Adipose tissue is an active endocrine tissue that stores a surplus energy in the form of lipids. While white adipose tissue mostly stores and releases lipids, brown adipose tissue (BAT) has a high abundance of mitochondria that can uncouple oxidation from ATP production, effectively generating heat instead of ATP bound energy (1). While the involvement of white adipose tissue in diabetes, obesity, and metabolism is much studied, only recently has involvement of BAT regained attention mainly because of identification of BAT in humans through the use of 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET) and X-ray computerized tomography (2). Studies identified BAT in humans and also showed that the amount of BAT exhibits a negative correlation with body mass, especially in older subjects (e.g., (3)). This suggests that increased BAT associates with decreased obesity. Also in mice and rat models, altered BAT functioning and thermogenesis has been associated with obesity phenotypes (see refs 3,4).

Circadian (~24-h) rhythms are important for a balance between predictable temporal changes in the internal and external environment and physiological activities of organ systems. For example, daily rhythms in metabolism are associated with catabolism and anabolism, and daily cycles in energy utilization are predicted by behavioral rest–activity cycles. More than 5,000 genes show daily rhythmic expression patterns in murine BAT, including genes associated with the circadian clock, adipose function, and metabolism (5). Conversely, metabolic phenotypes have been observed in several rodent models with perturbed canonical clock genes (6).

BAT receives norepinephrinic sympathetic innervation that acts primarily at the β3-adrenoceptor (7), and catecholamines can activate BAT glucose utilization (7,8). Testosterone decreases BAT activity, whereas estrogens and progesterone increase BAT activity, which is in line with an increased amount of BAT deposits in females (3,7,8). Circulating levels of thyroid hormone and corticosteroids have been associated with BAT activity and metabolic phenotypes; also growth hormone, insulin-like growth factor 1, and endocannabinoids have been associated with BAT function (8). Activity of the autonomic nervous system and plasma hormone levels show strong circadian variation (e.g., see ref. 9) and therefore could potentially transmit circadian control of BAT activity. Indeed, excitatory activation by glutamate of the hypothalamic suprachiasmatic nucleus, which contains the "master" central circadian clock, raises thermogenesis in BAT (10).

Because of the known rhythms in clock gene expression in BAT, and the rhythms in nervous and endocrine input to BAT, we hypothesized that there would be a rhythm in glucose metabolism. Using a micro-PET/computerized tomography scanner, we have imaged and measured the uptake of a radiolabeled sugar molecule, FDG, in interscapular BAT (iBAT) in C57Bl/6 mice at intervals across a 24-h light–dark cycle. Our data reveal a strong 24-h profile of glucose uptake of iBAT, peaking at ~9h into the light phase of the 12-h light, 12-h dark day. BAT is increasingly gaining attention as being involved in metabolic phenotypes and obesity, where BAT, as observed by PET analysis, negatively correlates with obesity and age. Conversely, animals that show perturbations in circadian clocks, behavior, and physiology show metabolic phenotypes. The observation of a 24-h rhythm in glucose uptake in iBAT makes this tissue a candidate site of interaction between metabolic and circadian systems.
METHODS
Male and female C57Bl/6 mice (N = 34; age = 93 ± 5 days; body mass = 22.7 ± 0.7 g) were kept under conditions of an alternating 12 h of light, 12 h of dark (LD) cycle for at least 3 weeks. Half of the animals were kept on a LD cycle 12 h out of phase, allowing for scanning at two opposite time points simultaneously. Food was removed 5 h prior the time of scanning for each group. Animals were anesthetized with 1.5% isoflurane, retro-orbitally injected with ~200 µCi of FDG (Spectron MRC, South Bend, IN)), and returned to their cages where they awoke from the light anesthesia within 2 min; 30 min after injection, during which animals were not under anesthesia, animals were re-anesthetized and PET images were acquired, alternating between mice at different phases of the light–dark cycle at the time of scanning. Measurements were taken on three separate occasions, first measuring time points centered on Zeitgeber time (ZT, ZT 0 = lights on; ZT 12 = lights off) 6, and ZT 18, second on time points centered on ZT 0 and ZT 12, and finally a third day during which images were acquired for all four different time points. PET images were acquired on a trimodal Albira PET/SPECT/CT image station (Carestream Health, Woodbridge, CT). High-density PET (voxel size 0.65 × 0.65 × 0.944 mm (xyz)) images were reconstructed, and FDG uptake in iBAT was quantified in standard uptake values, g/ml, which accounts for body mass and minor variations in injected dose, using PMOD version 3.2 (PMOD technologies, Zurich, Switzerland). FDG uptake was quantified as the mean voxel value within a visually determined volume of interest. Using the mean value minimizes the small bias that may be introduced by mice having different quantities of iBAT. Experiments were approved by the University of Notre Dame Animal Care and Use Committee no. 14-080.

For statistical analysis, time-of-day effects (ZT 0, 6, 12, and 18; N = 8–9 per group) were tested by fitting mixed linear models (PROC mixed, SAS version 9.2 (SAS Institute, Cary, NC)) with post hoc pairwise comparison based on least square means (LSMEANS). Absence of effects of age, gender, iBAT volume, and body mass were confirmed using Kruskal–Wallis one-way ANOVA, Mann–Whitney U and Spearman’s rank order correlation tests. A cosinor curve is fitted through individual measurements of iBAT uptake to determine peak phase and amplitude.

RESULTS
Figure 1a,b show representative PET acquired images of FDG uptake in each of the four groups of animals. FDG uptake in iBAT was unaffected by age, body mass, sex of the mice or total iBAT volume, nor did these variables differ significantly between animals measured at the different times of the day (one-way ANOVAs and Mann–Whitney U test, P > 0.05). No significant correlations were found between FDG uptake and the different variables (Spearman tests, P > 0.05). While there was no effect of body mass on iBAT FDG uptake, it is noteworthy that the four highest FDG uptake measurements (one at ZT 6, three at ZT 12) were obtained from some of the leanest

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**Figure 1** PET imaging reveals time-of-day–specific variation in interscapular brown adipose tissue (iBAT) FDG uptake. (a) shows a representative PET acquired image at ZT 12 of FDG uptake in a sagittal plane. From rostral to caudal, tissues that exhibit high uptake are the injections site (right eye)/brain (dorsal), tongue (ventral), iBAT (dorsal), heart (ventral), paraspinal muscle, and bladder. (b) shows four representative coronal (middle) and transverse (bottom) sections at the level of the iBAT for animals imaged at four alternative times of 24-h day: ZT 0, 6, 12, and 18. Kidneys can also be observed in these images. (c) Individual values and (d) group means of the average voxel value in the iBAT are plotted against time of measurement. Values for FDG uptake in those animals that are part of the group of animals that are measured around the dark to light transition (ZT 0/24), but fall in the light phase are plotted both early in the light phase and after the end of the dark phase. Cosinor fitting of the data predicts a peak phase of FDG uptake at ZT 9.3 and a trough at ZT 21.3. Grey area indicates the dark phase of the 24-h LD cycle. The data did not show any effects of age, gender, iBAT volume, and body mass on average voxel value FDG uptake (P > 0.05). **P < 0.01 against ZT 0 and 18. FDG, 18F-fluorodeoxyglucose; LD, light–dark; PET, positron emission tomography; SUV, standard uptake value; ZT, Zeitgeber time.**
animals in the cohort (20.2 ± 0.5 (mean ± s.e.m.) gr. and 22.7 ± 0.7 gr. body mass for the four individuals with the highest FDG measurements and the full cohort, respectively).

FDG uptake in iBAT for individual mice is shown in Figure 1c and a cosinor curve is fitted through individual values. There was a significant effect of time-of-day of scanning on FDG uptake (PROC mixed, P < 0.001). Figure 1d shows mean FDG uptake at ZT 0, 6, 12, and 18, showing that the FDG uptake is significantly higher from the mid-light phase (~ZT 6) through to the light to dark transition at ZT 12 (LSMEANS, P < 0.01 at least for comparisons of ZT 6 and ZT 12 against ZT 0 and ZT 18). Fitting of the cosinor curve predicted a peak phase of FDG uptake at ZT 9.3 and a trough at ZT 21.3.

DISCUSSION

Our observations reveal a diurnal pattern in iBAT FDG uptake, peaking during the mid- to late light phase of the light–dark cycle. This finding is consistent with reports of other physiological rhythms in BAT. In rats, a 24-h variation in BAT glycogen concentration has been documented, with peaks of concentration occurring during the late light phase and early dark phase (11,12). Interestingly, the glycogen content in BAT is lower in fasted than in ad libitum fed rats (11,12). Also in mice, fasting has been shown to reduce BAT FDG uptake by more than 30%, and mice that are warmed have a more than 50% reduction in FDG uptake in BAT (13). Thus, under conditions of ad libitum food availability, the 24-h pattern of FDG uptake observed in the current study may have shown a higher amplitude.

The here reported peak phase of the 24-h pattern in FDG uptake in mouse iBAT is markedly different from that of the brain which shows strong peaks in FDG uptake at the middle of the dark phase, and of cardiac tissue, which does not show significant temporal variation under these conditions (14). A 24-h profile of iBAT glucose uptake could in part be generated by signals from the autonomic nervous system (7,10) and plasma hormones (8). BAT expresses receptors for various endocrine factors (8), and circadian rhythms, for example in circulating cortisol, testosterone, and growth hormone (see ref. 9), could potentially regulate BAT activity and glucose uptake. A role for local intrinsic clock activity is also plausible, as expression of canonical clock genes and clock-controlled genes do show circadian activity in BAT (5).

In nonhuman biology, BAT is often studied in relation to nonshivering thermogenesis. Arousal from torpor (a hypoactive, hypometabolic state, inducible for example by cold ambient temperatures and food shortage) shows a clear circadian regulation, and most animals emerge in anticipation of the active phase of the 24-h cycle (15). In cold conditions, BAT-driven thermogenesis is important for increasing core body temperature. The peak in iBAT FDG uptake observed here precedes timing of emergence and may indicate the anticipation of the thermogenesis needed for arousal.

Reports on links between the circadian clock and obesity and diabetes are becoming more frequent, and perturbations of genes encoding canonical components of the circadian clock mechanism in mouse models are associated with both increased and decreased risks of metabolic syndromes (6). Indeed, BAT appears to express many components of the molecular circadian clock (5). With the renewed attention on BAT in relation to a possible protection against obesity (2), the 24-h profile in iBAT FDG uptake reported here further highlights the association between the circadian and metabolic systems.

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DISCLOSURE

The authors declared no conflict of interest.

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