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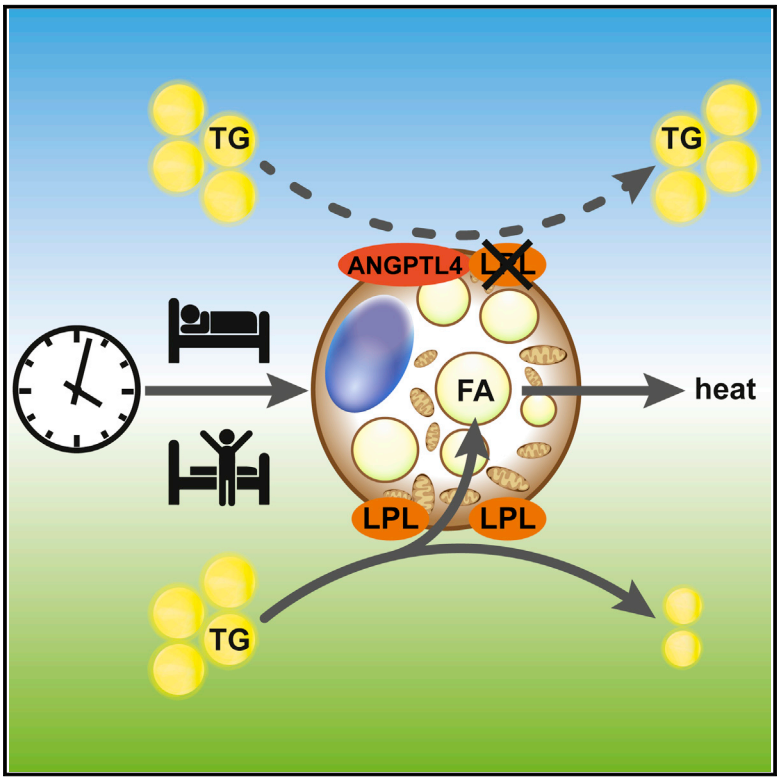
Cell Reports

A Diurnal Rhythm in Brown Adipose Tissue Causes Rapid Clearance and Combustion of Plasma Lipids at Wakening

Graphical Abstract



Highlights

- Fatty acid uptake by BAT is rhythmic and highest at the onset of wakening
- Circadian pattern of fatty acid uptake by BAT depends on light exposure duration
- Time of day dictates the capacity of BAT to clear postprandial lipids

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In Brief

van den Berg et al. show a strong circadian rhythm in fatty acid uptake by brown adipose tissue that peaks at wakening regardless of the light exposure period. Consequently, postprandial lipid handling by brown adipose tissue is highest at wakening, resulting in the lowest postprandial plasma lipid excursions.

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A Diurnal Rhythm in Brown Adipose Tissue Causes Rapid Clearance and Combustion of Plasma Lipids at Wakening

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SUMMARY

Many favorable metabolic effects have been attributed to thermogenic activity of brown adipose tissue (BAT). Yet, time of day has rarely been considered in this field of research. Here, we show that a diurnal rhythm in BAT activity regulates plasma lipid metabolism. We observed a high-amplitude rhythm in fatty acid uptake by BAT that synchronized with the light/dark cycle. Highest uptake was found at the onset of the active period, which coincided with high lipoprotein lipase expression and low angiopoietin-like 4 expression by BAT. Diurnal rhythmicity in BAT activity determined the rate at which lipids were cleared from the circulation, thereby imposing the daily rhythm in plasma lipid concentrations. In mice as well as humans, postprandial lipid excursions were nearly absent at waking. We anticipate that diurnal BAT activity is an important factor to consider when studying the therapeutic potential of promoting BAT activity.

INTRODUCTION

Brown adipose tissue (BAT) is a key site of mammalian thermogenesis, and its activity is critical for the survival of small mammals in cold environments and for arousal in hibernators. Heat arises primarily from mitochondrial uncoupling during the combustion of intracellularly stored lipids. To replenish these lipid stores, brown adipocytes predominantly take up triglyceride (TG)-derived fatty acids (FAs) from the circulation in a lipoprotein lipase (LPL)-dependent matter as shown in mice (Hoeke et al., 2016; Khedoe et al., 2015). Activation of BAT results in increased energy expenditure and a marked lowering of fasted and postprandial plasma TG levels (Bartelt et al., 2011; Berbée et al., 2015). Therefore, BAT activation is considered a target for the treatment of metabolic and cardiovascular disorders. The rediscovery of active BAT in human adults (Nedergaard et al., 2007; van Marken Lichtenbelt et al., 2009) and the identification of pharmacological targets to stimulate human BAT activity (Cypess et al., 2015) have made this a conceivable possibility.

Circadian rhythms in physiology and behavior are indispensable for life. They are orchestrated by a hierarchical system in which the master clock located in the hypothalamus synchronizes the cell-autonomous clocks and metabolic processes in peripheral tissues. The molecular clockwork involves transcriptional and translational feedback loops, in which the BMAL1:CLOCK complex promotes transcription of clock genes *Per1-3*, *Cry1-2*, and *Rev-Erbα* and β. In turn, the protein products PER and CRY repress their own transcription, and REV-ERB inhibits *Bmal1* transcription. A wide range of other proteins contribute to the fine-tuning of this system. Both central and peripheral circadian clocks, including the cell-autonomous clocks in BAT, are entrained to the external environment by ambient cues ("zeitgebers") that include light input and food availability (Reppert and Weaver, 2002). Recent studies have linked the

circadian rhythm in body temperature and cold adaptation of mice to a daily rhythm in BAT activity (Chappuis et al., 2013; Gerhart-Hines et al., 2013). This is consistent with earlier reports demonstrating circadian expression of clock genes in BAT (Zvonic et al., 2006). How these rhythms in BAT activity may impact metabolism, including fasting and postprandial lipid concentrations, is not known.

In rodents, plasma TG and FA levels show diurnal variations reaching their lowest levels at the start of the dark, active period (Pan et al., 2010; Rudic et al., 2004; Shostak et al., 2013). We (van den Berg et al., 2017) and others (Chua et al., 2013) have shown that, also in humans, plasma lipid concentrations vary over the day, which could only partly be explained by food intake, suggesting a contribution of oscillations in the clearance of lipids by metabolic organs. We hypothesized that BAT contributes to the diurnal regulation of plasma lipids. In the present study, we aimed to assess the diurnal rhythm of the plasma clearance of TG-derived FA by metabolic organs in mice and reveal a pronounced diurnal rhythm in lipid uptake, specifically by BAT, with the highest uptake at the onset of the dark, active period. We further explored the consequences for postprandial lipid handling and showed that, in both mice and humans, postprandial lipid excursions were nearly absent at waking.

RESULTS

BAT Displays a Pronounced Daily Rhythm in FA Uptake

First, we set out to determine the time-dependent uptake of FA by BAT and other metabolic organs in male 12-week-old C57BL/6J mice. Since FAs are predominantly transported in plasma as a constituent of TGs within lipoproteins, we assessed the kinetics of intravenously injected glycerol tri[^{3}H]oleate-labeled TG-rich lipoprotein-like particles (Rensen et al., 1995) at six different times of the day (Supplemental Experimental Procedures for a complete description of the particles). Time of day is denoted as zeitgeber time (ZT), where ZT0 is the start of the light period. Of note, as mice are nocturnal, ZT0 corresponds to the start of the resting phase, in which food intake and physical activity are minimal. In mice kept under a standard 12-hr:12-hr light:dark cycle, which is referred to as normal light exposure (normal LE), a pronounced rhythm in [^{3}H]oleate uptake was found for perivascular adipose tissue (representing a mixture of white and brown adipocytes; Brown et al., 2014), subscapular BAT, and interscapular BAT, with 15-, 12- and 4-fold differences between minimum and maximum uptake, respectively (Figures 1A–1C). The peak in [^{3}H]oleate uptake by interscapular BAT and subscapular BAT was reached around the onset of the dark period. While time of day was also a determinant of FA uptake in liver ($p_{ZT} < 0.001$), subcutaneous ($p_{ZT} < 0.001$) and gonadal ($p_{ZT} = 0.001$) white adipose tissue, heart ($p_{ZT} = 0.010$), and muscle ($p_{ZT} < 0.001$), only a 1.7- to 3.3-fold difference between minimum and maximum FA uptake could be identified in these metabolic tissues (Figures 1D–1H). In addition, the time point at which the uptake of FA peaked was different for these organs. In the middle of the dark period, uptake by liver and white adipose tissue (WAT) was high (Figures 1D–1F), while FA uptake by heart and skeletal muscle was low (Figures 1G and 1H).

Rhythm in FA Uptake by BAT Is Synchronized with the Light/Dark Cycle

Previously, we reported that uptake of FA by BAT is attenuated when mice are exposed to prolonged daily light (Kooijman et al., 2015a). Consequently, we assumed that diurnal FA uptake by BAT is subjected to environmental changes in light exposure duration. Analysis of time-dependent FA uptake in mice adapted to either a short LE (8-hr:16-hr light:dark cycle) or a long LE (16-hr:8-hr light:dark cycle), indeed, revealed specific adaptations in FA uptake by brown adipocyte depots (Figures 1A–1C) and not in other metabolic organs (Figures 1D–1H). Regardless of the light:dark cycle, highest [^{3}H]oleate uptake by interscapular BAT and subscapular BAT was reached around the onset of the dark period (ZT8 for short LE, ZT16 for long LE). Of note, short LE increased the area under the curve for [^{3}H]oleate uptake (Figure S1A) and increased the average daily [^{3}H]oleate uptake of all time points by interscapular BAT (+90%; $p = 0.016$), subscapular BAT (+107%; $p = 0.028$), and perivascular adipose tissue (+130%; $p = 0.030$) when compared to long LE (Figure S1B). Light exposure duration negatively correlated with the average [^{3}H]oleate uptake by interscapular BAT ($p = 0.006$), subscapular BAT ($p = 0.010$), and perivascular adipose tissue ($p = 0.011$) (Figures S1C–S1E), but not with [^{3}H]oleate uptake by other metabolic organs (data not shown). These data suggest that total daily BAT activity may be higher in "winter-like" short LE and lower in "summer-like" long LE.

Since it has repeatedly been reported that daily energy expenditure in small rodents fluctuates according to season in association with BAT thermogenic capacity, we determined the effect of the duration of light exposure on food intake, energy expenditure, and the respiratory exchange ratio (RER) from O_2 consumption and CO_2 production in an additional group of mice. Mice were first housed at normal LE and then subjected to either short LE or long LE. Behavioral adaptations to either short or long LE resulted in an expected clear shift in diurnal food intake, without affecting total daily intake (Figure S2A). In both settings, the peak in energy expenditure shifted to the beginning of the dark phase, and the average 24-hr energy expenditure tended to be increased in short LE compared to long LE (Figure S2B). Likewise, RER was increased in short LE compared to long LE (Figure S2C). Together, these data indicate that energy intake and expenditure adapt to seasonal changes in light exposure, with energy expenditure being highest in short LE (winter), likely in association with adaptation in daily BAT activity.

Diurnal FA Uptake by BAT Is Reflected by Rhythms in LPL and ANGPTL4 Levels

Next, we investigated possible regulatory pathways of TG-derived FA uptake in BAT. At the cellular level, clock proteins can drive tissue-specific rhythmic gene expression. We confirmed diurnal expression of the clock genes *Rev-erbα* (Figure 2A) and *Per2* (Figure 2B), both of which have been described to be involved in the regulation of BAT thermogenesis (Chappuis et al., 2013; Gerhart-Hines et al., 2013). In addition, expression of the core clock genes *Bmal1*, *Cry1*, and *Clock* were found to be rhythmic (Figure S3). Expression of the key thermogenic gene uncoupling protein 1 (*Ucp1*) was rhythmic, compared to normal LE and an advanced peak in short LE (Figure 2C).

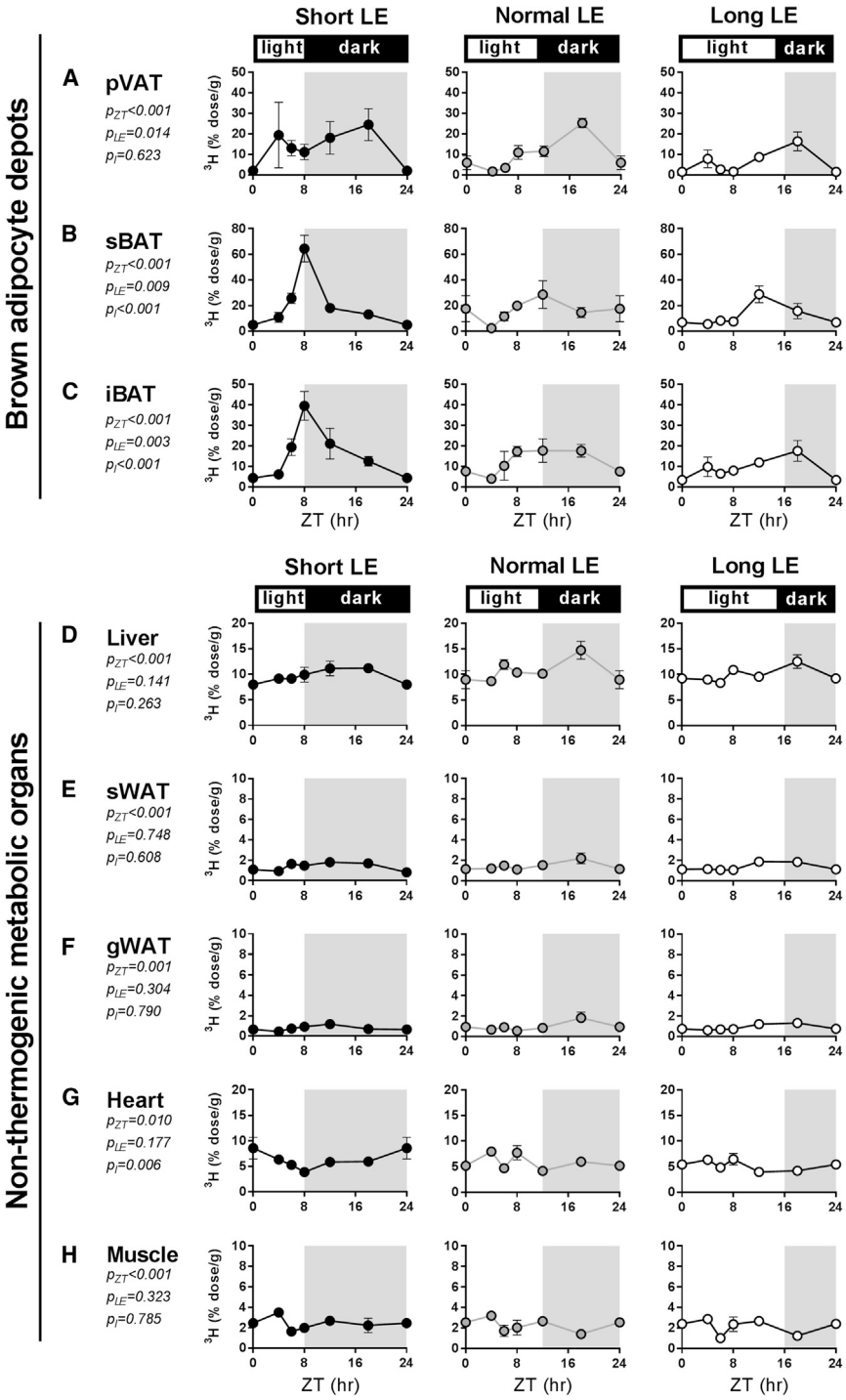

Figure 1. 24-Hr Rhythm of TG-Derived FA Uptake by BAT

(A–H) Wild-type mice subjected to short LE, normal LE, or long LE were injected with glycerol tri[^{3}H]oleate-labeled lipoprotein-like particles at 6 time points (n = 4 per group) and killed 15 min after the injection. The uptake of [^{3}H]oleate was determined for perivascular adipose tissue (pVAT) (A), subscapular brown adipose tissue (sBAT) (B), interscapular BAT (iBAT) (C), liver (D), subcutaneous white adipose tissue (sWAT) (E), gonadal white adipose tissue (gWAT) (F), heart (G), and skeletal muscle (H). Times are given as zeitgeber times (ZTs) in hours, with onset of light period at ZT0. Data are presented as means ± SEM, and ZT0/ZT24 was double plotted for visualization purposes. p_{ZT}, p_{LE}, and p_I represent p values for the factors zeitgeber time, daily LE, and interaction, respectively (two-way ANOVA).
See also Figures S1 and S2.

Importantly, expression of the LPL (*Lpl*), the enzyme that is crucial for the uptake of TG-derived FA (Bartelt et al., 2011), was found to be rhythmic (Figure 2D). Furthermore, *Lpl* expression correlated with the [^{3}H]FA uptake by interscapular BAT (R^2 = 0.183; p < 0.001; Figure S3G) and was inverse to the LPL inhibitor angiopoietin-like 4 (*Angptl4*) (Dijk et al., 2015) (Figure 2E). Consistent with the gene expression pattern, protein levels of total LPL and active LPL were highest at the onset of the dark period, and the diurnal pattern adapted to the light exposure duration. Total LPL protein levels correlated with the [^{3}H]oleate uptake by interscapular BAT (R^2 = 0.526; p < 0.001; Figure S3H), while ANGPTL4 levels displayed an inverse rhythm, reaching a peak at the onset of the light period (Figures 2F–2I). In particular, the levels of mature glycosylated LPL, representing LPL located in the Golgi apparatus or on the cell surface sensitive to inhibition by ANGPTL4 (Dijk et al., 2016), were regulated by the daily light exposure duration (Figure 2I).

Diurnal FA Uptake by BAT Is Affected by Prolonged Fasting and Sympathetic Denervation of the Tissue

We further investigated potential mechanisms underlying diurnal FA uptake by BAT in C57BL/6J mice housed under normal LE. We first addressed the effect of prolonged fasting, as the activity of LPL is dependent on nutritional status (Carneheim and Alexson, 1989; Ong and Kern, 1989). Overnight fasting resulted in increased FA uptake by subscapular BAT (sBAT) at the onset of the light phase, but not in interscapular BAT (iBAT) (Figure 3A), suggesting at least a contributing role for fasting/feeding in diurnal FA uptake by BAT. Other oscillating factors known to be involved in the regulation of BAT activity are glucocorticoids and sympathetic outflow. In the absence of glucocorticoids upon adrenalectomy, which stimulates BAT activity (Vander Tuig et al., 1984), uptake of FA by sBAT and iBAT seemed increased when compared to

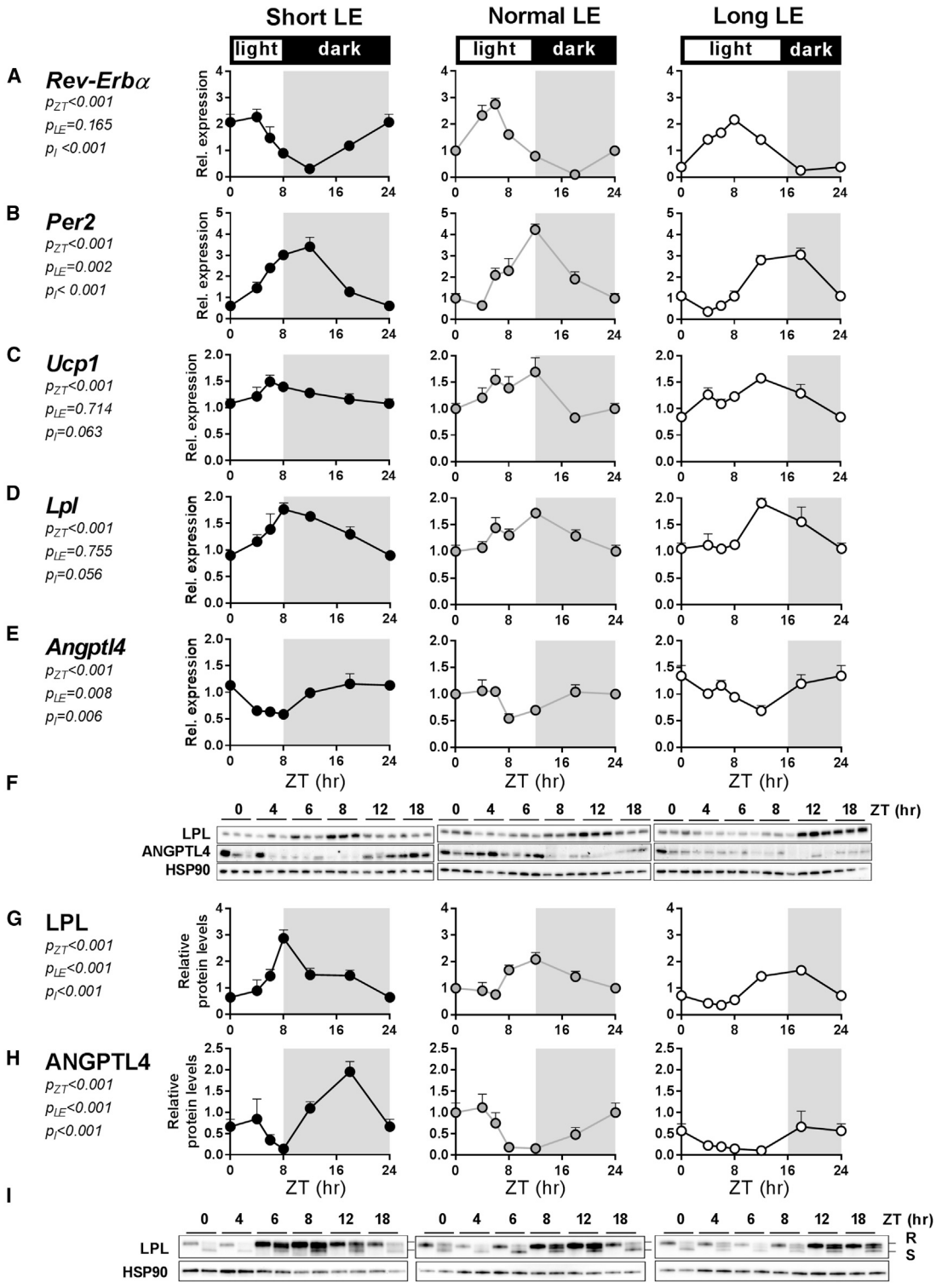

Figure 2. 24-Hr Rhythm in BAT LPL and Angiopoietin-like 4

(A–E) Interscapular brown adipose tissue (iBAT) of wild-type mice subjected to short LE, normal LE, or long LE was harvested at six time points (n = 4 per group). Gene expression was determined by qPCR, calculated relative to *Rplp0* and *Hprt* expression, and normalized to mean expression of ZT0 of the normal LE group (A–E). *Rev-Erbα* (A), *Per2* (B), *Ucp1* (C), *Lpl* (D), and *Angptl4* (E).

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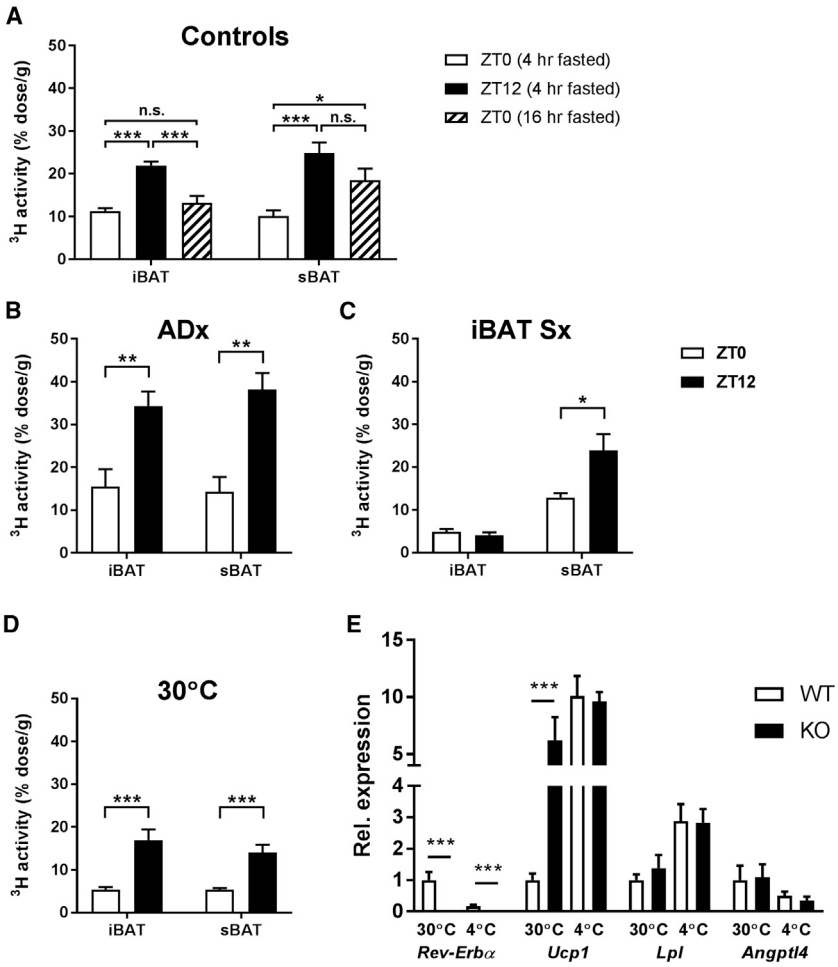


Figure 3. 24-Hr Rhythm of TG-Derived FA Uptake by BAT after Prolonged Fasting, Adrenalectomy, and Sympathetic Denervation at Thermoneutrality and in Rev-erbα Null Mice

(A–D) Wild-type mice were subjected to normal LE and injected with glycerol tri[^{3}H]oleate-labeled lipoprotein-like particles at lights on (ZT0) or lights off (ZT12). The uptake of [^{3}H]oleate was determined for interscapular BAT (iBAT) and subscapular brown adipose tissue (sBAT) after prolonged fasting (n = 8 per group; A), after adrenalectomy (n = 4–5 per group; B), after sympathetic denervation of iBAT (n = 8 per group; C), or after housing at thermoneutrality (n = 7 per group; D).

(E) Gene expression in Rev-Erbα null mice was compared to that of littermates housed at thermoneutrality or cold (n = 4–5 per group).

Data are presented as means ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001, ANOVA with Tukey's post hoc test in (A), independent-samples t test in (B)–(D), and two-way ANOVA with Tukey's post hoc test in (E). n.s., not significant. See also Figure S4.

uptake of FA in normal mice, but the diurnal rhythm in FA uptake was maintained (Figure 3B). In contrast, sympathetic denervation of iBAT completely abolished diurnal activity in this tissue (Figure 3C). However, these data should be interpreted with caution, as denervation of iBAT led to a strong reduction in FA uptake when compared to sBAT. When mice were housed at thermoneutrality (30°C), which is a more physiological way to reduce sympathetic outflow toward BAT, despite overall reduced FA uptake, diurnal rhythmicity was maintained (Figure 3D).

To identify whether diurnal FA uptake by BAT could be a direct consequence of glucose fluxes into BAT, a [^{14}C]deoxyglucose label was included in the experiments described earlier. Like FA uptake, glucose uptake by BAT was higher at ZT12 than at ZT0 and mostly unaffected by prolonged fasting in both iBAT and sBAT (Figure S4A). Interestingly, both adrenalectomy (Figure S4B) and sympathetic denervation of iBAT (Figure S4C) abolished the diurnal uptake of glucose by iBAT, indicating that FA uptake by BAT does not simply follow or reflect glucose uptake. Housing mice at thermoneutrality lowered overall glucose uptake but did not affect the diurnal rhythm in glucose uptake (Figure S4D).

We next investigated whether diurnal FA uptake might be regulated through the cell-autonomous clock machinery in BAT. To this end, we measured expression of *Lpl* and *Angptl4* in BAT of REV-ERBα null mice and wild-type littermates at ZT10 when REV-ERBα is abundant (Gerhart-Hines et al., 2013). While transcriptional repression of *Ucp1* is relieved in the REV-ERBα null mice, expression of *Lpl* and *Angptl4* was not different from that of the littermates (Figure 3E), suggesting that REV-ERBα is most likely not directly involved in the circadian regulation of FA uptake by BAT. Further studies are needed to investigate the role of other clock genes in circadian BAT function.

In summary, there is a potential contributing role for fasting/feeding and sympathetic innervation in diurnal FA uptake by BAT, while glucocorticoids may be involved in the regulation of diurnal glucose uptake by BAT.

Diurnal FA Uptake by BAT Imposes Diurnal Variations in Fasting Lipid Levels

Stimulation of BAT thermogenic activity leads to a reduction in plasma TG levels within TG-rich lipoproteins (i.e., chylomicrons and very low-density lipoproteins) (Bartelt et al., 2011; Berbée

(F–H) Protein levels of total LPL and ANGPTL4 were measured by western blot (F) and quantified normalized to HSP90; LPL and ANGPTL4 data are shown in (G) and (H), respectively. The amount of mature glycosylated LPL protein was determined in a subset of the samples by EndoH digestion.

(I) EndoH-sensitive LPL (ER LPL) is indicated with an S; EndoH-resistant active LPL (Golgi apparatus and cell-surface LPL) is indicated with an R. p_{ZT}, p_{LE}, and p_I represent p values for the factors zeitgeber time (ZT), LE, and interaction, respectively (two-way ANOVA).

Data are presented as means ± SEM, and ZT0/ZT24 was double plotted for visualization purposes. See also Figure S3.

et al., 2015). As wild-type mice generally have very low levels of circulating TG-rich lipoproteins, which are thus not prone to increased clearance, we tested whether diurnal BAT activity is accompanied by variations in (postprandial) lipid levels and handling in female APOE*3-Leiden.CETP mice, representing a well-established model for human-like lipoprotein metabolism (Berbée et al., 2015; Kühnast et al., 2015). We first confirmed that these mice adapt to light exposure regimes in a similar fashion as that of wild-type mice. Mice, of which a subgroup was equipped with telemetric transmitters to monitor rhythms in ambulatory activity and core body temperature, were housed for 5 weeks with short LE or long LE. In both groups, the timing of ambulatory activity (Figures 4A and 4B) and core body temperature (Figures 4C and 4D) adapted to the new light:dark cycles without affecting total 24-hr ambulatory activity (Figure 4B) or the average body temperature (Figure 4D). To study body temperature as a function of ambulatory activity, a Reitman plot was generated (Lateef et al., 2014). The difference between the light and dark curves represents ambulatory activity-independent heat production, arising from either central modulation of heat loss (Fischer et al., 2016; Warner et al., 2013) or thermogenesis (Abreu-Vieira et al., 2015). In mice adapted to short LE, there was a large light-dark difference (Figure 4E), possibly reflecting the diurnal amplitude in BAT activity. In line with our observation that total daily BAT activity may be lower in long LE, the difference between the curves for the light and dark periods were lost in mice adapted to long LE (Figure 4F).

Next, non-fasted plasma lipid levels were measured every 4 hr within a single day. Plasma TG and free FA levels were found to be rhythmic and synchronized with the light:dark cycle (Figures 4G–4J). Lowest TG and free FA levels were reached at the onset of the dark period (i.e., ZT8 for short LE and ZT16 for long LE). To evaluate whether these rhythms in plasma lipid levels were determined by the adaptive rhythm in FA uptake by BAT, we assessed the TG-derived FA uptake at three time points to confirm the high morning-evening amplitude of BAT activity. In APOE*3-Leiden.CETP mice, like in wild-type mice, the uptake of [^{3}H] oleate by the BAT depots was high at the onset of the dark period (ZT8 for short LE and ZT16 for long LE) (Figures 5A and 5B). This coincided with a faster clearance of plasma FA at the onset of the dark period for mice adapted to short LE (Figures 5C and 5D) and long LE (Figures 5E and 5F). In iBAT, expression of clock genes *Rev-erbα* and *Per2* showed adaptation to short or long LE (Figure S5). Gene expression of *Lpl* was higher at the onset of the dark period, compared to the light period, while expression of *Angptl4* was non-significantly reduced (Figure S5). Just like in wild-type mice, protein levels of (active glycosylated) LPL peaked around the onset of the respective dark periods (Figure 5G). Together, these data strongly suggest that diurnal rhythms in BAT activity are responsible for the time-dependent clearance of TG from the circulation.

Diurnal FA Uptake by BAT Modulates Postprandial Lipid Levels

To further investigate the consequence of the diurnal BAT activity for plasma lipid metabolism, we determined postprandial excursions of TG and free FA following an oral bolus of olive oil given at ZT0, ZT8, or ZT16 (Figure 6A). Comparable to the unfasted state, baseline 4-hr-fasted plasma TG and free FA levels were low at the start of the dark period (Figures 6B and 6C). The ZT at which the oral TG bolus was administered was associated with postprandial increase of plasma TG (Figures 6D and 6E) and free FA (Figures 6G and 6H) for both short LE and long LE. The area under the curve (AUC) was lowest when the oral TG bolus was given at the onset of the dark period (ZT8 for short LE and ZT16 for long LE) for TG (Figure 6F) and free FA (Figure 6I). Thus, regardless of the daily LE duration, the highest removal rate of TG-derived FA from plasma was observed at the start of the dark period, when FA uptake by BAT is highest.

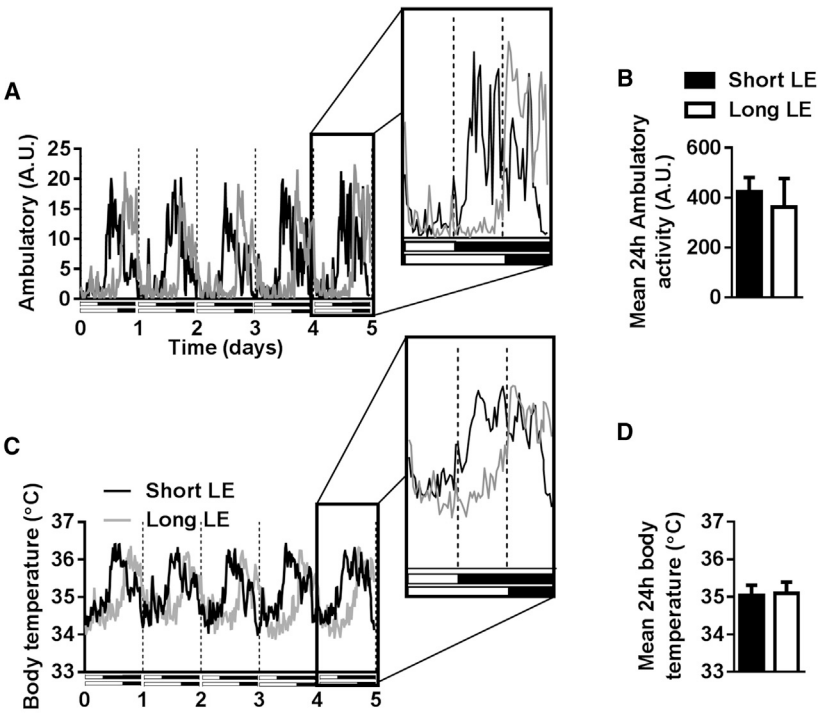
Postprandial Lipid Response in Humans Is Dependent on the Time of the Day

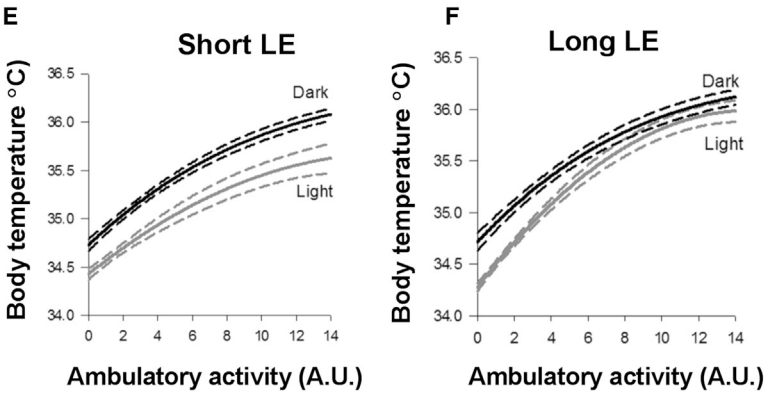
To evaluate whether a diurnal rhythm in FA uptake by BAT could exist in humans, we chose to determine the lipid response following isocaloric meals at three time points of the day in a cohort of 37 healthy individuals (Jansen et al., 2015) (characteristics are shown in Table S1). As human activity is in the opposite phase as compared to mice, we would expect postprandial lipids to be lowest around the onset of the light. Participants were allowed to sleep from 2300 hr to 0800 hr, during which lights were off, and consumed identical standard liquid test meals at clock times 0900, 1200, and 1800 hr. TG levels of this cohort were reported before and were found to be rhythmic (van den Berg et al., 2017). Postprandial AUCs at 9–12 hr (3.2 ± 0.2 mM • hr) were lower compared to 12–15 hr (5.0 ± 0.3 mM • hr; $p < 0.0001$) and 18–21 hr (4.0 ± 0.2 mM • hr; $p < 0.001$) (Figure S4). Postprandial free FA levels were low in the morning and highest in the evening (Figure 6J). The AUC in the 3 hr following the meal were lowest at 9–12 hr (1.64 ± 0.09 mM • hr) and increased at 12–15 hr (1.89 ± 0.13 mM • hr; $p = 0.004$) and 18–21 hr (2.52 ± 0.17 mM • hr; $p < 0.0001$) (Figure 6K). Collectively, these data show that, both in mice and humans, the postprandial lipid response is lowest at the start of the wakeful period.

DISCUSSION

In this study, by using wild-type and APOE*3-Leiden.CETP mice, we show that BAT displays a strong diurnal rhythm with respect to uptake of TG-derived FA from the circulation, likely regulated through a diurnal rhythm in LPL activity within the tissue. In addition, FA uptake specifically by BAT, but not by other organs, was found to readily adapt to modulation of the light exposure duration. The peak in FA uptake by BAT was aligned to the light:dark cycles and consistently occurred at the onset of the wakeful period (dark phase for mice). The daily pattern of BAT activity inversely predicted daily patterns of plasma TG and FA levels and determined the postprandial handling of TG. In parallel, in healthy middle-aged human subjects, postprandial FA excursions to identical meals were also lowest at the early wakeful period (light phase for humans), suggesting that the daily variation in BAT activity can be clinically relevant. However, diurnal variation in FA uptake by human BAT has to be verified first.

By investigating time-dependent differences in the uptake of FA by metabolically active organs, we observed a steep rise in the uptake of FA by BAT activity in anticipation of the dark period,





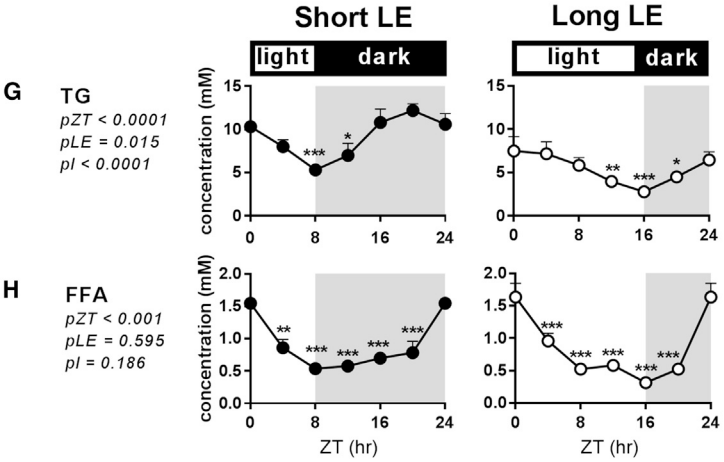


Figure 4. 24-Hr Rhythms in Ambulatory Activity, Body Temperature, and Plasma Lipids in Dyslipidemic APOE*3.Leiden-CETP Mice

APOE*3-Leiden.CETP mice, fed a western-type diet, were subjected to short LE or long LE and implanted with telemetric transmitters (n = 4–5 per group) to measure body temperature and ambulatory activity.

(A–D) Five consecutive representative days of ambulatory activity rhythms (A) (black lines indicate short LE, and gray lines indicate long LE) and body temperature rhythms (C) are indicated. Mean 24-hr ambulatory activity (B) and body temperature (D) were calculated.

(E and F) The quadratic fit of the body temperature to the ambulatory activity was calculated separately for the dark and light periods for mice adapted to short LE (E) or long LE (F). Dotted lines show the 95% confidence intervals of the fitted curves.

(G and H) 7 consecutive blood samples were drawn, and TG (G) and free FA (H) levels were determined (black circles indicate short LE, and white circles indicate long LE). p_{ZT} and p_I represent p values for the factors zeitgeber time (ZT) and interaction of LE on ZT, respectively (two-way repeated measurements [RM]-ANOVA). *p < 0.05; **p < 0.01; ***p < 0.001 (compared to ZT0, Dunnett's post hoc test).

Data are presented as means ± SEM.

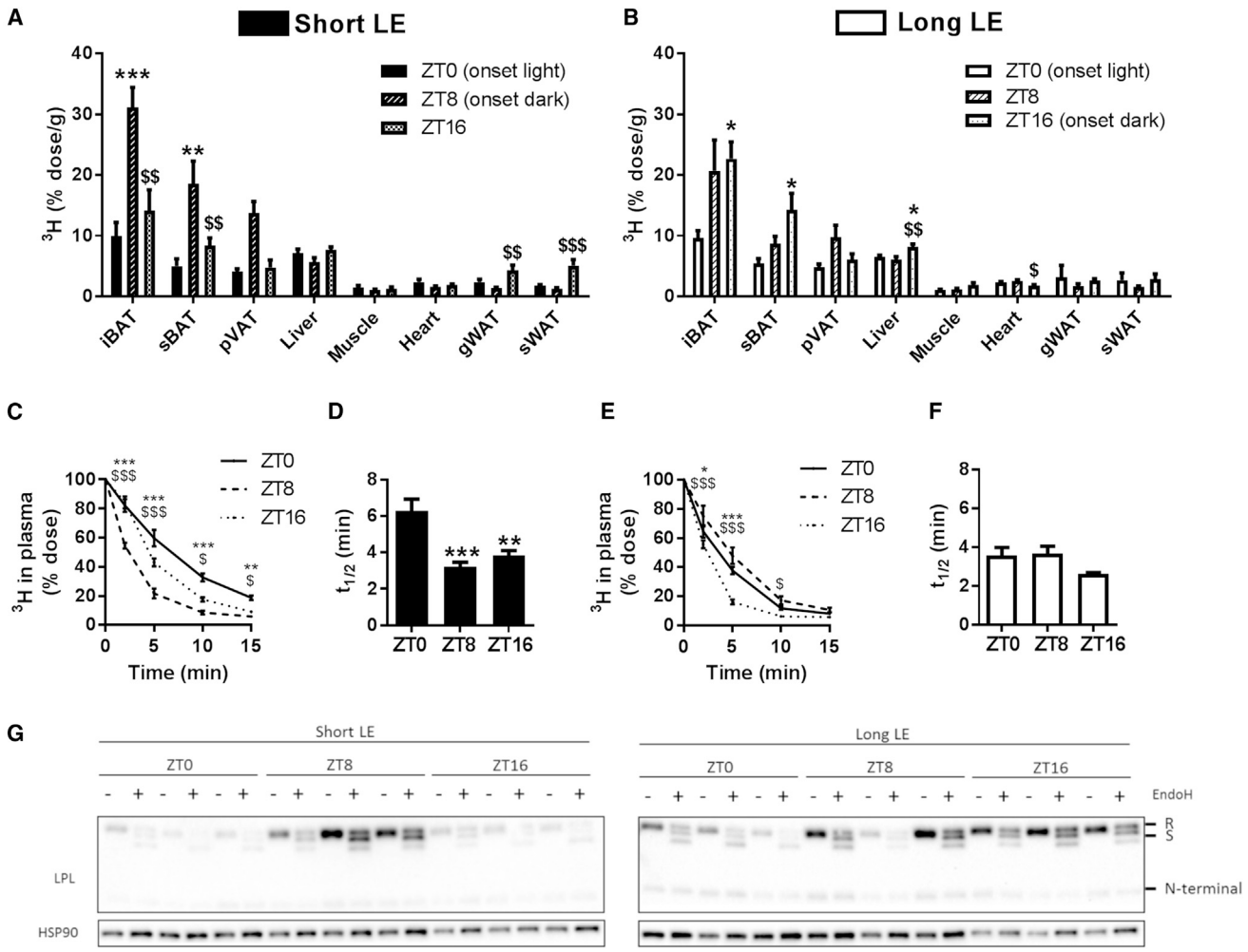


Figure 5. Diurnal Variation in TG-Derived FA Uptake by BAT and TG Clearance from the Circulation in Dyslipidemic APOE*3.Leiden-CETP Mice

(A and B) APOE*3-Leiden.CETP mice, fed a western-type diet, were subjected to short LE (A) or long LE (B) and were injected with glycerol tri[^{3}H]oleate-labeled lipoprotein-like particles at three time points (ZT0, ZT8, or ZT16) (n = 7–8 per group). Organ uptake of [^{3}H]oleate was determined.

(C–G) Plasma clearance of [^{3}H]oleate was determined (C and E), and half-life ($t_{1/2}$) was calculated (D and F). Protein levels of total LPL and mature glycosylated LPL protein were determined in a subset of the samples by EndoH digestion. EndoH-sensitive LPL (ER LPL) is indicated with S; EndoH-resistant active LPL (Golgi apparatus and cell-surface LPL) is indicated with an R (G).

Data are presented as means ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001 (lights on compared to lights off); $^{\$}$p < 0.05; $^{\$\$}$p < 0.01; $^{\$\$\$}$p < 0.001 (ZT8 compared to ZT16), ANOVA with Tukey's post hoc test for (A), (B), (D), and (F), and RM-ANOVA with Dunnett's post hoc test for (C) and (E). Black symbols/bars indicate short day, and white symbols/bars indicate long day. iBAT, interscapular BAT, sBAT, subscapular BAT, pVAT, perivascular adipose tissue; gWAT, gonadal white adipose tissue; sWAT, subcutaneous white adipose tissue. See also Figure S5.

which corresponded with a rise in the core body temperature and energy expenditure. Within the tissue, we identified rhythms in the core clock genes, including *Rev-erbα* and *Per2*, as well as the key thermogenic gene *Ucp1*. In line with a previous report, *Ucp1* expression was in anti-phase with the expression of its transcriptional repressor *Rev-erbα* (Gerhart-Hines et al., 2013). Importantly, we identified a diurnal rhythm in mRNA expression and protein levels of LPL, critical for TG-derived FA uptake (Khedoe et al., 2015; Olivecrona et al., 1997), which correlated with FA uptake by BAT. In addition, mRNA and protein levels of ANGPTL4 were found to be rhythmic and almost undetectable at the onset of the dark period. Since we previously demonstrated that ANGPTL4 is a potent inhibitor of LPL function (Dijk et al., 2015), we anticipate that the net effect, i.e., high levels of active LPL at the onset of the dark period, was causing the peak in FA uptake by BAT.

How diurnal BAT activity is regulated exactly remains to be determined. The central biological clock, located in the suprachiasmatic nucleus of the hypothalamus, orchestrates circadian and diurnal anticipatory adaptations of energy metabolism through a complex interplay of endocrine, neuronal, and behavioral factors. These oscillating factors can influence BAT activity either directly or indirectly through synchronization of the cell-autonomous clock. In a first attempt to delineate underlying mechanisms,

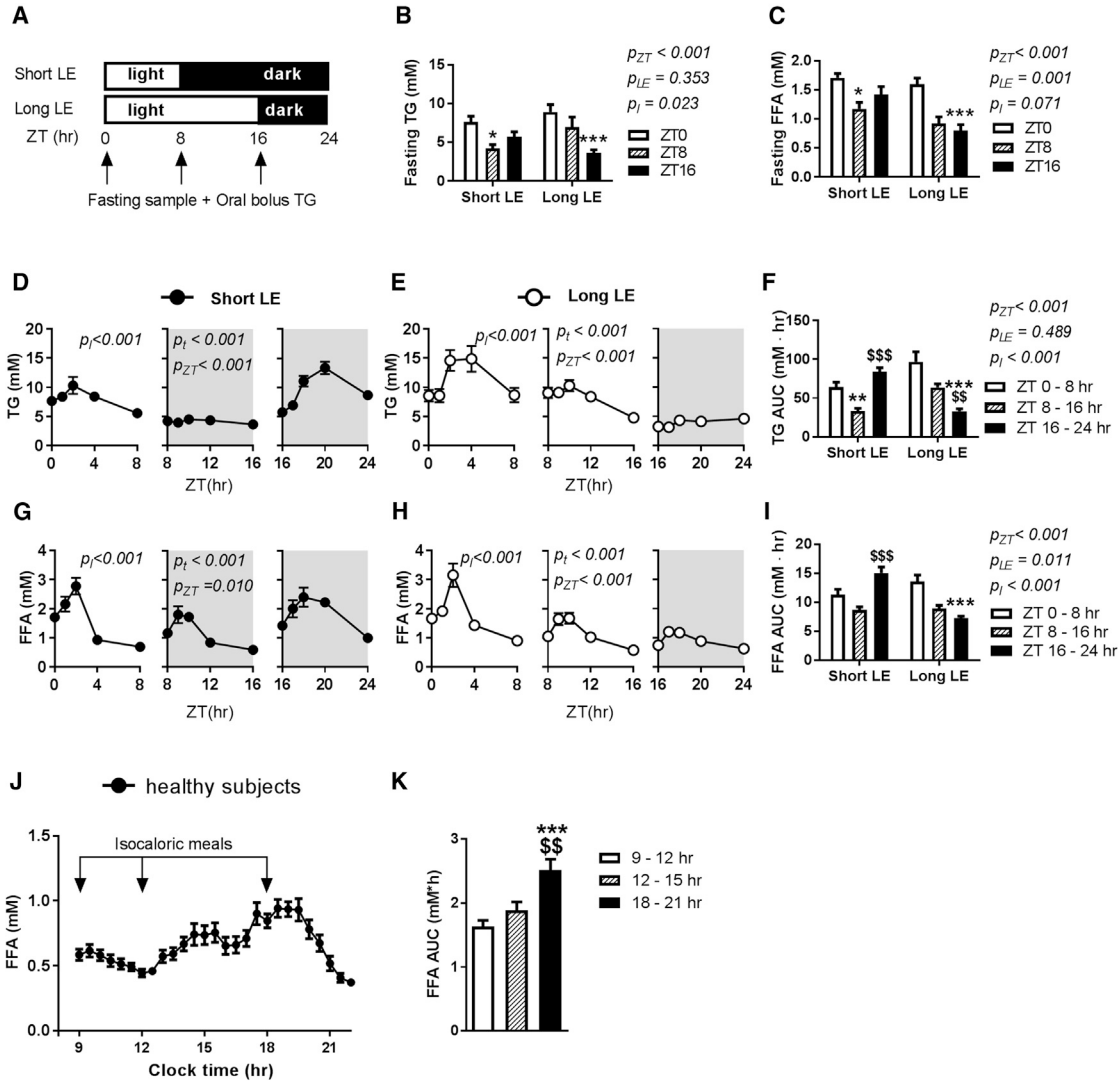


Figure 6. Diurnal Variations in Postprandial Lipid Responses in Mice and Humans

(A) APOE*3-Leiden.CETP mice, fed a western-type diet, were subjected to short LE or long LE. Mice were fasted for 4 hr, blood was drawn, and oral TG bolus was administered at three time points (ZT0, ZT8, or ZT16) (n = 7–8 per group).

(B and C) Fasting TG (B) and FFA (C) were determined.

(D–I) Postprandial plasma TG excursion for short LE in (D) and long LE in (E) and FFA for short LE in (G) and long LE in (H) were determined at time (t) = 1, 2, 4, and 8 hr after oral TG bolus. Postprandial AUC was calculated for TG (F) and FFA (I). 37 healthy individuals were fasted overnight before a diurnal venous blood sampling. At 9 hr, 12 hr, and 18 hr, a standard liquid meal was consumed.

(J and K) Free FA (FFA) levels were determined every 30 min (J), and postprandial AUC was calculated 3 hr after the ingestion of isocaloric meal (K).

Data are presented as means ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001 (lights on compared to lights off); $^{\$}$p < 0.05; $^{\$\$}$p < 0.01; $^{\$\$\$}$p < 0.001 (ZT8 compared to ZT16). P_t, p_{ZT}, and p_I, represent p values for the factors postprandial time point, ZT (time of day of oral gavage), and the interaction, respectively, based on 2-way repeated-measures ANOVA in (D), (E), (G), and (H). p_{ZT}, p_{LE}, and p_I represent p values for the factors zeitgeber time, LE, and interaction, respectively; two-way ANOVA with Tukey's post hoc test for (B), (C), (F), (I), and (K). See also Figure S6 and Table S1.

we measured the diurnal uptake of FA by BAT after prolonged fasting and in the absence of sympathetic innervation (thermoneutrality and sympathetic denervation) or in the absence of glucocorticoids (adrenalectomy). Fasting/feeding and in diurnal FA uptake by BAT both adapted to changes in the light:dark cycles. Prolonged fasting during the night period resulted in increased uptake of FA by sBAT but not iBAT. A possible explanation for the involvement of feeding patterns in diurnal FA uptake by BAT is that the activity of LPL and the expression of ANGPTL4 are known to be regulated by feeding and by insulin and glucose levels (Carneheim and Alexson, 1989; Ong and Kern, 1989; van Raalte et al., 2012). Thus, it is plausible that rhythms in feeding at least contribute to the diurnal activity of BAT. Interestingly, sympathetic denervation of iBAT completely abolished its diurnal activity, but, as mentioned before, interpretation is difficult, as denervation of this tissue generally results in a very strong

reduction of thermogenic activity. Also, diurnal rhythmicity in BAT was maintained upon the housing of mice at thermoneutrality (30°C), which is consistent with literature (Gerhart-Hines et al., 2013). Absence of glucocorticoids did not affect diurnal FA uptake, but, interestingly enough, did abolish rhythms in glucose uptake, suggesting a separated level of regulation for diurnal glucose and FA uptake by BAT. Furthermore, we showed that REV-ERBα deficiency, while releasing transcriptional repression of *Ucp1*, did not affect the rhythmicity of *Lpl* or *Angptl4* levels. In conclusion, we identified sympathetic outflow as the potential driving force of diurnal BAT activity, which is in line with our previous study (Kooijman et al., 2015a). In addition, we found some evidence for a contributing role of fasting/feeding in diurnal FA uptake by BAT. Further studies are needed to firmly establish these suspected relationships.

We investigated the adaptation of the tissue to changes in the environment—in this case, daily light exposure duration, a physiological signal of the time of year. Interestingly, we observed a specific adaptation in the activity of BAT with respect to FA uptake and not in that of other metabolic organs. It is tempting to speculate on the reasons why BAT is relatively more sensitive to changes in daily light exposure. BAT has evolved as a natural defense system against hypothermia, and the physiological importance of a seasonal and diurnal rhythm in BAT activity likely relates to this. From an evolutionary perspective, it would be a waste of energy to have BAT constitutively active. We suggest that BAT activity contributes to an increase in body temperature before waking. This could be analogous to the indispensable rise in BAT activity during arousal in hibernating mammals (Kitao and Hashimoto, 2012; Oelkrug et al., 2011). Furthermore, mice with dysfunctional BAT through genetic modification or denervation display an aberrant sleep phenotype (Szentirmai and Kapás, 2014). Since short LE signals winter, which is accompanied by lower temperatures, it stands to reason that short LE is an anticipatory signal to increase overall thermogenesis, while long LE results in diminished heat production.

Importantly, changes in daily light exposure duration are pertinent to metabolic health. Human studies have shown that exposure to light at night correlates to a higher body weight (McFadden et al., 2014; Rybnikova et al., 2016) and prolonged duration of environmental light exposure predicts increased weight gain in children (Pattinson et al., 2016). We previously identified impaired BAT activity as the potential missing link in the established association between perturbations in circadian rhythms and metabolic disorders by demonstrating that prolonged daily light exposure reduces BAT activity and induces adiposity in mice (Kooijman et al., 2015a). Moreover, we anticipate that diurnal BAT activity can be exploited to our benefit by adjusting the timing of food intake. We showed that, in humans, postprandial lipids are lower in the morning than in the afternoon, consistent with previous reports (Schlierf and Dorow, 1973; Schlierf et al., 1979), and we propose that diurnal BAT activity contributes to the complex process regulating these lipid levels. In favor of this hypothesis and in line with our data, others have demonstrated that postprandial thermogenesis in humans (Morris et al., 2015) and insulin-stimulated glucose uptake by human BAT explants (Lee et al., 2016) are higher in the morning than in the evening.

Our data help to explain previous findings that time-restricted feeding contributes to metabolic phenotypes. Feeding mice at the biologically "wrong" time (i.e., the resting phase) increases body weight (Arble et al., 2009), while feeding at the "right" time (i.e., the active phase) prevents diet-induced obesity and related health problems (Hatori et al., 2012). Humans eat virtually whenever they are awake (Gill and Panda, 2015). Early eaters were shown to be more successful at weight-loss therapy than late eaters, independent of caloric intake (Garaulet et al., 2013), and limited caloric intake in the evening has been associated with lower body mass index (Baron et al., 2011; Jakubowicz et al., 2013). Indeed, when overweight individuals were asked to limit the duration of food intake to a maximum of 11 hr daily (Gill and Panda, 2015), they were metabolically healthier and subjectively more energetic. Taken together, these data suggest that BAT has a higher capacity to take up FA and combust calories in the morning than in the evening, defining the fate of consumed calories. This might be (part of the) mechanistic basis for early timing of food intake as a potential strategy to improve metabolic health.

Promotion of BAT activity has shown great potential as treatment for metabolic disorders and cardiovascular disease in numerous preclinical models (Berbée et al., 2015; Boon et al., 2014; Kooijman et al., 2015b; Laurila et al., 2016) and, to some extent, in humans (Cypess et al., 2015). We anticipate that diurnal BAT activity is an important factor to consider when studying the therapeutic potential of promoting BAT activity for metabolic disorders.

EXPERIMENTAL PROCEDURES

Animal Experiments

All animal experiments were approved by the institutional ethics committee on animal care and experimentation at the Leiden University Medical Center (LUMC), Leiden, the Netherlands. Mice were single housed at 22°C room temperature and fed *ad libitum*, unless indicated otherwise. Wild-type mice were fed standard laboratory chow (Special Diets Services, Essex, UK), APOE*3-Leiden.CETP mice were fed a Western-type diet (WTD; 35% energy from fat supplemented with 0.1% cholesterol; AB Diets, Woerden, the Netherlands). Details are available in the Supplemental Experimental Procedures.

12-week-old male wild-type mice (C57BL/6J background; Charles River Laboratories) were housed on a 12-hr:12-hr light:dark cycle (normal daily LE, 8-hr:16-hr light:dark cycle [short daily LE] or a 16-hr:8-hr light:dark cycle [long daily LE];n = 24 per group). After 5 weeks, the uptake of TG-derived FAs by metabolic organs was assessed (discussed later) at the following time points (time after lights on): ZT0, -4, -6, -8, -12, and -18 hr. At the end of this experiment, mice were killed, and organs were collected for gene/protein expression analysis (discussed later). Additional cohorts of mice were used for investigating the effects of sympathetic denervation and adrenalectomy (see the Supplemental Experimental Procedures for details) or the determination of 24-hr rhythms in food intake and energy expenditure. Thereto, mice were housed in metabolic cages (Phenomaster, TSE Systems) at normal LE for 1 week. After 4 weeks of adaptation to either short or long LE (n = 15–16 per group), mice were again housed in metabolic cages. After 4 days of habituation, food intake rhythms, energy expenditure, and RER rhythms were obtained.

Female APOE*3-Leiden.CETP mice (C57BL/6J background), 9–12 weeks old, were adapted to either short or long daily LE (n = 27 per group). A subset of mice (n = 5 per group) was equipped with a subcutaneous telemetric transmitter, and after 4 weeks, 24-hr unfasted and stress-free venous blood samples were drawn. Fasting and postprandial lipids were determined either at ZT0, -8 or -16 (discussed later). After 5 weeks in short or long LE, the uptake

of TG-derived FAs by metabolic organs and clearance from the circulation were determined (discussed later) at the same time points.

TG-Derived FA Uptake

Glycerol tri[^{3}H]oleate-labeled lipoprotein-like particles (80 nm) were prepared as described in the Supplemental Experimental Procedures. Mice were fasted for 4 hr prior to the start of the experiment, which started with an intravenous injection of radiolabeled particles (1.0 mg TG in 200 μL PBS). Blood was collected after 2, 5, 10, and 15 min to determine plasma decay of the radiolabel. After 15 min, mice were killed by cervical dislocation and perfused with ice-cold PBS, and organs were harvested. The uptake of [^{3}H]oleate by metabolic organs was determined.

Gene and Protein Expression

Standard techniques were used for RNA and protein isolation. RNA and protein expression were determined by real-time PCR and western blot. Protocol details are supplied in the Supplemental Experimental Procedures.

Postprandial Lipid Response

To determine the postprandial lipid response, mice were fasted for 4 hr prior to the start of the experiment, after which a fasting blood sample via tail vein bleeding was drawn. Immediately thereafter, mice received an intragastric bolus of 200 μL olive oil (Carbonell, Cordoba, Spain). Blood was collected after 1, 2, 4, and 8 hr to determine plasma lipid concentrations (details are available in the Supplemental Experimental Procedures).

Human Study

For the present study, 37 healthy participants were studied under strictly standardized conditions (details are available in Supplemental Experimental Procedures). After an overnight fast of 10–14 hr, a catheter was inserted before the start of the study, and 10-min blood sampling started at 0900 hr. Participants received three standardized liquid meals at 0900, 1200, and 1800 hr (600 kcal containing fat [35%], carbohydrates [49%], and protein [16%]; Nutricia Advanced Medical Nutrition, Zoetermeer, the Netherlands).

Statistical Methods

Data are presented as means ± SEM. Contribution of the circadian time point (ZT), daily LE, and interaction effect was analyzed by two-way ANOVA. Differences between independent continuous variables were determined by t tests (for two groups) or by one-way ANOVA with Tukey's post hoc test (for more than two groups). In case of a time series within one subject, repeated-measures ANOVA was used, and Dunnett's post hoc test was used to compare time points within the series (relative to ZT0). Associations of variables with daily LE (expressed as hours of LE) as an independent variable were assessed by linear regression analysis. Differences at $p < 0.05$ were considered statistically significant. Analyses were performed using GraphPad software, v6.0 (Prism, La Jolla, CA, USA).

Study Approval

The ethics committee of the Leiden University Medical Center (Leiden, the Netherlands) approved all animal experiments. The medical ethical committee of the Leiden University Medical Center (Leiden, the Netherlands) approved the human study, which was performed according to the Declaration of Helsinki. Written informed consent was obtained from all study participants.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, six figures, and three tables and can be found with this article online at https://doi.org/10.1016/j.celrep.2018.03.004.

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AUTHOR CONTRIBUTIONS

Conceptualization, R.v.d.B., S. Kooijman, N.R.B., and P.C.N.R.; Methodology, R.v.d.B., S. Kooijman, N.R.B., and P.C.N.R.; Investigation, R.v.d.B., S. Kooijman, A.R., G.A.-V., I.M.M., P.R., B.K., W.D., P.R., L.L.T., R.C., L.S.P., E.M.d.R., J.K., M.H., R.J.v.d.S., and C.P.C.; Resources, R.N., C.C., L.W.M.v.K., F.K., S. Kersten, and Z.G.-H.; Writing – Original Draft, R.v.d.B. and S. Kooijman; Writing – Review & Editing, N.R.B. and P.C.N.R.; Supervision, O.C.M., K.W.v.D., J.H.M., D.v.H., N.R.B., and P.C.N.R.; Funding Acquisition, S. Kooijman, C.P.C., Z.G.-H., J.H.M., D.v.H., N.R.B., and P.C.N.R.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

Abreu-Vieira, G., Xiao, C., Gavrilova, O., and Reitman, M.L. (2015). Integration of body temperature into the analysis of energy expenditure in the mouse. Mol. Metab. *4*, 461–470.

Arble, D.M., Bass, J., Laposky, A.D., Vitaterna, M.H., and Turek, F.W. (2009). Circadian timing of food intake contributes to weight gain. Obesity (Silver Spring) *17*, 2100–2102.

Baron, K.G., Reid, K.J., Kern, A.S., and Zee, P.C. (2011). Role of sleep timing in caloric intake and BMI. Obesity (Silver Spring) *19*, 1374–1381.

Bartelt, A., Bruns, O.T., Reimer, R., Hohenberg, H., Ittrich, H., Peldschus, K., Kaul, M.G., Tromsdorf, U.I., Weller, H., Waurisch, C., et al. (2011). Brown adipose tissue activity controls triglyceride clearance. Nat. Med. *17*, 200–205.

Berbée, J.F., Boon, M.R., Khedoe, P.P., Bartelt, A., Schlein, C., Worthmann, A., Kooijman, S., Hoeke, G., Mol, I.M., John, C., et al. (2015). Brown fat activation reduces hypercholesterolaemia and protects from atherosclerosis development. Nat. Commun. *6*, 6356.

Boon, M.R., Bakker, L.E., van der Linden, R.A., Pereira Arias-Bouda, L., Smit, F., Verberne, H.J., van Marken Lichtenbelt, W.D., Jazet, I.M., and Rensen, P.C. (2014). Supraclavicular skin temperature as a measure of 18F-FDG uptake by BAT in human subjects. PLoS ONE *9*, e98822.

Brown, N.K., Zhou, Z., Zhang, J., Zeng, R., Wu, J., Eitzman, D.T., Chen, Y.E., and Chang, L. (2014). Perivascular adipose tissue in vascular function and disease: a review of current research and animal models. Arterioscler. Thromb. Vasc. Biol. *34*, 1621–1630.

Carneheim, C.M., and Alexson, S.E. (1989). Refeeding and insulin increase lipoprotein lipase activity in rat brown adipose tissue. Am. J. Physiol. *256*, E645–E650.

Chappuis, S., Ripperger, J.A., Schnell, A., Rando, G., Jud, C., Wahli, W., and Albrecht, U. (2013). Role of the circadian clock gene Per2 in adaptation to cold temperature. Mol. Metab. *2*, 184–193.

Chua, E.C., Shui, G., Lee, I.T., Lau, P., Tan, L.C., Yeo, S.C., Lam, B.D., Bulchand, S., Summers, S.A., Puvanendran, K., et al. (2013). Extensive diversity in circadian regulation of plasma lipids and evidence for different circadian metabolic phenotypes in humans. Proc. Natl. Acad. Sci. USA *110*, 14468–14473.

Cypess, A.M., Weiner, L.S., Roberts-Toler, C., Franquet Elía, E., Kessler, S.H., Kahn, P.A., English, J., Chatman, K., Trauger, S.A., Doria, A., and Kolodny, G.M. (2015). Activation of human brown adipose tissue by a β3-adrenergic receptor agonist. Cell Metab. *21*, 33–38.

Dijk, W., Heine, M., Vergnes, L., Boon, M.R., Schaart, G., Hesselink, M.K., Reue, K., van Marken Lichtenbelt, W.D., Olivecrona, G., Rensen, P.C., et al. (2015). ANGPTL4 mediates shuttling of lipid fuel to brown adipose tissue during sustained cold exposure. eLife *4*, e08428.

Dijk, W., Beigneux, A.P., Larsson, M., Bensadoun, A., Young, S.G., and Kersten, S. (2016). Angiopoietin-like 4 promotes intracellular degradation of lipoprotein lipase in adipocytes. J. Lipid Res. *57*, 1670–1683.

Fischer, A.W., Hoefig, C.S., Abreu-Vieira, G., de Jong, J.M.A., Petrovic, N., Mittag, J., Cannon, B., and Nedergaard, J. (2016). Leptin raises defended body temperature without activating thermogenesis. Cell Rep. *14*, 1621–1631.

Garaulet, M., Gómez-Abellán, P., Alburquerque-Béjar, J.J., Lee, Y.C., Ordovás, J.M., and Scheer, F.A. (2013). Timing of food intake predicts weight loss effectiveness. Int. J. Obes. *37*, 604–611.

Gerhart-Hines, Z., Feng, D., Emmett, M.J., Everett, L.J., Loro, E., Briggs, E.R., Bugge, A., Hou, C., Ferrara, C., Seale, P., et al. (2013). The nuclear receptor Rev-erbα controls circadian thermogenic plasticity. Nature *503*, 410–413.

Gill, S., and Panda, S. (2015). A smartphone app reveals erratic diurnal eating patterns in humans that can be modulated for health benefits. Cell Metab. *22*, 789–798.

Hatori, M., Vollmers, C., Zarrinpar, A., DiTacchio, L., Bushong, E.A., Gill, S., Leblanc, M., Chaix, A., Joens, M., Fitzpatrick, J.A., et al. (2012). Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. Cell Metab. *15*, 848–860.

Hoeke, G., Kooijman, S., Boon, M.R., Rensen, P.C., and Berbée, J.F. (2016). Role of brown fat in lipoprotein metabolism and atherosclerosis. Circ. Res. *118*, 173–182.

Jakubowicz, D., Barnea, M., Wainstein, J., and Froy, O. (2013). High caloric intake at breakfast vs. dinner differentially influences weight loss of overweight and obese women. Obesity (Silver Spring) *21*, 2504–2512.

Jansen, S.W., Akintola, A.A., Roelfsema, F., van der Spoel, E., Cobbaert, C.M., Ballieux, B.E., Egri, P., Kvarta-Papp, Z., Gereben, B., Fekete, C., et al. (2015). Human longevity is characterised by high thyroid stimulating hormone secretion without altered energy metabolism. Sci. Rep. *5*, 11525.

Khedoe, P.P., Hoeke, G., Kooijman, S., Dijk, W., Buijs, J.T., Kersten, S., Havekes, L.M., Hiemstra, P.S., Berbée, J.F., Boon, M.R., and Rensen, P.C. (2015). Brown adipose tissue takes up plasma triglycerides mostly after lipolysis. J. Lipid Res. *56*, 51–59.

Kitao, N., and Hashimoto, M. (2012). Increased thermogenic capacity of brown adipose tissue under low temperature and its contribution to arousal from hibernation in Syrian hamsters. Am. J. Physiol. Regul. Integr. Comp. Physiol. *302*, R118–R125.

Kooijman, S., van den Berg, R., Ramkisoensing, A., Boon, M.R., Kuipers, E.N., Loef, M., Zonneveld, T.C., Lucassen, E.A., Sips, H.C., Chatzispyrou, I.A., et al. (2015a). Prolonged daily light exposure increases body fat mass through attenuation of brown adipose tissue activity. Proc. Natl. Acad. Sci. USA *112*, 6748–6753.

Kooijman, S., Wang, Y., Parlevliet, E.T., Boon, M.R., Edelschaap, D., Snaterse, G., Pijl, H., Romijn, J.A., and Rensen, P.C. (2015b). Central GLP-1 receptor signalling accelerates plasma clearance of triacylglycerol and glucose by activating brown adipose tissue in mice. Diabetologia *58*, 2637–2646.

Kühnast, S., van der Tuin, S.J., van der Hoorn, J.W., van Klinken, J.B., Simic, B., Pieterman, E., Havekes, L.M., Landmesser, U., Lüscher, T.F., Willems van Dijk, K., et al. (2015). Anacetrapib reduces progression of atherosclerosis, mainly by reducing non-HDL-cholesterol, improves lesion stability and adds to the beneficial effects of atorvastatin. Eur. Heart J. *36*, 39–48.

Lateef, D.M., Abreu-Vieira, G., Xiao, C., and Reitman, M.L. (2014). Regulation of body temperature and brown adipose tissue thermogenesis by bombesin receptor subtype-3. Am. J. Physiol. Endocrinol. Metab. *306*, E681–E687.

Laurila, P.P., Soronen, J., Kooijman, S., Forsström, S., Boon, M.R., Surakka, I., Kaiharju, E., Coomans, C.P., Van Den Berg, S.A., Autio, A., et al. (2016). USF1 deficiency activates brown adipose tissue and improves cardiometabolic health. Sci. Transl. Med. *8*, 323ra13.

Lee, P., Bova, R., Schofield, L., Bryant, W., Dieckmann, W., Slattery, A., Govendir, M.A., Emmett, L., and Greenfield, J.R. (2016). Brown adipose tissue exhibits a glucose-responsive thermogenic biorhythm in humans. Cell Metab. *23*, 602–609.

McFadden, E., Jones, M.E., Schoemaker, M.J., Ashworth, A., and Swerdlow, A.J. (2014). The relationship between obesity and exposure to light at night: cross-sectional analyses of over 100,000 women in the Breakthrough Generations Study. Am. J. Epidemiol. *180*, 245–250.

Morris, C.J., Garcia, J.I., Myers, S., Yang, J.N., Trienekens, N., and Scheer, F.A. (2015). The human circadian system has a dominating role in causing the morning/evening difference in diet-induced thermogenesis. Obesity (Silver Spring) *23*, 2053–2058.

Nedergaard, J., Bengtsson, T., and Cannon, B. (2007). Unexpected evidence for active brown adipose tissue in adult humans. Am. J. Physiol. Endocrinol. Metab. *293*, E444–E452.

Oelkrug, R., Heldmaier, G., and Meyer, C.W. (2011). Torpor patterns, arousal rates, and temporal organization of torpor entry in wildtype and UCP1-ablated mice. J. Comp. Physiol. B *181*, 137–145.

Olivecrona, T., Hultin, M., Bergö, M., and Olivecrona, G. (1997). Lipoprotein lipase: regulation and role in lipoprotein metabolism. Proc. Nutr. Soc. *56*, 723–729.

Ong, J.M., and Kern, P.A. (1989). The role of glucose and glycosylation in the regulation of lipoprotein lipase synthesis and secretion in rat adipocytes. J. Biol. Chem. *264*, 3177–3182.

Pan, X., Zhang, Y., Wang, L., and Hussain, M.M. (2010). Diurnal regulation of MTP and plasma triglyceride by CLOCK is mediated by SHP. Cell Metab. *12*, 174–186.

Pattinson, C.L., Allan, A.C., Staton, S.L., Thorpe, K.J., and Smith, S.S. (2016). Environmental light exposure is associated with increased body mass in children. PLoS ONE *11*, e0143578.

Rensen, P.C., van Dijk, M.C., Havenaar, E.C., Bijsterbosch, M.K., Kruijt, J.K., and van Berkel, T.J. (1995). Selective liver targeting of antivirals by recombinant chylomicrons–a new therapeutic approach to hepatitis B. Nat. Med. *1*, 221–225.

Reppert, S.M., and Weaver, D.R. (2002). Coordination of circadian timing in mammals. Nature *418*, 935–941.

Rudic, R.D., McNamara, P., Curtis, A.M., Boston, R.C., Panda, S., Hogenesch, J.B., and Fitzgerald, G.A. (2004). BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. PLoS Biol. *2*, e377.

Rybnikova, N.A., Haim, A., and Portnov, B.A. (2016). Does artificial light-at-night exposure contribute to the worldwide obesity pandemic? Int. J. Obes. *40*, 815–823.

Schlierf, G., and Dorow, E. (1973). Diurnal patterns of triglycerides, free fatty acids, blood sugar, and insulin during carbohydrate-induction in man and their modification by nocturnal suppression of lipolysis. J. Clin. Invest. *52*, 732–740.

Schlierf, G., Jessel, S., Ohm, J., Heuck, C.C., Klose, G., Oster, P., Schellenberg, B., and Weizel, A. (1979). Acute dietary effects on plasma lipids,

lipoproteins and lipolytic enzymes in healthy normal males. Eur. J. Clin. Invest. *9*, 319–325.

Shostak, A., Meyer-Kovac, J., and Oster, H. (2013). Circadian regulation of lipid mobilization in white adipose tissues. Diabetes *62*, 2195–2203.

Szentirmai, É., and Kapás, L. (2014). Intact brown adipose tissue thermogenesis is required for restorative sleep responses after sleep loss. Eur. J. Neurosci. *39*, 984–998.

van den Berg, R., Noordam, R., Kooijman, S., Jansen, S.W.M., Akintola, A.A., Slagboom, P.E., Pijl, H., Rensen, P.C.N., Biermasz, N.R., and van Heemst, D. (2017). Familial longevity is characterized by high circadian rhythmicity of serum cholesterol in healthy elderly individuals. Aging Cell *16*, 237–243.

van Marken Lichtenbelt, W.D., Vanhommerig, J.W., Smulders, N.M., Drossaerts, J.M., Kemerink, G.J., Bouvy, N.D., Schrauwen, P., and Teule, G.J. (2009). Cold-activated brown adipose tissue in healthy men. N. Engl. J. Med. *360*, 1500–1508.

van Raalte, D.H., Brands, M., Serlie, M.J., Mudde, K., Stienstra, R., Sauerwein, H.P., Kersten, S., and Diamant, M. (2012). Angiopoietin-like protein 4 is differentially regulated by glucocorticoids and insulin in vitro and in vivo in healthy humans. Exp. Clin. Endocrinol. Diabetes *120*, 598–603.

Vander Tuig, J.G., Ohshima, K., Yoshida, T., Romsos, D.R., and Bray, G.A. (1984). Adrenalectomy increases norepinephrine turnover in brown adipose tissue of obese (ob/ob) mice. Life Sci. *34*, 1423–1432.

Warner, A., Rahman, A., Solsjö, P., Gottschling, K., Davis, B., Vennström, B., Arner, A., and Mittag, J. (2013). Inappropriate heat dissipation ignites brown fat thermogenesis in mice with a mutant thyroid hormone receptor α1. Proc. Natl. Acad. Sci. USA *110*, 16241–16246.

Zvonic, S., Ptitsyn, A.A., Conrad, S.A., Scott, L.K., Floyd, Z.E., Kilroy, G., Wu, X., Goh, B.C., Mynatt, R.L., and Gimble, J.M. (2006). Characterization of peripheral circadian clocks in adipose tissues. Diabetes *55*, 962–970.