometabolic health. In our study, removal of gonadal sex hormones in males reduced lean mass and voluntary activity, although glucose and lipid metabolism were unaltered during exposure to regular light–dark cycles. This discrepancy may be explained by the depletion of all endogenous gonadal sex hormones by degarelix injection, rather than solely testosterone. In our study, gonadal sex hormone depletion in males seemed to protect against effects of CD on glucose homeostasis, suggesting differential involvement of male and female gonadal sex hormones in the effects of CD on glucose homeostasis.

We conclude that CD sex dependently impairs glucose homeostasis, in which male gonadal sex hormones underlie this impairment and female gonadal sex hormones partly protect against this impairment. Provided these data can be translated to humans, we propose that sex could be an important factor underlying the high interindividual variability in shift work tolerance and disturbance of metabolism as a consequence of shift work.[47,48] Future studies on CD should consider the inclusion of both sexes, which may eventually contribute to personalized advice for shift workers.

AUTHOR CONTRIBUTIONS

Wietse In het Panhuis, Milena Schönke, Jan Kroon, Patrick C. N. Rensen, and Sander Kooijman conceived and designed the research; Wietse In het Panhuis, Milena Schönke, Ricky Siebeler, Dorien Banen, Amanda C. M. Pronk, Trea C. M. Streefland, Salwa Afkir, Hetty C. M. Sips, Jan Kroon, and Sander Kooijman performed the research and acquired the data, Wietse In het Panhuis analyzed the data, Wietse In het Panhuis, Milena Schönke, Jan Kroon, Patrick C. N. Rensen, and Sander Kooijman interpreted the data, and Patrick C. N. Rensen and Sander Kooijman acquired funding. All authors were involved in drafting and revising the manuscript.

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DISCLOSURES

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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