Report

Current Biology

Human Resting Energy Expenditure Varies with Circadian Phase

Highlights

- Resting energy expenditure varies with circadian phase in humans
- Respiratory quotient varies with circadian phase in humans
- Resting energy expenditure is lowest in the late biological night
- Circadian variation in energy expenditure exceeds that caused by sleep deprivation

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In Brief

Zitting et al. demonstrate that resting energy expenditure varies with circadian phase and is lowest in the late biological night. This may contribute to weight gain in people with irregular sleep schedules and highlights the importance of controlling for circadian phase and sleep-wake behavior when assessing energy expenditure.

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Report

Human Resting Energy Expenditure Varies with Circadian Phase

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SUMMARY

There is emerging evidence that circadian misalignment may alter energy expenditure, leading to obesity risk among those with irregular schedules [1–5]. It has been reported that energy expenditure is affected by the timing of sleep, exercise, and meals [6]. However, it is unclear whether the circadian system also modulates energy expenditure, independent of behavioral state and food intake. Here, we used a forced desynchrony protocol to examine whether fasted resting energy expenditure (REE) varies with circadian phase in seven participants. This protocol allowed us to uncouple sleep-wake and activity-related effects from the endogenous circadian rhythm, demonstrating that REE varies by circadian phase. REE is lowest at circadian phase ~0°, corresponding to the endogenous core body temperature (CBT) nadir in the late biological night, and highest at circadian phase ~180° in the biological afternoon and evening. Furthermore, we found that respiratory quotient (RQ), reflecting macronutrient utilization, also varies by circadian phase. RQ is lowest at circadian phase ~240° and highest at circadian phase ~60°, which corresponds to biological morning. This is the first characterization of a circadian profile in fasted resting energy expenditure and fasted respiratory quotient (with rhythmic profiles in both carbohydrate and lipid oxidation), decoupled from effects of activity, sleep-wake cycle, and diet in humans. The rhythm in energy expenditure and macronutrient metabolism may contribute to greater weight gain in shift workers and others with irregular schedules.

RESULTS AND DISCUSSION

The potential influence of circadian timing on energy expenditure and macronutrient metabolism and/or utilization independent of behavioral state and food intake has not been investigated in humans. Here, we investigated the influence of circadian timing on resting energy expenditure (REE) and respiratory quotient (RQ) across 3 weeks of controlled diet and activity within a 37-day inpatient research protocol. Participants experienced either 3 weeks of recurrent circadian disruption (RCD) on a 28-hr rest-activity schedule (see Figure 1, circadian disruption group; n = 7) or 3 weeks on a regular 24-hr schedule (Figure 1, control group; n = 6).

Circadian Variation of Fasting Resting Energy Expenditure

There is now emerging evidence that an irregular sleep-wake and fasting-feeding cycle, common in people working night or rotating shifts, can lead to disrupted circadian timing, which in turn may alter energy balance and lead to increased obesity risk [2, 4, 5, 7]. The positive energy balance associated with night work (which leads to weight gain and obesity over time) may be caused by increased energy intake, changes in the timing and/or frequency of food intake [8–11], or decreased energy expenditure [1, 12].

REE, also known as resting metabolic rate (RMR), is the largest component of daily energy expenditure and a major determinant of changes in weight [13]. REE accounts for 60%–70% of all calories burned at rest each day to support basic physiological functions such as ventilation, circulation, temperature regulation, and brain activity. We show that fasted REE, decoupled from behavioral state and food intake, varies with circadian phase (Figure 2; linear mixed model: n = 7, F = 10.76, $p < 0.0001$) in a protocol that kept caloric intake, time-in-bed sleep opportunity, and exercise levels consistent across all phases and over the duration of the study. REE is lowest at what we have defined as circadian phase 0°, corresponding to the nadir of the endogenous circadian rhythm of core body temperature in the late biological night, and is highest about 12 hr later at circadian phase ~180°, corresponding to the biological afternoon and evening (cosinor model: amplitude = 55.2 kcal/day; acrophase = 160.8°; H_0: $p < 0.001$). Thus, while awake and resting, the human body burns the fewest calories during the late biological night and the most calories during the biological afternoon and evening.

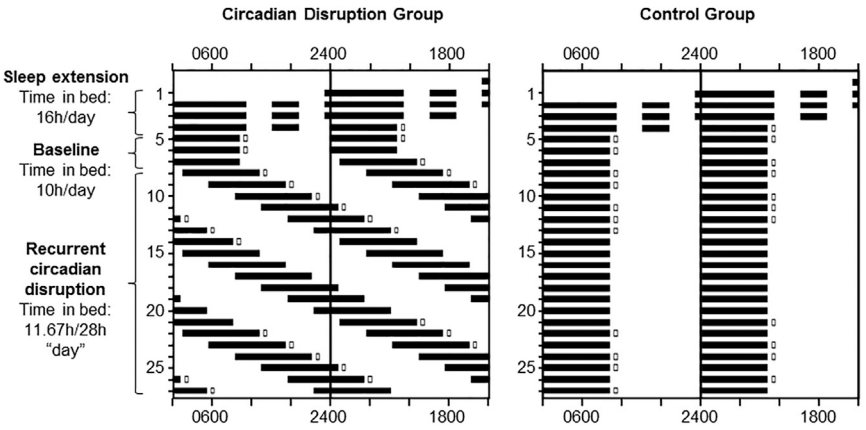

Figure 1. Experimental Protocol

Participants completed a 37-day laboratory protocol, beginning with 3 days of sleep extension with 16 hr/day time in bed then a baseline segment of 3 days with 8–10 hr/day time in bed. In the circadian disruption group (left), sleep opportunities (dark bars) were then spread across the circadian cycle on a 28-hr forced desynchrony protocol, with 11.67 hr time in bed and 16.33 hr of wake for 3 weeks. Open boxes indicate when RMR was measured via calorimetry. In both groups, days 28–37 consisted of a recovery and realignment segment (not depicted). Participants in the control group (right) had identical sleep extension and baseline segments but underwent 3 weeks of sleep and wake identical to the baseline schedule with 8–10 hr time in bed each day. See also Table S1.

Previous studies exploring diurnal changes in energy expenditure in humans [14–20] and animals [21–27] have reported mixed results. However, in attempting to evaluate the diurnal or circadian variation of REE, previous studies likely encountered confounding meal effects (e.g., breakfast measurement obtained after an overnight fast, whereas lunch and dinner measurements were obtained after prior meals). In the current study, each fasted recording during the 3-week forced desynchrony protocol was performed shortly after a scheduled wake time, following an overnight fast of at least 12 hr. The caloric and macronutrient content of the meals consumed throughout the day after the fasted recording were similar, with calories varying by <5% from day to day within each participant.

We found no significant difference in REE values between the first and third weeks in the recurrent circadian disruption group (Figure S1), suggesting that 3 weeks of recurrent circadian disruption does not change the overall REE or affect the circadian modulation of REE. There was also no significant difference in REE values between the first and third weeks in the control group (Figure S3), in which participants lived on a regular 24-hr schedule. These findings are in contrast to the results from our previous study, when we found that 3 weeks of sleep restriction combined with recurrent circadian disruption led to an 8% drop in RMR [28]. Sleep restriction alone either decreases fasting energy expenditure [28–30] or leads to no change, when measured at a consistent time of day [31–33]. Because we observed no change with circadian disruption alone (while minimizing sleep restriction) in the current study, the 8% reduction observed in our previous study [28] was likely due to sleep restriction or the combined effects of sleep restriction and circadian disruption.

Interestingly, while there was a significant effect on REE of day-to-day variability in body weight in both groups when tested independently (recurrent circadian disruption group: $n = 7$, $p = 0.004$; control group: $n = 6$, $p = 0.009$), this effect disappeared in the final statistical model for the recurrent circadian disruption group (but not for the control group), suggesting that circadian timing is a stronger determinant of REE than small changes in weight. In our previous study, which included a recent history of sleep restriction combined with circadian disruption, changes in bodyweight were unrelated to changes in RMR [28]. This difference may be due to fewer RMR recordings (3 versus >10) over the course of the previous study compared to the current study. In the present study, baseline REE value predicted the REE during the 3 weeks of circadian disruption in the recurrent circadian disruption group only ($n = 7$, $F = 36.16$, $p < 0.0001$).

Circadian Variation of Fasting Respiratory Quotient and Carbohydrate and Lipid Oxidation

The mean respiratory quotient, which is the ratio between carbon dioxide production and oxygen consumption, reflects the amount of energy derived from carbohydrates as opposed to lipids and tends to be higher in individuals who are obese or who have type 2 diabetes [13, 34–37]. The respiratory quotient is around 0.8 for a mixed diet and typically varies between 0.7 (100% lipid oxidation [LO]) and 1 (100% carbohydrate oxidation [CHO]), depending on the proportion of substrates being metabolized.

We show that, similar to REE, fasted respiratory quotient also varies by circadian phase (Figure 3; linear mixed model: $n = 7$, $p = 0.036$). While mean respiratory quotient measured in the biological morning at baseline was 0.84 in both the recurrent circadian disruption and control groups (Table S1), it varied by circadian phase and was 2.5% lower in the biological evening compared to the biological morning in the recurrent circadian disruption group (cosinor model: amplitude = 0.012; acrophase = 80.4°; H_0: $p = 0.019$). Fasting carbohydrate oxidation and lipid oxidation also varied by circadian phase; carbohydrate oxidation was highest during the biological morning and lowest during the biological evening (cosinor model: amplitude = 0.012; acrophase = 99.6°; H_0: $p = 0.012$), whereas LO was highest during the biological evening and lowest during the biological morning (cosinor model: amplitude = 0.004; acrophase = 226.2°; H_0: $p = 0.019$). There was also a significant effect of sex on respiratory quotient in the recurrent circadian disruption group ($n = 7$, $F = 14.31$, $p < 0.001$).

Duration of exposure to recurrent circadian disruption had no significant influence on the effect of circadian phase on respiratory quotient (Figure S2). This circadian variation in respiratory quotient and macronutrient utilization suggests that the body favors carbohydrate oxidation in the biological morning and lipid oxidation in the biological evening. We did not

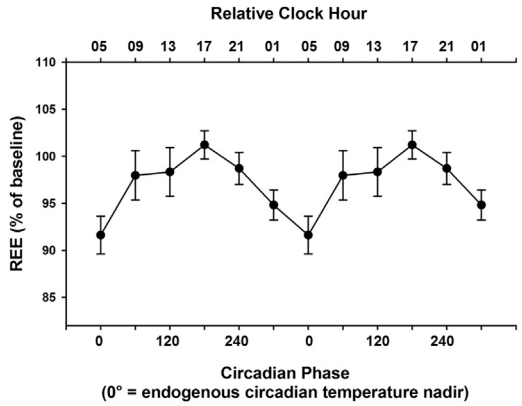

Figure 2. Circadian Variation of Fasting Resting Energy Expenditure

Fasting levels of resting energy expenditure (REE) are plotted with respect to the circadian phase at which they were recorded. Fasting REE is lowest at circadian phase 0°, which corresponds to the endogenous core body temperature nadir (late biological night), and highest approximately 12 hr later at circadian phase 180°, corresponding to biological afternoon and evening. Data are double plotted and represented as mean ± SEM. For reference, a relative clock hour timescale illustrating the approximate time of day is shown across the upper axis, with 05:00 referenced to the endogenous circadian temperature nadir in this group of older individuals. See also Figures S1 and S3.

observe a significant difference in respiratory quotient between the first and third weeks in the recurrent circadian disruption group (Figure S2) or in the control group (Figure S3), nor did we observe a sex difference in respiratory quotient in the control group. However, given that our power was limited due to small sample size, further studies will be needed to investigate variables that did not show significant differences. Our observations of circadian rhythms in fasting respiratory quotient, carbohydrate oxidation, and lipid oxidation are similar to those reported by Morris et al. [38]. While they did not find an overall effect of circadian phase on fasted respiratory quotient, when their analysis was confined to the acute effect of circadian misalignment, Morris et al. observed a 4% decrease in fasted respiratory quotient in the biological evening compared to the morning ($p = 0.006$) and a 26% increase in fasted lipid oxidation in the biological evening compared to the morning ($p = 0.006$), both of which are consistent with our current findings.

REE had a robust circadian rhythm with little variability in the timing of the peak between individuals (circular variance = 0.264), suggesting that it parallels changes in core body temperature [39, 40]. In contrast, the circadian pattern in respiratory quotient was less consistent, showing a larger spread in the timing of the peak between individuals (circular variance = 0.461). The circadian pattern of carbohydrate oxidation was robust (circular variance = 0.282) and tended to follow the pattern in respiratory quotient, whereas the pattern for lipid oxidation showed larger inter-individual variability in the timing of peaks (circular variance = 0.750). In summary, we found that REE varies with circadian phase and is highest during the biological day and lowest during the late biological night. This rhythm was robust; 3 weeks of circadian disruption had no measurable influence on the circadian variation of REE. Furthermore, we found that respiratory quotient, carbohydrate oxidation, and lipid oxidation also vary with circadian phase, with inter-individual variability in circadian patterns. These results may have important implications for understanding weight gain and obesity among night-shift workers. They may also have relevance to non-shift workers; there is evidence that many individuals keep irregular schedules, including a large portion who have social jet lag. When taken together with evidence that many US adults eat throughout their entire wake episodes [41], the circadian variation in metabolic functions we have observed may impact weight gain more broadly. Future studies are required to investigate the contribution of circadian misalignment-induced changes in energy expenditure to weight gain and adverse metabolic consequences in night-shift workers and in the general public.

Our observations also highlight the importance of controlling for the effect of circadian phase (misalignment) when carrying out recordings of energy expenditure in clinical and research settings. Per clinical guidelines and recommendations, REE should be measured in controlled conditions, including resting posture, after an 8-hr sleep, post-12-hr fast, in a thermoneutral room, with dimmed lighting and quiet ambient conditions (e.g., [42–44]). However, those guidelines do not consider the biological time at which REE should be assessed. The difference of ~129 kcal/day (amplitude of the fitted curve 110 kcal/day) we found between the circadian peak and trough in REE is similar to or greater in magnitude to that caused by acute sleep deprivation (one night of sleep deprivation lowered REE by 103 kcal/day [29]) and chronic sleep restriction (five nights of 4 hr time in bed lowered REE by 31–42 kcals/day [30, 33]). Given the magnitude of REE change due to circadian phase alone, it is of utmost importance for research studies using calorimetry as a key outcome to assess energy expenditure to control for the circadian time at which calorimetry recordings are made. Circadian phase timing is influenced by many factors, including recent sleep-wake schedule (e.g., timing and duration of sleep as well as regularity of schedule), light exposure history, travel across time zones, history of night work or rotating shift work, age, sex, and diurnal preference of the participant. At present, there are no reliable methods to approximate circadian phase in individuals whose schedules are irregular and no single-point biomarkers of circadian timing. Researchers can minimize differences in circadian timing by ensuring all participants are on very regular sleep-wake schedules with the same amount of adequate sleep each night for a week prior to assessment and by carrying out calorimetry relative to the individual's habitual schedule or to assess circadian time using 24-hr melatonin profiles. Alternatively, researchers should consider using methods such as doubly labeled water, which provide an integrated measure of energy expenditure around the clock, rather than at one discrete phase, when phase cannot be estimated.

Limitations: In addition to the small number of participants, a potential limitation of our study is that the identity of the clocks driving circadian changes in energy expenditure and macronutrient metabolisms is not known, although evidence suggests that they are driven by the hypothalamic master circadian pacemaker [21].

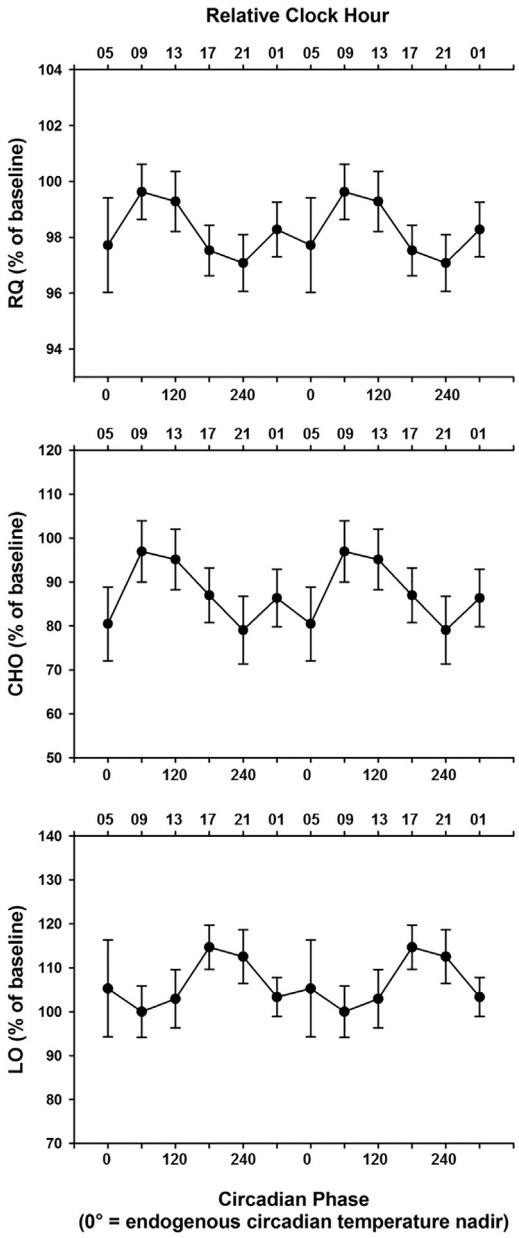

Figure 3. Circadian Variation of Fasting Respiratory Quotient and Carbohydrate and Lipid Oxidation

Top: Fasting respiratory quotient (RQ), the ratio of oxygen consumption and carbon dioxide production, is plotted with respect to the circadian phase at which it was measured. Respiratory quotient is lowest at circadian phase 240° (corresponding to late biological evening) and highest at circadian phase 60°, which corresponds to biological morning. Changes in respiratory quotient level reflect macronutrient metabolism; higher respiratory quotient favors carbohydrate oxidation, whereas lower respiratory quotient favors lipid oxidation. Middle: Fasting carbohydrate oxidation (CHO) is plotted with respect to the circadian phase at which it was assessed. Carbohydrate oxidation is highest at circadian phase 60° (corresponding to biological morning) and lowest at circadian phase 240° (corresponding to late biological evening). Bottom: Lipid oxidation (LO) is plotted with respect to the circadian phase at which it was measured. Lipid oxidation is highest at circadian phase 180° and lowest at circadian phase 60°. Data are double plotted and represented as mean ± SEM. For reference, a relative clock hour timescale illustrating the approximate time of day is shown on the upper axis, with 05:00 referenced to the endogenous circadian temperature nadir in this group of older individuals. See also Figures S2 and S3.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- QUANTIFICATION AND STATISTICAL ANALYSIS
- DATA AND SOFTWARE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures and one table and can be found with this article online at https://doi.org/10.1016/j.cub.2018.10.005.

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AUTHOR CONTRIBUTIONS

C.A.C., O.M.B., J.F.D., and J.S.W. designed the experiments. K.-M.Z., N.V., R.K.Y., C.M.I., and J.E.M. conducted the experiments. K.-M.Z., N.V., J.E.M., and W.W. analyzed the data. K.-M.Z. and R.K.Y. prepared the figures and tables. K.-M.Z., N.V., R.K.Y., C.M.I., J.E.M., J.S.W., J.F.D., and C.A.C. wrote the paper. All authors reviewed the paper.

DECLARATION OF INTERESTS

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Novartis; Purdue Pharma; Quest Diagnostics, Inc.; Samsung Electronics; Sleep Multimedia, Inc.; Teva Pharmaceuticals; Vanda Pharmaceuticals; Washington State Board of Pilotage Commissioners; and Zurich Insurance Company, Ltd. C.A.C. has also received education/research support from Cephalon, Inc.; Jazz Pharmaceuticals; Mary Ann & Stanley Snider via Combined Jewish Philanthropies; National Football League Charities; Optum; Philips Respironics; ResMed Foundation; San Francisco Bar Pilots; Schneider, Inc.; Simmons; Sysco; and Vanda Pharmaceuticals, Inc. The Sleep and Health Education Program of the Harvard Medical School Division of Sleep Medicine (which C.A.C. directs) has received Educational Grant funding from Cephalon, Inc.; Jazz Pharmaceuticals; Takeda Pharmaceuticals; Teva Pharmaceuticals Industries, Ltd.; Sanofi-Aventis, Inc.; Sepracor, Inc.; and Wake Up Narcolepsy. C.A.C. is the incumbent of an endowed professorship provided to Harvard University by Cephalon, Inc. and holds a number of process patents in the field of sleep/circadian rhythms (e.g., photic resetting of the human circadian pacemaker). Since 1985, C.A.C. has also served as an expert on various legal and technical cases related to sleep and/or circadian rhythms including those involving the following commercial entities: Bombardier, Inc.; Complete General Construction Company; Continental Airlines; FedEx; Greyhound; HG Energy LLC; Purdue Pharma, L.P.; South Carolina Central Railroad CO; StricLan Companies LLC; Texas Premier Resource LLC; and United Parcel Service (UPS). C.A.C. owns or owned an equity interest in Somnus Therapeutics, Inc. and Vanda Pharmaceuticals. He received royalties from the New England Journal of Medicine, McGraw Hill, Houghton Mifflin Harcourt, and Philips Respironics, Inc. for the Actiwatch-2 and Actiwatch-Spectrum devices. C.A.C.'s interests were reviewed and managed by Brigham and Women's Hospital and Partners HealthCare in accordance with their conflict of interest policies.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and Algorithms		
NOSA (non-orthogonal spectral analysis)	[45]	N/A
Vmax Encore 29N	CareFusion	http://www.carefusion.com/
Other		
Rectal thermistor	Measurement Specialties, Dayton, OH	Product# 861526TP

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Jeanne Duffy (jduffy@research.bwh.harvard.edu).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

10 participants completed the study protocol, 7 in the Recurrent Circadian Disruption (RCD) group and 6 in the Control group. One female participant completed the study three times and one male participant completed the study twice. No participant completed the study more than once in the same condition. Thus, we have data from 13 separate trials (mean ± sd, 57.6 + 7.24 years, range 38-69 years; 5 female, all post-menopausal). There is evidence that high-fat diet disrupts circadian rhythms and exacerbates the adverse effects of circadian disruption on metabolism [22, 46]. To study the interaction between circadian disruption and macronutrient composition on metabolism, we gave a subset of participants in each experimental condition a high-fat diet during the study (RCD group n = 3, Control group n = 2). However, there was no significant effect of diet on REE or RQ in the circadian disruption group or in the control group so we combined the diet groups for our analyses. See Table S1 for details.

Ethical Approval

The Partners Health Care Institutional Review Board reviewed and approved this study (#2014P000243). The study conduct adhered to the ethical principles outlined in the Declaration of Helsinki and each participant provided written informed consent.

METHOD DETAILS

Recruitment and Screening

Healthy adult participants were recruited using online and newspaper advertisements, recruitment letters, and flyers. Participants completed a screening consisting of medical history evaluation, physical examination, electrocardiogram, clinical blood tests, and urinalysis to rule out medical disorders; an all-night at-home polysomnogram to rule out sleep disorders; psychological questionnaires (MMPI, Beck Depression Inventory) and a semi-structured clinical psychological interview to rule out psychological disorders. Exclusion criteria included current or chronic medical conditions, regular use of prescription or over-the-counter medication, BMI > 32; current or past psychiatric or psychological disorders; smoking, excessive caffeine consumption; regular night shift work within the last 3 years, travel across more than one time zone within 3 months, significant sleep complaints, and habitual sleep duration shorter than 6.5 hours or longer than 9 hours.

Pre-Study Conditions

Participants maintained a self-selected regular sleep-wake schedule with a 10-hour nighttime sleep opportunity for at least 21 days prior to the inpatient study to ensure they were well-rested before the study began. Compliance was verified by wrist actigraphy, sleep diaries, and time-stamped voicemails at bed time and wake time. Participants were instructed not to use drugs (prescription, over-the-counter, recreational), alcohol, nicotine, or caffeine during this pre-study segment, and had a urine toxicology screen upon study admission to verify their compliance.

Inpatient Study Conditions

Participants were admitted to the Intensive Physiological Monitoring Unit of the Center for Clinical Investigation at Brigham & Women's Hospital for the 37-day inpatient portion of the study, during which time they lived in an individual study room free of time cues. Light levels were maintained at ~90 lux during scheduled wake episodes and all lights were turned off throughout scheduled sleep opportunities. On three occasions during the study, the participant had a constant posture (CP) procedure, during which they were restricted to bed for the entire wake episode and the room lighting was maintained at a dim (~4 lux) level. To ensure all

participants were well-rested before the intervention segment of the study began, the first three inpatient days included a 12-hour nighttime sleep opportunity as well as a 4-hour daytime nap opportunity (sleep extension segment of the study). Days five through seven (referred to as baseline) included an 8- or 10-hour nighttime sleep opportunity (see Figure 1 for details). For participants in the Control group, these baseline conditions were maintained for the remainder of the study (days 8-37). In the RCD group, the next 21 days were under forced desynchrony (FD) conditions consisting of eighteen, 28-hour "days" with 16.33-hour wake episodes and 11.67-hour sleep opportunities, corresponding to 10 hours sleep opportunity / 24 hours. Core body temperature (CBT) was recorded continuously throughout the RCD segment of the study with a rectal thermistor (Measurement Specialties, Inc., Dayton, OH) and was used to estimate circadian phase.

Study Diet

Participants were provided controlled meals throughout the inpatient study consisting of 58%–60% carbohydrates, 15%–17% protein, and 25%–27% fat, with 800-1000 mg of calcium, 100 mEq (+/− 20%) of potassium, and 150 mEq (+/− 20%) of sodium. The 24-hour kilocalorie (kcal) target for each participant was calculated using the Mifflin-St. Joer equation with an activity factor 1.3, and was distributed evenly across breakfast, lunch, and dinner (33% ± 30 kcals/meal). During the forced desynchrony segment in the RCD group, participants were given a snack after dinner which contained an additional 16.7% of the 24-hour kcal target to account for the 28-hour day. Participants who received a high-fat diet were provided meals containing 30%–40% carbohydrates, 15%–20% protein, and 45%–50% fat, with 800-1250mg of calcium, 100 mEq (+/− 30%) of potassium, and 150 mEq (+/− 30%) of sodium. The Mifflin-St. Joer equation with an activity factor of 1.6 was used to calculate the 24-hour kcal target for these participants, and kcals were distributed across breakfast, lunch, dinner (25% +/− 5% daily target per meal) and 2 separate snacks (after lunch and after dinner, 12.5% +/− 5% daily kcal target per snack). If a participant's REE measurements on the baseline days varied by more than 100 kcals from expected values, the kcal target per 24 hours was adjusted to match the measured REE value (See Table S1 for individuals with adjusted intervention kcal targets).

Indirect Calorimetry measurements

Fasting indirect calorimetry measurements were performed within ~2 hours of scheduled wake times (VMAX Encore Metabolic Cart, CareFusion, CA). Baseline recordings were taken on study days 5 and 6 across all groups. Additional recordings were taken on most wake episodes during weeks 1 and 3 of the intervention phase. See Figure 1 for details. All participants were in bed and resting for at least 20 minutes before the start of each recording. REE was calculated on a minute-by-minute basis in kcals/day from gas expiration. Measurements from each recording were averaged to estimate REE in kilocalories per day (kcals/day) and RQ (dimensionless number), which is the amount of expired carbon dioxide (VCO_2) per the amount of oxygen consumed (VO_2). Carbohydrate oxidation and lipid oxidation rates were calculated as grams per minute (g/min) according to the formulae of Frayn [47], assuming negligible protein oxidation. Recordings lasted between 14 and 25 minutes. The first five minutes and the last minute of each recording were excluded from analysis [48]. Only data points with an associated fraction of exhaled carbon dioxide ($FECO_2$) between 0.6%–0.9% were included in the analysis [49]. Weight was measured each study day except on the constant posture days and the most recent weight was used for each recording.

Circadian Phase

Core body temperature (CBT) data were edited to remove sensor slips and removals. Intrinsic circadian period of the CBT data from the FD segment of the protocol was estimated for each participant in the RCD group using non-orthogonal spectral analysis (NOSA) [45]. NOSA takes into account the 28-hour periodicity in the data resulting from the imposed rest-activity schedule and searches for an unknown periodicity in the circadian range (search period 20-30 hours). From this estimate, a circadian phase (from 0 to 359°) was assigned to each minute of the study, with 0° corresponding to the minimum of the waveform fit to the entire temperature data series (endogenous circadian temperature nadir). For visualization purposes and to assess the effect of circadian phase on REE, RQ, CHO, and LO, data were averaged within each participant and across participants and binned into six 60-degree (~4-hour) circadian phase bins (0°, 60°, 120°, 180°, 240°, and 300°).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses were carried out using SAS version 9.4 (SAS Institute, Cary, NC). The primary outcome variables REE and RQ were analyzed using Linear Mixed Models. Model diagnostics did not show a significant violation of the model assumptions for either outcome variable. Variables including age (young versus older), gender (male versus female), diet (regular diet versus high-fat diet), weight (lbs), week (week 1 versus week 3), baseline REE/RQ value, and circadian phase (RCD group only; 0°, 60°, 120°, 180°, 240°, and 300°) were treated as fixed effects, with a random intercept statement incorporated into the models to allow for means to vary between participants. We first tested each fixed effect independently and only included significant variables in the final multivariable model for each dataset. Finally, a cosinor model (period of 24 h) was fitted to the REE and RQ data and to the data from the two secondary outcome variables CHO and LO, averaged across weeks 1 and 3, to estimate amplitude (one-half peak-to-trough variation) and acrophase (peak time) and to test the null hypothesis that the amplitude of the fitted curve is zero, i.e., no rhythm detected [50]. To quantify the spread in the distribution of peak values, a cosinor model was fitted to each individual's data and the peak values were used to calculate circular variance [51]. Circular variance ranges from 0 to 1, with higher values indicating a larger spread of the data.

All results are reported as mean ± SEM and expressed as percentage of the baseline value in the figures. The critical significance level was set to $\alpha = 0.05$ for all tests and only significant results are reported.

DATA AND SOFTWARE AVAILABILITY

Execution of a materials transfer agreement is required by our institution for transfer of data.

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Supplemental Information

Human Resting Energy Expenditure Varies with Circadian Phase

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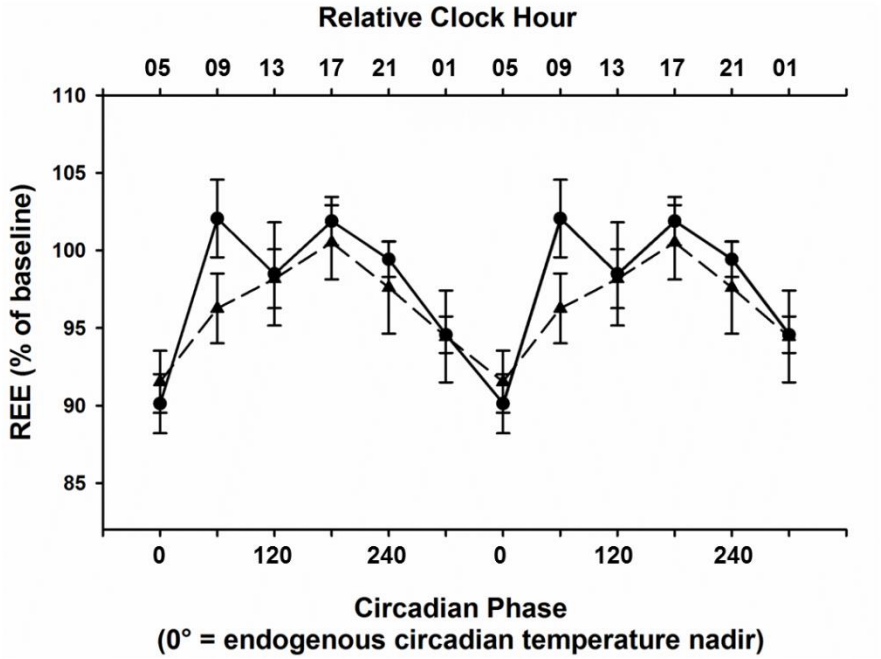

Figure S1. Circadian Variation of Fasting Resting Energy Expenditure during Week 1 and Week 3. Related to Figure 2.

The REE from week 1 (black circles) of recurrent circadian disruption is plotted with respect to the circadian phase at which the measurements were taken. REE from Week 3 (triangles and dashed line) are also plotted with respect to the circadian phase at which it was assessed. No significant difference in fasting levels or timing of REE was detected between week 1 and week 3. Data are double-plotted and represented as mean ± SEM. For reference, a relative clock hour time scale illustrating the approximate time of day is shown on the upper axis, with 05:00 referenced to the endogenous circadian temperature nadir.

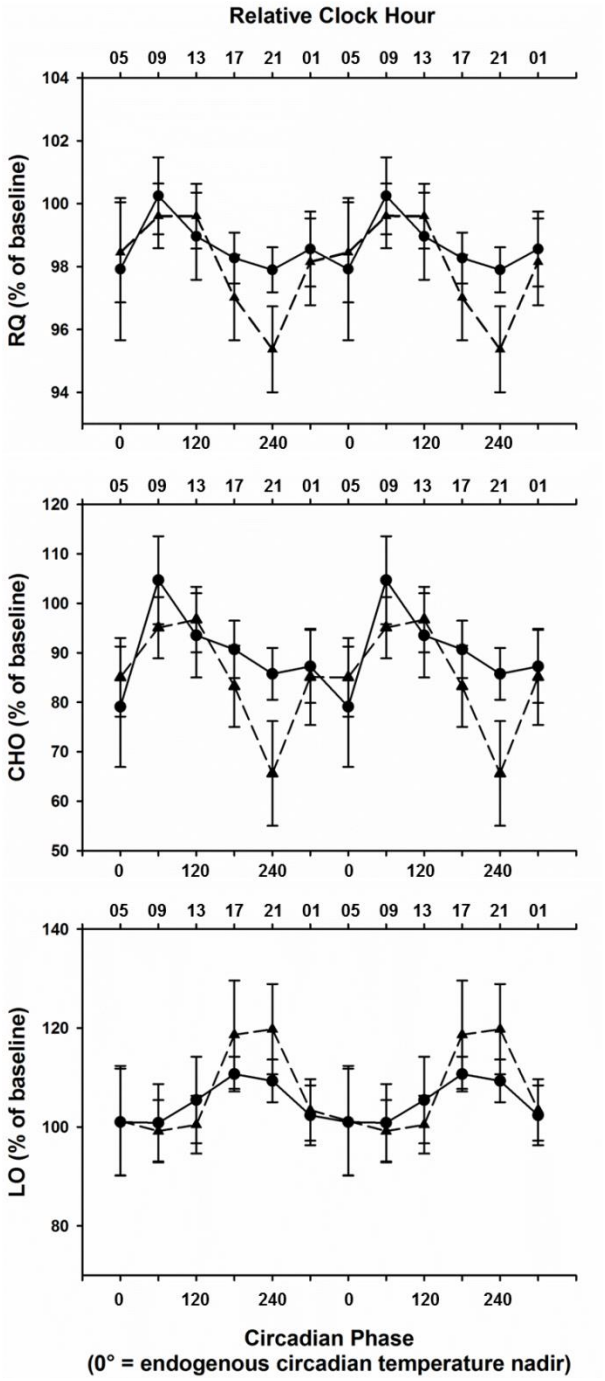

Figure S2. Circadian Variation of Fasting Respiratory Quotient, Carbohydrate, and Lipid Oxidation during Week 1 and Week 3. Related to Figure 3.

Fasting RQ (Top panel), CHO (Middle panel), and LO (Bottom panel) from week 1 (black circles) and week 3 (triangles and dashed line) of recurrent circadian disruption is plotted with respect to the circadian phase at which the measurements were taken. No significant difference in fasting levels or timing of RQ were detected between week 1 and week 3. Data in all three panels are double-plotted and represented as mean ± SEM.

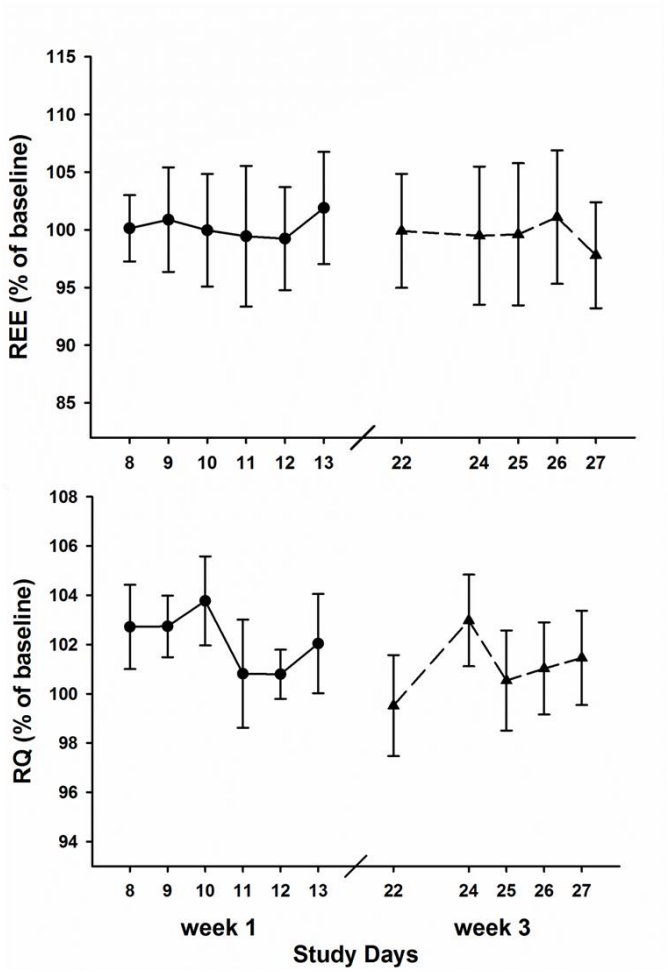

Figure S3. Day-to-Day Variation of Fasting Resting Energy Expenditure and Respiratory Quotient During Week 1 and Week 3 in the Control Group. Related to Figure 2 and Figure 3.

Fasting REE (Top panel) and RQ (Bottom panel) from week 1 (black circles) and week 3 (triangles and dashed line) of control participants is plotted with respect to the study day at which the measurements were taken. All measurements were taken within ~2 hours of the scheduled wake time, which remained consistent from baseline through the end of the study. No significant differences in fasting levels of REE or RQ were detected between week 1 and week 3. Data in both panels are single-plotted and presented as mean ± SEM.

Participant	Age	Gender	BMI	Group	Diet	Baseline kcal/day	Intervention kcal/day	Baseline REE (kcal/day)	Baseline RQ	Baseline phase
3436HY	60	female	19.8	control	regular	1288	1288	1059	0.82	n/a
3369HY42	58	male	27.0	control	regular	1952	1952	1137	0.82	n/a
3547HY	55	male	24.9	control	regular	2079	1853	1426	0.81	n/a
3552HY*	55	female	31.0	control	regular	1822	1822	1607	0.85	n/a
3776HY	60	male	29.2	control	HF	2737	2487	1396	0.83	n/a
3789HY	57	female	24.4	control	HF	2280	2280	1279	0.91	n/a
3453HY52**	63	male	30.1	RCD	regular	2102	2102	1537	0.86	39.3
3539HY	69	female	20.5	RCD	regular	1356	1509	1152	0.81	70.0
3536HY52	57	female	30.8	RCD	regular	1930	1930	1406	0.83	45.8
3552HY62*	56	female	32.1	RCD	regular	1877	2035	1551	0.83	48.1
3453HY73**	65	male	30.5	RCD	HF	2715	2715	1465	0.84	35.0
3552HY73*	56	female	31.4	RCD	HF	2314	2501	1564	0.83	54.0
2056HY75	38	male	21.0	RCD	HF	2728	2728	1452	0.87	4.0

Table S1. Demographics. Related to Figure 2, Figure 3 and Figure 4.
10 participants completed 13 studies. Participants were randomized into circadian disruption (RCD) and control groups, and were given either a regular diet or a high fat (HF) diet during their study. One female participant (3552*) completed the study three times (control regular diet; RCD regular diet; RCD high fat diet) and one male participant (3453**) completed the study twice (RCD regular diet; RCD high fat diet). No participant completed the study more than once in the same condition.