Circadian Rhythms, Metabolism, and Chrononutrition in Rodents and Humans[1–3]

Jonathan D Johnston,[4]* José M Ordovás,[5,6] Frank A Scheer,[7,8] and Fred W Turek[9]

[4]University of Surrey, Guildford, Surrey, United Kingdom; [5]Tufts University, Boston, MA; [6]Madrid Institutes of Advanced Studies-Food, Madrid, Spain; [7]Brigham and Women's Hospital, Boston, MA; [8]Harvard Medical School, Boston, MA; and [9]Northwestern University, Evanston, IL

ABSTRACT

Chrononutrition is an emerging discipline that builds on the intimate relation between endogenous circadian (24-h) rhythms and metabolism. Circadian regulation of metabolic function can be observed from the level of intracellular biochemistry to whole-organism physiology and even postprandial responses. Recent work has elucidated the metabolic roles of circadian clocks in key metabolic tissues, including liver, pancreas, white adipose, and skeletal muscle. For example, tissue-specific clock disruption in a single peripheral organ can cause obesity or disruption of whole-organism glucose homeostasis. This review explains mechanistic insights gained from transgenic animal studies and how these data are being translated into the study of human genetics and physiology. The principles of chrononutrition have already been demonstrated to improve human weight loss and are likely to benefit the health of individuals with metabolic disease, as well as of the general population. *Adv Nutr* 2016;7:399–406.

Keywords: dietary patterns, eating behavior, obesity, chronobiology, genetics, meal timing, misalignment, clock gene, diabetes, metabolic syndrome

Introduction to the Mammalian Circadian System

Circadian rhythms are found throughout the living world (1). They are generated by clocks that are endogenous in nature and oscillate even in the absence of environmental cues. Circadian clocks influence a broad range of biological processes, including neuronal, endocrine, metabolic, and behavioral function. This breadth of physiologic influence can be clearly demonstrated in humans maintained in laboratory conditions without 24-h rhythms in environmental conditions and behaviors, including maintaining constant room temperature, dim light, wakefulness, posture, rest, wakefulness, and equally spaced isocaloric snacks. These human volunteers exhibit circadian rhythms in hormone concentration (e.g., melatonin, varying between ~0 and 50 pM; cortisol, varying between ~100 and 400 nM), plasma lipid concentration, core body temperature, plasma glucose concentrations, heart rate, autonomic nervous system activity, blood pressure, subjective alertness, and objective reaction time, independent of the sleep/wake and fasting/feeding cycles (2). This ability of organisms to temporally regulate diverse biological functions enables them to maximize their ability to cope with and anticipate predictable 24-h changes in the environment.

In mammals, the suprachiasmatic nuclei (SCN)[10] of the hypothalamus are known to play a key role in circadian rhythm generation. The SCN express a robust circadian rhythm of electrophysiologic activity, even when isolated from the rest of the brain (3, 4). Classic neuronal lesion studies demonstrated the importance of intact SCN for behavioral rhythmicity (5, 6). Powerful evidence of the SCN's importance in circadian function later came from studies in which fetal SCN tissue was transplanted into the hypothalamus of SCN-lesioned animals. Not only did these transplants restore behavioral rhythms, but the circadian period of the recipient also was determined by the period of the donor animal and not the previous period of the host (7).

[1] This article is a review from the symposium "Is 'When' We Eat as Important as 'What' We Eat? Chronobiological Aspects of Food Intake," held 31 March 2015 at the ASN Scientific Sessions and Annual Meeting at Experimental Biology 2015 in Boston, MA. The symposium was sponsored by the American Society for Nutrition (ASN) Energy and Macronutrient Metabolism Research Interest Section (RIS), the ASN Nutrition Translation RIS, and supported by Kellogg Company and PepsiCo, Inc.

[2] Supported in part by the United Kingdom Biotechnology and Biological Sciences Research Council (grant BB/I008470/1), contracts 53-K06-5–10 and 58-1950-9-001 from the USDA, and grants R01-HL118601 and R01-DK099512 from the NIH.

[3] Author disclosures: JD Johnston, JM Ordovás, FA Scheer, and FW Turek, no conflicts of interest.

*To whom correspondence should be addressed. E-mail: j.johnston@surrey.ac.uk.

[10] Abbreviations used: *Cry*, *Cryptochrome*; GOLDN, Genetics of Lipid Lowering Drugs and Diet Network; MetS, metabolic syndrome; *Per*, *Period*; SCN, suprachiasmatic nuclei; *SIRT1*, *Sirtuin*; SNP, single-nucleotide polymorphism; TRF, time-restricted feeding; TTFL, transcription translation feedback loop.

It is now recognized that large multicellular organisms contain numerous clocks found within all major tissues and possibly within most individual cells of the body. Circadian rhythmicity is thus generated by complex networks of circadian clocks, referred to as the circadian timing system. The mammalian circadian system comprises the SCN and "peripheral clocks" located both within other brain regions and outside the brain. These peripheral clocks drive local tissue-specific processes, as demonstrated by both in vitro studies and the characterization of transgenic mice that lack functional clocks in a given tissue or cell type. For example, the hepatic clock regulates fasting glycemic control and glucose clearance (8), the pancreatic clock regulates insulin secretion and its response to glucose (9–11), the adipose clock regulates lipid storage and mobilization (12, 13), and the skeletal muscle clock regulates glucose uptake and metabolism (14).

In order for clocks to benefit an organism, they must be synchronized to each other and the outside environment. This synchronization (termed entrainment) is caused by factors external to a given clock, called zeitgebers. For most terrestrial organisms, the key zeitgeber is light. In mammals, light detection requires the retina and occurs via melanopsin-positive retinal ganglion cells working in conjunction with the rod and cone cells (15). This photic information travels via the retinohypothalamic tract to regulate a specific group of retinorecipient cells within the SCN. The SCN maintain coordination of peripheral clocks via a range of different pathways. Tone of the autonomic nervous system, secretion of hormones such as melatonin and cortisol, and core body temperature exhibit SCN-driven rhythms that can synchronize peripheral clocks (16). Furthermore, the SCN influence sleep-wake cycles that themselves influence circadian rhythmicity (17) and partially dictate feed-fast behavior, which is believed to be a key synchronizer of peripheral clocks (see below). Recent evidence also suggests that peripheral clocks may synchronize to photic cues in the absence of a functional molecular clock in the SCN (18), although the mechanisms involved are not yet clear.

Molecular Basis of Circadian Rhythms

Work in multiple species has led to the development of a transcriptional-translational feedback loop (TTFL) molecular model of circadian rhythms. The mammalian TTFL model consists of multiple interlocking loops that are described in detail elsewhere (19). At the center of this model is a primary loop in which transcription factors CLOCK and BMAL1 stimulate the transcription of 3 Period (*Per*) genes and 2 Cryptochrome (*Cry*) genes. The translated PER and CRY proteins then form protein complexes that translocate into the nucleus and repress the transcriptional activation of their own genes by CLOCK and BMAL1. The precise temporal dynamics of this loop are regulated by posttranscriptional and posttranslational modifications (19, 20).

Interlinked with the primary loop are multiple secondary loops, many of which involve important biochemical components of cellular metabolism. The best characterized of these secondary loops involves the circadian transcription of the nuclear receptor *Rev-erbα* (*Nr1d1*) by CLOCK-BMAL1 dimers acting through E-box regulatory elements; the resulting REVERBα/NR1D1 protein then inhibits *Bmal1* transcription through retinoic acid-related orphan receptor response elements, resulting in circadian rhythms of *Bmal1* mRNA expression (21). Additional loops involving nuclear receptors have been reported, for example, the interaction between clock genes and PPARα in mouse liver (22). More recently, redox-sensing molecules have been linked to the circadian clock machinery. For example, CLOCK-BMAL1 heterodimers drive rhythmic expression of nicotinamide phosphoribosyltransferase, which is a rate-limiting enzyme involved in the NAD+ salvage pathway; NAD+ acts as a cofactor for Sirtuin (SIRT1), which regulates the activity of CLOCK-BMAL1 among its other metabolic functions (23, 24). In addition, cellular redox state and core signaling pathways are also closely interlinked with the TTFL model (25, 26).

Soon after the discovery of mammalian clock genes came the identification of rhythmic clock output genes, called clock-controlled genes. Early work focused on individual output genes that are transcriptionally regulated by core clock proteins (27, 28). Soon afterward, multiple groups used microarray technology to identify the extent of circadian rhythmicity within the transcriptome (29). Although there is some variability between studies and methods, it is generally accepted that ~10% of the transcriptome in any given mouse tissue is under circadian control. Many of these genes are rhythmic in a tissue-specific context, and recent analysis of mouse transcriptome in 12 organs revealed that 43% of all protein coding genes exhibit circadian rhythmicity in one or more tissues (30). Analysis of multiple human and mouse transcriptomic data sets highlights the close linkage between circadian rhythms and metabolic gene expression (17). Moreover, the manipulation of sleep in studies of human circadian transcriptomics has enabled identification of genes involved in metabolism, cancer, transcription, and translation that are regulated by sleep timing per se (17, 31, 32).

Other research has extended our understanding of molecular rhythmicity by employing approaches such as proteomic and metabolomic analyses to samples collected across circadian cycles. Similar to the reported proportion of rhythmic transcripts, it has been estimated that 6–20% of the murine proteome exhibits circadian rhythms in tissues such as the SCN (33, 34) and liver (35–37). Some of these studies have reported inconsistent rhythmicity in transcripts and proteins derived from the same gene, a feature that is likely to be explained by rhythms in posttranscriptional mechanisms such as degradation rate (38). Daily rhythms in the metabolome have been described in mouse blood (39) and tissue samples (40–42). Genetic disruption of the molecular clock also has profound effects on metabolite profiles (43), thus strengthening the functional links between clocks and the metabolome. In addition to these mouse studies, human metabolome analyses also reveal that up to 20% of detectable metabolites in matrices such as plasma and saliva exhibit daily rhythms (44–46). It is therefore clear that molecular rhythmicity occurs at multiple levels of organization, from genes to protein and metabolites, in both rodents and humans.

Transgenic Animal Models for Studying Interactions between Circadian Rhythms and Metabolism

Most of the mammalian studies that have been performed to examine how the disruption of normal circadian rhythmicity affects metabolic function have been carried out in mice. In general, 2 different approaches have been taken to elucidate if disrupting the circadian clock system has an impact on metabolism, in particular body weight and glucose metabolism: *1*) a genetic approach whereby core circadian clock genes have been mutated or deleted and *2*) an environmental approach that involves abnormal patterns of exposure to light-dark cycles or the timing of food availability. In this section, we discuss only one genetic model to demonstrate how body weight is in part regulated by circadian signals.

The primary genetic model for examining linkage between the circadian and metabolic systems has been the *Clock* mutant mouse, in which the core circadian gene, *Clock*, is mutated such that the animal's endogenous circadian period is lengthened (47). In an initial report, *Clock* mutant mice were fed a high-fat diet and observed to develop obesity at a young age, as well as a variety of metabolic and endocrine abnormalities consistent with the metabolic syndrome (MetS) (e.g., hyperphagia and obesity, hyperleptinemia, hyperlipidemia, hyperglycemia). In addition, the normal diurnal feeding rhythms present in mice were significantly blunted in the mutant mice: on a standard laboratory 12-h light:12-h dark cycle, (nocturnal) mice typically consume ~75–80% of their total daily calories during the dark phase; in contrast, *Clock* mutant mice consume ~50% during the dark phase (48). The *Clock* mutants were also shown to exhibit reduced overall expression levels and a blunted diurnal rhythm of orexin mRNA, a hypothalamic neuropeptide involved in energy regulation (49).

A striking feature of the metabolic phenotype of the *Clock* mutant mice was the presence of hyperglycemia and hypoinsulinemia, a pattern suggestive of a defect along the insulin axis. Examination of isolated pancreatic islets, containing the insulin-secreting β cells, from *Clock* mutant mice, as well as from mice carrying a null mutation of *Bmal1*, revealed profound defects in insulin secretion, both at basal levels and in response to glucose stimulation, compared with wild-type mice (9). Furthermore, mice lacking a functional circadian clock in the pancreatic islets were shown to develop diabetes at an early age due to insufficient insulin secretion (49).

The description of the first genetic evidence linking the circadian clock system to energy regulation and metabolism provided a critical step forward for the field, enabling a flurry of biochemical, genetic, molecular, and physiologic studies capable of addressing the underlying mechanisms and pathways (49). There are of course both strengths and weaknesses to using animal models to study circadian metabolism and chrononutrition. Weaknesses include the fact that most animal models are nocturnal and lack some of the psychologic and social complexity of human eating behavior. Nonetheless, animal models permit molecular and genetic manipulation and more invasive studies, combined with the ability to precisely control food intake and environmental conditions during long (weeks to months) experiments, whereas such long-term controlled laboratory studies in humans are very expensive and demanding. Taken together, results indicate profound metabolic dysfunction and energy imbalance in mice that have a genetically defective circadian clock.

Human Genetics Linking Circadian Rhythms and Metabolism

The influence of genetic variance in the regulation of circadian rhythmicity has been long known from animal models; however, its demonstration in humans did not take place until the 1990s, when Linkowski and colleagues (50) analyzed the 24-h profile of plasma cortisol in 11 monozygotic and 10 dizygotic pairs of normal male twins. Their analysis supports the notion that, despite the increased impact of the social environment, genetic factors still define a significant component of the human circadian rhythmicity. Since then, multiple studies have examined associations between clock genes, sleep, and neurologic disorders; however, it was not until recently that investigators started paying attention to the potential relation between clock genes and metabolism [e.g., Scott et al. (51) and Monteleone et al. (52)].

To understand the role between human clock genes and metabolism, we conducted a series of studies to evaluate the influence of clock genes on the MetS and its individual components (obesity, dysglycemia, dyslipidemia, and hypertension) using both observational and intervention approaches. Moreover, we also investigated the relation between clock genes and the success of nutritional and behavioral interventions aimed for weight loss. Initial observational studies focused on the relation between genetic variation at the *CLOCK* gene and MetS using as a test population of participants (n = ~1100) from the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) study (53). We investigated 5 selected *CLOCK* single-nucleotide polymorphisms (SNPs) (rs 4864548, rs4580704, rs1464490, rs1801260, and rs3749474), of which 3 (rs4580704, rs1801260, and rs3749474) yielded statistically significant associations with BMI (in kg/m^2), blood pressure, glycemic traits, and markers of dietary intake (red blood cell membrane fatty acids). One of these SNPs, rs4580704, appeared to be protective; minor allele carriers had a 46% lower risk of hypertension than did noncarriers. Similar findings were obtained for glycemic traits. However, when we investigated further the potential modulating effect of diet, we observed that the protective effect of the minor allele on insulin sensitivity was only present when MUFA intake was above the median (>13.2% of energy in this population). Conversely, the detrimental effect of the minor allele at the rs1801260 SNP on waist circumference was only found with SFA intakes above the median (>11.8% of energy in GOLDN). Another finding came from the analysis of the MUFA content of red blood cell membranes, particularly oleic acid, which was significantly different according to *CLOCK* genetic variants. Finally, we also observed significant associations with markers of chronic inflammation (i.e., IL-6). In a more recent study, we have shown associations between clock genes and blood pressure, supporting the importance of the circadian rhythm in cardiac physiology

(54). Moreover, we demonstrated that whereas temperature was associated with blood pressure, it did not modulate the associations with genetic markers in either whites or Hispanics. Next, we posed additional questions related to food intake and the role of cytokines (55). In this same population, we demonstrated an association between the *CLOCK* gene and energy intake, particularly for the rs3749474 SNP. Total energy intake and fat, protein, and carbohydrate intakes were significantly higher in minor allele carriers than in noncarriers. Moreover, all *CLOCK* SNPs were associated with plasma cytokine concentrations, in particular with those that were highly correlated with energy intake (i.e., monocyte chemotactic protein 1, IL-6, and adiponectin).

On the basis of the observational evidence related to obesity, cytokines, and energy intake obtained from the GOLDN study, we continued our queries in the context of interventional studies, which allowed us to investigate whether the *CLOCK* gene, as well as other clock genes, plays a significant role in the individual variability in response to a weight reduction program (56). For this purpose, we investigated the *CLOCK* SNPs listed above, in relation to weight loss in response to a behavioral weight reduction program based on the Mediterranean diet. Moreover, we investigated associations with baseline anthropometric and metabolic traits. Five hundred overweight/obese subjects were studied. The *CLOCK* rs1801260 SNP was associated with obesity at baseline and also affected weight loss. Patients with the variant allele (G) lost significantly less weight compared with wild type during the 28 wk of follow-up. The behavioral (i.e., sleep duration, eating patterns, and chronobiological features) and hormonal (plasma ghrelin and leptin concentrations) factors potentially responsible for this difference were further investigated in a larger population consisting of 1495 overweight/obese subjects (BMI: 25–40) aged 20–65 y who attended outpatient obesity clinics in Murcia in Spain (57). We detected an association between the CLOCK rs1801260 SNP and weight loss, which was particularly evident after 12–14 wk of treatment. Specifically, carriers of the minor C allele were more resistant to weight loss than TT individuals. In addition, our data show that compared with TT subjects, those carrying the minor C allele had statistically significant *1*) shorter sleep duration, *2*) higher plasma ghrelin concentrations, *3*) delayed breakfast time, *4*) evening preference, and *5*) less compliance with a Mediterranean diet pattern.

We also investigated whether some of the interactions observed for *CLOCK* genetic variants were also present for other clock genes. We analyzed interactions between the *CRY1* polymorphism, rs2287161, and carbohydrate intake on insulin resistance in 2 of the populations described above (GOLDN and Murcia) and showed consistently across populations that an increase in carbohydrate intake (percentage of energy) was associated with a significant increase in HOMA-IR and fasting insulin and a decrease in the quantitative insulin sensitivity check index only among homozygotes for the minor C allele (58). Another significant interaction, this time with *PER2*, was demonstrated in the LIPGENE Study (59) after analysis of the *PER2* SNPs rs934945 and rs2304672. In particular, the rs2304672 SNP interacted with plasma SFAs to modulate a series of plasma lipoprotein-related biomarkers. Carriers of the minor allele (G) with the highest SFA concentration (>median) had higher plasma triglyceride concentrations and higher triglyceride-rich lipoproteins-triglyceride than CC subjects. Similar findings were reported for plasma concentrations of apolipoprotein C-II, apolipoprotein C-III, and apolipoprotein B-48.

In terms of response to dietary and behavioral intervention, we investigated whether the *PER2* gene was associated with weight-loss success in the same patients tested above for the *CLOCK* gene (60). Our results show that *PER2* SNPs rs2304672 and rs4663302 were associated with abdominal obesity. Moreover, carriers of the minor allele at these *PER2* SNPs had a greater probability of dropping out from the weight-loss program as well as displaying extreme snacking, experiencing stress with dieting, eating when bored, and skipping breakfast than noncarriers. Therefore, *PER2*, like its counterpart *CLOCK*, is implicated in attrition in weight-loss treatment and may modulate eating behavior–related phenotypes.

The complex interplay between the clock genes was made evident from another study in which we examined the combined effect of the *CLOCK* and Sirtuin (*SIRT1*) genes (61). In this study, carried out in subjects attending the same outpatient obesity clinics, we analyzed the combined effects of the SIRT1-rs1467568 and the *CLOCK*-rs1801260 SNPs on the effectiveness of the weight-loss program and demonstrated that *SIRT1* and *CLOCK* SNPs have an additive effect on resistance to weight loss that could be driven by effects on chronotype, plasma ghrelin concentrations, and adherence to a Mediterranean diet.

An interesting finding between clock genes and type 2 diabetes mellitus was reported recently in a case (n = 302 type 2 diabetes mellitus) control (n = 300) design (62). A *PER3*, variable number tandem repeat polymorphism was investigated. This variable number tandem repeat consists of 2 alleles of 4 and 5 repeats. These authors found that those with the 5-repeats allele had a greater risk of type 2 diabetes mellitus compared with those carrying the 4-repeats allele.

The current literature therefore supports the notion that clock genes are significantly associated with different features of the MetS as well as diabetes. Moreover, significant interactions between clock genes and dietary factors modulate the expression of these traits. Finally, intervention studies have shown that the success of behavioral interventions aimed to lose weight may be predicted in part by genetic variability of clock genes.

Circadian Misalignment Is Detrimental to Human Metabolic Physiology

Shift work is a risk factor for diabetes, obesity, and cardiovascular disease (63–66). This effect cannot be fully explained by traditional risk factors. Important early studies have shown decreased glucose tolerance, a risk factor for diabetes, under simulated shift work conditions and in shift workers [e.g., Hampton et al. (67) and Lund et al. (68)]. However, these studies could not distinguish the separate influences of circadian

phase, circadian misalignment, and the behavioral cycle (including sleep/wake and fasting/feeding cycle). Human studies have shown that misalignment between the central circadian system (as assessed by melatonin and cortisol profiles) and the behavioral cycle, typical of shift workers, can cause physiologic changes that are risk factors for these adverse health consequences of shift work.

In an initial study, we showed that circadian misalignment causes decreased glucose tolerance, decreased concentrations of the satiety hormone leptin, and increased wake time blood pressure (69). The effects were already observed after just a few days of circadian misalignment. These findings suggest that circadian misalignment is a mechanism that could increase the risk of diabetes, obesity, and cardiovascular disease in shift workers if these effects are sustained chronically. A second study showed that a history of 3 wk of circadian misalignment plus sleep restriction caused a decrease in glucose tolerance and a decrease in insulin release, with recovery after a week of sleep extension (70). A third study showed that a history of 1 wk of circadian misalignment with sleep restriction resulted in a reduction of insulin sensitivity that was worse than that observed after 1 wk of the same degree of sleep restriction without circadian misalignment in male subjects (71). Recently we showed, using a realistic simulated night work protocol including several consecutive shifts of night work under room light intensity (90 lux), that circadian misalignment decreased glucose tolerance, independent of effects of the circadian phase and of the behavioral cycle (72). This study further supported the notion that the effects of circadian misalignment cannot be fully explained by disturbances in sleep and showed that these effects were sustained for several days upon repeated exposure. The links between circadian misalignment and metabolic dysfunction are discussed in more detail elsewhere [e.g., Dibner and Schibler (73) and Perelis et al. (74)].

Effects of Meal Timing on Circadian Synchronization and Metabolism

Although there has been a tremendous amount of interest in the role of clocks in regulating biochemical pathways and metabolic processes, less effort has been expended examining how metabolic inputs themselves influence the circadian system. It has been known for many decades that restriction of food availability to a short time window will cause the manifestation of behavioral and physiologic changes, including food anticipatory activity. The circadian nature of food anticipatory activity led to development of the concept of a food-entrainable oscillator (75, 76). The anatomic (77) and molecular (78, 79) nature of the food-entrainable oscillator is not yet well understood. However, progress has been made understanding the effects of timed feeding on circadian clocks in rodent tissues.

Under normal circumstances, gross fasting/feeding cycles are determined by sleep/wake behavior, which is influenced by the circadian system. However, by restricting the temporal availability of food, it is possible to experimentally determine the effect of meal timing on circadian rhythms in different parts of the body. In a key experiment, animals maintained under a 12-h:12-h light-dark cycle were allowed access to food only during the light or dark phase of each 24-h cycle for 8 d (80). Despite clock gene expression rhythms in the SCN retaining the same phase relation to the light-dark cycle, the expression of the same genes in the liver synchronized to mealtimes, generating rhythms that peaked ~12 h apart in the 2 locations. As reviewed elsewhere, it is now known that timed feeding regulates clock gene rhythms in most peripheral tissues and may also regulate SCN rhythmicity when combined with hypocaloric feeding paradigms (81, 82). Compared with rodent studies, very little is known about how timed feeding regulates the human circadian system.

In addition to studies of clock gene rhythms, a growing number of experiments are using controlled feeding regimens over multiple weeks to investigate changes in metabolic physiology. In an initial study, mice kept on a 12-h:12-h light-dark cycle and provided with a high-fat diet were fed exclusively during the dark phase (i.e., the "right" time of day for nocturnal mice) or the light phase (i.e., the "wrong" time of day for nocturnal mice) for 6 wk (83). The group fed during the light phase gained more weight, despite the absence of any significant differences in calorie intake or activity over the course of the experiment. This finding suggested that temporal rhythms in energy intake are relevant for energy balance, perhaps identifying approaches involving timed feeding schedules, designed according to the properties of the circadian clock system, as possible strategies for weight management and the treatment of obesity (49). Other experiments have reduced the daily duration of daily feeding behavior, which is often referred to as time-restricted feeding (TRF). Restriction of feeding to either the middle of the light (84) or dark (42) phase reduces body weight and improves markers of metabolic health in mice compared with ad libitum fed controls. Importantly, these effects occurred despite equivalent calorie consumption in the TRF and ad libitum groups. Later mouse studies have demonstrated benefits of TRF when using multiple variations of dietary and temporal control and also suggest that TRF can even stabilize or reverse the progression of metabolic diseases (85). Interestingly, the ability of TRF to prevent body weight gain and decelerate cardiac aging in *Drosophila* (86) indicates that it may represent a beneficial aspect of chrononutrition across species.

The role of feeding time on energy balance and metabolism is currently an active area of investigation. Although much work remains to be done, human studies already suggest that timed feeding may translate into a beneficial approach to enhance weight loss and glycemic control in humans (87–89). Energy balance is determined by the relation between energy intake and energy expenditure, with energy expenditure being the sum of basal metabolic rate, energy expenditure due to physical activity, and diet-induced thermogenesis. In the human studies mentioned above, there were no differences in self-reported physical activity level and caloric intake. As of now, the mechanism underlying the effect of the timing of caloric intake on weight-loss success is

unknown. To assess one potential mechanism, we recently tested whether the timing of food intake influences diet-induced thermogenesis (also known as the thermic effect of food, or the specific dynamic action of food) within the first 2 h after consumption of an identical test meal. In healthy research participants under highly controlled laboratory conditions, including fixed caloric intake, fixed sleep/wake cycles, and an absence of exercise, we found that early phase diet-induced thermogenesis was approximately twice as large in the morning at 0800 h compared with in the evening at 2000 h (90). This result is consistent with an earlier study that showed larger diet-induced thermogenesis assessed over 6 h in the morning (0900 h) compared with the afternoon (1700 h) and at night (0100 h) (91), although another study did not find a difference between morning and afternoon (92). Unique in our study, we could assess whether the morning-evening difference was due to the behavioral cycle or due to the endogenous circadian phase. Many behavioral/environmental differences between assessments performed in the morning (1 h after scheduled awakening) and evening (12 h later) theoretically could cause this morning-evening difference: the morning assessments were performed shortly after an 8-h sleep opportunity, supine posture, behavioral rest, and complete darkness, whereas the evening assessment was performed after extended wakefulness, upright body posture, behavioral activity, and exposure to room light intensity (90 lux). However, remarkably, we found no effect of the behavioral cycle but a strong effect of the endogenous circadian phase, with diet-induced thermogenesis again being approximately twice as large in the biological morning (0800 h) compared with the biological evening (2000 h), independent of the behavioral cycle. We found no difference in basal metabolic rate. Although diet-induced thermogenesis only contributes to ~10% of the daily energy expenditure, our data suggest that the influence of the circadian system on diet-induced thermogenesis could lead to decreased energy expenditure when the same meal is consumed late in the day compared with early in the day. Future controlled laboratory studies closely fixing or directly measuring caloric intake and assessing total energy expenditure across full 24-h periods in respiratory chambers are required to determine the combined effect of shifts in the timing of caloric intake on energy balance and weight regulation.

In conclusion, evidence to date suggests that chrononutrition may be an important tool, not only to enhance the metabolic health of the general population but also to benefit the health of particular population groups (e.g., shift workers) and treatment of certain metabolic diseases. Obesity and diabetes are prototypical examples of complex, polygenic diseases influenced by numerous environmental factors, including diet and physical activity, among others. As such, a variety of approaches and measures will likely be necessary for effective prevention and treatment. Given the growing body of evidence linking the circadian clock system to energy regulation and metabolic physiology, circadian organization emerges as a clinically significant factor that should be considered in the understanding of the pathophysiology of these diseases and in potential targeted strategies for treating them (49, 63, 81).

Although animal models will continue to be of importance to inform our understanding of this research field, methods described above indicate how complex protocols can be employed to dissociate circadian from behavioral factors in human physiology. Application of circadian genomics, transcriptomics, and metabolomics, coupled with the development of serial biopsy procedures (93, 94) and genetic analysis, will enable detailed molecular analysis of such experiments. Moreover, in vitro assays (95, 96) provide additional tools for future mechanistic studies in human cells derived from specific tissues in targeted population groups.

Acknowledgments

All authors read and approved the final manuscript.

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