Cell Metabolism
Short Article

High-Fat Diet Disrupts Behavioral and Molecular Circadian Rhythms in Mice

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SUMMARY

The circadian clock programs daily rhythms and coordinates multiple behavioral and physiological processes, including activity, sleep, feeding, and fuel homeostasis. Recent studies indicate that genetic alteration in the core molecular clock machinery can have pronounced effects on both peripheral and central metabolic regulatory signals. Many metabolic systems also cycle and may in turn affect function of clock genes and circadian systems. However, little is known about how alterations in energy balance affect the clock. Here we show that a high-fat diet in mice leads to changes in the period of the locomotor activity rhythm and alterations in the expression and cycling of canonical circadian clock genes, nuclear receptors that regulate clock transcription factors, and clock-controlled genes involved in fuel utilization in the hypothalamus, liver, and adipose tissue. These results indicate that consumption of a high-calorie diet alters the function of the mammalian circadian clock.

INTRODUCTION

An important component of nutrient homeostasis in many terrestrial vertebrates is the coordination of daily rhythms in rest and activity, feeding behavior, energy utilization, and energy storage across the daily 24 hr light/dark (LD) cycle. The molecular machinery underlying the generation of circadian rhythms involves a transcriptional/translational feedback loop that is now recognized to cycle in the suprachiasmatic nucleus (SCN) of the hypothalamus, as well as in most of the cells of the body (Kohsaka and Bass, 2007). While the SCN coordinates the timing of behavioral processes, recent molecular analyses have uncovered 24 hr variation in the expression of RNAs encoding genes involved in lipogenesis, lipid catabolism, sterol metabolism, and gluconeogenesis (Oishi et al., 2003; Yang et al., 2006; Zvonic et al., 2006; Panda et al., 2002). These findings suggest that the capacity of cells to engage in energy utilization and/or storage may be closely tied to the periodicity of RNA transcription programmed by the circadian clock.

We and others have recently uncovered a link between the molecular circadian clock and metabolism by demonstrating that *Clock* mutant mice exhibit hyperphagia, obesity, and hyperleptinemia (Turek et al., 2005; Oishi et al., 2006a). While the effects of the molecular circadian clock on metabolic processes have now been well documented, much less is known about how metabolic processes may alter the circadian clock. Previous studies have demonstrated that changes in cellular redox potential may affect the activity of clock transcription factors (Rutter et al., 2001). Furthermore, several metabolic transcription factors have been shown to bind to the promoters of *Clock* and *Bmal1*, a gene encoding the partner of CLOCK. For example, *Bmal1* transcription is inhibited by REV-ERBα, a transcription factor regulated by adipogenesis (Preitner et al., 2002; Chawla and Lazar, 1993), and activated by RORα, a nuclear receptor involved in both lipogenesis and lipid storage (Sato et al., 2004; Lau et al., 2004). Links between nutrient status and circadian transcription pathways are also likely strain-, gender-, and age-specific (Yanagihara et al., 2006). Here, we sought to examine whether diet-induced obesity per se might lead to altered circadian behavioral and molecular rhythms in postpubertal C57BL/6J male mice.

RESULTS

To test the hypothesis that changes in metabolic state associated with obesity and diabetes affect circadian rhythms of behavior and physiology, we examined the effect of a high-calorie diet in mice on patterns of activity, feeding, and hormone production and the cycling of clock genes and their downstream clock-controlled targets.

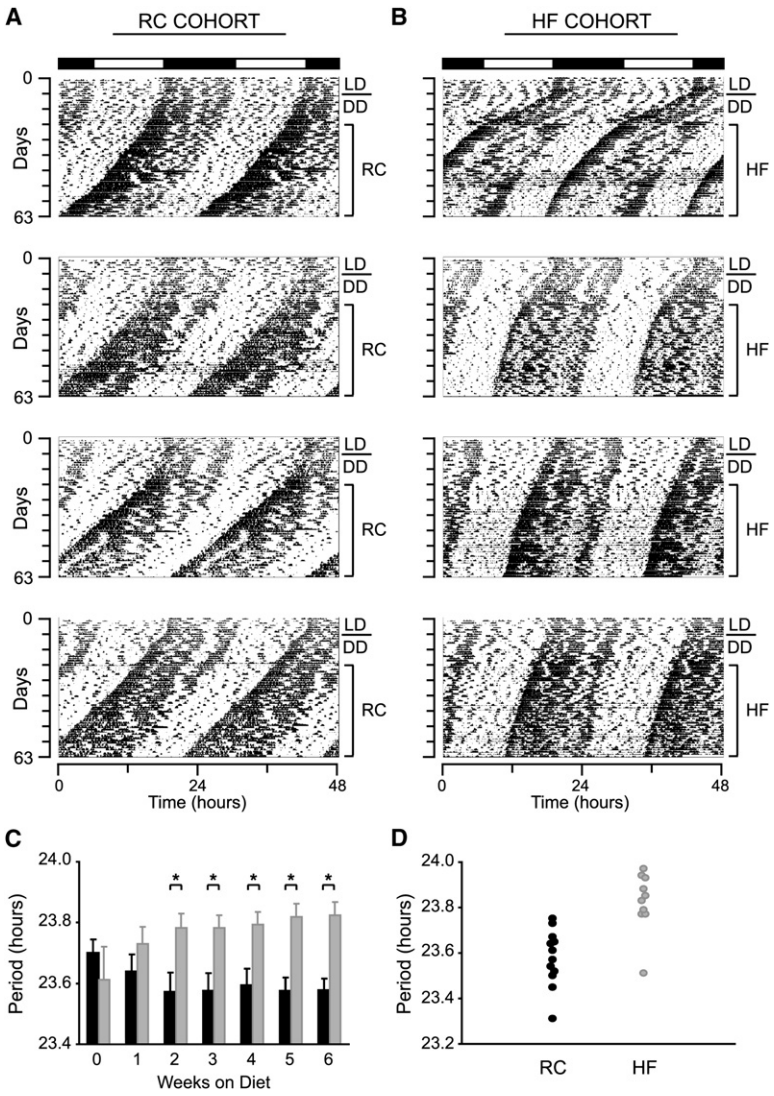

Figure 1. High-Fat Diet Lengthens the Free-Running Period in Mice

(A and B) Four representative locomotor activity records from mice fed regular chow (RC) (A) or a high-fat (HF) diet (B). Activity counts are indicated by the vertical black marks in the activity record. The records are double plotted so that each day's record is presented both to the right of and beneath that of the previous day. Mice were maintained on a 12:12 light/dark (LD) cycle (white and black horizontal bars above the records) for the first 7 days and then transferred to constant darkness (DD) on the day indicated by a horizontal line at the right margin. Fourteen days after the onset of DD, one group of mice was maintained on RC, whereas the other group was switched to a HF diet.

(C) Weekly comparison of the free-running period in mice fed RC (black bars, n = 12) and a HF diet (gray bars, n = 10). Note that week 0 represents the second week of DD, when all animals were still receiving RC. Results are expressed as means ± SEM (*$p < 0.05$).

(D) Distribution of the free-running period during week 6 in individual animals on RC and HF diets.

High-Fat Diet Lengthens Circadian Period

Mice fed a regular chow (RC) diet were maintained on a 12:12 LD cycle for 1 week before being transferred to constant darkness (DD) for 2 weeks (Figure 1). RC-fed mice showed a characteristic free-running rhythm of activity of ~23.6 hr (Figures 1A and 1C), whereas mice switched to a high-fat (HF) chow showed an increase in the free-running period as early as 1 week following the start of the calorie-dense chow (Figures 1B and 1C). This effect persisted throughout weeks 2–6 on the diet (Figures 1B and 1C) and was consistent across individual animals (Figure 1D). Neither the amplitude of the circadian rhythm nor the overall activity counts differed between the RC-fed and HF-fed groups (see Figures S1A and S1B in the Supplemental Data available with this article online). Interestingly, whereas period lengthening occurred after just 1 week on the HF diet, differences in mean body weight did not occur until the third week of HF feeding; thus, the increase in period length was independent of body weight (Figure S1C). Furthermore, there was no significant correlation between body weight change and period change for any week of the study comparing RC-fed and HF-fed groups (Pearson correlation; data not shown).

High-Fat Diet Attenuates the Diurnal Pattern of Feeding Behavior

We next examined the effect of a HF diet on the diurnal rhythm of food intake and locomotor activity in animals under entrained 12:12 LD conditions. Following acclimation to food- and activity-monitoring cages for 3 weeks, half of the animals were switched to a HF diet, while the other half remained on RC for 6 weeks. As expected, the RC-fed animals exhibited a robust diurnal rhythm of food intake and locomotor activity throughout the entire experiment, with most activity and feeding (~80%) occurring during the dark period (Figure 2).

Even though the HF-fed mice exhibited an overall diurnal rhythm in feeding, attenuation in the rhythm of this

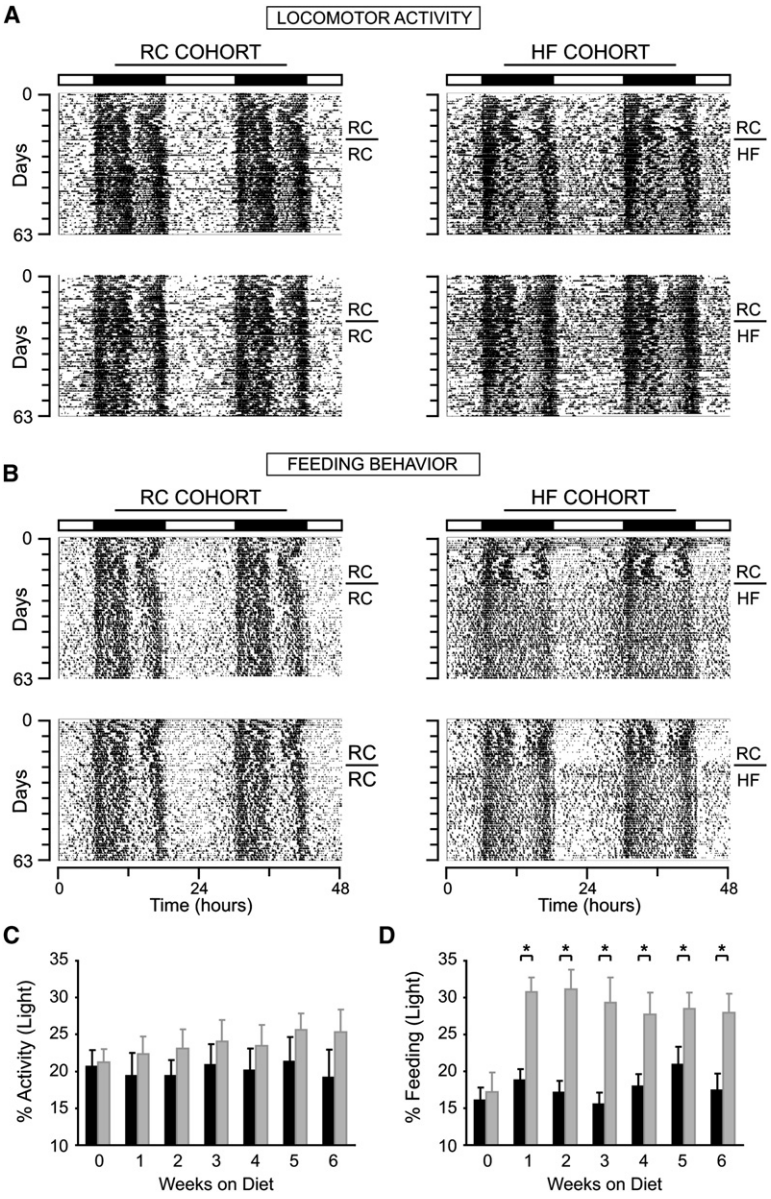

Figure 2. High-Fat Diet Attenuates Diurnal Rhythms of Feeding and Locomotor Activity in Mice

(A and B) Locomotor (A) and feeding (B) activities were recorded simultaneously in 6 min increments in each animal. Two representative animals from the RC-fed (left column) and HF-fed (right column) groups are shown. All animals were fed RC for the first 21 days and were then either maintained on RC or switched to a HF diet starting on the day indicated by a horizontal line at the right margin. All animals were maintained on a 12:12 LD cycle and were provided food and water ad libitum.

(C and D) Weekly comparisons were made for the diurnal rhythm (% of total activity occurring in the light period) of locomotor activity (C) and feeding behavior (D) in mice fed either RC (black bars, n = 6) or HF diet (gray bars, n = 7). Note that week 0 represents days 15–21 on RC and was used as the baseline measurement for each group. Results are expressed as means ± SEM (*$p < 0.05$).

behavior was observed (Figures 2B and 2D). As early as the first week on the HF diet, mice consumed a higher percentage of daily food intake during the rest (light) period (Figure 2D). The altered diurnal rhythm of food intake in HF-fed mice was due to an increase in the amount (in grams) of food consumed in the light period and a decrease in the dark period (Figure S2A). Interestingly, the change in feeding rhythm in HF-fed mice occurred well before the onset of significant weight gain (Figures S2A and S2C).

In parallel with the changes in feeding behavior, HF-fed animals had a dampened diurnal rhythm of locomotor activity compared to RC-fed animals (Figure 2C). While not statistically significant at any given week, the HF-fed group consistently showed a higher percent of total activity during the light period (Figure 2C). With respect to absolute activity levels, a reduction in total activity counts first appeared during the dark period on the third week of HF feeding, whereas no absolute difference in activity was observed during the light period between HF-fed and RC-fed groups (Figure S2B). It is also interesting that the reduction in overall activity counts observed in the HF-fed mice was selective for LD conditions since we observed equivalent levels of activity and preserved amplitude in activity rhythms between RC-fed and HF-fed mice under DD conditions (Figures S1A and S1B).

Effect of High-Fat Diet on Expression of Core Clock Genes

To test the hypothesis that a HF diet affects clock gene expression, we analyzed the diurnal expression of transcripts encoding CLOCK, BMAL1, and PER2 in the

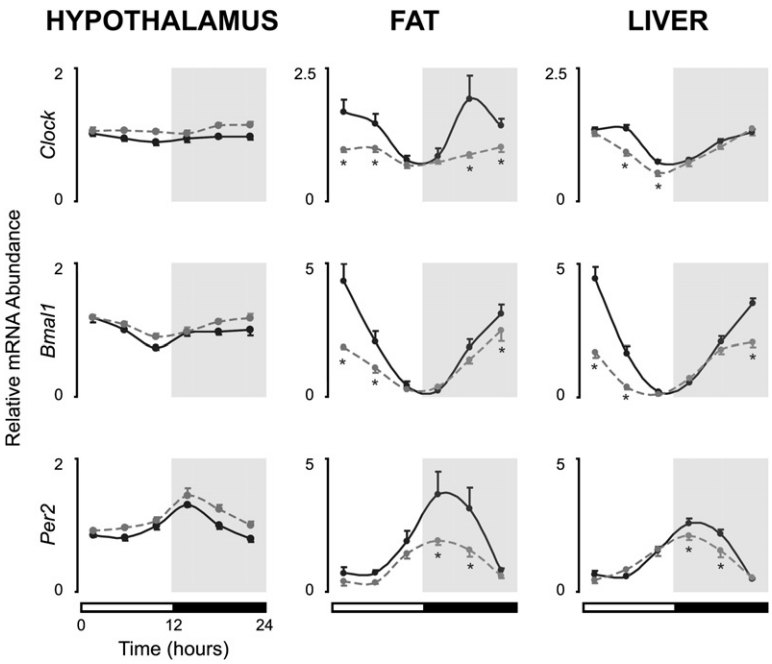

Figure 3. High-Fat Diet Attenuates Amplitude of Clock Gene Expression

Transcripts of the core circadian clock genes *Clock*, *Bmal1*, and *Per2* in the mediobasal hypothalamus, fat, and liver were analyzed by real-time PCR. Tissues were harvested every 4 hr from mice fed either RC (black lines) or HF diet (gray dotted lines) for 6 weeks; values are displayed as relative abundance (mean ± SEM) after normalization to *Gapdh* (*$p < 0.05$ between groups at each time point).

mediobasal hypothalamus (MBH), fat, and liver after 6 weeks on RC or HF diet in a cohort of mice independent from those represented in Figure 1 and Figure 2. *Clock* RNA expression lacked a diurnal rhythm in the MBH (Figure 3), consistent with previous findings in other CNS regions, including the SCN (Shearman et al., 2000). However, in both RC-fed and HF-fed groups, there was clear cycling of both *Bmal1* and *Per2* RNAs in the MBH, and the HF diet did not affect expression or rhythmicity of these core clock genes.

In contrast to the MBH, diurnal rhythmicity in *Clock* gene expression was observed in both fat and liver in the RC-fed mice (Figure 3). In response to HF diet, the amplitude of *Clock* rhythm in fat tissue was severely attenuated, with a smaller effect in liver. In both fat and liver, the amplitude of *Bmal1* expression was reduced in both the light and dark periods, whereas *Per2* expression was decreased selectively during the dark period. Collectively, these findings reveal that HF diet generates both tissue- and gene-specific changes in expression levels of circadian clock genes.

High-Fat Diet Alters Circadian Regulation of Humoral and CNS Metabolic Systems

We analyzed the effects of HF diet on the 24 hr profiles of leptin, glucose, insulin, free fatty acids (FFA), and corticosterone (Figure 4A). All five of these metabolic parameters were altered in animals on the HF diet. In particular, changes included (1) increased levels of leptin and glucose during both the light and dark periods, (2) increased insulin and FFA levels during the dark period, and (3) decreased amplitude of the corticosterone rhythm. Interestingly, each marker displayed changes not only in absolute expression but also in the temporal pattern of expression.

We also examined the effects of a HF diet on the 24 hr expression profiles of RNAs encoding the primary orexigenic and anorexigenic neuropeptides within the MBH (Figure 4B). As anticipated under diet-induced obesity conditions, the overall levels of transcripts encoding AGRP and NPY were reduced (Chavez et al., 1998), whereas RNAs encoding POMC and CART were increased. Interestingly, we also observed a dampened diurnal rhythm in *Orx* gene expression in HF-fed animals due to decreased *Orx* levels toward the end of the light period in anticipation of the onset of activity.

High-Fat Diet Impairs Circadian Regulation of Nuclear Receptor Networks

To further explore the impact of HF feeding on the temporal regulation of transcription networks, we also studied 24 hr patterns of the expression of nuclear hormone receptors (NRs) that control the expression and function of core clock genes (Figure 4C) (McNamara et al., 2001; Sato et al., 2004; Canaple et al., 2006; Fontaine et al., 2003). In RC-fed mice, levels of *Rev-erbα* displayed diurnal variation in both fat and liver, with a peak during the light period and low levels throughout the dark period (Figure 4C). Similar time-dependent cycling was observed for levels of *Rorα*, *Rxrα*, *Pparα*, and *Pparγ* in both fat and liver in RC-fed mice, although the patterns of expression were different between the two tissues (Figure 4C).

In fat tissue, levels of expression of *Rorα*, *Rxrα*, and *Pparγ* were decreased in HF-fed compared to RC-fed mice, resulting in attenuated diurnal variation in each of these transcripts (Figure 4C, left panel). Interestingly, reduced levels of expression of the lipogenic NRs *Rxrα* and *Pparγ* during the dark period (Figure 4C) corresponded with the observed increase in circulating levels of FFA during the dark period (Figure 4A). The diurnal rhythm of *Rev-erbα* was largely preserved after HF feeding; however, expression levels were reduced in the

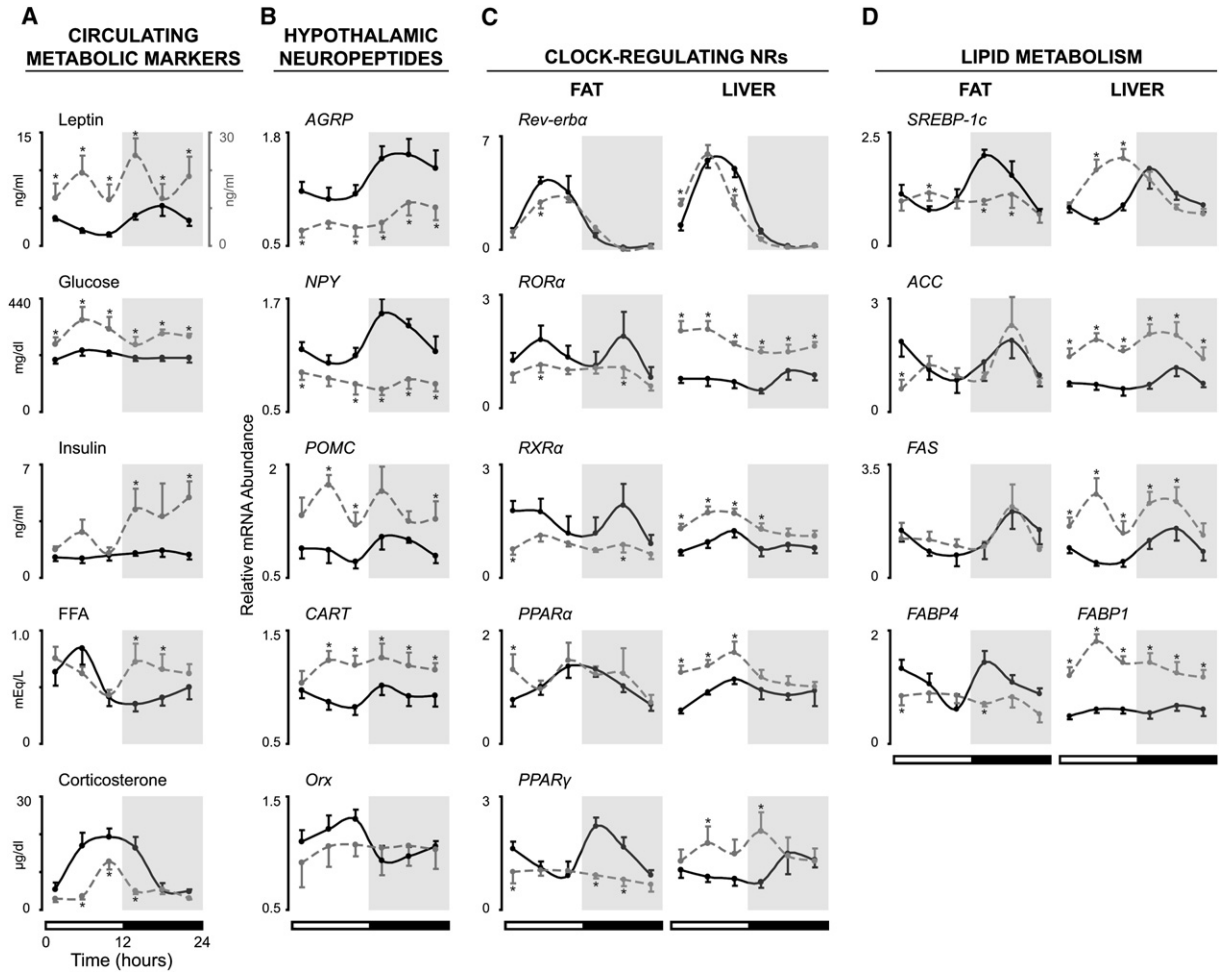

Figure 4. High-Fat Diet Alters Diurnal Patterns of Metabolic Markers and Transcription Networks

Mice were maintained on a 12:12 LD cycle and fed either RC (black lines) or HF diet (gray dotted lines) ad libitum for 6 weeks (n = 6–8 per group per time point).

(A) Diurnal variation in serum leptin, glucose, insulin, free fatty acid (FFA), and corticosterone levels.

(B) Mediobasal hypothalamus sections were used to measure the mRNA expression levels of orexigenic (*Agrp* and *Npy*) and anorexigenic (*Pomc* and *Cart*) neuropeptides, as well as orexin (*Orx*) transcript levels.

(C) The diurnal distribution of various circadian clock gene-regulating nuclear receptors (NRs; *Rev-erbα*, *Rorα*, *Rxrα*, *Pparα*, and *Pparγ*) was measured in fat and liver.

(D) Transcripts involved in lipid metabolism (*Srebp-1c*, *Acc*, *Fas*, and *Fabp*) were measured in fat and liver.

Results are expressed as means ± SEM (*p < 0.05).

middle of the light period. The HF diet led to a variable pattern of *Pparα* expression across the LD cycle, and there was a significant increase above RC levels at the beginning of the light period. HF-fed mice also displayed an increase in the hepatic expression of *Rorα*, *Rxrα*, *Pparα*, and *Pparγ* across multiple time points in both the light and dark periods (Figure 4C, right panel). Elevation of *Rorα*, *Rxrα*, *Pparα*, and *Pparγ* during the light period also corresponded with increased activity and feeding during this time period in HF-fed mice.

We also examined the expression of RNAs controlling downstream metabolic pathways that are known to cycle within adipose tissue and liver (Figure 4D; Figure S3) (Brewer et al., 2005; Yang et al., 2006; Horton et al., 2002). In fat tissue from RC-fed mice, *Srebp-1c* RNA levels displayed a diurnal rhythm of expression with a peak in the first part of the dark period. Similar patterns were observed for the downstream lipogenic genes *Acc*, *Fas*, and *Fabp4* (fatty acid binding protein 4) (Figure 4D, left panels). In contrast, in fat tissue from HF-fed mice, the nighttime peak in *Srebp-1c* was decreased, and there was no longer a diurnal pattern in its expression (Figure 4D). Although there was not a significant difference in the expression of *Acc* and *Fas* in HF-fed mice, *Fabp4* levels were decreased at the beginning of the light and dark periods (Figure 4D).

In the liver of HF-fed mice, we observed several changes in the diurnal pattern of expression of lipogenic

genes, including a shift in the peak of *Srebp-1c* from the dark period to the light period and an increase in *Acc*, *Fas*, and *Fabp1* at virtually all times during the day and night (Figure 4D, right panels). In addition, we found that levels of *Lxrα*, *Lxrβ*, and *Rarα*, three cycling NRs involved in regulation of hepatic carbohydrate and lipid turnover (Kalaany and Mangelsdorf, 2006), were significantly higher across the entire LD cycle in liver, but not fat, of HF-fed animals (Figure S3).

Analysis of RNA expression of both clock-interacting nuclear receptors (Figure 4C) and downstream metabolic pathway regulators (Figure 4D) also revealed changes in the synchronization of gene expression in liver and fat following HF feeding. For example, in RC-fed mice, we found that levels of *Pparγ*, *Srebp-1c*, *Acc*, and *Fas* each peaked at the same time of day in both fat and liver (Figures 4C and 4D). However, in HF-fed animals, expression of each of these factors was no longer synchronous in liver and fat.

DISCUSSION

Interrelationship between Nutritional Status and Circadian Synchrony

Our observation that HF diet alters the period of the central clock under free-running unentrained conditions indicates that changes in energy homeostasis must either directly or indirectly affect the molecular machinery of the clock. Importantly, we did not find a correlation between the change in body weight and change in period length in individual animals, indicating that the circadian effect was not simply due to body mass. Mice fed a HF diet consumed more food during the light period and less food during the dark period, resulting in an attenuated diurnal rhythm of food intake. It is intriguing that the extra calorie intake on the HF diet occurred exclusively during the light period, a time when mice normally eat very little. Disorganization in the feeding rhythm may contribute to alterations in body weight regulation under high-calorie conditions. Indeed, other studies have demonstrated that genetically obese animals exhibit attenuated diurnal feeding rhythms and that, if food availability is restricted to the dark period, these animals gain less weight despite eating the same amount of food (Masaki et al., 2004). In our HF-fed mice, the diurnal rhythm in locomotor activity was dampened, but this was much less pronounced than for feeding. The increase in food intake during the light period was not accompanied by increased activity (or activity-related energy expenditure). It should be noted that the effects of the HF diet on circadian clock gene expression may be gender-specific since recent studies in female mice have not demonstrated the same magnitude of effects on clock gene expression (Yanagihara et al., 2006).

Impact of Nutrient Excess on Circadian Synchrony of Clock Output Pathways

Our results corroborate several observations of nuclear receptor cycling in liver versus fat (Yang et al., 2006) and provide additional evidence that metabolic cycles affect diurnal cycles. A corollary of these findings is that not only do conditions commonly associated with diabetes and obesity result in altered rhythms of metabolic pathways, dysregulation of metabolism in turn leads to alterations in circadian cycles. For example, in RC-fed mice, we observed 24 hr oscillations in fat of two sterol-sensing NRs (*Rorα* and *Rxrα*) that interact with *Bmal1*, whereas in HF-fed mice, the circadian variation of *Rorα* and *Rxrα* was reduced in fat. Of note, we also observed that selective loss of the positive regulator *Rorα* in fat was in the same direction as the observed decrease in *Bmal1* expression (Figure 3 and Figure 4C). It is striking that the direction of change in NR receptor expression in HF-fed animals differed in liver and fat tissue. For example, whereas we found that hepatic expression of *Srebp-1c* was shifted to an earlier time of day in liver, we found that *Srebp-1c* was markedly decreased during the nighttime in fat. An intriguing question is whether more frequent sampling would reveal effects on circadian phase for both clock genes and clock-controlled genes (e.g., those suggested by the subtle shift in *Srebp-1c* gene expression in Figure 4D in the liver of HF-fed mice compared to RC-fed mice).

It is possible that local physiologic factors within each tissue may influence the cycling and expression of RNAs encoding both the core clock components and nuclear receptors involved in regulation of clock gene expression. Of note, work by McKnight and colleagues (Rutter et al., 2001) has suggested that the redox state itself may act as a nutrient sensor and may link cell metabolism with the transcriptional activity of CLOCK and its homolog NPAS2.

Circadian Gene Function in Metabolic Homeostasis

Genetic models have also indicated a close association between the molecular events underlying metabolism and those involved in the generation of circadian rhythms. For example, animals with mutations in the *Clock* gene become overweight on a HF diet and have features associated with insulin resistance at the level of fat on the C57BL/6J background (Turek et al., 2005). Furthermore, the *Clock* mutation introgressed onto C57BL/6J *ob/ob* mice also results in exaggerated adiposity (Oishi et al., 2006b). On the ICR background, the *Clock* gene mutation disrupts lipid absorption and, as a consequence, attenuates the diet-induced obesity observed in the C57BL/6J background (Oishi et al., 2006a). Clock genes have been further associated with the pathophysiology of the metabolic syndrome since deficiency of *Bmal1* impairs adipogenesis (Shimba et al., 2005; Wang et al., 2006). Ablation of *Bmal1* also leads to arrhythmicity, myopathy, arthropathy, and altered hepatic carbohydrate metabolism (Bunger et al., 2005; Kondratov et al., 2006; Rudic et al., 2004). Thus, CLOCK, BMAL1, and other core clock transcription factors function within both brain and peripheral tissues to affect metabolic physiology. While these results indicate that mutations in factors involved in circadian control increase susceptibility to metabolic disease, the

results of the present studies using diet-induced obesity suggest that nutrient state affects the control of the molecular circadian clock. Detailed analysis of temporal changes induced by nutrient excess may provide insight into the onset and progression of disorders such as obesity, diabetes, and sleep and circadian disruption.

EXPERIMENTAL PROCEDURES

Animals

Male C57BL/6J mice were purchased from the Jackson Laboratory and maintained at the Center for Comparative Medicine at Northwestern University. All animal care and use procedures were in accordance with guidelines of the Northwestern University Institutional Animal Care and Use Committee.

Behavioral Analyses

Three-week-old mice were placed in standard mouse cages equipped with infrared sensors to detect locomotor activity. For the first week of activity recording, mice were maintained on a 12:12 LD cycle and fed regular chow (RC; 16% kcal from fat, 27% kcal from protein, and 57% kcal from carbohydrate; 7012, Harlan Teklad) diet. Starting at 4 weeks of age, animals were maintained in constant darkness (DD) for 2 weeks and then fed either RC (n = 12) or a high-fat (HF; 45% kcal from fat, 20% kcal from protein, and 35% kcal from carbohydrate; D12451, Research Diets, Inc.; n = 10) diet for 6 weeks. The free-running period was calculated as the duration of time between the major activity periods on consecutive days. The amplitude of the locomotor activity rhythm was determined by fast Fourier transformation (FFT) analysis. Total activity counts were quantified as the total number of infrared sensor beam breaks (The Chronobiology Kit, Stanford Software Systems).

For analyses under entrained 12:12 LD conditions, 3-week-old mice were placed in combined feeding and activity monitors, provided regular chow ad libitum for 3 weeks, and then fed either RC (n = 6) or HF (n = 7) diet for 6 weeks. Food intake (Feed-Scale, Columbus Instruments) and locomotor activity were continuously monitored throughout the experiment.

Tissue and Blood Collection and Serum Analysis

All mice were maintained on a 12:12 LD cycle with free access to RC diet and water and were provided at 6 weeks of age with either RC or HF diet for 6 additional weeks. Mice were then sacrificed at 4 hr intervals across the LD cycle to obtain tissue (mediobasal hypothalamus, liver, and epididymal fat) and blood samples as previously described (Turek et al., 2005). Serum insulin and leptin levels were determined by ELISA (Crystal Chem Inc.), and glucose (Analox Instruments USA), free fatty acid (Wako NEFA C microtiter procedure), and corticosterone (MP Biomedicals, LLC) levels were determined according to the manufacturers' instructions.

RNA Extraction and Quantitative Real-Time PCR

Total RNA was extracted from frozen tissue with TRI Reagent (Molecular Research Center, Inc.), and real-time PCR was performed and analyzed using an Applied Biosystems 7900HT Fast Real-Time PCR System with 1× SYBR green master mix (Applied Biosystems) and 1 μM primers (see sequences in Table S1). PCR conditions were 10 min at 95°C followed by 35 cycles of 10 s at 95°C, 15 s at 60°C. Relative expression levels were determined using the comparative C_T method to normalize target gene mRNA to *Gapdh*.

Statistical Analysis

All results are expressed as means ± SEM. Data were analyzed by unpaired two-tailed Student's t test (body weight and behavioral data) or two-way analysis of variance (ANOVA) (time of day × diet) followed by Scheffe's post hoc tests (time course data of transcripts and serum metabolic parameters). $p < 0.05$ was considered to be statistically significant.

Supplemental Data

Supplemental Data include three figures and one table and can be found with this article online at http://www.cellmetabolism.org/cgi/content/full/6/5/414/DC1/.

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