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June 2023 | HQ23RYB00040

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Received: 5 June 2023 | Revised: 6 September 2023 | Accepted: 26 September 2023

DOI: 10.1002/oby.23940

ORIGINAL ARTICLE

Obesity Biology and Integrated Physiology

Obesity A Research Journal | THE OBESITY SOCIETY | WILEY

Obesity alters the circadian profiles of energy metabolism and glucose regulation in humans

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Funding information
National Center for Advancing Translational Sciences, Grant/Award Numbers: TR000128, TR002369, TR002370; National Heart, Lung, and Blood Institute, Grant/Award Numbers: HL131308, HL125893, HL146992, HL151745, HL155681, HL156948, HL163232, HD109477; Oregon Institute of Occupational Health Sciences via funds from the State of Oregon, Grant/Award Number: Oregon Revised Statutes (ORS) 656.630

Abstract

Objective: Given the complex interaction among the circadian system, energy metabolism, and obesity, the authors tested whether having obesity impacts the circadian variation in energy and glucose metabolism in humans.

Methods: Participants with BMI either in the healthy weight or obesity ranges were studied in a 5-day, in-laboratory protocol that equally distributed behaviors (i.e., sleep, eating, exercise) across 24 h. Energy metabolism was measured at rest and during a standardized exercise bout and blood was sampled before and after each identical study meal to assess glucose and insulin levels.

Results: In those with a healthy weight, the circadian nadir of energy expenditure, during both rest and exercise, occurred when participants would normally be asleep. However, in those with obesity, this nadir appears to occur during the habitual wake period. Differences in glucose regulation also depended on the circadian phase, such that individuals with obesity appeared to have relatively greater glucose intolerance during the circadian day and produced less insulin during the circadian night.

Conclusions: Obesity is associated with altered circadian energy and glucose metabolism. Understanding and addressing these associations could lead to strategies that improve body weight and metabolic health in people with obesity.

INTRODUCTION

Approximately half of the adults in the United States have obesity [1], diabetes [2], or both, accounting for high rates of comorbidities and mortality [3] and over $327 billion in annual health care costs [4]. Increasing evidence has suggested that circadian timing contributes to energy metabolism [5–7] and glucose regulation [8, 9] in humans, which, if the timing of behaviors with circadian timing is disturbed,

may promote or exacerbate these health conditions [10]. However, these findings have been primarily documented in participants with a healthy weight or under conditions that cannot separately assess the effects of the endogenous circadian system from those caused by diurnal patterns of behaviors, such as eating and sleeping. Unraveling these factors is important because obesity rates continue to rise, especially in shift-working populations in whom the daily patterns of behaviors often occur during unusual circadian phases [11].

Energy levels and glucose metabolism differ between individuals who are lean and those with obesity [12, 13]. For example, a review of the literature suggested that individuals with obesity expend, on average, an increase in absolute resting energy expenditure (EE) of approximately 360 kcal/day [14] and an increase in absolute total daily EE of 370 kcal/day compared with those with lean body composition [15], although these expenditures may be similar when controlling for differences in fat-free mass. However, variations in metabolism across the circadian cycle, using intensive circadian protocols, have typically only been measured in resting conditions and not when the system has been challenged (e.g., exercise) in participants with lean body weight [5, 6]. When 24-h energy metabolism has been measured with exercise in individuals of differing body compositions [16], conclusions regarding circadian contributions are unclear because the experimental protocols used could not disentangle contributions of the circadian system from behaviors (eating, sleep). Therefore, there is a fundamental gap in our knowledge regarding the influence of obesity, exercise, and their combination on metabolic processes and glucose regulation across the circadian cycle.

We therefore examined changes to energy metabolism at rest and during exercise, including glucose regulation in response to an identical study meal, in a group of healthy individuals of varying body compositions in two protocols designed to evenly distribute all activity and meals across all circadian phases (Figure 1). These 5-day, in-laboratory protocols, both free of time cues and in dim lighting, consisted of 10 identical recurring 5-h and 20-min sleep/wake behavioral cycles in dim light throughout which EE and substrate utilization (i.e., respiratory exchange ratio [RER]) were measured via indirect calorimetry immediately prior to ("resting") and during a 15-min cycle ergometer exercise bout at ~50% of estimated heart rate maximum. Additionally, subjective hunger was measured throughout each protocol using visual analog scales, and plasma glucose and insulin levels were measured before and after an identical meal provided during each wake episode. Using this "forced desynchrony" protocol allowed for examining responses to identical stimuli across all circadian phases in groups with healthy weight (HW) and with obesity (OB). Specifically, we tested the hypothesis that the circadian effects on energy and glucose would differ between HW and OB groups, both at rest and during exercise.

Study Importance

What is already known?

- There are robust circadian variations in energy metabolism and glucose regulation in humans.
- Disrupting the normal circadian timing of eating, as occurs with shift work, can cause weight gain and diabetes.

What does this study add?

- We discovered that having obesity alters the circadian variation in energy metabolism. In individuals categorized as having a healthy-weight BMI, the circadian nadir of energy expenditure, during both rest and exercise, occurred during the circadian night, when participants would normally be asleep. However, in those with obesity, this nadir appeared to occur during habitual wake timing. Moreover, individuals with obesity did not adjust substrate utilization during rest as readily as individuals with healthy weight during the circadian night.
- Differences in glucose regulation also depended on the circadian phase, such that individuals with obesity displayed relatively greater glucose intolerance during the circadian day and produced less insulin during the circadian night.

How might these results change the direction of research or the focus of clinical practice?

- These findings may enable clinicians and researchers to consider circadian timing when developing treatment strategies for individuals depending on body adiposity. This approach could potentially increase the effectiveness of interventions aimed at weight control.

METHODS

Study participants

Participants (total, $n = 30$; age [mean ± SD], 48.4 ± 9.2 years; 14 female) were recruited from the local community using flyers, internet research recruitment websites, and word of mouth. Participants were categorized into HW (body mass index [BMI] < 25 kg/m^2; $n = 13$) or OB (BMI ≥ 30 kg/m^2; $n = 17$) categories, with both groups otherwise being comparable in baseline health characteristics with the exception of body weight and composition (Table 1). These data were collected toward the fulfillment of the objectives of a Career Development Award KL2TR002370 (to Andrew W. McHill) as part of two larger studies examining the effects of the circadian system on cardiovascular function in humans (ClinicalTrials.gov NCT02202811 and NCT03388788). Data from these protocols pertaining to cardiovascular function, mood, and cortisol have been previously published [17–22]. This is the first analysis from these protocols to examine energy and glucose metabolism and to separate the participants into HW and OB groups. Both protocols were similar in regard to the number of sleep/wake episodes, the timing of meals, blood draws, and

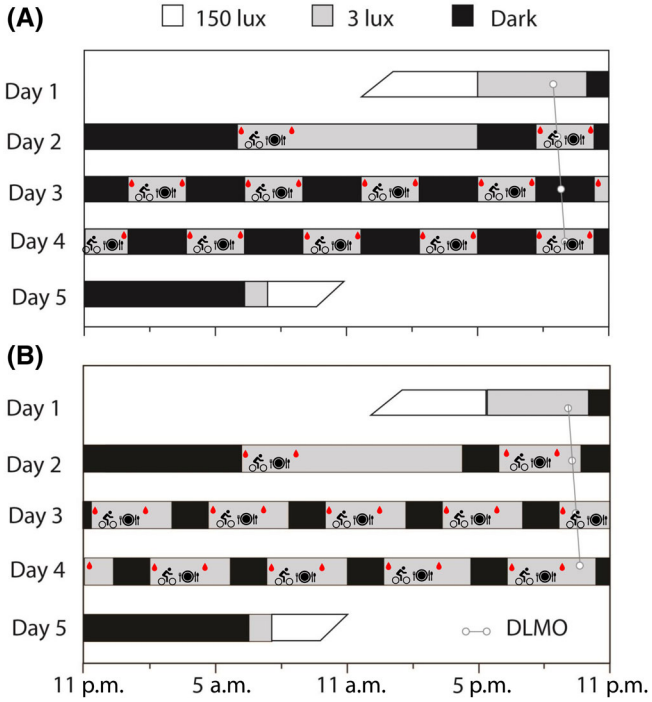

FIGURE 1 Circadian forced desynchrony protocols designed to spread activities equally across the 24-h day. After a baseline 8-h night and a baseline day, participants ($n = 30$) completed 10 alternating sleep/wake intervals of either 2 h and 40 min of sleep opportunity and 2 h and 40 min of scheduled wakefulness (Protocol A; $n = 22$) or 1 h and 47 min of sleep opportunity and 3 h and 33 min of scheduled wakefulness (Protocol B; $n = 8$), evenly spaced across the 24-h day. White bars in the figure represent wakefulness in typical room lighting (150 lux), black bars represent sleep opportunities (darkness), and gray bars represent dim-light waking times (1.5 ± 0.5 lux). Red drops denote the timing of blood draws for plasma glucose and insulin, bikes denote exercise bouts of ~50% heart rate maximum, and plates denote the standardized study meal. Indirect calorimetry was performed immediately prior to (rested) and during exercise. Open circles denote an example of the free-running rhythm of the dim-light melatonin onset (DLMO) for an individual with a longer-than-24-h clock. Note, indirect calorimetry and exercise testing were randomly omitted in three wake episodes during Protocol B to enable other cardiovascular testing (see NCT03388788 for additional details).

exercise but differed slightly in sleep opportunity duration and study procedures not included in the current analysis (Figure 1A, $n = 22$, Figure 1B, $n = 8$; note: indirect calorimetry and exercise testing sessions were randomly omitted in 3 wake episodes during one protocol, i.e., Figure 1B, to enable timing for other cardiovascular testing; see NCT02202811 and NCT03388788 for differences in study procedures not pertinent to the current study outcomes). Because some of the data were collected before the initiation of grant KL2TR002370, only subsets of participant data were available for each analysis, and the number of participants in each analysis is denoted throughout. All experimental procedures were approved by the Oregon Health & Science University Institutional Review Board (Portland, Oregon) for human subject protection. All participants provided written informed consent prior to participation in study procedures.

Screening procedures

Participants underwent an initial 12-lead electrocardiogram measurement, a blood draw for hematologic and metabolic measures (i.e., hemoglobin, hematocrit levels, basic blood chemistry, and blood glucose), blood pressure measurement while resting in the laboratory and for ~48 h in the at-home environment (Spacelabs Healthcare, Snoqualmie, Washington), dual-energy x-ray absorptiometry scan for percent body fat ($n = 15$, OB; $n = 8$, HW), and a psychological and physical evaluation by a physician. Exclusion criteria included a history of chronic disease such as diabetes or cardiovascular disease, pregnancy, history of ≥5 "pack years" of smoking or currently smoking, or use of any prescription or nonprescription medications as verified by urinalysis of drugs (Drugsmart 12 panel cup; Speares Medical, Inc., Irmo, South Carolina) and cotinine (NicAlert; Nymox Pharmaceutical Corporation, Saint-Laurent, Quebec, Canada). To ensure stability of the circadian clock prior to entering the protocol, participants were also excluded if they had traveled across ≥three time zones in the past 3 months or participated in shift work within the past 6 months prior to study procedures.

Pre-inpatient procedures

Each participant abstained from caffeine, alcohol, and over-the-counter and recreational pharmaceuticals and was asked to maintain an 8-h time-in-bed sleep opportunity per night for at least 1 week immediately prior to entering the in-laboratory protocol to further ensure stability of the circadian clock. Compliance with these sleep schedules was assessed using wrist actigraphy (ActiGraph wGT3X-BT; ActiGraph LLC, Pensacola, Florida), a sleep diary, and daily bedtime and wake-time calls to a time-stamped voicemail system.

Circadian protocol ("forced desynchrony")

Upon admission to the Oregon Clinical and Translational Research Institute at Oregon Health & Science University, a drug screen and pregnancy test were performed to ensure inclusion criteria. Participants were then trained on testing procedures, instrumented with study equipment (i.e., intravenous catheter to allow for blood sampling), and were provided with an 8-h sleep opportunity at their habitual sleep timing as determined by the prior week of actigraphy. Upon awakening after the first sleep period, participants had a "baseline day" and then entered a "forced desynchrony" protocol [23] in dim light designed to desynchronize sleep-wakefulness cycles from the circadian cycles by altering the length of the daily sleep-wakefulness schedule beyond the range of circadian entrainment [24]. This protocol thereby allows the human circadian pacemaker to "free-run" at its intrinsic rate of ~24.1 h [25]. Throughout the forced desynchrony protocols (Figure 1), lighting levels were kept dim (<3 lux) during scheduled wakefulness and were dark (<0.1 lux) during scheduled sleep opportunities. The actual forced desynchrony portion of

TABLE 1 Baseline health, sleep, and circadian timing of study participants

	HW ($n = 13$)	OB ($n = 17$)	p value
Male (n, %)	8 (61.6)	8 (47.1)	-
Female (n, %)	5 (38.5)	9 (52.9)	-
Age (y, SEM)	47.6 (3.4)	48.3 (1.4)	0.83
Body composition			
BMI (kg/m^2, SEM)	23.4 (0.4)	34.7 (0.8)	<0.0001
Body fat (%, SEM)	23.8 (2.7)	45.6 (2.8)	<0.0001
Waist-hip ratio (ratio, SEM)	0.86 (0.03)	0.87 (0.02)	0.59
Fasted blood concentrations			
Glucose (mg/dL, SEM)	88.7 (2.2)	94.5 (2.3)	0.09
Triglycerides (mg/dL, SEM)	92.3 (14.7)	121.5 (9.7)	0.15
Total cholesterol (mg/dL, SEM)	196.7 (16.3)	166.7 (9.5)	0.17
High-density lipoprotein cholesterol (mg/dL, SEM)	66.0 (4.3)	52.5 (6.2)	0.09
Low-density lipoprotein cholesterol (mg/dL, SEM)	112.5 (17.7)	89.8 (8.4)	0.32
Sleep and circadian timing			
Sleep onset week prior to in-laboratory study (hh:mm, SEM)	23:12 (00:16)	22:35 (00:14)	0.09
Sleep efficiency week prior to in-laboratory study (%, SEM)	87.19 (2.2)	88.49 (1.1)	0.56
Circadian period (hh:mm, SEM)	24:04 (00:06)	24:06 (00:05)	0.79
DLMO (hh:mm, SEM)	21:04 (00:22)	20:58 (00:16)	0.81
Morningness-eveningness score (score, SEM)	55.8 (2.6)	50.8 (2.5)	0.59

Note: Sleep efficiency was calculated as actigraphic sleep/the diary-determined time-in-bed.
Abbreviations: DLMO, dim-light melatonin onset; HW, individuals with healthy weight; OB, individuals with obesity.

the protocol began on the evening of experimental day 2 (end of baseline) and continued for the next 2 days, ending in the evening of experimental day 4 and followed by a recovery sleep opportunity. Each participant's diet was standardized to meet daily caloric requirements and divided into small meals (22.2% of the 24-h total isocaloric requirement, thus equating to 100% of the predetermined isocaloric need across the 4.5 forced desynchrony days occurring within each 24-h day). The meals were identical (macronutrient composition of 55% carbohydrate, 30% fat, and 15% protein), and participants were instructed to fully consume the meal within 20 minutes during each 5-h and 20-min cycle. Subjective hunger was measured via visual analog scales that were provided upon awakening, before exercise testing, after exercising testing, and before sleep (for analysis, these ratings were averaged across each wake period and plotted against circadian phase). Participants were instructed to denote, on a 100-mm horizontal line, how hungry they felt at that moment, with each end of the line labeled with "I am not hungry at all" to "I have never been more hungry." Study staff were present 24 h/day to monitor data acquisition, collect biologic specimens, and administer study testing procedures.

EE and substrate utilization (RER) assessment

In a subset of participants ($n = 16$ [9 male]; 9 HW, 7 OB), indirect calorimetry (Vmax Enterprises LLC, Franklinville, New Jersey) was assessed during each scheduled wake episode period (total of 9–12 tests for each participant) for assessment of resting EE, exercising EE, and macronutrient substrate utilization (calculated using standard equations) [26]. Throughout the forced desynchrony protocols, all measurements were conducted in an identical manner, with all activities/study procedures and time into wakefulness prior to the indirect calorimetry measurement being the same. Participants sat and wore a fitted breathing mask for assessment of resting EE and RER across a period of 15 min. Immediately following, participants began exercising on a cycle ergometer (Cybex 525R Recumbent Bike; Life Fitness, Franklin Park, Illinois), for ~20 min while EE and RER were again measured. Only steady-state (<10% fluctuation in samples measured every 10 s in oxygen consumption) [27] values were used in the analysis.

Exercise testing

The workload on the cycle was tailored to induce ~50% heart rate maximum (predicted heart rate using the Karvonen formula) [28] for the duration of the exercise; this intensity of exercise was chosen for the following reasons: 1) to reflect the type of workload placed on the cardiovascular system during normal daily activities (e.g., walking); 2) to not cause an anaerobic state or training effect; and 3) to elicit changes in substrate utilization [29, 30]. The power output of the cycle ergometer at 50% of heart rate maximum for each individual was determined during an initial exercise test on admit day and was

kept constant for subsequent tests to enable quantification of within-participant changes to the same exercise stimulus.

Circadian phase and period assessment

Saliva was collected at least hourly during each wake episode via Salivette (Sarstedt, Inc., Newton, North Carolina) and assayed for melatonin using the radioimmunoassay from Bühlmann Laboratories (Schönenbuch, Switzerland). Circadian phase was determined via the dim-light melatonin onset (DLMO), defined as the linear interpolated time point in which melatonin exceeded 3 pg/mL [31–34], and circadian period was calculated from the average differences among consecutive DLMOs. The in-house interassay coefficient of variation (CV) was 11.5%.

Glucose and insulin assessment

In another subset of participants ($n = 19$ [11 male]; 7 HW, 12 OB), blood samples for the assessment of glucose and insulin concentrations were drawn upon awakening (pre-meal) and ~45 min after the consumption of the standardized meal (postprandial) from an indwelling venous catheter. The response to a standardized meal was calculated as the difference in glucose and insulin levels from pre- to post-meal. Although participants were instructed to eat the entirety of the provided meal, compliance was inconsistent. Participants variously reported low hunger, distaste for particular aspects of a meal, and other reasons for this. Therefore, glucose and insulin data in response to a meal were only included if the participant had consumed >85% of the provided meal between blood draws (≥1 meal removed in $n = 4$ participants).

After the blood draw, samples were placed on ice until centrifugation at 2500 g at 4°C for 15 min. Glucose was measured in triplicate by analyzing heparinized plasma using the enzymatic Trinder method with reagents from EKF Diagnostics (Stanbio Laboratory, Boerne, Texas). The interassay CV was 2.6% at 117 mg/dL. Insulin was measured in duplicate by analyzing heparinized plasma using an ultrasensitive enzyme-linked immunosorbent assay from Mercodia AB (Uppsala, Sweden). The interassay CV was 7.2% and 4.7% at 8.5 mU/L and 45.4 mU/L, respectively.

Analysis

In order to determine any circadian rhythmicity in EE, RER, hunger, glucose, and insulin and to account for potential differences in the absolute levels due to differences in body composition, data were first transformed to the percent of the mean for each individual. Data were then assigned a circadian phase (DLMO = 0°) and binned for each participant in 60° circadian phase bins. To uncover circadian variation in outcomes in participants in the HW or OB categories, outcomes were analyzed using mixed-effects models with circadian phase as a fixed effect. Additionally, we performed a mixed-model Cosinor analysis on the energy and glucose metabolism data, including the fundamental frequency (24-h component) and the first harmonic (12-h component). Data were then analyzed using mixed-effects models with circadian phase and group as fixed effects and circadian phase-by-group as an interaction to compare the circadian variation between the two groups. To examine circadian day/night differences within each individual, the raw values of each outcome variable were binned according to whether they occurred during the usual sleep period (here, the start of sleep ranged from 7 to 54 circadian degrees, and the end of sleep ranged from 123 to 174 circadian degrees) or the usual wake period (all other circadian phases not included within the sleep interval) and were compared using paired Student t tests. Differences in circadian day/night responses between groups were analyzed using independent Student t tests. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina), and the Cosinor models were performed in Stata version 14 (StataCorp LLC, College Station, Texas).

RESULTS

Within the HW group, both resting EE ($F_{5,43} = 4.48$, $p < 0.01$, Figure 2A; Cosinor model 24-h component: $p = 0.04$, Figure S1A) and exercising EE ($F_{5,41} = 2.78$, $p = 0.03$, Figure 2B; model: $p = 0.10$) differed by circadian phase, with peaks for both EE measures in the late evening (melatonin phase of 0°, relative clock hour ~21:00; modeled fit peak of 345°, ~20:00) and nadirs in the early morning (120°, ~05:00; modeled 90°, ~03:00). There was no significant difference in resting RER by circadian phase for the HW group ($p = 0.30$, Figure 2C; model: $p = 0.43$, Figure S1C), but a significant circadian phase effect was found for exercising RER ($F_{5,41} = 2.95$, $p = 0.02$; Figure 2D; model: $p = 0.04$, Figure S1D) with a similar peak to that of EE in the late evening and a nadir in the early morning. In contrast, there were no significant circadian phase effects for the OB group in any of these four energy metabolism metrics (Figures 2, S1, and S2).

When examining interactions among circadian phase, group, and energy metabolism, there was a significant group-by-circadian-phase interaction for resting EE ($F_{5,77} = 2.62$, $p = 0.03$) and for exercising EE ($F_{5,72} = 2.37$, $p = 0.04$). The nadir of EE in the HW group occurred during the participants' habitual sleep periods, but this was relatively delayed by ~4 h in the OB group, whose EE minimum visually occurred during a time of typical wakefulness (Figure 2A,B). In regard to substrate utilization, there were no significant differences in RER between groups during rest ($F_{5,76} = 1.50$, $p = 0.20$). However, there was a significant group-by-circadian-phase interaction during exercise ($F_{5,75} = 2.68$, $p = 0.03$) such that individuals in the OB group had a higher RER, representing a higher utilization of carbohydrates over fat, during the morning exercise (Figure 2D). When data were separated to examine both resting and exercising EE either during the circadian day or night (defined as when a participant would habitually be awake or asleep), EE was significantly lower in individuals in the HW group during both rest (Figure 2E) and exercise (Figure 2F) at night

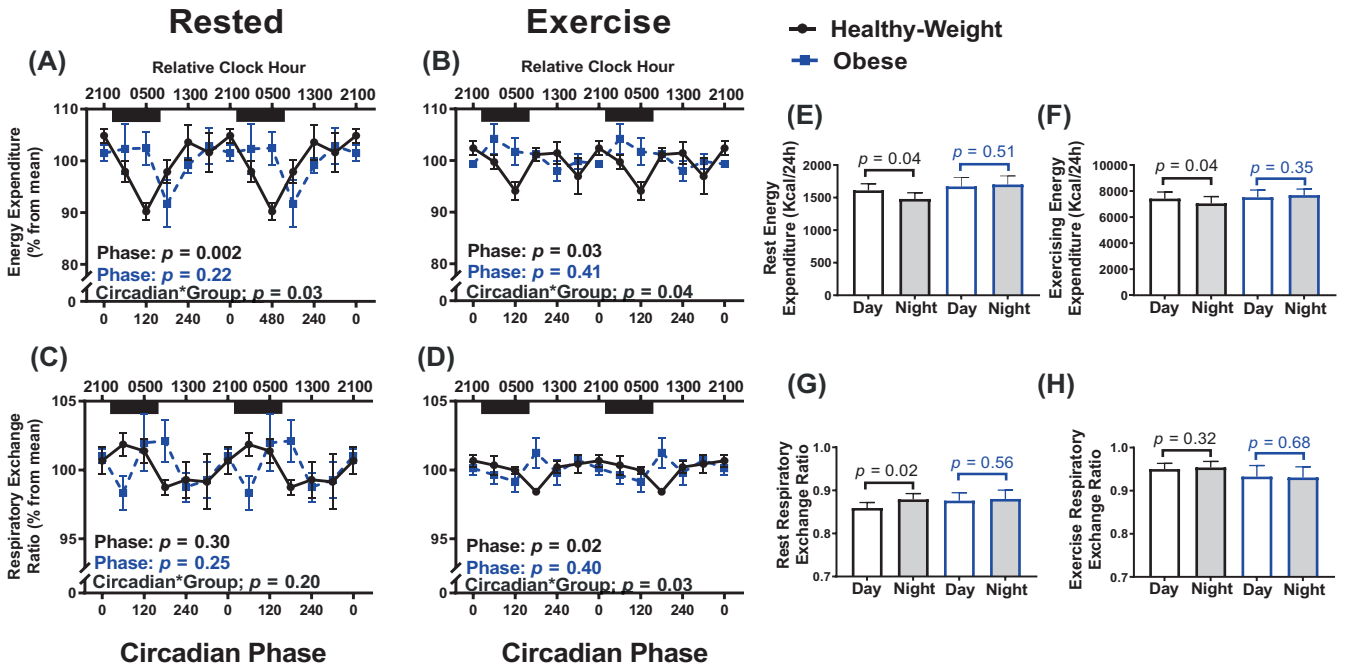

FIGURE 2 Influence of circadian timing and obesity on energy expenditure and respiratory exchange ratio. Black circles and blue square symbols denote individuals with BMI categorized as either healthy weight ($n = 9$) or obesity ($n = 7$), respectively. The black bars along the top x-axis represent the circadian night, when habitual sleep would occur, and correspond to the night designation in the bar graphs (other phases without the black bar correspond to the day designation). Note, these times are not when sleep occurred in the laboratory but are based on the timing the week prior to the in-laboratory study. Data are double-plotted, and circadian phase is derived from the dim-light melatonin onset, wherein melatonin onset equates to 0°. *P* values denote either mixed-model (A–D) or paired Student *t* test results (E–H). Circadian phase of day/night designations are described in the methods.

compared with the day, with no differences within the OB group. Additionally, the HW group significantly increased their RER at rest (Figure 2G), but not during exercise (Figure 2H), across the night compared with the day ($p = 0.02$). There were no significant differences across circadian phase for either HW ($F_{5,72} = 1.54$, $p = 0.19$) or OB groups ($F_{5,96} = 1.81$, $p = 0.12$) in subjective hunger. Moreover, there were no significant interaction ($F_{5,168} = 1.06$, $p = 0.38$) or group effects ($F_{5,168} = 0.01$, $p = 0.94$) between the HW and OB groups.

Glucose levels in response to a standardized meal changed by circadian phase in the HW group ($F_{5,28} = 4.33$, $p < 0.01$, model: $p = 0.03$) and with a nonsignificant trend in the same direction in the OB group ($F_{5,52} = 2.16$, $p = 0.07$, model: $p = 0.68$), such that the peaks occurred ~8 h apart (Figures 3A and S3A). Insulin levels varied by circadian phase only in the OB group ($F_{5,55} = 4.23$, $p < 0.01$, Figure 3B; model: $p < 0.01$, Figure S3B). When comparing glucose and insulin responses to the standardized meal between groups, there were significant interactions for both glucose ($F_{5,80} = 3.64$, $p < 0.01$) and insulin ($F_{5,87} = 3.20$, $p = 0.01$). The nadirs for both glucose and insulin were ~8 h later in the OB group relative to the HW group (Figure 3A,B and S4). Neither group exhibited significant day/night differences in glucose response (Figure 3C). However, individuals in the OB group displayed a higher change in insulin during the circadian day ($p = 0.03$) despite similar glucose levels compared with the HW group, who did not display day/night changes in insulin. Moreover, the meal elicited significantly less insulin secretion during the night compared with the day in the OB group (−24.2%, $p = 0.03$, Figure 3D).

DISCUSSION

Using a protocol designed to equally distribute standardized meals and standardized exercise bouts across the endogenous circadian cycle, we found that obesity alters metabolic parameters dependent on circadian phase. Specifically, the HW group had their lowest resting and exercising EE across the circadian time when they would normally be asleep. In contrast, the OB group did not differ significantly in EE metrics across circadian timing. However, those in the OB group favored higher carbohydrate over fat utilization in response to exercise during the day and were less responsive to the circadian day/night changes in resting EE (HW group decreased by 8.9% during the night, OB group displayed no significant change), exercising EE (HW group decreased by 5.1% during the night, OB group displayed no significant change), and rest RER (HW group increased by 2.3% during the night, OB group displayed no significant change), potentially suggesting a circadian-based metabolic inflexibility [35]. We did not find any differences between groups in subjective hunger. In addition to energy metabolism differences, we also found differences in glucose and insulin responses to a standardized meal depending on the circadian phase, such that individuals in the HW group displayed

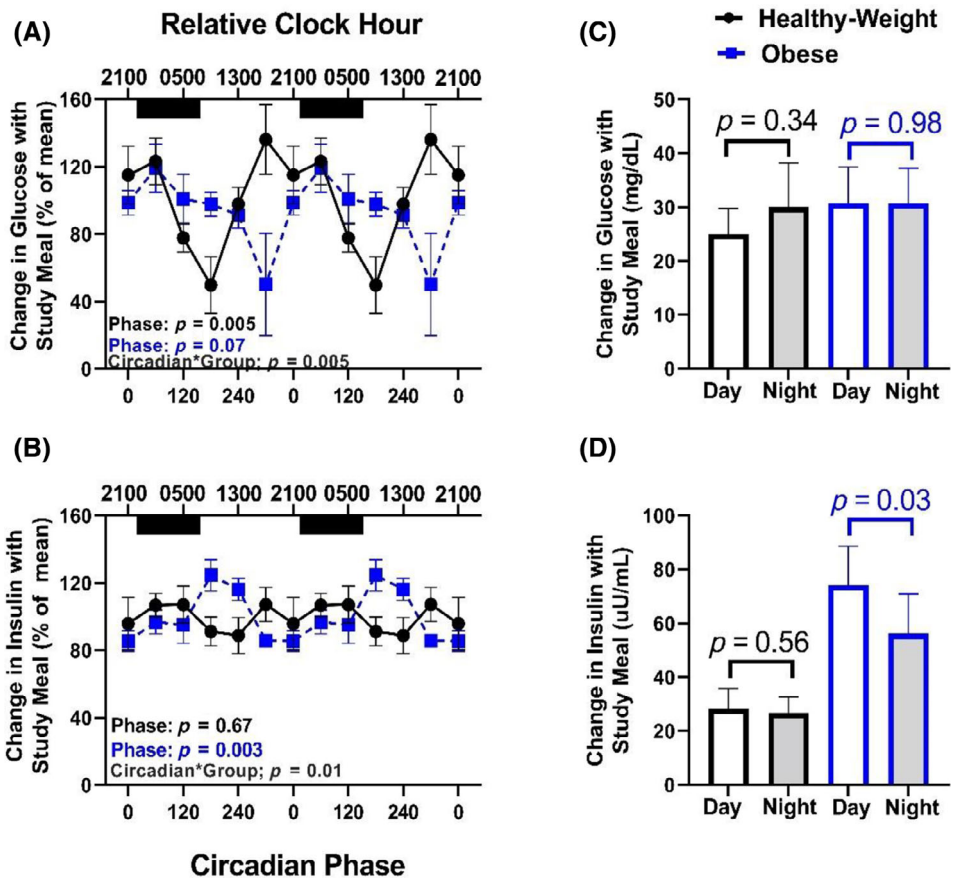

FIGURE 3 Influence of circadian timing and obesity on the glucose and insulin responses to a standardized meal. Black circles and blue square symbols denote individuals with BMI categorized as either healthy weight ($n = 7$) or obesity ($n = 12$), respectively. The black bars along the top x-axis represent the circadian night, when habitual sleep would occur, and correspond to the night designation in the bar graphs (other phases without the black bar correspond to the day designation). Note, these are not when sleep occurred in the laboratory but are based on the timing the week prior to the in-laboratory study. Data are double-plotted, and circadian phase is derived from the dim-light melatonin onset, wherein melatonin onset equates to 0°. *P* values denote either mixed-model (A,B) or paired Student *t* test results (C,D). Circadian phase of day/night designations are described in the methods.

an ~8-h advanced glucose rhythm and an almost antiphasic insulin rhythm compared with those in the OB group, with robust circadian day/night differences in response to a meal (OB group insulin decreased by 24.2% during the night, HW group displayed no significant change).

Our findings of circadian variation in EE in individuals in the HW group are similar to that of previous reports of circadian variation in resting EE in individuals without obesity [5, 6]; we now add the circadian rhythm of exercise EE and demonstrate how individuals with obesity may have a different profile depending on circadian phase. These differences may suggest a role for circadian timing in the expression and maintenance of body weight and metabolic health. Moreover, our findings that subjective hunger did not differ between the two groups might suggest that food intake (when food is freely available outside of the laboratory) may not differ between groups across circadian timing, which could have implications for energy balance. Future work is needed to elucidate whether these circadian differences in EE contribute to, or are caused by, changes in body composition, how metabolism may change under an ad-libitum and/or timed-controlled diet, or whether sex differences may play a role in the interaction between circadian timing and obesity [36].

It has been well-established that glucose tolerance differs by circadian phase [9] and that eating during the circadian night in lean individuals impairs insulin sensitivity [8]. It has also been established that body composition alters glucose tolerance [12, 13]. Thus, our findings of circadian variation in glucose response to a meal align with previous reports. However, our findings of significant differences in response to a standardized meal between those in the HW and OB groups being dependent on circadian phase are novel and may have implications for those eating across the 24-h day depending on body composition, as well as for the diagnosis and treatment of impaired glucose tolerance and diabetes. Indeed, previous work has demonstrated that individuals with obesity display a blunted diurnal response (morning to evening differences in response to a glucose tolerance test) in insulin sensitivity and β-cell responsivity to glucose [37], which may be similar to our finding of a significant decrease in insulin response (−24.2%) during the circadian night. However, it could also be interpreted that those in the OB group needed additional insulin during

the circadian day to maintain similar glucose levels, indicative of glucose intolerance. More specific and sensitive methods (i.e., frequently sampled intravenous glucose tolerance test) are needed to uncover mechanistic pathways and how they differ by circadian phase between the two groups.

Despite the rigorous study design used to uncover differences in the circadian patterns of energy and glucose metabolism between the HW and OB groups, there are several limitations to consider. First, although use of the forced desynchrony design is necessary in order to uncouple behaviors from endogenous patterns in energy and glucose metabolism, use of these protocols may limit generalizability to other environments. Future work is needed to understand whether metabolic differences persist between those with lean and obese body compositions in shift-working populations that must be active and eat at all times of the day. Second, our sample size may have limited our ability to detect differences between groups in outcomes. To increase sample size, we combined two protocols that were similar in design and outcomes but may have introduced variability that we are unable to detect. Nevertheless, markers of the central circadian clock, e.g., timing of DLMO and circadian period, are not dissimilar between these two protocols [17–22]. Last, although the use of individual indirect calorimetry tests enabled the examination of both EE and substrate utilization at all circadian phases, we were unable to examine changes during sleep or continuously throughout the protocol.

In summary, we have discovered that, compared with lean individuals, those with obesity exhibit altered resting and exercising EE across the circadian cycle and an altered circadian pattern of glucose regulation. Causal directionality remains to be elucidated, and mechanisms could include circadian effects on peripheral metabolism, glucose absorption, peripheral adipocyte insulin sensitivity, or hepatic glucose production. Understanding these differences could help optimize weight-management approaches and reduce the adverse impact of shift work on body weight and metabolism to those with obesity.O

AUTHOR CONTRIBUTIONS

All authors designed research; Andrew W. McHill, Saurabh S. Thosar, Nicole P. Bowles, Matthew P. Butler, and Steven A. Shea secured funding; all authors performed research; Andrew W. McHill and Saurabh S. Thosar analyzed data; and all authors wrote the paper.

ACKNOWLEDGMENTS

The authors thank Noal Clemons and all research staff involved with this investigation, the research volunteers, and the Oregon Clinical and Translational Research Institute staff for their support in conducting these studies. Data supporting the conclusions of this manuscript will be made available by the authors, upon request, to any qualified researcher.

FUNDING INFORMATION

This work was supported by National Institutes of Health (NIH) grants HL125893, HL156948, HL146992, HL155681, HL131308, HL163232, TR002370, HL151745, HD109477, TR000128, and TR002369 and by the Oregon Institute of Occupational Health Sciences via funds from the State of Oregon (Oregon Revised Statutes [ORS] 656.630).

CONFLICT OF INTEREST

Andrew W. McHill reports consulting for Pure Somni Corporation. The other authors declared no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: McHill AW, Thosar SS, Bowles NP, et al. Obesity alters the circadian profiles of energy metabolism and glucose regulation in humans. *Obesity (Silver Spring)*. 2023;1-9. doi:10.1002/oby.23940