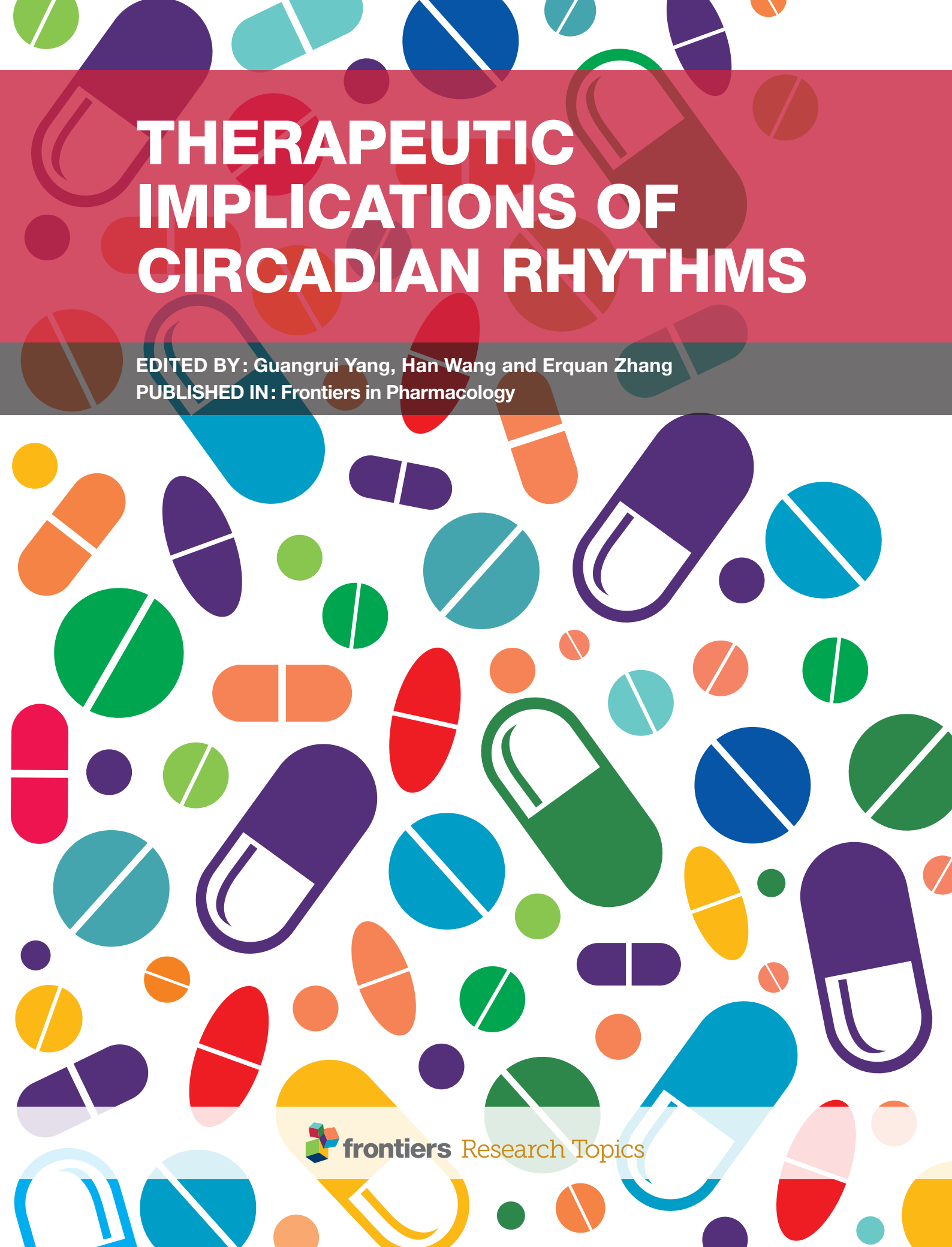
THERAPEUTIC IMPLICATIONS OF CIRCADIAN RHYTHMS

EDITED BY: Guangrui Yang, Han Wang and Erquan Zhang
PUBLISHED IN: Frontiers in Pharmacology



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ISSN 1664-8714
ISBN 978-2-88919-665-4
DOI 10.3389/978-2-88919-665-4

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THERAPEUTIC IMPLICATIONS OF CIRCADIAN RHYTHMS

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Circadian rhythms are biological processes displaying endogenous and entrainable oscillations of about 24 hours. They are driven by a group of genes called clock genes that have been widely observed in plants, animals and even in bacteria. In mammals, the core clock genes are rhythmically expressed in both the suprachiasmatic nucleus (SCN), the master clock residing in the hypothalamus, and almost all peripheral tissues where they control numerous target genes in a circadian manner, and thus affect many physiological and biochemical processes. Evidence suggests that disruption of the circadian rhythms (or desynchronization) is a significant risk factor for the development of metabolic diseases, cardiovascular diseases, cancer and sleep disorders. Evidence also suggests that the disruption suppresses immune function and increases vulnerability to infectious diseases. Restoring or strengthening the circadian rhythm may be therapeutic for these conditions. This becomes exceptionally important in modern societies because many people are suffering from frequent desynchronization due to shift working, exposure to artificial light, travel by transmeridian air flight, and involvement in social activities. Besides, the temporal variations in the incidence and severity of many diseases, such as the onset of cardiovascular events, chronic obstructive pulmonary disease (COPD), inflammatory diseases and mental disorders have also drawn increasing attention to the circadian clock. The circadian rhythms affect not only the health status, but also the drug efficiency. The effects (and side effects) of many drugs vary with biological timing. The tolerance of many medications displays circadian variation as well. The timing of medical treatment in coordination with the body clock may significantly increase the desired effects of drugs, and lower the dose and toxicity. In addition, circadian rhythms can also be modulated by some therapeutic drugs, for example, melatonin and modafinil, which are used to treat circadian rhythm sleep disorders. In this Research Topic, we assembled a series of critical review and research articles that focus on the therapeutic implications of circadian rhythms. Topics include, but are not limited to:

- Circadian disruption caused diseases or disorders and related intervention
- Temporal manifestation of diseases or disorders and therapeutic implications
- The effects of circadian rhythms on drugs
- The effects of drugs on circadian rhythms

Citation: Yang, G., Wang, H., Zhang, E., eds. (2015). Therapeutic Implications of Circadian Rhythms. Lausanne: Frontiers Media. doi: 10.3389/978-2-88919-665-4

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frontiers in Pharmacology

EDITORIAL
published: 19 August 2015
doi: 10.3389/fphar.2015.00175

Editorial: Therapeutic implications of circadian rhythms

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Keywords: circadian rhythm, circadian clocks, circadian disruption, therapeutic implications, metabolism, cardiovascular diseases, central nervous system, clinical relevance

Circadian rhythms are biological processes displaying endogenous and entrainable oscillations of about 24 h. In mammals the sleep/wake cycle, core body temperature fluctuation, and diurnal variation of blood pressure and heart rate are among the most well-known circadian rhythms. These rhythms are not just the consequence of activity/rest cycles, but are also controlled by molecular clocks, a biological network of fundamental value in the harmonization of physiological and biochemical processes with the external environment. Substantial evidence suggests that:

1. Dysregulation of the circadian system is a significant risk factor for many health problems such as metabolic disorders, cardiovascular diseases, impaired immune function, and accelerated aging. Restoring or strengthening the circadian rhythm may be therapeutic for these conditions.
2. The incidence and severity of many diseases, such as the onset of cardiovascular events, chronic obstructive pulmonary disease (COPD), inflammatory diseases, and mental disorders, are time-dependent.
3. The efficiency and side effects of many drugs has temporal variations.
4. Circadian rhythms can be modulated by some drugs.

Despite the large amount of experimental and epidemiological evidence, the importance of circadian rhythms has not been paid much attention in real clinical settings. The aim of this Research Topic in Frontiers is to highlight the therapeutic implications of circadian rhythms.

The mating behavior or close-proximity (CP) displays day/night variation in some insects including *Drosophila melanogaster*, which is controlled by molecular clocks and affected by food consumption. For example, CP rhythm is abolished in *per* or *tim*-null flies, and dampened under low-nutrient conditions. In the research article, *Inositols affect the mating circadian rhythm of Drosophila melanogaster* (Sakata et al., 2015), Sakata et al. found that CP rhythm significantly enhanced by feeding the flies with powdered ice plant, a little-known vegetable that may improve hyperglycemia in a streptozotocin-induced diabetic rat model. Among various components of ice plant, myo-inositol could increase the amplitude and shorten the period of CP rhythm. Real-time reporter assays showed that myo-inositol also shortened the period of the circadian reporter gene Per2-luc in the mouse cell line NIH3T3. Their data suggested that ice plant and myo-inositol may be beneficial to insect reproduction, while its potential role in mammals need to be carefully investigated.

There's obvious day/night variation in urinary voiding with much more during the day than at night in human. However, a large portion of human beings suffer excessive urination at night (nocturia), which dramatically decreases quality of life. Therefore, understanding the underlying mechanism has significant clinical relevance. Most studies on the circadian rhythm of micturition were focused on urine production by the kidneys. Although smooth muscle cells from mouse bladder express a functional and autonomous circadian clock at the molecular level, very few studies show circadian rhythms in the bladder function. In the research article, *Evaluation of*

OPEN ACCESS

Edited and reviewed by:
Dominique J. Dubois, Université Libre de Bruxelles, Belgium

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Specialty section:
This article was submitted to Pharmaceutical Medicine and Outcomes Research, a section of the journal Frontiers in Pharmacology

Received: *28 July 2015*
Accepted: *03 August 2015*
Published: *19 August 2015*

Citation:
Yang G, Wang H and Zhang E (2015) Editorial: Therapeutic implications of circadian rhythms. Front. Pharmacol. 6:175. doi: 10.3389/fphar.2015.00175

mouse urinary bladder smooth muscle for diurnal differences in contractile properties (White et al., 2014), White et al. measured spontaneous (phasic) and nerve-evoked contractions of mouse bladder tissue strips collected from multiple time points during 24 h and found phasic contraction, but not nerve-evoked contraction displayed diurnal rhythm.

Circadian rhythm has significant therapeutic implications in the central nervous system. It has long been known that circadian disruption by frequent shift work, jet lag, or exposure to artificial light is a risk factor for several neurodegenerative diseases, including Alzheimer's disease. Conversely, many neurodegenerative diseases result in circadian abnormalities. Besides, mice lacking clock genes, such as Bmal or Clock/Npas2, developed marked astrogliosis. In the mini-review, *Circadian clock disruption in neurodegenerative diseases: cause and effect?* (Musiek, 2015), Musiek reviewed recent studies implicating circadian rhythms and neurodegeneration and emphasized future research directions and potential therapeutic strategies for neurodegenerative diseases.

Cardiovascular disease (CVD) is a leading cause of death worldwide and new approaches in the management of CVD are clearly warranted. Since cardiovascular function and the onset of many CVDs display obvious diurnal variations, novel pharmacologic compounds that target the circadian mechanism may have potential clinical applications. Two review articles in current research topic were focused on the cardiovascular system and circadian rhythms from different views. In *Recent advances in circadian rhythms in cardiovascular system* (Chen and Yang, 2015), Chen and Yang summarized recent advances in the understanding of the relationship between circadian rhythm and cardiovascular physiology and diseases including blood pressure regulation and myocardial infarction. In *Therapeutic applications of circadian rhythms for the cardiovascular system* (Tsimakouridze et al., 2015), Tsimakouridze et al. mainly focused on circadian biomarkers, chronotherapy for CVDs and new drugs targeting circadian clocks.

Biological clock and metabolism are tightly intertwined. On one hand, the disturbance of circadian rhythms negatively affects metabolic homeostasis, and thus may promote the development of obesity and diabetes. On the other hand, high fat consumption alters circadian behavior in mice, while temporal restriction of food consumption limits mouse weight gain on a high fat diet via restoring the robustness of clock gene oscillation. In mini-review, *Circadian clocks, feeding time, and metabolic homeostasis* (Paschos, 2015), Paschos collected evidence about the association between circadian misalignment and metabolic homeostasis and discussed the role of feeding time in energy metabolism. In another review paper, *Rodent models to study the metabolic effects of shiftwork in humans* (Opperhuizen et al., 2015), Opperhuizen et al. provided a thorough view of animal models that are used to mimic human shiftwork. They divided published models in four categories, i.e., altered timing of food intake, activity, sleep, or light exposure and scored and compared their effects on metabolic parameters. They also discussed the drawback of animal studies and evaluated the translatability to human beings.

Mothers who experience breastfeeding problems in the early post-partum period are more likely to discontinue breastfeeding within 2 weeks. A major risk factor for shorter breastfeeding duration is delayed lactogenesis II (DLII). Based on the facts that circadian clocks coordinate hormonal and metabolic changes to support lactation in rodent studies, and disruption of the circadian system intervenes the initiation of lactation and negatively impacts milk production, Fu et al. (2015) hypothesized that DLII is related to disruption of the mother's circadian system. Authors reviewed literatures that support this hypothesis, and described interventions that may help to increase breastfeeding success.

The treatment of circadian disorders has drawn attention recently. However, the development of pertinent drugs has a high failure rate possibly due to the variations in chronotype. Therefore, similar to treatment of given cancers, personalized medicine might become a standard for drug development in the field of chronobiology. In *Personalized medicine for pathological circadian dysfunctions* (Skelton et al., 2015), Skelton et al. reviewed the current clinical trials of circadian drugs and the history of personalized medicine in oncology, and discussed how personalized medicine can be used in future clinical trials for circadian disorders.

As presented above, we recruited two research articles, six review articles, and a hypothesis and theory article that covered multiple aspects of *Therapeutic Implications of Circadian Rhythms*, from model organisms to human, from central nervous system to peripheral tissues, and from clinical study to drug development. Although much attention has been paid to this field in recent years, we are still far from our goal, especially the translation of basic science into clinical applications. Therefore, we encourage researchers to continue contributing to our understanding of the clinical relevance of circadian systems. Finally, we would like to thank all authors who contributed papers to our research topic. We would also like to thank all reviewers and editorial board for helping us to underscore the importance and organization of this Research Topic.

References

Chen, L., and Yang, G. (2015). Recent advances in circadian rhythms in cardiovascular system. *Front. Pharmacol.* 6:71. doi: 10.3389/fphar.2015.00071

Fu, M., Zhang, L., Ahmed, A., Plaut, K., Haas, D. M., Szucs, K., et al. (2015). Does circadian disruption play a role in the metabolic-hormonal link to delayed lactogenesis II? *Front. Nutr.* 2:4. doi: 10.3389/fnut.2015.00004

Musiek, E. S. (2015). Circadian clock disruption in neurodegenerative diseases: cause and effect? *Front. Pharmacol.* 6:29. doi: 10.3389/fphar.2015.00029

Opperhuizen, A. L., van Kerkhof, L. W., Proper, K. I., Rodenburg, W., and Kalsbeek, A. (2015). Rodent models to study the metabolic effects of shiftwork in humans. *Front. Pharmacol.* 6:50. doi: 10.3389/fphar.2015.00050

Paschos, G. K. (2015). Circadian clocks, feeding time, and metabolic homeostasis. *Front. Pharmacol.* 6:112. doi: 10.3389/fphar.2015.00112

Sakata, K., Kawasaki, H., Suzuki, T., Ito, K., Negishi, O., Tsuno, T., et al. (2015). Inositols affect the mating circadian rhythm of *Drosophila melanogaster*. *Front. Pharmacol.* 6:111. doi: 10.3389/fphar.2015.00111

Skelton, R. L., Kornhauser, J. M., and Tate, B. A. (2015). Personalized medicine for pathological circadian dysfunctions. *Front. Pharmacol.* 6:125. doi: 10.3389/fphar.2015.00125

Tsimakouridze, E. V., Alibhai, F. J., and Martino, T. A. (2015). Therapeutic applications of circadian rhythms for the cardiovascular system. *Front. Pharmacol.* 6:77. doi: 10.3389/fphar.2015.00077

White, R. S., Zemen, B. G., Khan, Z., Montgomery, J. R., Herrera, G. M., and Meredith, A. L. (2014). Evaluation of mouse urinary bladder smooth muscle for diurnal differences in contractile properties. *Front. Pharmacol.* 5:293. doi: 10.3389/fphar.2014.00293

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in
PHARMACOLOGY

ORIGINAL RESEARCH ARTICLE
published: 09 January 2015
doi: 10.3389/fphar.2014.00293

Evaluation of mouse urinary bladder smooth muscle for diurnal differences in contractile properties

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Most physiological systems show daily variations in functional output, entrained to the day–night cycle. Humans exhibit a daily rhythm in urinary voiding (micturition), and disruption of this rhythm (nocturia) has significant clinical impact. However, the underlying mechanisms are not well-understood. Recently, a circadian rhythm in micturition was demonstrated in rodents, correlated with functional changes in urodynamics, providing the opportunity to address this issue in an animal model. Smooth muscle cells from mouse bladder have been proposed to express a functional and autonomous circadian clock at the molecular level. In this study, we addressed whether a semi-intact preparation of mouse urinary bladder smooth muscle (UBSM) exhibited measurable differences in contractility between day and night. UBSM tissue strips were harvested at four time points over the diurnal cycle, and spontaneous (phasic) and nerve-evoked contractions were assessed using isometric tension recordings. During the active period (ZT12-24) when micturition frequency is higher in rodents, UBSM strips had no significant differences in maximal- (high K^+) or nerve-evoked contractions compared to strips harvested from the resting period (ZT0-12). However, a diurnal rhythm in phasic contraction was observed, with higher amplitudes at ZT10. Consistent with the enhanced phasic amplitudes, expression of the BK K^+ channel, a key suppressor of UBSM excitability, was lower at ZT8. Higher expression of BK at ZT20 was correlated with an enhanced effect of the BK antagonist paxilline (PAX) on phasic amplitude, but PAX had no significant time-of-day dependent effect on phasic frequency or nerve-evoked contractions. Overall, these results identify a diurnal difference for one contractile parameter of bladder muscle. Taken together, the results suggest that autonomous clocks in UBSM make only a limited contribution to the integrated control of diurnal micturition patterns.

Keywords: UBSM, BK channel, *Kcnma1*, circadian rhythm, peripheral rhythm, urodynamics, isometric tension, lower urinary tract

INTRODUCTION

Most physiological systems, including the urinary system, exhibit daily (24-hr) variations in functional output that are entrained to the day–night cycle. Humans exhibit a daily rhythm in urinary voiding (micturition), and nocturia, excessive urination at night, is a persistent disorder affecting >50% of people in some age groups and significantly decreasing quality of life (Ticher et al., 1994; Hetta, 1999; Neveus et al., 1999; Weiss et al., 2008). The circadian variation in urination depends on daily urine production, the physical properties of the bladder, and neural control. Dysfunction in these pathways may contribute to nocturia, but the identification of causal relationships has been limited. The diurnal variation in glomerular filtration rate (GFR) in the kidney is well-documented in humans and animals (Koopman et al., 1985; Zuber et al., 2009), and in some cases, nocturia in humans is associated with a loss of the diurnal variation in GFR (De Guchtenaere et al., 2007). However, not all cases of nocturia are caused by polyuria. Diminished bladder capacity is a major contributor to nocturia and can result from nocturnal detrusor overactivity and neurogenic bladder (Weiss et al., 2008). Few direct comparisons have been made between the physical properties of the bladder during the day and night under controlled conditions (Herrera and Meredith, 2010). Thus the aspects of the lower urinary tract that influence normal circadian micturition patterns, and consequently that contribute to nocturia, are essentially unknown.

Recently, rodents have been found to be an appropriate model for addressing the basis for daily rhythm in micturition. In rodent models, the day–night difference in urine voiding is in part driven by urine production by the kidney, coordinated through hormonal control via aldosterone and vasopressin linked to the circadian clock (Jin et al., 1999; Zuber et al., 2009). Rats and mice demonstrate a circadian rhythm in micturition frequency and volume, correlated with daily changes in functional bladder capacity (Herrera and Meredith, 2010; Negoro et al., 2012). At night, the rodent's active period, bladder capacity is reduced and micturition frequency is increased compared to day, when rodents sleep. Both renal and micturition rhythms are disrupted by mutations

in 'clock genes' that abolish circadian rhythms (Zuber et al., 2009; Negoro et al., 2012; Noh et al., 2014).

To dissect the mechanism of circadian rhythms in micturition, the validation of daily changes in urodynamic properties established the bladder as a target for circadian regulation (Herrera and Meredith, 2010). Like many other peripheral tissues in the body, smooth muscle has been shown to possess intrinsic rhythms (Reilly et al., 2008; Paschos and FitzGerald, 2010; Su et al., 2012). Cultured bladder smooth muscle cells show circadian rhythms in gene expression, suggesting there is an autonomous circadian clock at the level of bladder muscle. Daily oscillations have been observed in several transcription factors previously demonstrated to drive the core clock mechanism in SCN and other peripheral tissues (Negoro et al., 2012; Noh et al., 2014). These transcription factors are linked to Cx43 expression in bladder cells, a gap junction channel that regulates bladder storage capacity, as well as other genes associated with smooth muscle contractility (Negoro et al., 2012). These data predict that UBSM possesses robust autonomous rhythmicity, yet no direct evidence demonstrating daily variations in baseline UBSM contractility has been reported to date.

To address this issue, in this study we recorded contractile activity from urinary bladder smooth muscle (UBSM) strips harvested at four time points to identify any differences in spontaneous and evoked contractile amplitudes over the circadian cycle. The expression pattern of the BK K^+ channel ($K_{Ca}1.1$, *Kcnma1*), a potent regulator of smooth muscle excitability (Meredith et al., 2004) and output of the central circadian clock (Meredith et al., 2006), was also assessed in UBSM, and contractile activity was recorded in the presence of a BK channel blocker to determine whether the diurnal difference in contractility was reduced.

MATERIAL AND METHODS

MICE

All procedures involving mice were conducted in accordance with The University of Maryland School of Medicine animal care and use guidelines. C57BL6/J WT mice were group housed on a standard 12:12 h light:dark cycle (LD) until experimental procedures. Time points over the circadian cycle are referred to as zeitgeber time (ZT), denoting time in hours relative to the 24 h cycle. Lights on is defined as ZT0, and lights off is ZT12. Mice were euthanized by inhalation of saturating isoflurane vapors, followed by rapid decapitation.

ISOLATION OF UBSM AND WESTERN BLOTTING

For Western blots, mouse (3–4 mo) urinary bladders were solubilized in lysis buffer (137 mM NaCl, 1% Triton X-100, 0.5% deoxycholate, 40 mM HEPES, pH 7.4, 1 mM EDTA, pH 7.4, 2 μg/ml aprotinin, 1 μg/ml leupeptin, 2 μg/ml antipain, 10 μg/ml benzamidine, and 0.5 mM phenylmethylsulfonyl fluoride). The insoluble fraction was separated by centrifugation (14,000 *g* for 5 min). 5 μg of soluble supernatant protein was loaded per lane and subjected to SDS-PAGE on a 7.5% acrylamide gel. Proteins were transferred to a nitrocellulose membrane, and membranes were blocked (4% dry non-fat milk, 2% normal goat serum, 10 mM Tris (pH 8), 0.15 M NaCl, and 0.1% Tween 20) for 1-hr. Primary antibodies in blocking solution were incubated overnight at 4°C each of mouse monoclonal α-*Slo* (1 μg/ml L6.60, Neuromab, University of California at Davis, Davis, CA, USA) and mouse monoclonal DM1a α-tubulin (1:10,000, T-9026, Sigma). Membranes were labeled with 1:500 SuperSignal West Dura horseradish peroxidase-conjugated goat α-rabbit and α-mouse secondary antibodies (Pierce), and proteins were visualized by SuperSignal chemiluminescence detection (Pierce). Densitometry of BK band to DM1α anti-tubulin was performed as described previously (Meredith et al., 2006).

ISOMETRIC TENSION RECORDINGS

After euthanasia, urinary bladders were removed and placed in ice-cold dissection solution composed of (in mM) 80 monosodium glutamate, 55 NaCl, 6 KCl, 10 glucose, 10 HEPES, and 2 $MgCl_2$, with pH adjusted to 7.3 with NaOH. The bladder was cut open to expose the urothelial surface and rinsed several times with dissection saline to remove residual traces of urine. The urothelial layer was carefully dissected away from the smooth muscle layer and discarded. Small strips of detrusor (2–3 mm wide and 5–7 mm long) were cut from the bladder wall. Silk threads were attached to each end of the strips, and the strips were transferred to cold (4°C) physiological saline solution (PSS) containing (in mM) 119 NaCl, 4.7 KCl, 24 $NaHCO_3$, 1.2 KH_2PO_4, 2.5 $CaCl_2$, 1.2 $MgSO_4$, and 11 glucose and aerated with 95% O_2–5% CO_2 to obtain pH 7.4. Each strip was mounted in a tissue bath (15-ml volume) containing aerated PSS (95% O_2–5% CO_2, 37°C; MyoMED myograph system; Catamount Research and Development Inc., St. Albans, VT). Initial tension was applied as indicated, and strips were equilibrated for 45 min with bath solution exchanges every 15 min. 60 mM KCl in PSS was delivered for 5 min to produce a maximal contraction, and then washed out with two 10 min PSS washes. KCl-induced contractions were repeated twice. Strips with no baseline contractile activity were not included in the dataset. KCl-induced contractile amplitudes were determined from the third KCl application, either the maximal contractile amplitude (peak) or 5 min post-KCl (steady-state). Area under the curve (AUC) values were obtained from the integral of the contractile response covering the initial rise to 5 min post-KCl. All time points indicate the time of contractile assays.

For phasic contractions, force transducers were calibrated for 1 g and contractile activity was recorded for 30 min after the KCl applications and wash out (Herrera et al., 2003; Meredith et al., 2004). Frequency and amplitude were determined for each strip from 5 min of continuous spontaneous activity within the 30 min recording window (MiniAnalysis, Synaptosoft, Inc.). Phasic amplitude values were normalized to the KCl-evoked amplitude to account for any variability in cutting the strips. AUC and rise time values were obtained from each contractile event in the 5 min period (MiniAnalysis, Synaptosoft, Inc.) and averaged for each strip. For pharmacology experiments, Paxilline (PAX; 10 μM; Sigma) or DMSO (0.1% vehicle control) was added in each chamber after 30 min. Analysis of phasic activity after drug or vehicle was performed on 5 min of continuous spontaneous activity, 30 min after Pax or DMSO application.

For nerve-evoked contractions, frequency-response curves were constructed by measuring the electric field stimulation (EFS)-induced contraction amplitude at stimulus frequencies of 0.5, 2, 3.5, 5, 7.5, 10, 12.5, 15, 20, 30, 40, and 50 Hz. Pulse amplitude was 20–30 V of alternating polarity. Pulse width was 0.2 ms, and stimulus duration was 2 s. Stimuli were given every 3 min using a model PHM-152V stimulator (Catamount Research and Development Inc; Herrera et al., 2005; Werner et al., 2007). Amplitude was determined in Myograph software (Catamount Research and Development, Inc.). EFS-evoked amplitude values were normalized to the KCl-evoked amplitude. EFS-evoked amplitudes normalized to the 50-Hz amplitude value were fit with a standard exponential function to derive the frequency of half maximal activation (OriginLab, Northampton, MA, USA). For pharmacology experiments, one 5 min PSS wash was conducted after the first EFS, followed by addition of Pax (10 μM) or DMSO (0.1%) and a post-drug EFS after 30 min.

STATISTICS

Group averages are reported ±SE. Reported n's are the number of animals, with 1–4 strips averaged together for each animal as indicated in figure legends. Statistical significance was determined across time points at $p < 0.05$ by one-way ANOVA (or repeated measures ANOVA across frequencies EFS-evoked contractions across time points) with Bonferroni *post hoc* tests in SPSS v19 (IBM Corp., Armonk, NY, USA). Cosinor analysis was performed with software available at http://www.circadian.org/software.html (Refinetti et al., 2007).

RESULTS

BASELINE AND PHASIC CONTRACTILE ACTIVITY IN MOUSE UBSM AT DIFFERENT TIMES OF DAY

In nocturnal rodents, micturition frequency is higher during the night (active) period, compared to daytime. We hypothesized that strips of UBSM tissue harvested during the dark period would demonstrate stronger contractile activity than strips harvested during the day, when micturition frequency is low and the bladder relaxes to store urine (Herrera and Meredith, 2010; Negoro et al., 2012). Thus, to determine whether contractile properties of UBSM varied by time of day, isometric tension recordings were performed at ZT4, 10, 16, and 22 (**Figure 1A**). Isolated strips were denuded of the urothelium, but nerve terminals are retained in this prep, enabling both spontaneous and nerve-evoked contractions (Kullmann et al., 2014). UBSM strips were affixed to a solid support, and an initial stretch was applied (1.5 g). After the initial relaxation, 60 mM KCl was applied to induce depolarization of the muscle and elicit a maximal contraction (**Figure 1B**).

To characterize whether a daily rhythm was present contractile activity, the KCl-induced responses were compared across time points. No significant differences were found in the peak, steady-state, or integrated KCl-induced amplitudes between timepoints (**Figures 1C–E**). Application of higher initial tension (2.5 g) also did not reveal any significant difference in maximal KCl-induced amplitude (**Table 1**). These data suggest that the basic contractile apparatus does not undergo daily alterations that have a major consequence on function.

Next, we addressed whether phasic activity in UBSM strips differed by time of day. Phasic contractions result from the spontaneous action potential activity of smooth muscle cells within the UBSM strip (Brading, 1997). Phasic contractions are proposed to be important in maintaining bladder tone, and reduction of phasic contractility is correlated with bladder relaxation to accommodate filling (Herrera et al., 2003; Kullmann et al., 2014). Greater than 80% of strips exhibited phasic contractions, similar to previous results on this mouse strain background (Herrera et al., 2003). There was no significant difference in the number of strips with phasic activity at each time point ($p > 0.05$, Fisher's Exact test, *n*'s as indicated in **Figure 2** legend). These results show that phasic activity is generated throughout the daily cycle.

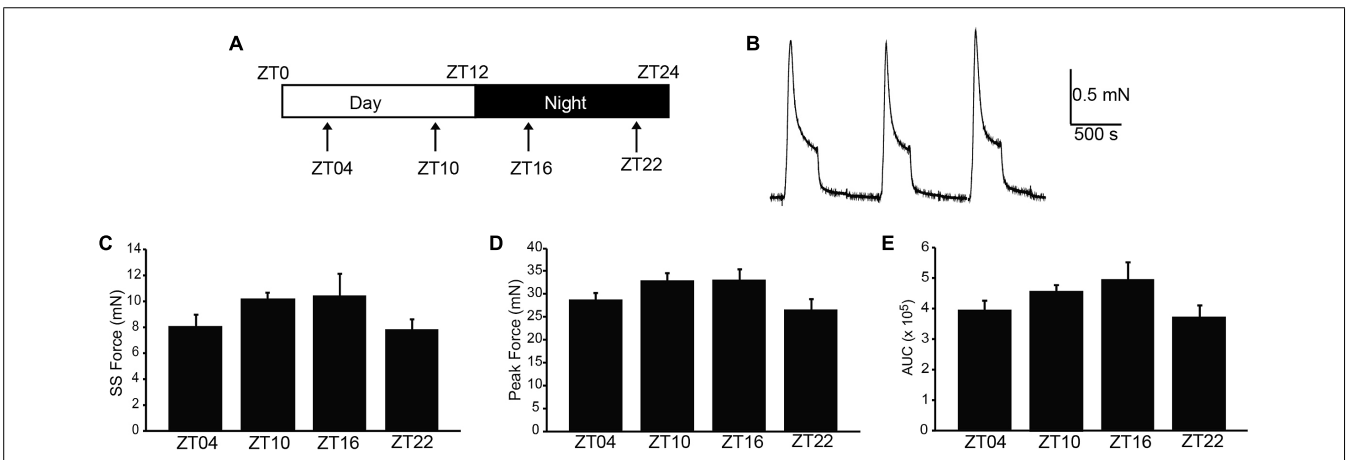

FIGURE 1 | High K^+-induced excitation–contraction responses are similar across the circadian cycle in isolated UBSM strips. (A) Schematic of the circadian cycle and four time points examined in this study. Mice are nocturnal, resting during the daytime light period, zeitgeber time (ZT) 0–12, and being active at night during the nighttime dark period, ZT12-24. **(B)** Representative 60 mM KCl-induced contractions. KCl was applied for 5 min, followed by a 10 min washout. Contraction values were obtained from the third KCl application. **(C)** Peak amplitude. **(D)** Steady-state amplitude. **(E)** Area under the curve (AUC). All data are mean ± SE, $n = 5$–6 mice (20–24 UBSM strips) per timepoint. For all comparisons, $p > 0.05$ (one-way ANOVA).

Table 1 | Effect of time of day on UBSM contractility at two initial tensions.

	1.5 g	2.5 g
Initial tension		
ssKCl-Induced amplitude	ns	ns
Phasic amplitude	*ZT4/ZT10	*ZT4/ZT10
Phasic frequency	ns	ns
EFS amplitude	ns	ns
Half-max frequency	ns	ns
Paxilline		
Phasic amplitude	*ZT17	
Phasic frequency	ns	
EFS amplitude	ns	

*For 1.5 g initial tension, n's are reported in the previous figure legends. For 2.5 g initial tension, n = 8 animals (1–2 UBSM strips averaged per animal). ssKCl, steady-state KCl-induced amplitude. ns, no significant difference across timepoints ($p > 0.05$, one-way ANOVA). *$p < 0.05$ (one way ANOVA, and the indicated post hoc comparison (Bonferroni) was significant at $p < 0.05$.*

Urinary bladder smooth muscle strips isolated at ZT4 had the lowest phasic amplitudes (**Figures 2A,B**). By ZT10, phasic amplitude was fourfold greater than ZT4 (**Figure 2B**). The amplitudes decreased at ZT16 and ZT22 (**Figure 2B**). Fitting the data to a cosine function also established ZT10 as the peak contractile amplitude of the 24-hr rhythm ($p = 0.01$, Refinetti et al., 2007). Similarly, the integrated area of the phasic contraction was greater at ZT10 (**Figure 2D**). Although not significant, the time to peak contraction (rise time) was shorter on average at ZT10. Furthermore, in independent experiments, phasic activity from UBSM with a higher initial tension applied also showed a significant difference between ZT4 and ZT10 contractile amplitudes (**Table 1**). Taken together, these data suggest that a daily rhythm in phasic contractile amplitude is present in UBSM. In contrast, there was no significant difference in the frequency of phasic contractions across the daily cycle (**Figure 2C**; **Table 1**).

NERVE-EVOKED CONTRACTILE ACTIVITY IN UBSM AT DIFFERENT TIMES OF DAY

Coordinated bladder contraction during micturition is controlled by the parasympathetic nerves encapsulated in the bladder wall (Andersson and Arner, 2004). To investigate diurnal differences in nerve-evoked contractile activity, nerve-mediated release of neurotransmitter was elicited by electrical field stimulation (EFS). Physiological frequencies from 0.5 to 50 Hz, mimicking the excitation that occurs during micturition *in vivo*, were applied to strips harvested at different times of day, and the peak contractile responses were measured (**Figure 3A**). In the presence of 1 μM tetrodotoxin, no contractile response could be elicited ($n = 3$), validating that the contractions in response to EFS at each frequency were entirely derived from nerve activity.

Increasing stimulation frequencies produced greater contractile force (**Figures 3A,B**). However, no significant differences in EFS-evoked contractions across time points were found (**Figure 3B**; **Table 1**). To reveal any frequency-dependent differences across timepoints, contraction amplitudes at each frequency were normalized to the maximal EFS-evoked response at 50 Hz (**Figure 3C**). While no significant differences were obtained, ZT10 showed a slight reduction in the frequency of half-maximal contraction (**Figure 3D**), suggesting a trend toward enhanced sensitivity to nerve-mediated stimulation at ZT10. Nevertheless, on the whole, no substantial differences were found that would provide clear

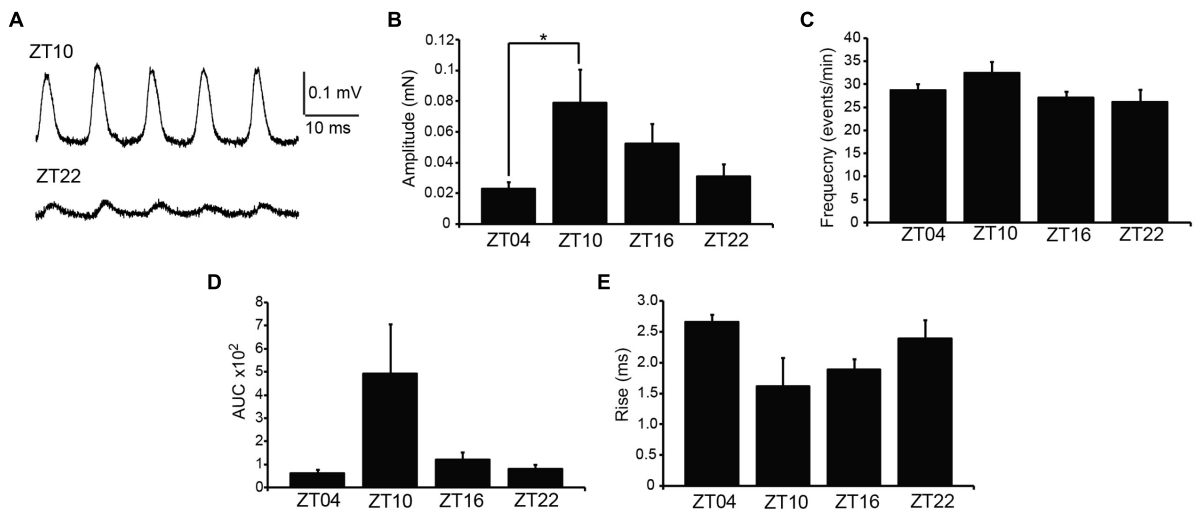

FIGURE 2 | Spontaneous (phasic) contractions are larger at ZT10. **(A)** Representative phasic contractile activity at ZT10 and ZT22. **(B)** Phasic amplitude differs by time of day. $p = 0.03$ (one-way ANOVA), *Bonferroni *post hoc*, $p < 0.04$. **(C)** Phasic frequency is not different across time points, $p = 0.17$ (one-way ANOVA). **(D)** AUC. $p = 0.03$ (one-way ANOVA). **(E)** Rise time of phasic events. $p = 0.08$ (one-way ANOVA). All data are mean ± SE, $n = 6$–7 mice (10–12 UBSM strips) per timepoint.

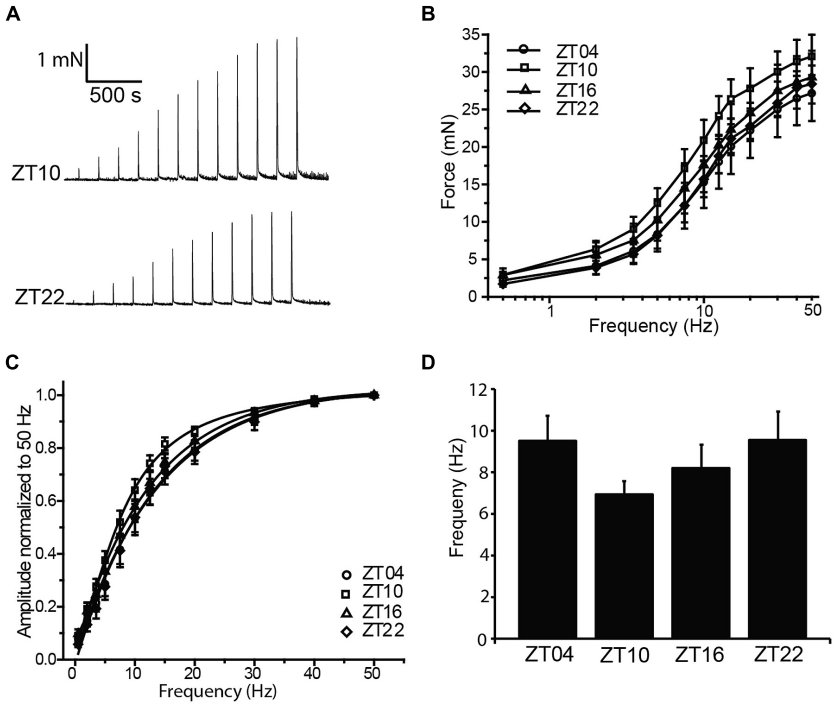

FIGURE 3 | Nerve-evoked (EFS) contractions are not different between timepoints. (A) Representative EFS-evoked contractions at ZT10 and ZT22. **(B)** EFS-evoked contractions, elicited by 0–50 Hz stimulation, are not different between timepoints. **(C)** EFS-evoked amplitudes normalized to the maximal amplitude at 50 Hz. **(D)** Frequency of half-maximal activation, derived from fits of data in **(C)**, was not different between timepoints. All data are mean ± SE, $n = 6$ mice (12 UBSM strips) per timepoint. For all comparisons, $p > 0.05$ (one-way ANOVA).

evidence of a daily rhythm in nerve-mediated contraction of UBSM tissue.

BK CHANNEL EXPRESSION AND FUNCTION IN UBSM AT DIFFERENT TIMES OF DAY

BK channels are major regulators of UBSM excitability, and block or loss of BK channel activity in UBSM leads to increased phasic and EFS-evoked contractile amplitude and frequency (Meredith et al., 2004; Thorneloe et al., 2005). In addition, BK channels are also key regulators of the circadian rhythm in pacemaker excitability in the brain (Meredith et al., 2006; Kent and Meredith, 2008; Montgomery et al., 2013). To determine whether there was any evidence for BK channel involvement in the daily variation in UBSM phasic contractility, we first assessed the expression of BK from bladders harvested at ZT8 versus ZT20. BK expression was low at ZT8 (**Figure 4A**), similar to the time window with the highest phasic contractile amplitudes (**Figures 3A,B**). Conversely, BK expression was higher at ZT20, when phasic amplitudes were lower. The 2.3-fold increase in BK expression at ZT20 compared to ZT8 was similar to the difference in magnitude between the peak and trough of BK expression in the SCN circadian pacemaker (Meredith et al., 2006).

To determine the functional impact of blocking BK channels at different times of day, we applied a BK channel blocker, PAX, to UBSM strips and recorded phasic and EFS-evoked contractile responses. The results are plotted as the proportional change after PAX from baseline. We found an increase in both the phasic amplitude and frequency after application of PAX (**Figures 4B–D**), but not after application of DMSO (control). The effect of PAX to enhance phasic contractions is consistent with previous data showing the BK channel to be a critical suppressor of UBSM contractility (Meredith et al., 2004). The PAX-induced increase in phasic frequency did not vary by time of day (**Figure 4D**). However, the PAX-induced increase in phasic amplitude was highest at ZT17 (**Figures 4B,C**), parallel to the increased BK protein expression observed at ZT20 (**Figure 4A**). These data suggest that inhibition of BK channel activity has a limited diurnal effect on phasic contractile amplitude, and the time window of the enhanced effect of PAX is consistent with the phase of increased BK protein expression in bladder.

Application of PAX also resulted in an enhancement of EFS-evoked amplitudes (**Figures 4E,F**). The PAX-induced increase in EFS-evoked amplitude was frequency dependent, with a larger proportional increase at low compared to high frequencies. Nevertheless, the PAX-induced increase in EFS-evoked amplitudes was not found to significantly differ by time of day (**Figure 4F**). Thus the results obtained with PAX generally corroborate the pattern of diurnal changes observed in baseline contractility – i.e., an effect on phasic amplitude, but not phasic frequency or EFS-evoked contractions.

DISCUSSION

Mice have recently been shown to express a bona fide circadian rhythm in micturition, and this rhythm has been proposed to rely

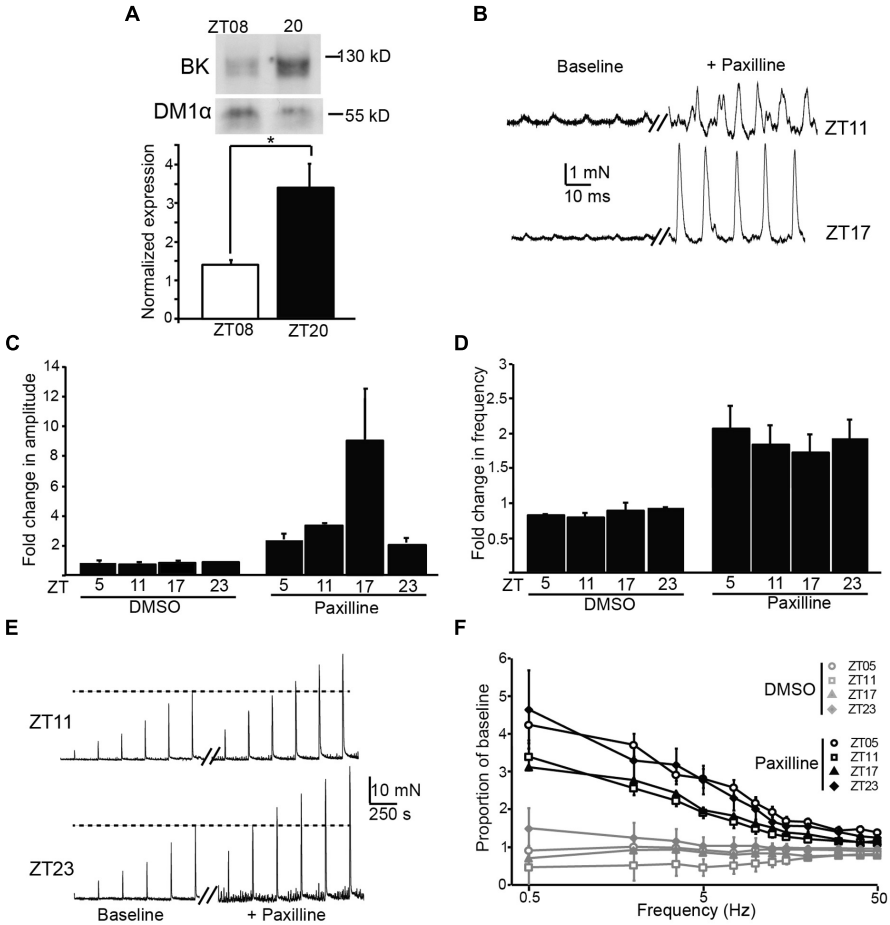

FIGURE 4 | Expression and functional impact of the BK channel on UBSM contractility at different times of day. (A) Representative Western blot showing BK channel and DM1α protein expression at ZT8 and ZT20 (top). Average BK expression normalized to DM1α (bottom). ZT8 expression is significantly lower than ZT20 ($p = 0.03$, *t*-test, $n = 4$ mice per timepoint). **(B)** Representative phasic contractile activity at ZT11 and ZT17, at baseline and after Paxilline (PAX) application. **(C,D)** Fold-increase in phasic amplitude **(C)** and frequency **(D)** after PAX or DMSO (control) application. The effect of PAX and time were significant for phasic amplitude (factorial ANOVA, $p = 10^{-4}$ and 0.01, respectively), but only the effect of PAX was significant on phasic frequency ($p = 10^{-8}$). $n = 3$–6 mice (6–12 UBSM strips) per timepoint and condition. DMSO had no effect on either parameter. **(E)** Representative EFS-evoked contractile activity at ZT11 and ZT23, at baseline and after PAX application. **(F)** Increase in EFS-evoked amplitude after PAX or DMSO (control) as a proportion of baseline. The effect of PAX was significant (factorial ANOVA, $p = 10^{-5}$), but the effect of time was not ($p = 0.99$). $n = 5$–6 mice (10–12 UBSM strips) per timepoint and condition. All data are mean ± SE.

on an intrinsic clock housed within UBSM (Negoro et al., 2012; Noh et al., 2014). This hypothesis predicts that strips of UBSM tissue harvested across circadian timepoints would demonstrate cyclic alterations in contractile properties. The central finding of this study was that acutely isolated UBSM tissue exhibits only a limited diurnal difference in contractile properties. We did not find significant evidence for rhythms in the output of the basic (KCl-induced) contractile apparatus in UBSM (**Figure 1**), or in nerve-evoked contractions (**Figure 3**). Instead, we found a single major difference in the amplitude, but not frequency, of phasic contractions (**Figure 2**). Notably, the observations were consistent across datasets from UBSM strips with two different initial tensions applied (**Table 1**). Taken together, these data did not show the expected co-variance of related parameters that would provide strong support for a diurnal rhythm in UBSM contractile properties. Furthermore, these data suggest the conclusion that UBSM possesses only limited intrinsic machinery for functional autonomous control of contractility.

Although limited, the time of day-dependent difference in phasic contraction identified here could potentially involve the activity of the BK K^+ channel. The BK channel has been previously shown to regulate phasic contractions in UBSM (Meredith et al., 2004). The increased phasic amplitude at ZT10, compared to other timepoints, parallels the lower expression of BK protein in UBSM at ZT8. A reduction of BK expression at this time could facilitate the observed increase in phasic amplitude. Similarly, application of PAX, an inhibitor of BK channel activity, produced the largest effect on phasic amplitude at ZT17, near the timepoint of higher BK protein expression (ZT20, **Figure 4**). Although these data are suggestive of BK channel involvement in the daily difference in phasic activity, not all the results fit this hypothesis. For example, BK channel antagonists are also known to significantly enhance

phasic frequency and EFS-evoked contractions (Meredith et al., 2004). Yet no significant time of day difference could be detected in these parameters at baseline or after BK inhibition with PAX. It is not clear how BK channel function would contribute selectively to suppressing phasic amplitude at ZT17, when PAX has a maximal effect, but not have an impact on phasic frequency or EFS-evoked contractions. Future studies that directly address the nature of excitation–contraction coupling at different times of day will be required to address this dilemma.

One question that remains outstanding is the functional significance of the diurnal rhythm in phasic amplitude. Phasic contractions are thought to be important for maintaining bladder tone, decreasing with bladder relaxation to accommodate filling (Herrera et al., 2003; Kullmann et al., 2014). The increase in phasic contractile amplitude at ZT10, a timepoint which occurs at the end of the rest (light) phase, may indicate the bladder is intrinsically programmed to switch out of a urine storage mode (light phase) to facilitate increased micturition when entering the active (dark) phase. Recordings of bladder capacity from rats in the day or night are consistent with this idea (Herrera and Meredith, 2010), but concomitant measurements of UBSM and bladder properties in the same animal model across timepoints will be required to correlate the precise phase relationship.

From a clinical perspective, understanding the underlying pathology of nocturia will require identifying the circadian mechanisms that are deranged in the pathophysiological state. Systemic disruption of the mechanism for encoding circadian rhythm, via mutation of *Cry1/Cry2* or *Per1/Per2* double knockout mice, alters both the circadian pattern of micturition and gene expression (Negoro et al., 2012; Noh et al., 2014). However, tissue-specific deletions will be necessary to parse out the relative contributions of central, renal, and peripheral clocks to the circadian rhythm in urodynamics. To date, the lower urinary tract has not been comprehensively investigated as a contributor to nocturia. However, the results reported here showing minimal diurnal differences in USBM contractility contrast with recent reports of robust circadian oscillations reported in cultured bladder cells expressing a *Per2*-luciferase reporter and clock gene expression in acutely harvested bladder tissue (Negoro et al., 2012). Our data suggest the possibility that these oscillations in gene expression may not drive intrinsic rhythms in UBSM contractile activity in a meaningful way.

The only other study to provide data directly addressing the presence of intrinsic rhythms in contractility found a circadian rhythm in muscarinic-stimulated UBSM contraction, but no clear rhythm in either nerve-evoked or direct muscle-stimulation evoked responses (Wu et al., 2014). Although this study differed methodologically from the data presented here, where Wu et al. (2014) cultured the bladder strips and applied dexamethasone to synchronize circadian rhythmicity, it could be interpreted as consistent with our data with respect to a lack of rhythmicity in EFS-evoked contractions. Taking these initial investigations together, the lack of a robust circadian rhythm in UBSM contractility in our study, and the emergence of a circadian difference only with muscarinic-stimulated UBSM contraction (Wu et al., 2014), underscores the importance of continued investigation of alternative mechanisms focusing on both descending outflow through autonomic control of the bladder, as well as the kidney and polyuria, in the treatment of nocturia.

AUTHOR CONTRIBUTIONS

Rachel S. White, Betsir G. Zemen, Zulqarnain Khan, Andrea L. Meredith, and Gerald M. Herrera designed the experiments. Rachel S. White, Betsir G. Zemen, and Zulqarnain Khan performed the experiments. Jenna R. Montgomery assisted with data analysis, performed statistical analysis, and prepared figures. Andrea L. Meredith wrote the manuscript. All authors approved the final version of the manuscript.

ACKNOWLEDGMENTS

We wish to thank Michael Lai and Adrian Sackheim for assistance with the Myomed setup and Josh Whitt for assistance with circadian analysis and statistics.

This work was supported by grants from NIDDK R21-089337 (Andrea L. Meredith), NHLBI R01-HL102758 (Andrea L. Meredith), NSF IOS-0956237 (Andrea L. Meredith), The American Physiological Society's Ryuji Ueno award, sponsored by the S&R Foundation (Andrea L. Meredith), and an *American Physiological Society* Undergraduate Summer Research Fellowship to Zulqarnain Khan.

Rachel S. White, Betsir G. Zemen, Zulqarnain Khan, Jenna R. Montgomery, and Andrea L. Meredith declare no conflict of interest. Gerald M. Herrera is a full-time employee and co-owner of Catamount Research and Development, Inc., and was involved in study design and data analysis and interpretation.

REFERENCES

Andersson, K. E., and Arner, A. (2004). Urinary bladder contraction and relaxation: physiology and pathophysiology. *Physiol. Rev.* 84, 935–986. doi: 10.1152/physrev.00038.2003

Brading, A. F. (1997). A myogenic basis for the overactive bladder. *Urology* 50, 57–67. doi: 10.1016/S0090-4295(97)00591-8

De Guchtenaere, A., Vande Walle, C., Van Sintjan, P., Raes, A., Donckerwolcke, R., Van Laecke, E., et al. (2007). Nocturnal polyuria is related to absent circadian rhythm of glomerular filtration rate. *J. Urol.* 178, 2626–2629. doi: 10.1016/j.juro.2007.08.028

Herrera, G. M., Etherton, B., Nausch, B., and Nelson, M. T. (2005). Negative feedback regulation of nerve-mediated contractions by KCa channels in mouse urinary bladder smooth muscle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289, R402–R409. doi: 10.1152/ajpregu.00488.2004

Herrera, G. M., and Meredith, A. L. (2010). Diurnal variation in urodynamics of rat. *PLoS ONE* 5:e12298. doi: 10.1371/journal.pone.0012298

Herrera, G. M., Pozo, M. J., Zvara, P., Petkov, G. V., Bond, C. T., Adelman, J. P., et al. (2003). Urinary bladder instability induced by selective suppression of the murine small conductance calcium-activated potassium (SK3) channel. *J. Physiol.* 551, 893–903. doi: 10.1113/jphysiol.2003.045914

Hetta, J. (1999). The impact of sleep deprivation caused by nocturia. *BJU Int.* 84(Suppl. 1), 27–28. doi: 10.1046/j.1464-410X.84.s1.3.x

Jin, X., Shearman, L. P., Weaver, D. R., Zylka, M. J., De Vries, G. J., and Reppert, S. M. (1999). A molecular mechanism regulating rhythmic output from the suprachiasmatic circadian clock. *Cell* 96, 57–68. doi: 10.1016/S0092-8674(00)80959-9

Kent, J., and Meredith, A. L. (2008). BK channels regulate spontaneous action potential rhythmicity in the suprachiasmatic nucleus. *PLoS ONE* 3:e3884. doi: 10.1371/journal.pone.0003884

Koopman, M. G., Krediet, R. T., and Arisz, L. (1985). Circadian rhythms and the kidney. A review. *Neth. J. Med.* 28, 416–423.

Kullmann, F. A., Daugherty, S. L., de Groat, W. C., and Birder, L. A. (2014). Bladder smooth muscle strip contractility as a method to evaluate lower urinary tract pharmacology. *J. Vis. Exp.* 90:e51807. doi: 10.3791/51807

Meredith, A. L., Thorneloe, K. S., Werner, M. E., Nelson, M. T., and Aldrich, R. W. (2004). Overactive bladder and incontinence in the absence of the BK large conductance Ca^{2+}-activated K^+ channel. *J. Biol. Chem.* 279, 36746–36752. doi: 10.1074/jbc.M405621200

Meredith, A. L., Wiler, S. W., Miller, B. H., Takahashi, J. S., Fodor, A. A., Ruby, N. F., et al. (2006). BK calcium-activated potassium channels regulate circadian behavioral rhythms and pacemaker output. *Nat. Neurosci.* 9, 1041–1049. doi: 10.1038/nn1740

Montgomery, J. R., Whitt, J. P., Wright, B. N., Lai, M. H., and Meredith, A. L. (2013). Mis-expression of the BK K^+ channel disrupts suprachiasmatic nucleus circuit rhythmicity and alters clock-controlled behavior. *Am. J. Physiol. Cell Physiol.* 304, C299–C311. doi: 10.1152/ajpcell.00302.2012

Negoro, H., Kanematsu, A., Doi, M., Suadicani, S. O., Matsuo, M., Imamura, M., et al. (2012). Involvement of urinary bladder Connexin43 and the circadian clock in coordination of diurnal micturition rhythm. *Nat. Commun.* 3:809. doi: 10.1038/ncomms1812

Neveus, T., Hetta, J., Cnattingius, S., Tuvemo, T., Lackgren, G., Olsson, U., et al. (1999). Depth of sleep and sleep habits among enuretic and incontinent children. *Acta Paediatr.* 88, 748–752. doi: 10.1111/j.1651-2227.1999.tb00036.x

Noh, J. Y., Han, D. H., Kim, M. H., Ko, I. G., Kim, S. E., Park, N., et al. (2014). Presence of multiple peripheral circadian oscillators in the tissues controlling voiding function in mice. *Exp. Mol. Med.* 46:e81. doi: 10.1038/emm.2013.153

Paschos, G. K., and FitzGerald, G. A. (2010). Circadian clocks and vascular function. *Circ. Res.* 106, 833–841. doi: 10.1161/CIRCRESAHA.109.211706

Refinetti, R., Lissen, G. C., and Halberg, F. (2007). Procedures for numerical analysis of circadian rhythms. *Biol. Rhythm Res.* 38, 275–325. doi: 10.1080/09291010600903692

Reilly, D. F., Curtis, A. M., Cheng, Y., Westgate, E. J., Rudic, R. D., Paschos, G., et al. (2008). Peripheral circadian clock rhythmicity is retained in the absence of adrenergic signaling. *Arterioscler. Thromb. Vasc. Biol.* 28, 121–126. doi: 10.1161/ATVBAHA.107.152538

Su, W., Xie, Z., Guo, Z., Duncan, M. J., Lutshumba, J., and Gong, M. C. (2012). Altered clock gene expression and vascular smooth muscle diurnal contractile variations in type 2 diabetic db/db mice. *Am. J. Physiol. Heart Circ. Physiol.* 302, H621–H633. doi: 10.1152/ajpheart.00825.2011

Thorneloe, K. S., Meredith, A. L., Knorn, A. M., Aldrich, R. W., and Nelson, M. T. (2005). Urodynamic properties and neurotransmitter dependence of urinary bladder contractility in the BK channel deletion model of overactive bladder. *Am. J. Physiol. Renal Physiol.* 289, F604–F610. doi: 10.1152/ajprenal.00060.2005

Ticher, A., Sackett-Lundeen, L., Ashkenazi, I. E., and Haus, E. (1994). Human circadian time structure in subjects of different gender and age. *Chronobiol. Int.* 11, 349–355. doi: 10.3109/07420529409057252

Weiss, J. P., Weinberg, A. C., and Blaivas, J. G. (2008). New aspects of the classification of nocturia. *Curr. Urol. Rep.* 9, 362–367. doi: 10.1007/s11934-008-0063-7

Werner, M. E., Knorn, A. M., Meredith, A. L., Aldrich, R. W., and Nelson, M. T. (2007). Frequency encoding of cholinergic- and purinergic-mediated signaling to mouse urinary bladder smooth muscle: modulation by BK channels. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 292, R616–R624. doi: 10.1152/ajpregu.00036.2006

Wu, C., Sui, G., Archer, S. N., Sassone-Corsi, P., Aitken, K., Bagli, D., et al. (2014). Local receptors as novel regulators for peripheral clock expression. *FASEB J.* 28, 4610–4616. doi: 10.1096/fj.13-243295

Zuber, A. M., Centeno, G., Pradervand, S., Nikolaeva, S., Maquelin, L., Cardinaux, L., et al. (2009). Molecular clock is involved in predictive circadian adjustment of renal function. *Proc. Natl. Acad. Sci. U.S.A.* 106, 16523–16528. doi: 10.1073/pnas.0904890106

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 07 November 2014; accepted: 17 December 2014; published online: 09 January 2015.

Citation: White RS, Zemen BG, Khan Z, Montgomery JR, Herrera GM and Meredith AL (2015) Evaluation of mouse urinary bladder smooth muscle for diurnal differences in contractile properties. Front. Pharmacol. ***5****:293. doi: 10.3389/fphar.2014.00293*

This article was submitted to Pharmaceutical Medicine and Outcomes Research, a section of the journal Frontiers in Pharmacology.

frontiers in
NUTRITION

HYPOTHESIS AND THEORY ARTICLE
published: 23 February 2015
doi: 10.3389/fnut.2015.00004

Does circadian disruption play a role in the metabolic–hormonal link to delayed lactogenesis II?

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Breastfeeding improves maternal and child health. The American Academy of Pediatrics recommends exclusive breastfeeding for 6 months, with continued breastfeeding for at least 1 year. However, in the US, only 18.8% of infants are exclusively breastfed until 6 months of age. For mothers who initiate breastfeeding, the early post-partum period sets the stage for sustained breastfeeding. Mothers who experience breastfeeding problems in the early post-partum period are more likely to discontinue breastfeeding within 2 weeks. A major risk factor for shorter breastfeeding duration is delayed lactogenesis II (DLII; i.e., onset of milk "coming in" more than 72 h post-partum). Recent studies report a metabolic–hormonal link to DLII. This is not surprising because around the time of birth the mother's entire metabolism changes to direct nutrients to mammary glands. Circadian and metabolic systems are closely linked, and our rodent studies suggest circadian clocks coordinate hormonal and metabolic changes to support lactation. Molecular and environmental disruption of the circadian system decreases a dam's ability to initiate lactation and negatively impacts milk production. Circadian and metabolic systems evolved to be functional and adaptive when lifestyles and environmental exposures were quite different from modern times. We now have artificial lights, longer work days, and increases in shift work. Disruption in the circadian system due to shift work, jet-lag, sleep disorders, and other modern life style choices are associated with metabolic disorders, obesity, and impaired reproduction. We hypothesize that DLII is related to disruption of the mother's circadian system. Here, we review literature that supports this hypothesis, and describe interventions that may help to increase breastfeeding success.

Keywords: breastfeeding, chronodisruption, circadian clocks, delayed onset of lactogenesis II, lactation, metabolism, pregnancy, sleep

INTRODUCTION

The World Health Organization recommends breast milk as the ideal food source for growth and development of infants (1). Human milk functions not only as food for the infant, but also protects against infection, promotes intestinal, immune, and cognitive development (2), and stimulates establishment of the unique gut microbiome (3, 4) of the breastfed infant. Breastfeeding also has beneficial effects on short- and long-term maternal and infant health outcomes. Teens and adults who were breastfed as babies are less likely to be overweight or obese and less likely to develop type-2 diabetes as well as perform better on intelligence tests (4). Mothers who breastfeed return to their pre-pregnancy weight faster, have lower rates of obesity, and lower risks of developing breast and ovarian cancers (1).

Due to the tremendous health benefits of breastfeeding, the American Academy of Pediatrics recommends exclusive breastfeeding (i.e., no supplementation with formula or solid food) for about 6 months, with continuation of breastfeeding for 1 year or longer as mutually desired by mother and infant (5). Economic analysis of breastfeeding benefits revealed that $13 billion in healthcare costs would be saved and 911 infant deaths prevented each year if 90% of families in the US complied with medical recommendations to breastfeed exclusively for 6 months (6). However, rates of adequate breastfeeding are far below national targets. The 2011 National Immunization Survey reported rates of breastfeeding initiation were at 79.2%, with breastfeeding rates dropping precipitously after that. Exclusive breastfeeding fell by 20%, to 59% at 1 week post-partum, 40.7% at 3 months, and only 18.8% of mothers exclusively breastfed for 6 months (7).

The most common reason mothers cite for stopping breastfeeding before their infant reached 2 weeks old, was that the baby was unsettled, a behavior often interpreted by mothers as indicating an insufficient milk supply (8). Delayed lactogenesis II (DLII), the onset of milk "coming in" more than 72 h post-partum, is a major contributor to early formula supplementation, inadequate breastfeeding, and breastfeeding cessation (9, 10). Further, infants

of mothers who experience DLII are seven times more likely to lose excessive weight the first 5 days after birth (11).

LACTOGENESIS IN WOMEN

Lactogenesis occurs in several stages. Lactogenesis I occurs during pregnancy and is the initiation of the synthetic capacity of the mammary glands. Lactogenesis II commences after delivery and is the initiation of plentiful milk secretion. Changes in milk composition from colostrum to mature milk in combination with a sudden feeling of breast fullness mark the onset of lactogenesis II, which normally occurs between 30 and 40 h following the birth of a full-term infant (10). Lactogenesis II is initiated post-partum by a fall in progesterone while prolactin levels remain high. The process does not depend on suckling of the infant until about the third or fourth day post-partum. Comparison between breastfeeding and non-breastfeeding women showed prolactin levels and milk secretion volumes are the same between groups of women the first 2 days post-partum (12, 13). Beginning day 3, post-partum prolactin levels begin to become significantly less in non-lactating women (12), and by day 4, secretion volume is lower in non-lactating women with lack of milk-removal initiating mammary involution and compositional differences in breast secretions between the groups (13). Thus, although breastfeeding is not necessary for initiation of lactogenesis II, it is essential for the continuation of lactation. The final stage of lactogenesis, lactogenesis III, also called galactopoiesis, is the production and maintenance of mature milk from day 9 post-partum, until weaning.

RISK FACTORS FOR DELAYED ONSET OF LACTOGENESIS II

Risk factors associated with DLII include primiparity, Cesarean delivery, longer duration of labor, and elevated blood cortisol concentrations (**Table 1**). The risk for low milk volume on day 4 post-partum was 4.3-fold (95% confidence interval-CI: 1.5–12.4) higher for mothers of pre-term infants delivered by Cesarean section versus vaginally (14). In this study, Cesarean delivery was associated with pregnancy-induced hypertension, delayed milk expression initiation, and low pumping frequency. Together, these findings suggest a composite of underlying risk factors contributes to the association of Cesarean delivery with DLII and low milk volume.

Studies of primiparous women revealed that independent risk factors for DLII were maternal age $\geq$30 years, body mass index (BMI) in the overweight or obese range, and infant birth weight >3600 g (15). A dose-response relation to BMI was evident, with risk of DLII being 1.84 (95% CI: 1.02–2.80) times higher in overweight and 2.21 (95% CI: 1.52–4.30) times higher in obese women, as compared with women with a BMI in the healthy range (15). In obese women, DLII was not associated with psychosocial factors, such as planned duration of breastfeeding or behavioral beliefs about breast- and bottle-feeding (16). Therefore, it is likely that there is a physiological basis for the delay. Older maternal age and higher BMI are known risk factors for gestational diabetes (17). Lower glucose tolerance in the antenatal period was associated with longer time to onset of lactation (18), and prolactin release in response to suckling in the early post-partum period was found to be significantly lower in the overweight/obese women compared to healthy weight women (19). Importantly, low prolactin levels in women, as described for Sheehan's syndrome, are associated with failed lactogenesis II (20). In addition, DLII often leads to failed lactogenesis II (14). Failed lactogenesis II is a condition wherein the mother is either able to achieve full lactation but an extrinsic factor has interfered with the process, or one or more factors results in failure to attain adequate milk production (10). Failed lactogenesis II can be described further in the context of two types of conditions: a primary inability to produce adequate milk volume, or a secondary condition as a result of improper breastfeeding management and/or infant-related problems (10).

Table 1 | Risk factors for delayed or failed lactogenesis II [Modified from Ref. (10)].

Delayed lactogenesis II
Primiparity
Psychosocial stress/pain
Maternal obesity
Diabetes
Hypertension
Stressful labor and delivery
Cesarean section
Delayed first breastfeed episode
Low perinatal breastfeeding frequency
Elevated cortisol
Failed lactogenesis II and/or low milk supply
Breast surgery/injury
Retained placental fragments
Cigarette smoking
Hypothyroidism, hypopituitarism
Ovarian theca-lutein cyst
Insufficient mammary glandular tissue
Polycystic ovarian syndrome

METABOLIC-HORMONAL ADAPTATIONS TO LACTATION

Lactation is the continuum of reproduction in mammals, and the most energetically demanding stage. Metabolically, the reproductive process in females can be divided into three periods which correspond to the energetic needs of the fetus and neonate. Period one spans the first two-thirds of pregnancy. There is little demand for nutrients by fetus during the first two trimesters, so the mother uses this time to store energy by increased consumption and enhanced lipogenesis (21). To support large gains in fetal growth, the mother transitions to a catabolic state in the last third of pregnancy, period two. Period two is characterized by increased gluconeogenesis, decreased peripheral tissue glucose utilization, increased fatty acid mobilization from adipose, and increased amino acid mobilization from muscle (22). Period three is lactation. During this period, the dam's metabolism changes to accommodate the even greater energetic demands of milk synthesis. All the lactose and protein and most lipids in milk are synthesized in mammary gland, and thus the mammary gland has a high requirement for circulating substrates (glucose, amino acids, free fatty acids, and triglycerides) (21–23). In addition to further increasing metabolic responses described for period 2, there are substantial increases in size and complexity of the maternal intestine, liver,

and cardiovascular system, including increased mammary blood flow, increased blood flow to liver and gastrointestinal tract, and higher cardiac output (24). Thus, the transition from pregnancy to lactation represents a major physiological change requiring on the one hand, coordinated changes in various body tissues, and on the other hand, mammary-specific changes to support a dominant physiological process (production of milk).

During pregnancy and at the onset of lactation, dramatic changes in circulating levels of reproductive and metabolic hormones (e.g., estrogen, progesterone, placental lactogen, prolactin, leptin, and cortisol) occur (12, 25). Hormonal changes stimulate metabolic changes in almost every organ of the body so that nutrients and energy can be diverted to the fetus to support growth before birth and then to the mammary gland to support milk synthesis post-partum (26, 27). Therefore, factors affecting metabolic-hormonal regulation (e.g., obesity, diabetes, hypothyroidism) during pregnancy, may also impact the ability of the mother to initiate lactation.

During pregnancy, the high levels of circulating progesterone enable differentiation of the mammary gland while inhibiting the secretory process of the mammary gland. Once the placenta is expelled after birth, progesterone levels decline rapidly, and increasing prolactin levels trigger the beginning of lactogenesis II (28). Neonatal suckling induces a neuroendocrine response that stimulates secretion of prolactin and glucocorticoids as well as oxytocin, which stimulates expulsion of milk from the gland (29). Increases in prolactin, estradiol, and cortisol levels during the periparturient period decrease peripheral tissue insulin sensitivity and responsiveness. These changes in insulin homeostasis result in increased rates of lipolysis and gluconeogenesis and decreased rates of glucose uptake by adipose and muscle, and decreased protein synthesis in muscle with concomitant increases in protein degradation and amino acid release (23, 30). Thyroid hormones are also essential for efficient milk production (31). A study of women with insufficient lactation found that the nasal administration of thyrotropin-releasing factor increased prolactin and daily milk volume (32).

HYPOTHESIS: METABOLIC–HORMONAL–CIRCADIAN CLOCK LINK TO DELAYED LACTOGENESIS II

As outlined above, maternal hormonal milieu stimulates metabolic adaptations to reproductive state and mammary gland responsiveness. Therefore, it follows that conditions with a hormonal etiology (e.g., diabetes, hypothyroidism, or obesity) may interfere with these adaptations and cause a delay in lactogenesis II (10). Furthermore, some delivery modes and conditions that result in a delay in breastfeeding initiation and/or breast stimulation (e.g., pre-term, Cesarean, or a prolonged second stage of labor) may impact periparturient hormonal milieu needed to stimulate metabolic and mammary-specific adaptations needed to initiate copious milk secretion. We hypothesize that disruption of the circadian timing system during pregnancy and peripartum play a role in DLII.

The circadian timing system is intimately linked and reciprocally regulated by hormones and metabolism, and below we describe our preliminary studies that support this hypothesis. In addition, we summarize findings from a comprehensive database search in PubMed used to further support our hypothesis. In searching the literature to investigate this hypothesis, we found one of the immediate challenges encountered was the lack of studies conducted relating to the circadian timing system in pregnant or lactating women (33–36). In addition, information about what was considered normal or abnormal for circadian rhythms in pregnancy and lactation was lacking. Thus, much of the evidence used to develop and support our hypothesis was drawn from studies conducted on a more general population or inferred from animal studies.

THE CIRCADIAN TIMING SYSTEM

Nearly all physiological and behavioral functions of animals are rhythmic including secretion patterns of hormones, sleep–wake cycles, metabolism, and core body temperature. These circadian rhythms, 24 h cycles in biochemical, physiological, or behavioral processes, evolved as a common strategy among animals to coordinate internal systems and synchronize these systems to the environment (37, 38). Circadian rhythms are generated at the molecular level by circadian clocks. In mammals, circadian clocks are regulated hierarchically, with the master circadian clock located centrally in the suprachiasmatic nuclei (SCN) of the hypothalamus. In addition to the SCN, there are peripheral clocks distributed in every organ. The intrinsic rhythmicity of the SCN is entrained by synchronization to the 24-h day to regularly occurring environmental signals. The light–dark cycle is the most important environmental cue for entraining the master clock (39). Other cues include exercise, food availability, temperature, and stress, which directly or indirectly entrain the SCN (40, 41). The SCN integrates this temporal information and translates it into hormonal and autonomic signals that influence and synchronize peripheral clocks in every tissue of the body (42). In turn, peripheral clocks drive the circadian expression of local transcriptomes, thereby coordinating metabolism and physiology of the entire animal.

The circadian timing system must continuously adapt to and synchronize with the environment and the body's internal signals in order to organize clocks into a coherent functional network that regulates behavior and physiology. Hallmarks of organization of circadian timing are the perception of environmental input, integration of time-related information into the circadian clock "device" (molecular clock), and transmission of adjusted timing information as output of metabolic and physiological processes (**Figure 1**). The molecular clock mechanism is based on a transcription-translation feedback loop. At the core of this loop are two transcription factors, CLOCK (or its ortholog NPAS2) and BMAL1, which in the form of a heterodimer drive rhythmic expression of output genes either directly via E-box regulatory element in their promoter regions, or indirectly by other transcription factors whose expression is under clock control (43). Among transcriptional targets of this complex are *Period* and *Cryptochrome* genes, whose products function as negative regulators of CLOCK/BMAL1-mediated transcription [**Figure 1**; (44)]. Approximately, 10–20% of genes expressed in a tissue exhibit circadian rhythms (45). Tissue-specific clock-controlled genes are involved in rate-limiting steps critical for organ function. For example, in the liver, molecules involved in metabolism of carbohydrate, lipid, and cholesterol encode genes that exhibit

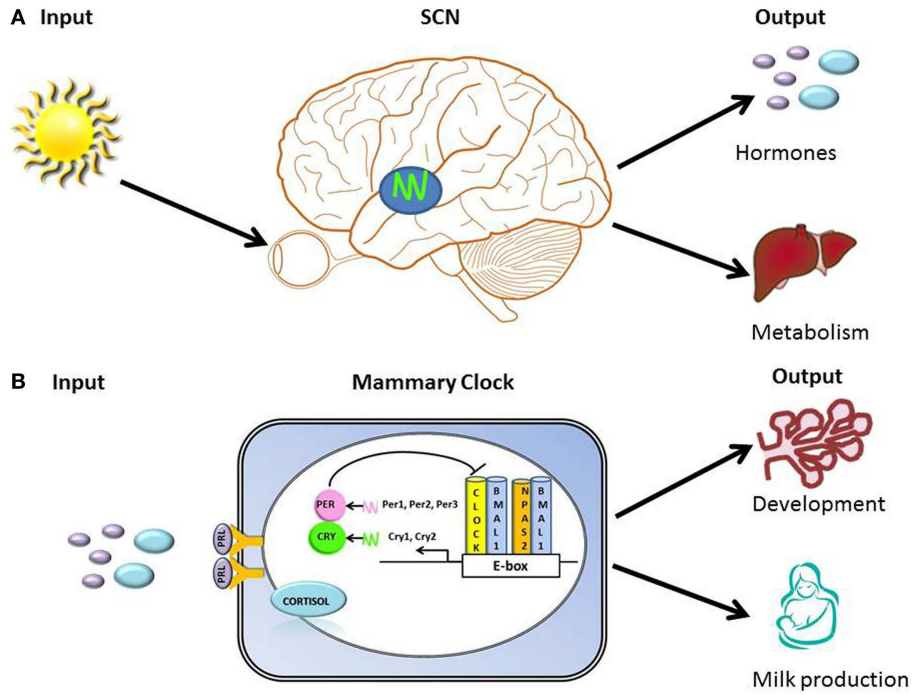

FIGURE 1 | Hallmarks of organization of clocks and circadian timing system. **(A)** Illustration of primary input (light) to the SCN and outputs that include regulation of hormones (e.g., prolactin and cortisol) and metabolism. **(B)** Illustration of the transcription-translation feedback loop of the core molecular clock device, with proposed inputs [prolactin (PRL) and cortisol] and outputs (regulation of breast development and milk production) of mammary clock. At the core of the transcription-translation feedback loop are two transcription factors, CLOCK (or its ortholog NPAS2) and BMAL1, which in the form of a heterodimer drive rhythmic expression of output genes either directly via E-box regulatory element in their promoter regions, or indirectly by other transcription factors whose expression is under clock control (43). Among transcriptional targets of this complex are *Period* and *Cryptochrome* genes, whose products function as negative regulators of CLOCK/BMAL1-mediated transcription.

coordinated circadian expression (45). We propose that the mammary clock functions to regulate gland development and metabolic output [**Figure 1**; (46)].

Intimate interactions and reciprocal regulation occur between metabolic and circadian systems. The endogenous circadian timing system coordinates daily patterns of feeding, energy utilization, and energy storage across the daily 24 h cycle (47). Many metabolic hormones exhibit circadian rhythms. For example, cortisol levels are highest in the early morning and lowest at the first part of the biological night (47). Further, the SCN is responsible for a 24-h rhythm in plasma glucose concentrations, with the highest concentrations occurring toward the beginning of the activity period (48).

CHRONODISRUPTION: CONSEQUENCES TO METABOLISM AND HEALTH

Disruptions of normal circadian timing can evoke a multitude of downstream effects, including reorganizing the entire physiological state. Depressive mood (41), light, activity, and eating at night [e.g., night-shift work and night-eating syndrome; (49–53)], excessive weight (54), stress, and sleep disturbances (55) have all been characterized as chronodisruptors, i.e., factors that disrupt circadian rhythms. Circadian disruption can result in disorders such as diabetes, obesity, and cardiac disease (56–58). In humans, living in modern industrialized societies with 24 h access to light coupled with work and social obligations often leads to behaviors that are inappropriately timed relative to endogenous circadian rhythms. Night-shift work is an example of severe circadian disruption, as workers are awake, active, and eating during their biological night and trying to sleep and fast during their biological day (59).

Animal studies demonstrated that being active and feeding during the usual rest phase leads to alterations in metabolism and weight gain, even with the same caloric intake (60). In humans, internal desynchronization can be induced by a forced 28-h sleep–wake cycle (8 h sleep, 20 h awake), which is outside the range of entrainment for the human circadian clock (61). After four cycles, this protocol results in circadian misalignment, in which the behavioral sleep–wake cycle is 12 h out of phase with the circadian cycle. In these misaligned conditions, leptin rhythms are blunted, postprandial glucose and insulin are increased, and cortisol rhythms are 180° out of phase with the behavioral rhythm. Nearly half of the participants undergoing the 28-h cycle exhibited a pre-diabetic state during circadian misalignment (62).

Epidemiological studies have shown night-shift work, which disrupts the circadian system, is associated with development of obesity. Studies of women with phase-delayed eating patterns, such as not eating breakfast or night-eating syndrome, are associated with increased BMI, altered metabolism, changes in plasma hormone concentrations and rhythms, and depressive mood (52, 53). At the molecular level in humans, a single

nucleotide polymorphism in CLOCK is associated with abnormal fatty acid metabolism and development of fatty liver, and a polymorphism in the BMAL1 core circadian clock gene is associated with susceptibility to hypertension and type-2 diabetes (63, 64).

In reciprocal, the over-fat state is characterized by alterations of circadian rhythms. In obese mice, there is attenuation of rhythmic gene expression patterns (65), and a delay in circadian entrainment to light-phase shift (66). Circadian rhythms of glucose and insulin are elevated in obese rats throughout the 24-h period. Levels of growth hormone, prolactin, and thyroxine are depressed. Serum levels of corticosterone do not exhibit distinct circadian rhythms and are elevated throughout the circadian cycle in obese rats (67). Similarly, in obese humans, basal levels of cortisol are higher with an attenuation of the circadian rhythm (68) and a lengthening of rhythm period (54).

With the advent of electric lighting, humans in industrialized societies are exposed to light at night. The natural light–dark cycle is the most salient cue for entraining the master clock to the 24-h day. The SCN communicates photoperiodic information to the pineal gland, where light inhibits melatonin secretion, such that melatonin secretion normally occurs at night. Melatonin has a fundamental role in regulating and timing several physiological functions, including glucose homeostasis, insulin secretion, and energy metabolism (69). As such, metabolism is impaired after a reduction in melatonin production, and the basic processes associated with acquisition and utilization of energy are functionally altered after exposure to extended periods of artificial lighting (70). Chronic light at night exposure suppresses melatonin levels as well as disrupts central clock rhythms, both of which are implicated in metabolic disturbances that predispose individuals to the development of type-2 diabetes, obesity, and metabolic syndrome (70). For example, a recent cross-sectional study of 500 people in Japan (71) found that elderly people sleeping in lighter rooms had higher body weight, waist circumference, and BMI; in that study, light exposure and obesity outcome variables were all objectively measured, although the BMI of participants was generally low (an average of 22.8). A large cohort study of 100,000 women revealed that the association between light at night exposure and obesity increased the odds of obesity with increasing levels of light at night exposure (72).

Sleep is cooperatively regulated by homeostatic and circadian factors. Voluntary sleep curtailment has become common in many modern life styles. For example, although the National Institutes of Health recommends that adults need 7–8 h of sleep per day (73), data from the National Health Interview Survey, found nearly 30% of adults reported an average of $\leq$6 h of sleep per day in 2005–2007 (74). Less than 1 week of sleep curtailment in healthy young men was associated with lower glucose tolerance, lower thyrotropin concentrations, and raised evening concentrations of cortisol (75). Poor sleep quality is also associated with increased risk for depression (76). Whereas short sleep duration is associated with increased incidence of diabetes, obesity (77), as well as increased all-cause mortality (78), there also appears to be a consistent association of poor sleep quality and short sleep duration with increased risk of cardiovascular disease, an association that is stronger in women than men (79, 80).

Disrupted sleep includes both abnormal sleep patterns and sleep deprivation. Studies have shown that disrupted sleep cycles impair the function of adipocytes, which regulate leptin levels (81). Abnormal leptin levels may lead to irregular meal times (82). This disrupts the balance between insulin and glucose cycles, causing reductions in insulin sensitivity and increases in glucose concentration, a prelude to diabetes (47, 81, 83). Lipid metabolism is similarly impaired, which may lead to increased lipogenesis, and by extension, obesity (82, 83). Impaired carbohydrate and lipid metabolism from disrupted circadian rhythms have also been linked to increased risk of cardiovascular disease (83, 84).

In human studies of shift work and atypical schedules, irregular sleep and disruptive circadian rhythms appear together, indicating that the two are closely related and that the presence of one usually entails the other (85, 86). In humans, sleep is normally timed to occur during the biological night, when body temperature is low and melatonin is synthesized. The sleep–wake cycle, and associated cycles of darkness and light and fasting and feeding, interacts with the circadian system and is a major driving factor of rhythms in physiology and behavior (87). Desynchrony of sleep-wake timing and other circadian rhythms, such as occurs in shift work and jet-lag, is associated with disruption of rhythmicity in physiology and endocrinology (87). Insufficient or mistimed sleep reduces the rhythmicity of clock-controlled transcripts and expression of core circadian clock genes. Thus, circadian disruption occurs as a result of irregular sleep patterns, (47, 82, 85, 86), and in converse circadian abnormalities can also result in sleep disturbances (88).

In summary, changes in glucose and lipid metabolism, abnormally high levels of cortisol at night, changes in melatonin, leptin, and thyroid hormone levels, as well as cardiovascular problems and development of type-2 diabetes are commonly associated with disruptions in circadian rhythms. Exposure to light, activity or eating at night, sleep disturbances/curtailment, depression, and stress are common chronodisruptors in many modern life styles and work schedules, and thus may be partly responsible for the rise in metabolic disease and obesity apparent in many industrialized societies (89).

CIRCADIAN SYSTEM REGULATION OF AND ADAPTATIONS TO PREGNANCY AND LACTATION

As highlighted in multiple recent review articles (33, 90–92), much more work is needed to understand interactions among circadian clocks, metabolism, and female reproductive cycles and states. What is known, is that the circadian system plays a key role in the timing of reproductive events and hormones important to the regulation of pregnancy and lactation. For example, neural mechanisms regulating ovulation are under circadian control in many species to ensure that the timing of greatest fertility coincides with the period of maximal sexual motivation (93). SCN lesions result in infertility in rodents, due to the lack of the ability to synchronize events for ovulation (94), and mice with mutant core clock genes or core clock-gene knocked-out mice exhibit reduced fertility and fecundity (95, 96).

The SCN has been shown to be necessary for normal functioning of the hypothalamic-pituitary-gonadal (HPG) axis, and rhythms of clock-gene expression have been recorded in brain

regions controlling both the HPG and hypothalamic-pituitary-adrenal (HPA) axis (97). Rhythmic gene expression of prolactin in pituitary mammotrophs was shown to be mediated by CLOCK–BMAL1 binding to clock-gene regulatory elements (98). In addition, ovariectomized and estradiol-treated rats fail to exhibit a prolactin surge following SCN lesions. Furthermore, SCN lesion also prevents the twice daily prolactin surge induced by mating in rodents, which maintains the corpus luteum and thus the secretion of progesterone and pregnancy maintenance [for review, see Ref. (93)], suggesting the central clock plays a direct or indirect role in regulation of prolactin secretion. Therefore, it is interesting to speculate that decreased blood prolactin observed in healthy, young non-pregnant women following exposure to partial sleep deprivation (99) is due to the disruption of the master clock.

Circadian rhythms in behavior and physiology change substantially as female mammals transition through the reproductive states of non-pregnancy, pregnancy, and lactation, with changes in circadian rhythms supporting physiological demands unique to each of these stages (100–104). For example, to compensate for increases in the daily temperature minimum during gestation studies of pregnant laboratory animals showed phase of body temperature rhythm was advanced and amplitude decreased relative to non-pregnant controls (100). Further, to compensate for the increased need for sleep in early pregnancy, sleep patterns are altered in pregnant rodents (103, 105).

Sleep is also significantly impacted by pregnancy in women. A study of 192 pregnant women surveyed retrospectively found 88% had alterations in sleep compared with their usual experience (106). Reported changes included insomnia, parasomnias (nightmares and night terrors), restless leg syndrome, snoring, and sleep apnea. Among the most frequent self-reported causes of sleep disturbance during pregnancy were urinary frequency, back or hip ache, and heartburn. A prospective, cohort study of healthy nulliparous women found compared with the baseline assessment done before 20 weeks gestation, mean sleep duration in the third trimester was significantly shorter (7.4 h compared with 7.0 h), and overall poor sleep quality became significantly more common as pregnancy progressed (107). Okun and Coussons-Read collected qualitative sleep data at 12, 24, and 36 weeks' gestation, and found as early as 12 weeks, pregnant women reported an increased number of naps, nocturnal awakenings, time spent awake during the night, and poorer sleep quality than non-pregnant women (108).

The dramatic fluctuations in reproductive hormones that occur during pregnancy and the transition from pregnancy to the post-partum period are accompanied by alterations in circadian rhythms of melatonin (109, 110) and cortisol (111). In seasonally breeding animals, melatonin regulates reproductive hormones and behavior (112). During pregnancy in humans, night time melatonin levels increase linearly with progressive weeks of gestation, and fall in the early post-partum period (110). Changes in cortisol dynamics during pregnancy are due in part to the remodeling of maternal HPA axis, which results in an altered maternal stress response and energy balance, as well as rising placental cortico-releasing hormone (CRH) levels (113). Change in the HPA axis and placental CRH result in attenuated rhythms of plasma cortisol and a period of hypercortisolism beginning in mid-gestation. Following birth of the neonate, maternal plasma levels of cortisol drop due to loss of placental CRH, if the mother breastfeeds, attenuation in cortisol rhythms and stress response continue throughout lactation (114, 115). Synchronization among the multitude of molecular clocks in the body is believed to be regulated in part by cortisol circadian rhythms which are regulated by the central clock (116). Thus, changes in cortisol secretion patterns during pregnancy and lactation have the potential to affect circadian rhythms across the entire body.

Timing of parturition in women also shows signs of being regulated by the circadian timing system. For example, the onset of labor and spontaneous membrane rupture peaks at night between midnight and 4:00 a.m. (117–119), and the timing of births peak around 1:00–2:00 p.m. for primiparous women (120). Further, a nested, randomized, controlled clinical trial that compared morning versus evening administration of prostaglandin and its success rate in inducing labor, reported no difference in rate of Cesarean delivery, however morning inductions required less oxytocin, had a shorter induction to birth interval, and were less likely to result in instrumental vaginal births for primiparous mothers (121).

During lactation in women, the potent lactogens, prolactin and cortisol, exhibit circadian variation in secretion. The prolactin-secretory response to nursing is superimposed on the endogenous circadian rhythm of prolactin secretion, thus the suckling stimulus elevates prolactin levels more effectively at certain times of day when the circadian input enhances the suckling stimulus-evoked secretory response (122). Studies in lactating rabbits revealed timing the single bout of daily suckling that occurs in this species shifted PER1 expression in SCN clock and in peripheral clocks of the brain (76, 77). Our *in vitro* studies showed prolactin and glucocorticoids can directly affect mammary clock, with prolactin inducing phase shifts in core clock genes expression, suggesting that external cues emanating from neonate can have effects on maternal circadian physiology.

Our rodent studies also demonstrated that during the transition from pregnancy to lactation, dynamic changes in core clocks occurred in multiple tissues. The amplitude of core clock genes' expression increased significantly in the SCN and liver (123). Work of others found that expression of PER2 expression shifted and amplitude increased in SCN in early pregnant versus diestrus rats (124). The central clock functions to synchronize the timing of metabolic and reproductive functions, and thus changes in the SCN during the transition in physiological states may function to mediate coordinated changes in tissue-specific metabolism needed to support pregnancy and lactation. Increases in amplitude of hepatic expression of core clock genes' rhythms during the transition from pregnancy to lactation, likely reflect the increase in liver metabolic output (123). In addition, changes revealed in mammary clock dynamics led us to hypothesize that differentiation-driven changes during the transition from pregnancy to lactation in the mammary clock are stimulated, in part, by peripartum changes in prolactin and glucocorticoids. Further, we envision that differentiation-associated changes in mammary clock mediate the increase in metabolic output of the gland during lactation (123).

Milk synthesis and composition shows circadian variation in lactating women (24). Approximately, seven percent of the genes expressed in the lactating breast show circadian oscillation; many

of these genes regulate cell growth and differentiation as well as metabolic pathways (125). Offspring of homozygous female *Clock-Δ19* mutant mice fail to thrive suggesting that the mutation affects the dam's ability to support milk production during lactation. Our studies of *ClockΔ19 mice* revealed poorer mammary development and evidence for delayed or failed lactogenesis II, with *in vitro* studies demonstrating a role for *Clock* in regulating mammary epithelial growth and differentiation (unpublished data). Miller et al. have evidence to suggest that prolactin release is altered in *ClockΔ19 mice* (126). Thus, both systemic and mammary-specific alterations likely account for negative impact of *ClockΔ19* mutation on lactation. The photoperiod effect on ruminant milk production (127) and our studies with cattle showing circadian disruption significantly decreases milk production (46), also support a role for the circadian timing system in mediating systemic metabolism and mammary metabolic output during lactation.

CONSEQUENCES OF CHRONODISRUPTION ON ABILITY OF MOTHER TO SUPPORT OFFSPRING

Several rodent studies have been designed to determine if circadian disruption impacts pregnancy outcome. These studies found that exposing mice immediately after confirmed mating to continuous shifts in the light-dark cycle (a chronic jet-lag model) resulted in a significant decrease in the number of full-term pregnancies (128). Rat dams exposed to chronic jet-lag throughout gestation gained 70% less weight during the first week of pregnancy than those housed in control conditions. In late pregnancy (gestation day 20), chronic jet-lag exposure profoundly disrupted timing of corticosterone, leptin, glucose, insulin, free fatty acids, triglycerides, and cholesterol concentrations in these dams. Further, expression of gluconeogenic and circadian clock genes in maternal and fetal liver was arrhythmic relative to controls (129). Offspring of rat dams exposed to a chronic jet-lag paradigm from the first day of pregnancy to lactation day 10 developed metabolic problems such as obesity, hyperleptinemia, and glucose tolerance/insulin insensitivity when they reached maturity (130). These studies demonstrate that exposure to chronic circadian disruption during pregnancy impacts the normal maternal metabolic-hormonal adaptations to this physiological state. Further, these perturbations may contribute to the programing of poor metabolic homeostasis in adult offspring.

In humans, a polymorphism in the circadian clock-gene BMAL1 was shown to be associated with increased risk of miscarriages (131). Studies of shift workers found night and rotating shift work during pregnancy increased the risk of pre-term birth, low birth weight, and miscarriage (132, 133). For example, a retrospective study of a large cohort in women (National Birth Cohort in Denmark) reported a fixed night work schedule increases risk of post-term birth (odds ratio, 1.35; 95% CI, 1.01–1.79). Fixed evening work had a higher risk of full-term low birth weight (odds ratio, 1.80; 95% CI, 1.10–2.94); and shift work as a group showed a slight excess of small-for-gestational-age babies (odds ratio, 1.09; 95% CI, 1.00–1.18) (134). A population-based prospective cohort study conducted in Sri Lanka found risk factors for small-for-gestational-age were shift work and exposure to physical and chemical hazards during second and third trimesters (odds ratio, 4.20; 95% CI, 1.10–16.0), as well as sleeping ≤8 h during second or third trimesters (odds ratio, 2.23; 95% CI, 1.08–4.59) (135).

A prospective cohort study of approximately 1,200 healthy pregnant women was used to evaluate the influence of maternal self-reported sleep duration during early pregnancy on blood pressure levels and risk of hypertensive disorders of pregnancy. Investigators found that the mean third trimester systolic blood pressure was higher for women reporting ≤6 and 7–8 h sleep compared with women reporting 9 h of sleep, with odds ratio for pre-eclampsia in very short (<5 h) sleepers being 9.52 (95% CI, 1.83–49.40) (136). Sleep disturbances in early pregnancy are also associated with higher risk for development of hyperglycemia (137). Moreover, gestational diabetes mellitus risk was increased among women sleeping <4 h compared with those sleeping 9 h per night during early pregnancy with relative risk for overweight women threefold higher (138). Snoring, which is associated with sleep disturbances, was associated with a 1.86-fold (95% CI, 0.88–3.94) increased risk of gestational diabetes, with the risk being 6.9-fold (95% CI, 2.87–16.6) higher in overweight women who snored compared with lean women (138). Hyperleptinemia is also an important clinical risk factor for adverse pregnancy outcomes such as pre-eclampsia and gestational diabetes mellitus (139–141). A cross-sectional study of 830 pregnant women found that shorter sleep (≤5 h) and longer sleep (≥9 h) were associated with elevated leptin among overweight or obese women (142).

Researchers have also linked abnormalities in circadian rhythms with development of mood disorders such as bipolar disorder, major depression, and seasonal affective disorder (143, 144). Individuals with major depression exhibit blunted or abnormal circadian rhythms in body temperature, plasma cortisol, norepinephrine, thyroid stimulating hormone, blood pressure, pulse, and melatonin (143). Studies of depressed pregnant women found significantly lower levels and phased-advanced melatonin secretion in pregnant women with personal and family histories of depression relative to women without history of depression (110). Further, in healthy women, plasma melatonin levels became increasingly elevated as pregnancy progressed but this increase did not occur in depressed women (110). Thus, it is interesting to speculate whether mothers with depression are at an increased risk for shorter breastfeeding duration and increased breastfeeding difficulties (145), in part, through physiological disruption of the circadian timing system, which in turn impacts her milk production (**Figure 2**).

The association of maternal obesity with DLII appears to have a physiological basis related to alterations in hormones and metabolic adaptations needed to initiate copious milk production (**Figure 2**). In rodent models of obesity, the normal hormonal response to the periparturient period is altered, with a lower rise in prolactin and insulin levels during the transition from pregnancy to lactation and significantly higher corticosterone levels (146–148). In obese humans, basal levels of cortisol are also higher with an attenuation of the circadian rhythm (68) and a lengthening of rhythm period (54). Circadian rhythms of plasma cortisol are believed to be a primary signal for synchronization of peripheral clocks (149). Glucocorticoids also regulate milk synthesis (150). However, antenatal treatment with glucocorticoids delays secretory activation in ewes (151), and treatment of animals with

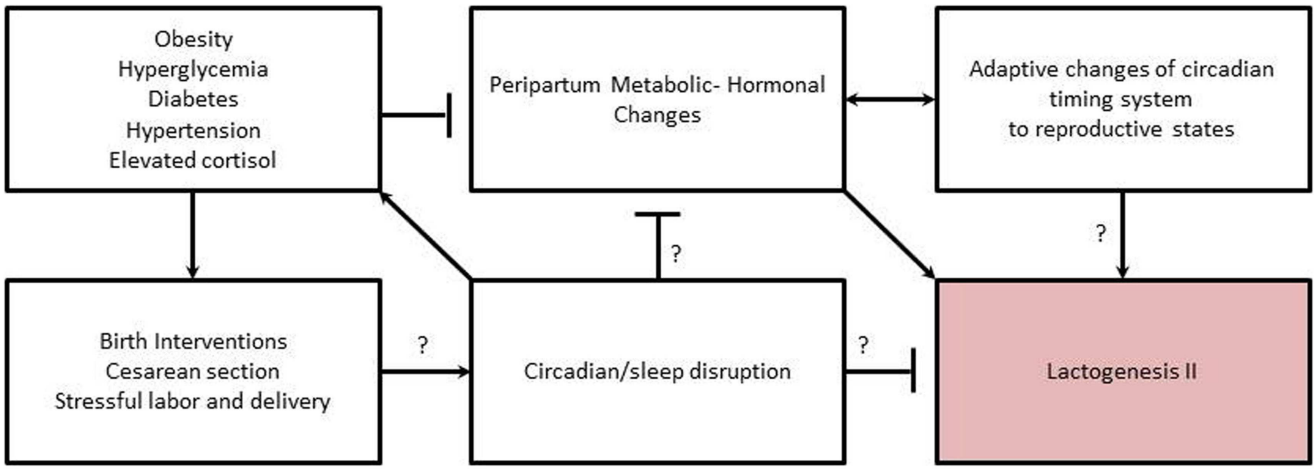

FIGURE 2 | Proposed relationships among metabolic-hormonal-circadian clock changes needed to initiate lactogenesis II during the periparturient period, and how related risk factors interfere with initiation. Arrows indicate inductive relationship. "T" indicates an inhibitory relationship. Question marks indicate areas needing further research to support proposed relationship/mechanism.

supra-physiological of glucocorticoids depresses milk production in an established lactation (150). Thus, the delay in onset of lactogenesis II experienced by obese women may be due to alterations in coordinated changes and interactions among circadian timing system, endocrine milieu, and metabolism needed to initiate copious milk secretion (**Figure 2**).

CONCLUSION AND POTENTIAL INTERVENTIONS

Although there is a paucity of information available to understand the role of the circadian timing system in mediating metabolic and hormonal adaptations to pregnancy and lactation, there is strong evidence that clocks play a role in regulating metabolic and hormonal homeostasis in animals. The circadian system functions to prepare physiological systems and behavioral activity for anticipated changes in the environment (e.g., day–night cycle). In addition, the circadian system also prepares for anticipated changes in physiological–reproductive state (e.g., seasonal fertility in some species). Chronic disruption of the circadian timing system has negative impacts on fertility and fecundity in females. Fertility and fecundity depend on precise hormonal timing and adequate metabolic adaptions to support the extra energy investment of reproduction (92, 152). Similarly, the initiation of lactogenesis II, requires timing coordinated changes in hormones and metabolism to initiate copious milk production in the early post-partum. Thus, we hypothesize that chronic disruption of the maternal circadian timing system during pregnancy and peripartum alters hormones and metabolic adaptations resulting in DLII (**Figure 2**).

Human lactation is a complex phenomenon and the initiation and duration of breastfeeding is influenced by many demographic, physical, social, and psychological variables. Interventions developed to increase the rates of successful breastfeeding target management strategies to ensure adequate milk supply (153–155). Although there is evidence to suggest that the circadian system plays a significant role in lactation and maternal behavior, current breastfeeding interventions do not encompass management strategies and education that take into account circadian disruptions. Depressive mood, light, activity, and eating at night (e.g., night-shift work and night-eating syndrome), excessive weight, and sleep disturbances are well characterized chronodisruptors. These chronodisruptors have also been associated with hormonal and metabolic alterations during pregnancy and inadequate breastfeeding outcomes.

Therefore, we propose the need to test interventions aimed at maintaining circadian alignment (e.g., limiting exposure to chronodisruption) during three stages that impact the ability of the mother to initiate and maintain lactogenesis: (1) during pregnancy; (2) in the hospital; and (3) after post-partum discharge from the hospital. Interventions during pregnancy may include, raising mothers' awareness of their sleep, eating and exercise patterns through diaries and self-monitoring, as well and educating mothers about sleep hygiene and consequences of sleep deprivation and interrupted sleep cycle and exposure to light at night.

Good sleep hygiene, together with circadian alignment of food intake, a regular meal frequency, as well as attention for protein intake or diets, may contribute to cure sleep abnormalities and overweight/obesity (47). Circadian alignment diminishes the urge to overeat, normalizes substrate oxidation, stress, and insulin and glucose metabolism. In addition, circadian alignment impacts leptin concentrations, lipid metabolism, blood pressure, appetite, energy expenditure, and substrate oxidation, and normalizes the experience of food reward (47). For example, a clinical trial investigated whether sleep extension under real-life conditions is a feasible intervention in 16 healthy non-obese adults who were chronically sleep restricted (156). The intervention was 2 weeks of habitual time in bed, followed by 6 weeks during which participants were instructed to increase their time in bed by 1 h per day. Continuous actigraphy monitoring and daily sleep logs during the entire study showed that sleep time during weekdays increased (mean actigraphic data: 44 ± 34 min, $P < 0.0001$;

polysomnographic data: 49 ± 68 min, $P = 0.014$), without any significant change during weekends. Changes from habitual time in bed to the end of the intervention in total sleep time correlated with changes in glucose and insulin levels, as well as with indices in insulin sensitivity.

In the hospital, interventions may include implementing light–dark cycles and/or light filters that help to maintain circadian alignment; educating families about the importance of limited visiting hours and number of visitors; and implementing Baby Friendly Hospital Initiative (157) to include quiet time and light-dark cycles. Discharge interventions may include providing education about sleep hygiene, diet, and activities important to maintain circadian alignment.

Exposure to lighting environments that more closely align to the Earth's natural light–dark cycles may prove to limit metabolic-hormonal disturbances during pregnancy and promote normal metabolic-hormonal adaptations during the peripartum needed to initiate lactation. An example of the recognition that hospital lighting environment impacts physiology, health, and development, comes from studies of infants in Neonatal Intensive Care Units (NICU) [for review, see Ref. (158)]. Providing a light–dark cycle in the NICU increased sleeping time in infants, decreased the time spent feeding, and increased weight gain resulting in earlier hospital discharge relative to infants exposed to constant lighting typical of some hospital nurseries (159, 160). In addition, exposure to a light–dark cycle promoted heart rate stability, improved oxygen saturation, establishment of daily melatonin rhythms, and a better tolerance to milk (160). These studies demonstrate that exposure to a light–dark cycle immediately after birth promotes beneficial effects on the development of infants, and thus support the need for research on the impact of hospital lighting environment on maternal physiology and maternal-offspring interactions in the peripartum that affect breastfeeding outcomes.

Recent studies using animal and clinical models have demonstrated that filtering short wavelengths (below 480 nm) for nocturnal lighting can attenuate alterations in hormone secretion (melatonin and glucocorticoids), and in central and peripheral clock-gene expression induced by nighttime light exposure (161). In humans, use of optical filters led to an improvement in mood and cognitive performance under controlled laboratory conditions as well as during field-based shiftwork studies. For example, studies found that use of optical filters during shift work increased sleep duration and quality on nights immediately following night shifts (161). Thus, a method to improve or prevent many of the health problems associated with circadian misalignment, including timing to onset of lactogenesis II may be to incorporate optical filters into glasses or as coverings for light bulbs in work places and hospitals for procedures that require night time exposure to light (161).

If these proposed interventions prove to mitigate the development of metabolic and hormonal imbalances that increase the risk of DLII, the rates of adequate breastfeeding may increase. Importantly, since many of the external factors that disrupt circadian clocks are modifiable by changes in lifestyle or external environment, the interventions we suggest are minimally invasive and thus are readily implementable during pregnancy and peripartum.

REFERENCES

1. *Ten Facts about Breastfeeding*. World Health Organization (2014). Available from: http://www.who.int/features/factfiles/breastfeeding/en/
2. Lassek WD, Gaulin SJC. Maternal milk DHA content predicts cognitive performance in a sample of 28 nations. *Matern Child Nutr* (2013). doi:10.1111/mcn.12060
3. Zivkovic AM, German JB, Lebrilla CB, Mills DA. Human milk glycobiome and its impact on the infant gastrointestinal microbiota. *Proc Natl Acad Sci U S A* (2011) **108**(Suppl 1):4653–8. doi:10.1073/pnas.1000083107
4. Neville M, Anderson S, McManaman J, Badger T, Bunik M, Contractor N, et al. Lactation and neonatal nutrition: defining and refining the critical questions. *J Mammary Gland Biol Neoplasia* (2012) **17**:167–88. doi:10.1007/s10911-012-9261-5
5. Johnston M, Landers S, Noble L, Szucs K, Viehmann L. Breastfeeding and the use of human milk. *Pediatrics* (2012) **129**:e827–41. doi:10.1542/peds.2011-3552
6. Bartick M, Reinhold A. The burden of suboptimal breastfeeding in the United States: a pediatric cost analysis. *Pediatrics* (2010) **125**:e1048–56. doi:10.1542/peds.2009-1616
7. Breastfeeding Among U.S. *Children Born 2001–2011, CDC National Immunization Survey*. (2014). Available from: http://www.cdc.gov/breastfeeding/data/NIS_data/index.htm
8. Colin WB, Scott JA. Breastfeeding: reasons for starting, reasons for stopping and problems along the way. *Breastfeed Rev* (2002) **10**:13–9.
9. Brownell E, Howard CR, Lawrence RA, Dozier AM. Delayed onset lactogenesis II predicts the cessation of any or exclusive breastfeeding. *J Pediatr* (2012) **161**:608–14. doi:10.1016/j.jpeds.2012.03.035
10. Hurst NM. Recognizing and treating delayed or failed lactogenesis II. *J Midwifery Womens Health* (2007) **52**:588–94. doi:10.1016/j.jmwh.2007.05.005
11. Dewey K, Nommsen-Rivers L, Heinig M, Cohen R. Risk factors for suboptimal infant breastfeeding behavior, delayed onset of lactation, and excess neonatal weight loss. *Pediatrics* (2003) **112**:607–19. doi:10.1542/peds.112.3.607
12. Neville MC, Morton J. Physiology and endocrine changes underlying human lactogenesis II. *J Nutr* (2001) **131**:3005S–8S.
13. Kulski JK, Hartmann PE. Changes in human milk composition during the initiation of lactation. *Aust J Exp Biol Med Sci* (1981) **59**:101–14. doi:10.1038/icb.1981.66
14. Murase M, Nommsen-Rivers L, Morrow AL, Hatsuno M, Mizuno K, Taki M, et al. Predictors of low milk volume among mothers who delivered preterm. *J Hum Lact* (2014) **30**:425–35. doi:10.1177/0890334414543951
15. Nommsen-Rivers LA, Chantry CJ, Peerson JM, Cohen RJ, Dewey KG. Delayed onset of lactogenesis among first-time mothers is related to maternal obesity and factors associated with ineffective breastfeeding. *Am J Clin Nutr* (2010) **92**:574–84. doi:10.3945/ajcn.2010.29192
16. Hilson JA, Rasmussen KM, Kjolhede CL. High prepregnant body mass index is associated with poor lactation outcomes among white, rural women independent of psychosocial and demographic correlates. *J Hum Lact* (2004) **20**:18–29. doi:10.1177/0890334403261345
17. Aviram A, Hod M, Yogev Y. Maternal obesity: implications for pregnancy outcome and long-term risks–a link to maternal nutrition. *Int J Gynecol Obstet* (2011) **115**(Suppl 1):S6–10. doi:10.1016/S0020-7292(11)60004-0
18. Nommsen-Rivers L, Dolan L, Huang B. Timing of stage II lactogenesis is predicted by antenatal metabolic health in a cohort of primiparas. *Breastfeed Med* (2012) **7**:43–9. doi:10.1089/bfm.2011.0007
19. Rasmussen KM, Kjolhede CL. Prepregnant overweight and obesity diminish the prolactin response to suckling in the first week postpartum. *Pediatrics* (2004) **113**:e465–71. doi:10.1542/peds.113.5.e465
20. Ramiandrasoa C, Castinetti F, Raingeard I, Fenichel P, Chabre O, Brue T, et al. Delayed diagnosis of Sheehan's syndrome in a developed country: a retrospective cohort study. *Eur J Endocrinol* (2013) **169**:431–8. doi:10.1530/EJE-13-0279
21. Herrera E. Metabolic adaptations in pregnancy and their implications for the availability of substrates to the fetus. *Eur J Clin Nutr* (2000) **54**(Suppl 1):S47–51. doi:10.1038/sj.ejcn.1600984
22. Bauman DE, Currie WB. Partitioning of nutrients during pregnancy and lactation: a review of mechanisms involving homeostasis and homeorhesis. *J Dairy Sci* (1980) **63**:1514–29. doi:10.3168/jds.S0022-0302(80)83111-0
23. Bell AW, Bauman DE. Adaptations of glucose metabolism during pregnancy and lactation. *J Mammary Gland Biol Neoplasia* (1997) **2**:265–78. doi:10.1023/A:1026336505343

24. Lawrence R, Lawrence R. *Breastfeeding Guide for the Medical Profession, Sixth Edition*. Philadelphia, PA: Elsevier Mosby (2005).
25. Augustine RA, Ladyman SR, Grattan DR. From feeding one to feeding many: hormone-induced changes in bodyweight homeostasis during pregnancy. *J Physiol* (2008) **586**:387–97. doi:10.1113/jphysiol.2007.146316
26. Speakman JR. The cost of living: field metabolic rates of small mammals. *Adv Ecol Res* (2000) **30**:177–297. doi:10.1242/jeb.099739
27. Johnson MS, Thomson SC, Speakman JR. Limits to sustained energy intake. I. Lactation in the laboratory mouse *Mus musculus*. *J Exp Biol* (2001) **204**:1925–35.
28. Nguyen DA, Beeman NE, Neville MC. Regulation of tight junction permeability in the mammary gland. In: Cereijido M, Anderson J, editors. *Tight Junctions*. Boca Raton: CRC Press (2001). p. 395–414.
29. Grattan DR, Steyn FJ, Kokay IC, Anderson GM, Bunn S. Pregnancy-induced adaptation in the neuroendocrine control of prolactin secretion. *J Endocrinol* (2008) **20**:497–507. doi:10.1111/j.1365-2826.2008.01661.x
30. Vernon RG. Endocrine control of metabolic adaptation during lactation. *Proc Nutr Soc* (1989) **48**:23–32. doi:10.1079/PNS19890006
31. Hapon MB, Simoncini M, Via G, Jahn GA. Effect of hypothyroidism on hormone profiles in virgin, pregnant and lactating rats, and on lactation. *Reproduction* (2003) **126**:371–82. doi:10.1530/rep.0.1260371
32. Peters F, Schulze-Tollert J, Schuth W. Thyrotropin-releasing hormone – a lactation-promoting agent? *Br J Obstet Gynaecol* (1991) **98**:880–5. doi:10.1111/j.1471-0528.1991.tb13509.x
33. Bailey M, Silver R. Sex differences in circadian timing systems: implications for disease. *Front Neuroendocrinol* (2014) **35**:111–39. doi:10.1016/j.yfrne.2013.11.003
34. Bolon B. Gender agenda: sex bias can be justified in animal research. *Nature* (2010) **466**:28. doi:10.1038/466028d
35. Putting gender on the agenda. *Nature* (2010) **465**:665. doi:10.1038/465665a
36. Baylis F. Pregnant women deserve better. *Nature* (2010) **465**:689–90. doi:10.1038/465689a
37. Plaut K, Casey T. Does the circadian system regulate lactation? *Animal* (2012) **6**:394–402. doi:10.1017/S1751731111002187
38. Casey TM, Plaut K. Lactation biology symposium: circadian clocks as mediators of the homeorhetic response to lactation. *J Anim Sci* (2012) **90**:744–54. doi:10.2527/jas.2011-4590
39. Reppert SM, Weaver DR. Coordination of circadian timing in mammals. *Nature* (2002) **418**:935–41. doi:10.1038/nature00965
40. Moore RY, Speh JC. Serotonin innervation of the primate suprachiasmatic nucleus. *Brain Res* (2004) **1010**:169–73. doi:10.1016/j.brainres.2004.02.024
41. Wirz-Justice A. Biological rhythm disturbances in mood disorders. *Int Clin Psychopharmacol* (2006) **21**(Suppl 1):S11–5. doi:10.1097/01.yic.0000195660.37267.cf
42. Sheward WJ, Maywood ES, French KL, Horn JM, Hastings MH, Seckl JR, et al. Entrainment to feeding but not to light: circadian phenotype of VPAC2 receptor-null mice. *J Neurosci* (2007) **27**:4351–8. doi:10.1523/JNEUROSCI.4843-06.2007
43. Hardin PE. Transcription regulation within the circadian clock: the E-box and beyond. *J Biol Rhythms* (2004) **19**:348–60. doi:10.1177/0748730404268052
44. Akhtar RA, Reddy AB, Maywood ES, Clayton JD, King VM, Smith AG, et al. Circadian cycling of the mouse liver transcriptome, as revealed by cDNA microarray, is driven by the suprachiasmatic nucleus. *Curr Biol* (2002) **12**:540–50. doi:10.1016/S0960-9822(02)00759-5
45. Panda S. Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* (2002) **109**:307–20. doi:10.1016/S0092-8674(02)00722-5
46. Casey T, Crodian J, Donkin SS, Plaut K. Continuously changing light-dark phase decreases milk yield, fat, protein and lactose in dairy cows. *J Adv Dairy Res* (2014) **2**:119. doi:10.4172/2329-888X.1000119
47. Gonnissen HK, Hulshof T, Westerterp-Plantenga MS. Chronobiology, endocrinology, and energy- and food-reward homeostasis. *Obes Rev* (2013) **14**:405–16. doi:10.1111/obr.12019
48. la Fleur SE, Kalsbeek A, Wortel J, Fekkes ML, Buijs RM. A daily rhythm in glucose tolerance: a role for the suprachiasmatic nucleus. *Diabetes* (2001) **50**:1237–43. doi:10.2337/diabetes.50.6.1237
49. Reiter RJ, Tan DX, Korkmaz A, Erren TC, Piekarski C, Tamura H, et al. Light at night, chronodisruption, melatonin suppression, and cancer risk: a review. *Crit Rev Oncog* (2007) **13**:303–28. doi:10.1615/CritRevOncog.v13.i4.30
50. Pauley SM. Lighting for the human circadian clock: recent research indicates that lighting has become a public health issue. *Med Hypotheses* (2004) **63**:588–96. doi:10.1016/j.mehy.2004.03.020
51. Díaz-Muñoz M, Vázquez-Martínez O, Aguilar-Roblero R, Escobar C. Anticipatory changes in liver metabolism and entrainment of insulin, glucagon, and corticosterone in food-restricted rats. *Am J Physiol* (2000) **279**:R2048–56.
52. Milano W, De Rosa M, Milano L, Capasso A. Night eating syndrome: an overview. *J Pharm Pharmacol* (2012) **64**:2–10. doi:10.1111/j.2042-7158.2011.01353.x
53. Goel N, Stunkard AJ, Rogers NL, Van Dongen HPA, Allison KC, O'Reardon JP, et al. Circadian rhythm profiles in women with night eating syndrome. *J Biol Rhythms* (2009) **24**:85–94. doi:10.1177/0748730408328914
54. Bass J, Takahashi JS. Circadian integration of metabolism and energetics. *Science* (2010) **330**:1349–54. doi:10.1126/science.1195027
55. Gimble JM, Bray MS, Young A. Circadian biology and sleep: missing links in obesity and metabolism? *Obes Rev* (2009) **10**:1–5. doi:10.1111/j.1467-789X.2009.00672.x
56. Karlsson B, Knutsson A, Lindahl B. Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27,485 people. *Occup Environ Med* (2001) **58**:747–52. doi:10.1136/oem.58.11.747
57. Lamia KA, Storch KF, Weitz CJ. Physiological significance of a peripheral tissue circadian clock. *Proc Natl Acad Sci U S A* (2008) **105**:15172–7. doi:10.1073/pnas.0806717105
58. Suwazono Y, Dochi M, Sakata K, Okubo Y, Oishi M, Tanaka K, et al. A longitudinal study on the effect of shift work on weight gain in male Japanese workers. *Obesity (Silver Spring)* (2008) **16**:1887–93. doi:10.1038/oby.2008.298
59. Boivin DB, Boudreau P. Impacts of shift work on sleep and circadian rhythms. *Pathol Biol (Paris)* (2014) **62**:292–301. doi:10.1016/j.patbio.2014.08.001
60. Froy O. The relationship between nutrition and circadian rhythms in mammals. *Front Neuroendocrinol* (2007) **28**:61–71. doi:10.1016/j.yfrne.2007.03.001
61. Foster RG, Wulff K. The rhythm of rest and excess. *Nat Rev Neurosci* (2005) **6**:407–14. doi:10.1038/nrn1670
62. Scheer FAJL, Hilton MF, Mantzoros CS, Shea SA. Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc Natl Acad Sci U S A* (2009) **106**:4453–8. doi:10.1073/pnas.0808180106
63. Sookoian S, Castano G, Gemma C, Gianotti TF, Pirola CJ. Common genetic variations in CLOCK transcription factor are associated with nonalcoholic fatty liver disease. *World J Gastroenterol* (2007) **13**:4242–8.
64. Woon PY, Kaisaki PJ, Braganca J, Bihoreau MT, Levy JC, Farrall M, et al. Aryl hydrocarbon receptor nuclear translocator-like (BMAL1) is associated with susceptibility to hypertension and type 2 diabetes. *Proc Natl Acad Sci U S A* (2007) **104**:14412–7. doi:10.1073/pnas.0703247104
65. Ando H, Yanagihara H, Hayashi Y, Obi Y, Tsuruoka S, Takamura T, et al. Rhythmic messenger ribonucleic acid expression of clock genes and adipocytokines in mouse visceral adipose tissue. *Endocrinology* (2005) **146**:5631–6. doi:10.1210/en.2005-0771
66. Mendoza J, Pévet P, Challet E. High-fat feeding alters the clock synchronization to light. *J Physiol* (2008) **586**:5901–10. doi:10.1113/jphysiol.2008.159566
67. Martin RJ, Wangsness PJ, Gahagan JH. Diurnal changes in serum metabolites and hormones in lean and obese Zucker rats. *Horm Metab Res* (1978) **10**:187–92. doi:10.1055/s-0028-1093431
68. Pasquali R, Vicennati V, Cacciari M, Pagotto U. The hypothalamic-pituitary-adrenal axis activity in obesity and the metabolic syndrome. *Ann N Y Acad Sci* (2006) **1083**:111–28. doi:10.1196/annals.1367.009
69. Dardente H, Wyse CA, Birnie MJ, Dupré SM, Loudon ASI, Lincoln GA, et al. A molecular switch for photoperiod responsiveness in mammals. *Curr Biol* (2010) **20**:2193–8. doi:10.1016/j.cub.2010.10.048
70. Navara KJ, Nelson RJ. The dark side of light at night: physiological, epidemiological, and ecological consequences. *J Pineal Res* (2007) **43**:215–24. doi:10.1111/j.1600-079X.2007.00473.x
71. Obayashi K, Saeki K, Iwamoto J, Okamoto N, Tomioka K, Nezu S, et al. Exposure to light at night, nocturnal urinary melatonin excretion, and obesity/dyslipidemia in the elderly: a cross-sectional analysis of the HEIJO-KYO study. *J Clin Endocrinol Metab* (2013) **98**:337–44. doi:10.1210/jc.2012-2874
72. McFadden E, Jones ME, Schoemaker MJ, Ashworth A, Swerdlow AJ. 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* (2014) **180**:245–50. doi:10.1093/aje/kwu117

73. Medicine IO. *Sleep Disorders and Sleep Deprivation: An Unmet Public Health Problem*. Washington, DC: The National Academies Press (2006).
74. Schoenborn CA, Adams PF. Health behaviors of adults: United States, 2005–2007 national center for health statistics. *Vital Health Stat* (2010) **10**(245). Available from: http://www.cdc.gov/nchs/data/series/sr_10/sr10_245.pdf
75. Spiegel KLR, Van Cauter E. Impact of sleep debt on metabolic and endocrine function. *Lancet* (1999) **354**:1435–9. doi:10.1016/S0140-6736(99)01376-8
76. Breslau N, Roth T, Rosenthal L, Andreski P. Sleep disturbance and psychiatric disorders: a longitudinal epidemiological study of young adults. *Biol Psychiatry* (1996) **39**:411–8. doi:10.1016/0006-3223(95)00188-3
77. Bjorvatn B, Sagen IM, Oyane N, Waage S, Fetveit A, Pallesen S, et al. The association between sleep duration, body mass index and metabolic measures in the Hordaland Health Study. *J Sleep Res* (2007) **16**:66–76. doi:10.1111/j.1365-2869.2007.00569.x
78. Heslop P, Smith GD, Metcalfe C, Macleod J, Hart C. Sleep duration and mortality: the effect of short or long sleep duration on cardiovascular and all-cause mortality in working men and women. *Sleep Med* (2002) **3**:305–14. doi:10.1016/S1389-9457(02)00016-3
79. Cappuccio FP, Stranges S, Kandala NB, Miller MA, Taggart FM, Kumari M, et al. Gender-specific associations of short sleep duration with prevalent and incident hypertension: the Whitehall II Study. *Hypertension* (2007) **50**:693–700. doi:10.1161/HYPERTENSIONAHA.107.095471
80. Meisinger C, Heier M, Lowel H, Schneider A, Doring A. Sleep duration and sleep complaints and risk of myocardial infarction in middle-aged men and women from the general population: the MONICA/KORA Augsburg cohort study. *Sleep* (2007) **30**:1121–7.
81. Broussard J, Brady MJ. The impact of sleep disturbances on adipocyte function and lipid metabolism. *Best Pract Res Clin Endocrinol Metab* (2010) **24**:763–73. doi:10.1016/j.beem.2010.08.007
82. Buxton OM, Cain SW, O'Connor SP, Porter JH, Duffy JF, Wang W, et al. Adverse metabolic consequences in humans of prolonged sleep restriction combined with circadian disruption. *Sci Transl Med* (2012) **4**:129ra143. doi:10.1126/scitranslmed.3003200
83. Bailey SM, Udoh US, Young ME. Circadian regulation of metabolism. *J Endocrinol* (2014) **222**:R75–96. doi:10.1530/JOE-14-0200
84. Karatsoreos IN, Bhagat S, Bloss EB, Morrison JH, McEwen BS. Disruption of circadian clocks has ramifications for metabolism, brain, and behavior. *Proc Natl Acad Sci U S A* (2011) **108**:1657–62. doi:10.1073/pnas.1018375108
85. Reutrakul S, Van Cauter E. Interactions between sleep, circadian function, and glucose metabolism: implications for risk and severity of diabetes. *Ann N Y Acad Sci* (2014) **1311**:151–73. doi:10.1111/nyas.12355
86. Husse J, Hintze SC, Eichele G, Lehnert H, Oster H. Circadian clock genes Per1 and Per2 regulate the response of metabolism-associated transcripts to sleep disruption. *PLoS One* (2012) **7**:e52983. doi:10.1371/journal.pone.0052983
87. Morris CJ, Aeschbach D, Scheer FA. Circadian system, sleep and endocrinology. *Mol Cell Endocrinol* (2012) **349**:91–104. doi:10.1016/j.mce.2011.09.003
88. Zee PC, Attarian H, Videnovic A. Circadian rhythm abnormalities. *Continuum (Minneap Minn)* (2013) **19**:132–47. doi:10.1212/01.CON.0000427209.21177.aa
89. Gangwisch JE. Epidemiological evidence for the links between sleep, circadian rhythms and metabolism. *Obes Rev* (2009) **10**:37–45. doi:10.1111/j.1467-789X.2009.00663.x
90. Miller BH, Takahashi JS. Central circadian control of female reproductive function. *Front Endocrinol* (2014) **4**:195. doi:10.3389/fendo.2013.00195
91. Olcese J. Circadian clocks and pregnancy. *Front Endocrinol* (2014) **5**:123. doi:10.3389/fendo.2014.00123
92. Sellix MT. Clocks underneath: the role of peripheral clocks in the timing of female reproductive physiology. *Front Endocrinol* (2013) **4**:91. doi:10.3389/fendo.2013.00091
93. Kriegsfeld LJ, Williams I, Wilbur P. Circadian control of the estrogenic circuits regulating GnRH secretion and the preovulatory luteinizing hormone surge. *Front Endocrinol (Lausanne)* (2012) **3**:60. doi:10.3389/fendo.2012.00060
94. Turek FW, Swann J, Earnest DJ. Role of the circadian system in reproductive phenomena. *Recent Prog Horm Res* (1984) **40**:143–83.
95. Dolatshad H, Campbell EA, O'Hara L, Maywood ES, Hastings MH, Johnson MH. Developmental and reproductive performance in circadian mutant mice. *Hum Reprod* (2006) **21**:68–79. doi:10.1093/humrep/dei313
96. Pilorz V, Steinlechner S. Low reproductive success in Per1 and Per2 mutant mouse females due to accelerated ageing? *Reproduction* (2008) **135**:559–68. doi:10.1530/REP-07-0434
97. Sellix M, Menaker M. Circadian clocks in mammalian reproductive physiology: effects of the "other" biological clock on fertility. *Discov Med* (2011) **11**:273–81.
98. Leclerc GM, Boockfor FR. Pulses of prolactin promoter activity depend on a noncanonical E-box that can bind the circadian proteins CLOCK and BMAL1. *Endocrinology* (2005) **146**:2782–90. doi:10.1210/en.2005-0100
99. Baumgartner A, Dietzel M, Saletu B, Wolf R, Campos-Barros A, Graf KJ, et al. Influence of partial sleep deprivation on the secretion of thyrotropin, thyroid hormones, growth hormone, prolactin, luteinizing hormone, follicle stimulating hormone, and estradiol in healthy young women. *Psychiatry Res* (1993) **48**:153–78. doi:10.1016/0165-1781(93)90039-J
100. Kittrell EM, Satinoff E. Diurnal rhythms of body temperature, drinking and activity over reproductive cycles. *Physiol Behav* (1988) **42**:477–84. doi:10.1016/0031-9384(88)90180-1
101. Scribner SJ, Wynne-Edwards KE. Disruption of body temperature and behavior rhythms during reproduction in dwarf hamsters (*Phodopus*). *Physiol Behav* (1994) **55**:361–9. doi:10.1016/0031-9384(94)90147-3
102. Schrader JA, Nunez AA, Smale L. Changes in and dorsal to the rat suprachiasmatic nucleus during early pregnancy. *Neuroscience* (2010) **171**:513–23. doi:10.1016/j.neuroscience.2010.08.057
103. Schrader JA, Smale L, Nunez AA. Pregnancy affects FOS rhythms in brain regions regulating sleep/wake state and body temperature in rats. *Brain Res* (2012) **1480**:53–60. doi:10.1016/j.brainres.2012.09.003
104. Schrader JA, Walaszczyk EJ, Smale L. Changing patterns of daily rhythmicity across reproductive states in diurnal female Nile grass rats (*Arvicanthis niloticus*). *Physiol Behav* (2009) **98**:547–56. doi:10.1016/j.physbeh.2009.08.012
105. Kimura M, Zhang SQ, Inoue S. Pregnancy-associated sleep changes in the rat. *Am J Physiol Regul Integr Comp Physiol* (1996) **271**:R1063–9.
106. Suzuki S, Dennerstein L, Greenwood KM, Armstrong SM, Satohisa E. Sleeping patterns during pregnancy in Japanese women. *J Psychosom Obstet Gynaecol* (1994) **15**:19–26. doi:10.3109/01674829409025625
107. Facco FL, Kramer J, Ho KH, Zee PC, Grobman WA. Sleep disturbances in pregnancy. *Obstet Gynecol* (2010) **115**:77–83. doi:10.1097/AOG.0b013e3181c4f8ec
108. Okun ML, Coussons-Read ME. Sleep disruption during pregnancy: how does it influence serum cytokines? *J Reprod Immunol* (2007) **73**:158–65. doi:10.1016/j.jri.2006.06.006
109. Posadas ES, Meliska CJ, Martinez LF, Sorenson DL, Lopez AM, Nowakowski S, et al. The relationship of nocturnal melatonin to estradiol and progesterone in depressed and healthy pregnant women. *J Womens Health (Larchmt)* (2012) **21**:649–55. doi:10.1089/jwh.2011.3191
110. Parry BL, Meliska CJ, Sorenson DL, Lopez AM, Martinez LF, Nowakowski S, et al. Plasma melatonin circadian rhythm disturbances during pregnancy and postpartum in depressed women and women with personal or family histories of depression. *Am J Psychiatry* (2008) **165**:1551–8. doi:10.1176/appi.ajp.2008.08050709
111. Allolio B, Hoffmann J, Linton EA, Winkelmann W, Kusche M. Diurnal salivary cortisol patterns during pregnancy and after delivery: relationship to plasma corticotrophin-releasing-hormone. *Clin Endocrinol (Oxf)* (1990) **33**:279–89. doi:10.1111/j.1365-2265.1990.tb00492.x
112. Goodman RL, Jansen HT, Billings HJ, Coolen LM, Lehman MN. Neural systems mediating seasonal breeding in the Ewe. *J Neuroendocrinol* (2010) **22**:674–81. doi:10.1111/j.1365-2826.2010.02014.x
113. Entringer S, Buss C, Shirtcliff EA, Cammack AL, Yim IS, Chicz-DeMet A, et al. Attenuation of maternal psychophysiological stress responses and the maternal cortisol awakening response over the course of human pregnancy. *Stress* (2010) **13**:258–68. doi:10.3109/10253890903349501
114. Lightman SL, Windle RJ, Wood SA, Kershaw YM, Shanks N, Ingram CD. Peripartum plasticity within the hypothalamo-pituitary-adrenal axis. *Prog Brain Res* (2001) **133**:111–29. doi:10.1016/S0079-6123(01)33009-1
115. Tu M, Lupien S, Walker C. Diurnal salivary cortisol levels in postpartum mothers as a function of infant feeding choice and parity. *Psychoneuroendocrinology* (2006) **31**:812–24. doi:10.1016/j.psyneuen.2006.03.006

116. Reddy AB, Maywood ES, Karp NA, King VM, Inoue Y, Gonzalez FJ, et al. Glucocorticoid signaling synchronizes the liver circadian transcriptome. *Hepatology* (2007) **45**:1478–88. doi:10.1002/hep.21571
117. Cooperstock M, England JE, Wolfe RA. Circadian incidence of labor onset hour in preterm birth and chorioamnionitis. *Obstet Gynecol* (1987) **70**:852–5.
118. Cooperstock M, England JE, Wolfe RA. Circadian incidence of premature rupture of the membranes in term and preterm births. *Obstet Gynecol* (1987) **69**:936–41.
119. Ngwenya S, Lindow SW. 24 hour rhythm in the timing of pre-labour spontaneous rupture of membranes at term. *Eur J Obstet Gynecol Reprod Biol* (2004) **112**:151–3. doi:10.1016/S0301-2115(03)00286-0
120. Mancuso PJ, Alexander JM, McIntire DD, Davis E, Burke G, Leveno KJ. Timing of birth after spontaneous onset of labor. *Obstet Gynecol* (2004) **103**:653–6. doi:10.1097/01.AOG.0000118309.70035.63
121. Dodd JM, Crowther CA, Robinson JS. Morning compared with evening induction of labor: a nested randomized controlled trial. A nested randomized controlled trial. *Obstet Gynecol* (2006) **108**:350–60. doi:10.1097/01.AOG.0000227746.35565.d9
122. Freeman ME, Kanyicska B, Lerant A, Nagy G. Prolactin: structure, function, and regulation of secretion. *Physiol Rev* (2000) **80**:1523–631.
123. Casey TM, Crodian J, Erickson E, Kuropatwinski KK, Gleiberman AS, Antoch MP. Tissue-specific changes in molecular clocks during the transition from pregnancy to lactation in mice. *Biol Reprod* (2014) **90**(6):127. doi:10.1095/biolreprod.113.116137
124. Schradera J, Nunez A, Smale L. Changes in and dorsal to the rat suprachiasmatic nucleus during early pregnancy. *Neuroscience* (2010) **171**:513–23. doi:10.1016/j.neuroscience.2010.08.057
125. Maningat PD, Sen P, Rijnkels M, Sunehag AL, Hadsell DL, Bray M, et al. Gene expression in the human mammary epithelium during lactation: the milk fat globule transcriptome. *Physiol Genomics* (2009) **37**:12–22. doi:10.1152/physiolgenomics.90341.2008
126. Miller BH, Olson SL, Turek FW, Levine JE, Horton TH, Takahashi JS. Circadian clock mutation disrupts estrous cyclicity and maintenance of pregnancy. *Curr Biol* (2004) **14**:1367–73. doi:10.1016/j.cub.2004.07.055
127. Dahl GE. Effects of short day photoperiod on prolactin signaling in dry cows: a common mechanism among tissues and environments? *J Anim Sci* (2008) **86**:10–4. doi:10.2527/jas.2007-0311
128. Summa KC, Vitaterna MH, Turek FW. Environmental perturbation of the circadian clock disrupts pregnancy in the mouse. *PLoS One* (2012) **7**:e37668. doi:10.1371/journal.pone.0037668
129. Varcoe TJ, Boden MJ, Voultsios A, Salkeld MD, Rattanatray L, Kennaway DJ. Characterisation of the maternal response to chronic phase shifts during gestation in the rat: implications for fetal metabolic programming. *PLoS One* (2013) **8**:e53800. doi:10.1371/journal.pone.0053800
130. Varcoe T, Wight N, Voultsios A, Salkeld M, Kennaway D. Chronic phase shifts of the photoperiod throughout pregnancy programs glucose intolerance and insulin resistance in the rat. *PLoS One* (2011) **6**:e18504. doi:10.1371/journal.pone.0018504
131. Kovanen L, Saarikoski ST, Aromaa A, Lönnqvist J, Partonen T. *ARNTL* (*BMAL1*) and *NPAS2* gene variants contribute to fertility and seasonality. *PLoS One* (2010) **5**:e10007. doi:10.1371/journal.pone.0010007
132. Knutsson A. Health disorders of shift workers. *Occup Med* (2003) **53**:103–8. doi:10.1093/occmed/kqg048
133. Zhu JL, Hjollund NH, Andersen AM, Olsen J. Shift work, job stress, and late fetal loss: the national birth cohort in Denmark. *J Occup Environ Med* (2004) **46**:1144–9. doi:10.1097/01.jom.0000145168.21614.21
134. Zhu JL, Hjollund NH, Olsen J. Shift work, duration of pregnancy, and birth weight: the national birth cohort in Denmark. *Am J Obstet Gynecol* (2004) **191**:285–91. doi:10.1016/j.ajog.2003.12.002
135. Abeysena C, Jayawardana P, de A Seneviratne R. Maternal sleep deprivation is a risk factor for small for gestational age: a cohort study. *Aust N Z J Obstet Gynaecol* (2009) **49**:382–7. doi:10.1111/j.1479-828X.2009.01010.x
136. Williams MA, Miller RS, Qiu C, Cripe SM, Gelaye B, Enquobahrie D. Associations of early pregnancy sleep duration with trimester-specific blood pressures and hypertensive disorders in pregnancy. *Sleep* (2010) **33**:1363–71.
137. Izci Balserak B, Jackson N, Ratcliffe S, Pack A, Pien G. Sleep-disordered breathing and daytime napping are associated with maternal hyperglycemia. *Sleep Breath* (2013) **17**(3):1093–102. doi:10.1007/s11325-013-0809-4
138. Qiu C, Enquobahrie D, Frederick I, Abetew D, Williams M. Glucose intolerance and gestational diabetes risk in relation to sleep duration and snoring during pregnancy: a pilot study. *BMC Womens Health* (2010) **10**:17. doi:10.1186/1472-6874-10-17
139. Mendieta Zeron H, Garcia Solorio VJ, Nava Diaz PM, Garduno Alanis A, Santillan Benitez JG, Dominguez Garcia V, et al. Hyperleptinemia as a prognostic factor for preeclampsia: a cohort study. *Acta Medica (Hradec Kralove)* (2012) **55**:165–71.
140. Henson MC, Castracane VD. Leptin in pregnancy: an update. *Biol Reprod* (2006) **74**:218–29. doi:10.1095/biolreprod.105.045120
141. Kocyigit Y, Bayhan G, Atamer A, Atamer Y. Serum levels of leptin, insulin-like growth factor-I and insulin-like growth factor binding protein-3 in women with pre-eclampsia, and their relationship to insulin resistance. *Gynecol Endocrinol* (2004) **18**:341–8. doi:10.1080/09513590410001704975
142. Qiu C, Frederick IO, Sorensen TK, Enquobahrie DA, Williams MA. Sleep duration and plasma leptin concentrations in early pregnancy among lean and overweight/obese women: a cross sectional study. *BMC Res Notes* (2014) **7**:20. doi:10.1186/1756-0500-7-20
143. McClung C. Circadian genes, rhythms and the biology of mood disorders. *Pharmacol Ther* (2007) **114**:222–32. doi:10.1016/j.pharmthera.2007.02.003
144. Monteleone P, Maj M. The circadian basis of mood disorders: recent developments and treatment implications. *Eur Neuropsychopharmacol* (2008) **18**:701–11. doi:10.1016/j.euroneuro.2008.06.007
145. Dennis CL, McQueen K. Does maternal postpartum depressive symptomatology influence infant feeding outcomes? *Acta Paediatr* (2007) **96**:590–4. doi:10.1111/j.1651-2227.2007.00184.x
146. Shaw MA, Rasmussen KM, Myers TR. Consumption of a high fat diet impairs reproductive performance in Sprague-Dawley rats. *J Nutr* (1997) **127**(1):64–9.
147. Flint DJ, Travers MT, Barber MC, Binart N, Kelly PA. Diet-induced obesity impairs mammary development and lactogenesis in murine mammary gland. *Am J Physiol Endocrinol Metab* (2005) **288**:E1179–87. doi:10.1152/ajpendo.00433.2004
148. Agius L, Rolls BJ, Rowe EA, Williamson DH. Obese rats develop hyperketonemia and a fatty liver during lactation. *Int J Obes* (1983) **7**:447–52.
149. Reddy TE, Gertz J, Crawford GE, Garabedian MJ, Myers RM. The hypersensitive glucocorticoid response specifically regulates period 1 and expression of circadian genes. *Mol Cell Biol* (2012) **32**:3756–67. doi:10.1128/MCB.00062-12
150. Casey T, Plaut K. The role of glucocorticoids in secretory activation and milk secretion, a historical perspective. *J Mammary Gland Biol Neoplasia* (2007) **12**:293–304. doi:10.1007/s10911-007-9055-3
151. Henderson JJ, Hartmann PE, Moss TJM, Doherty DA, Newnham JP. Disrupted secretory activation of the mammary gland after antenatal glucocorticoid treatment in sheep. *Reproduction* (2008) **136**:649–55. doi:10.1530/REP-08-0134
152. Amaral FG, Castrucci AM, Cipolla-Neto J, Poletini MO, Mendez N, Richter HG, et al. Environmental control of biological rhythms: effects on development, fertility and metabolism. *J Neuroendocrinol* (2014) **26**:603–12. doi:10.1111/jne.12144
153. Meedya S, Fahy K, Kable A. Factors that positively influence breastfeeding duration to 6 months: a literature review. *Women Birth* (2010) **23**(4):135–45. doi:10.1016/j.wombi.2010.02.002
154. Fairbank L, O'Meara S, Renfrew MJ, Woolridge M, Sowden AJ, Lister-Sharp D. A systematic review to evaluate the effectiveness of interventions to promote the initiation of breastfeeding. *Health Technol Assess* (2000) **4**:1–171. doi:10.3310/hta4250
155. Shealy KR, Li R, Benton-Davis S, Grummer-Strawn LM. *The CDC Guide to Breastfeeding Interventions.* U.S. Department of Health and Human Services, Centers for Disease Control and Prevention (2005). Available from: http://www.cdc.gov/breastfeeding/pdf/breastfeeding_interventions.pdf
156. Leproult R, Deliens G, Gilson M, Peigneux P. Beneficial impact of sleep extension on fasting insulin sensitivity in adults with habitual sleep restriction. *Sleep* (2014).
157. The Baby Friendly Hospital Initiative. *Guidelines and Evaluation Criteria for Facilities Seeking Baby-Friendly Designation.* (2010). Available from: www.babyfriendlyusa.org/
158. Blackburn S. Environmental impact of the NICU on developmental outcomes. *J Pediatr Nurs* (1998) **13**:279–89. doi:10.1016/S0882-5963(98)80013-4
159. Mann NP, Haddow R, Stokes L, Goodley S, Rutter N. Effect of night and day on preterm infants in a newborn nursery: randomised trial. *Br Med J (Clin Res Ed)* (1986) **293**:1265–7. doi:10.1136/bmj.293.6557.1265

160. Vásquez-Ruiz S, Maya-Barrios JA, Torres-Narváez P, Vega-Martínez BR, Rojas-Granados A, Escobar C, et al. A light/dark cycle in the NICU accelerates body weight gain and shortens time to discharge in preterm infants. *Early Hum Dev* (2014) **90**:535–40. doi:10.1016/j.earlhumdev.2014.04.015
161. Casper RF, Rahman S. Spectral modulation of light wavelengths using optical filters: effect on melatonin secretion. *Fertil Steril* (2014) **102**:336–8. doi:10.1016/j.fertnstert.2014.06.006

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 19 December 2014; accepted: 05 February 2015; published online: 23 February 2015.
*Citation: Fu M, Zhang L, Ahmed A, Plaut K, Haas DM, Szucs K and Casey TM (2015) Does circadian disruption play a role in the metabolic–hormonal link to delayed lactogenesis II? Front. Nutr. **2**:4. doi:10.3389/fnut.2015.00004*
This article was submitted to Pharmaceutical Medicine and Outcomes Research, a section of the journal Frontiers in Nutrition.

frontiers in
PHARMACOLOGY

MINI REVIEW ARTICLE
published: 27 February 2015
doi: 10.3389/fphar.2015.00029

Circadian clock disruption in neurodegenerative diseases: cause and effect?

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Disturbance of the circadian system, manifested as disrupted daily rhythms of physiologic parameters such as sleep, activity, and hormone secretion, has long been observed as a symptom of several neurodegenerative diseases, including Alzheimer disease. Circadian abnormalities have generally been considered consequences of the neurodegeneration. Recent evidence suggests, however, that circadian disruption might actually contribute to the neurodegenerative process, and thus might be a modifiable cause of neural injury. Herein we will review the evidence implicating circadian rhythms disturbances and clock gene dysfunction in neurodegeneration, with an emphasis on future research directions and potential therapeutic implications for neurodegenerative diseases.

Keywords: circadian clock, Bmal1, Per2, neurodegeneration, Alzheimer disease, Huntington disease

INTRODUCTION

Numerous studies over the past 30 years have described a wide variety of circadian and sleep-wake cycle aberrations which occur in aging and neurodegenerative diseases (Ju et al., 2014; Videnovic et al., 2014a). Many behavioral and physiologic processes oscillate with a 24 h period, including sleep–wake, activity, body temperature, blood pressure, and hormone secretion. These circadian rhythms are frequently disrupted in patients with neurodegenerative disease, including Alzheimer disease (AD), Parkinson disease (PD), Huntington disease (HD). Systemic circadian rhythms in mice and humans are maintained via the function of the body's master clock in the suprachiasmatic nucleus (SCN), which receives input from the retina and synchronizes oscillations in peripheral organs to the light:dark cycle. On a cellular level, circadian rhythms are generated by a transcriptional–translational feedback loop consisting of the bHLH/PAS transcription factors BMAL1 and CLOCK, which heterodimerize and drive transcription of many genes, including their own negative feedback repressors, including PERIOD (Per) and CRYPTOCHROME (Cry) and REVERB genes, which repress BMAL1/CLOCK-mediated transcription (Mohawk et al., 2012). This transcriptional machinery, which we will refer to herein as the core circadian clock, is present in most cells in the body, including neurons and astrocytes in the SCN and throughout the brain. The core circadian clock regulates the circadian expression of thousands of genes in a tissue-specific manner, and is a major regulator of cellular metabolism, stress response, and many other functions (Bass and Takahashi, 2010; Evans and Davidson, 2013). While whole-organism rhythms are known to be disrupted in many neurodegenerative diseases, far less information exists regarding specific alterations in clock protein expression and function in these conditions. Furthermore, it remains unclear if or how circadian disruption might influence the neurodegenerative process itself, or if the core clock represents a reasonable therapeutic target for the treatment or prevention of neurodegeneration. We will focus on these issues in this review.

PART 1: IS THERE EVIDENCE OF CORE CIRCADIAN CLOCK DYSFUNCTION IN NEURODEGENERATIVE DISEASES?

In AD, both sleep and circadian dysfunction are commonly reported. While sleep disturbances in AD are beyond the scope of this discussion and have been reviewed elsewhere (Ju et al., 2014; Peter-Derex et al., 2014), it is important to mention that subtle sleep disturbances appear to occur early in the disease process and may predict amyloid-beta (Aβ) plaque pathology and precede subsequent development of clinical dementia (Ju et al., 2013; Lim et al., 2013a; Spira et al., 2013). Disrupted circadian rhythms in activity, physiologic parameters, and melatonin secretion have been reported in AD reported by several groups (Witting et al., 1990; Skene and Swaab, 2003; Hatfield et al., 2004; Wu et al., 2006; Hu et al., 2009; Coogan et al., 2013). A proposed mechanism of circadian dysfunction in AD is degeneration of the SCN, as loss of critical vasopressin and vasoactive intestinal peptide (VIP)-expressing neurons in this region has been reported in AD patients (Swaab et al., 1985; Zhou et al., 1995; Farajnia et al., 2012). Transcriptional analysis of postmortem human brain tissue has revealed detectable oscillations in core clock genes in various brain regions based on time of death, and shown that in AD brains the phase of oscillation is dysregulated between various regions (Cermakian et al., 2011; Lim et al., 2013b). Rhythms in whole-genome DNA methylation could also be detected which appear to become less robust with age or in AD brains (Lim et al., 2014). Circadian oscillation of clock genes in the human pineal gland was disrupted even at very early pathological stages of AD, mirroring loss of rhythmic melatonin secretion in AD patients (Skene and Swaab, 2003; Wu et al., 2006). Thus, in human AD

there is evidence of disturbed rhythms of clock gene expression which appear to begin early in the disease course.

Animal models of AD also exhibit disturbances of behavioral and physiologic circadian rhythms. In mice, these disturbances appear to correlate with the degree of amyloid plaque burden, and can in some cases be rescued with anti-Aβ immunotherapy, suggesting that aggregated forms of Aβ might disrupt clock mechanisms (Wisor et al., 2005; Sterniczuk et al., 2010; Duncan et al., 2012; Roh et al., 2012). However, while one study described damped expression of *Per2* in the SCN of APP-PS1 transgenic mice (Duncan et al., 2012), more detailed molecular analysis of clock gene function in AD mouse models is lacking. In *Drosophila*, two groups have found that pan-neuronal expression of arctic mutant human Aβ causes marked degradation of behavioral circadian rhythms, despite preserved clock gene oscillation in the central pacemaker cells (Chen et al., 2014; Long et al., 2014). Chen et al. (2014) found that restricted Aβ expression in central clock (PDF) neurons did not disrupt clock gene oscillation or cause behavioral arrhythmicity, which Aβ expression in glia surrounding the clock neurons did both. Thus, in flies, Aβ does not directly disrupt clock gene function in the central pacemaker, but acts more peripherally (and perhaps on glia) to disrupt behavioral rhythms. While the biological relevance of this fly models of Aβ toxicity to humans is debatable, these findings provide leads for further research in mammalian models.

In the case of PD, the second most common age-related neurodegenerative condition, there is ample evidence of disrupted circadian rhythms and sleep–wake disturbance in humans and mouse models (Videnovic and Golombek, 2013). PD patients exhibit progressive disruption of activity rhythms (Niwa et al., 2011), as well as damped circadian oscillation of both melatonin release and *Bmal1* expression in peripheral blood monocytes (Cai et al., 2010; Breen et al., 2014; Videnovic et al., 2014b). Fly and mouse models of PD which expresses mutant human α-synuclein, a protein implicated in PD pathogenesis, develop behavioral circadian disruption early in its disease course (Gajula Balija et al., 2011; Kudo et al., 2011a). Synuclein transgenic mice display normal *Per2* oscillation in the SCN, but have damped electrical output from the SCN, again suggesting disordered SCN function or synchrony (Kudo et al., 2011a).

Huntington disease, unlike AD and PD, is an autosomal disorder caused by trinucleotide expansion within the *huntingtin* gene. Neurodegeneration occurs earlier in HD patients and initially involves the basal ganglia. Sleep and circadian rhythm dysfunction are common in HD (Morton et al., 2005; Aziz et al., 2010), though there is a paucity of studies on clock gene expression and function in human HD. Several mouse models of HD, which express expanded human *huntingtin*, develop pronounced impairment in behavioral circadian rhythms (Morton et al., 2005; Kudo et al., 2011b). The R6/2 line exhibits behavioral arrhythmicity as well as dysregulated clock gene oscillation *in vivo* in the liver and SCN (Morton et al., 2005; Maywood et al., 2010). Interestingly, clock gene oscillation appears to be normal in liver or SCN explants from R6/2 mice, suggesting that some other aspect of the internal milieu of the R6/2 mouse is causing arrhythmicity *in vivo* (Pallier et al., 2007; Maywood et al., 2010). In another HD mouse line (BACHD) the rhythmicity of electrical output of the SCN was disrupted, while the oscillation of *Per2* transcription was grossly intact (Kudo et al., 2011b). These findings suggest support the idea that dysfunction of the neural networks within the SCN, rather than the cell-intrinsic clock gene oscillation, underlies circadian impairment in HD model mice. Accordingly, decreased expression of the neuropeptide VIP and the VIP receptor VPAC2, which are critical for SCN function (Aton et al., 2005), was also observed in R6/2 mice (Fahrenkrug et al., 2007). It appears that in HD mice, disrupted peripheral rhythms may adversely impact SCN function, leading to further systemic circadian arrhythmicity, though the details of this mechanism are still being explored.

PART 2: IS THERE EVIDENCE THAT CLOCK DISRUPTION EXACERBATES NEURODEGENERATION?

While circadian disturbances in aging and neurodegenerative diseases have been duly noted, a key question is whether these disturbances influence disease pathology. This question is much more difficult to examine, and has received significantly less attention. In *Drosophila*, levels of oxidative stress markers, as well as cellular content of the critical antioxidant glutathione show circadian oscillation which is dependent on the clock gene Period (*Per*, Krishnan et al., 2008; Beaver et al., 2012). *Per* deletion exacerbates oxidative injury and shortens lifespan in *Drosophila* (Krishnan et al., 2008, 2009). Disruption of clock function via *Per* deletion also accelerates neurodegeneration in flies bearing a carbonyl reductase mutation which causes oxidative injury to neurons (Krishnan et al., 2012). However, in fly models of Aβ pathology which express different human Aβ isoforms, *Per* deletion did not impact neurodegeneration or behavior, though lifespan was decreased. Conversely, levels of Cryptochrome (*Cry*), which serves as a light-responsive modulator of clock function in *Drosophila*, decline in parallel with damped circadian rhythms in old flies, while *Cry* overexpression restores robust rhythms and enhances lifespan (Rakshit and Giebultowicz, 2013). Thus, the clock genes *Per* and *Cry* clearly appear to contribute to regulation of brain aging and neurodegeneration in fly models.

In mice, evidence linking circadian dysfunction and neurodegeneration is emerging. Chronic disruption of circadian rhythms in mice via housing in 20:4 light:dark conditions leads to decreased neuronal dendritic arborization and cognitive deficits, demonstrating that disturbed circadian rhythms have negative implications for the brain, though the exact degree of clock gene dysregulation and the role of other factors such as stress are unknown (Karatsoreos et al., 2011). Accordingly, studies in rats and hamsters have demonstrated cognitive impairment and decreased hippocampal neurogenesis following chronic "jet lag" protocols, during which circadian rhythms are disrupted by frequent shifting of the light:dark cycle (Gibson et al., 2010; Kott et al., 2012). Mechanistically, RevErbα-mediated regulation of fatty acid binding protein 7 (*Fabp7*), both of which are strongly controlled by the core clock, has been implicated in neurogenesis (Schnell et al., 2014). Similarly, RevErbα shows dynamic activity-dependent regulation in the dendrites of hippocampal neurons and interacts with oligophrenin-1, a regulator of dendritic spines (Valnegri et al., 2011). In a mouse model of AD, Aβ levels in the brain interstitial fluid show pronounced circadian oscillation

(Kang et al., 2009), though it unclear if this is a direct effect of the sleep–wake cycle or may be more directly clock-mediated.

In order to gain some appreciation of the role of clock genes in maintaining brain homeostasis, our group performed functional and neuropathologic analysis of mice with global deletion of *Bmal1*. *Bmal1* knockout (KO) mice developed marked astrogliosis which was evident by 2 months of age and progressed to involve the entirety of the cortex, striatum, and hippocampus (Musiek et al., 2013). These mice also had increased levels of oxidative damage in the cortex, and exhibited spontaneous degeneration of presynaptic terminals and diminished cortical functional connectivity. We found a similar phenotype in *Clock;Npas2* double KO mice, which like *Bmal1* KOs have completely disabled core clock transcriptional function (DeBruyne et al., 2007), but not in *Per1/Per2* double mutant mice, which are arrhythmic but have intact core clock-mediated transcription (Bae et al., 2001). We subsequently generated brain-specific *Bmal1* KO mice which have preserved SCN *Bmal1* expression and intact systemic circadian activity and sleep rhythms, but disrupted BMAL1-mediated transcription in cortical, striatal, and hippocampal neurons and astrocytes. These mice also developed severe astrogliosis, suggesting that positive-limb core clock function is required locally in neurons and/or astrocytes to prevent pathology, independent of the SCN or sleep–wake cycle (Musiek et al., 2013). Finally, we found that neurodegeneration induced by the mitochondrial complex II inhibitor 3-nitropropionic acid, which has been used to model HD (Beal et al., 1995), was exacerbated in *Bmal1* hemizygous mice, which have intact systemic rhythms but only half the normal level of BMAL1 protein expression in the brain. Thus, it appears that the core clock transcriptional machinery plays a critical role in protecting the brain from oxidative injury, and that this function is not entirely dictated by systemic circadian rhythms. Accordingly, we found that *Bmal1* directly regulates the transcription of several important redox defense genes in the brain, including *Nqo1* and *Aldh2*. We are currently working to identify novel mechanisms by which the core clock mediates neuroprotection or neurodegeneration, and to understand the relative importance of systemic circadian rhythms and clock gene oscillation versus static transcriptional function in this process.

Conversely, the effect of improving circadian function on pathology in a mouse model of neurologic disease has been demonstrated, at least initially. In the aforementioned R6/2 mouse model of HD, pharmacologic induction of rhythmic sleep normalized *Per2* oscillation in the SCN and lead to improvements in cognition (Pallier et al., 2007; Pallier and Morton, 2009). Furthermore, when food was provided to these mice only during a strategic window in the circadian cycle, rhythms in behavior were restored and metabolic abnormalities in these mice improved, suggesting that synchronizing the food-entrainable oscillator could overcome the circadian defect. Thus, correcting peripheral rhythms by imposing circadian sleep or feeding schedules can mitigate cognitive impairment in a mouse model of HD (Maywood et al., 2010). The application of these methods, or more specific molecular targeting of the core clock or its outputs, now needs to be evaluated in other animal models of neurodegeneration.

Human data demonstrating a contributory effect of circadian disruption to neurodegeneration is scarce, though several encouraging findings have emerged. Two observational studies examining young female flight attendants who routinely flew across multiple time zones found that the group that those afforded shorter recovery time between cross-time zone flights (who thus experienced more severe circadian disruption) had higher cortisol levels, smaller temporal lobe volumes on MRI, and performed more poorly on hippocampal-based cognitive testing than ground crew members or other flight attendants with less severe jet lag exposure (Cho et al., 2000; Cho, 2001). In the field of AD, a small amount of human data now also exists which supports a role for circadian disruption in disease pathogenesis. Three separate genetic polymorphism in the *Clock* gene have been linked to increased risk of AD in Han Chinese populations (Chen et al., 2013a,b; Yang et al., 2013), though these findings have not been reported by other large AD genetics consortiums. An epidemiologic study of daily activity data from of over 1,200 initially cognitively-normal older women demonstrated that diminished circadian rhythm amplitude, robustness, or phase delay were associated with increased risk of developing dementia during the 5 year follow-up period (Tranah et al., 2011). On a more mechanistic level, circadian oscillations in the level of the Aβ in cerebrospinal fluid of older adults has been described and suggest possible regulation of Aβ metabolism by the circadian clock, though it does not demonstrate a clear role for these oscillations in the disease process (Kang et al., 2009; Huang et al., 2012). Further research into circadian function in prodromal AD and other neurodegenerative disease, and how this relates to disease risk or progression is needed.

CONCLUSIONS AND THERAPEUTIC PERSPECTIVES

Taken in total, there is promising early data but not iron-clad proof that circadian clock disruption contributes to the pathogenesis of age-related neurodegenerative diseases. Two major challenges in this area are apparent. First, distinguishing the specific effects of alterations in sleep from those of the circadian clock is difficult but necessary. Activity data (actigraphy) in humans is often used as a biomarker of sleep or circadian rhythms, and the two processes are often lumped together, obscuring specific conclusions about either. Of further concern is the fact that disrupting sleep impacts core clock protein function (Mongrain et al., 2011), while deleting clock genes also alters sleep (Laposky et al., 2005). Disentangling these two processes is important if we hope to identify specific downstream pharmacologic targets from either pathway to treat or prevent neurodegenerative diseases.

The second major challenge involves distinguishing the specific importance of circadian oscillation versus the "static" function of clock proteins in the brain. While circadian oscillations are observed in thousands of transcripts in many tissues, including the brain, the physiological relevance of these oscillations remains in many cases unclear (Zhang et al., 2014). Clock proteins exert various functions in cells, some of which may have less dependence on these oscillations. Ultimately, the function of clock proteins is never completely disengaged from their oscillation, as the BMAL1/CLOCK DNA binding shows clear circadian variation (Koike et al., 2012), but the relative importance of rhythmic versus

static function remains a key therapeutic question. If restoration of robust systemic oscillations is the therapeutic goal, then therapies might target the SCN. Vasopressin V1a and V1b, as well as VIP VPAC2 receptors, play critical roles in synchronizing SCN neurons and their response to phase shift, and thus might represent tractable therapeutic targets to optimize systemic rhythms (Aton et al., 2005; An et al., 2013; Kudo et al., 2013; Yamaguchi et al., 2013). Behavioral manipulations such as imposed light:dark exposure, timed melatonin treatment, or rhythmic meal schedules might have shown promise in HD mouse models and might also be considered (Pallier et al., 2007; Maywood et al., 2010). Finally, novel small molecule modulators of clock oscillation are being developed which alter the period, amplitude, or frequency of SCN output (Hirota et al., 2010; Chen et al., 2012). However, if bolstering clock gene expression outside the SCN is the more advantageous strategy, then a new set of therapies would need to be developed. In this case, downstream neuroprotective targets of the core clock would need to be identified and screening pursued to identify compounds or strategies which enhance core clock transcription of these protective downstream targets. Because the clock serves as an orchestrator of a multitude of biological processes, there is great potential for clock-targeted therapeutics to simultaneously ameliorate multiple pathologic aspects of complex neurodegenerative diseases. Thus, it is important to more fully understand the mechanisms by which the circadian clock regulates brain function and neurodegeneration, such that rational strategies to target the clock for neuroprotection can be devised.

ACKNOWLEDGMENTS

ESM acknowledges funding from NIH grant K08NS079405 (ESM); Alzheimer's Association grant NIRP-14-305476 (ESM), and Donor's Cure Foundation (ESM).

REFERENCES

An, S., Harang, R., Meeker, K., Granados-Fuentes, D., Tsai, C. A., Mazuski, C., et al. (2013). A neuropeptide speeds circadian entrainment by reducing intercellular synchrony. *Proc. Natl. Acad. Sci. U.S.A.* 110, E4355–E4361. doi: 10.1073/pnas.1307088110

Aton, S. J., Colwell, C. S., Harmar, A. J., Waschek, J., and Herzog, E. D. (2005). Vasoactive intestinal polypeptide mediates circadian rhythmicity and synchrony in mammalian clock neurons. *Nat. Neurosci.* 8, 476–483. doi: 10.1038/nn1419

Aziz, N. A., Anguelova, G. V., Marinus, J., Lammers, G. J., and Roos, R. A. (2010). Sleep and circadian rhythm alterations correlate with depression and cognitive impairment in Huntington's disease. *Parkinsonism Relat. Disord.* 16, 345–350. doi: 10.1016/j.parkreldis.2010.02.009

Bae, K., Jin, X., Maywood, E. S., Hastings, M. H., Reppert, S. M., and Weaver, D. R. (2001). Differential functions of *mPer1*, *mPer2*, and *mPer3* in the SCN circadian clock. *Neuron* 30, 525–536. doi: 10.1016/S0896-6273(01)00302-6

Bass, J., and Takahashi, J. S. (2010). Circadian integration of metabolism and energetics. *Science* 330, 1349–1354. doi: 10.1126/science.1195027

Beal, M. F., Ferrante, R. J., Henshaw, R., Matthews, R. T., Chan, P. H., Kowall, N. W., et al. (1995). 3-Nitropropionic acid neurotoxicity is attenuated in copper/zinc superoxide dismutase transgenic mice. *J. Neurochem.* 65, 919–922. doi: 10.1046/j.1471-4159.1995.65020919.x

Beaver, L. M., Klichko, V. I., Chow, E. S., Kotwica-Rolinska, J., Williamson, M., Orr, W. C., et al. (2012). Circadian regulation of glutathione levels and biosynthesis in *Drosophila melanogaster*. *PLoS ONE* 7:e50454. doi: 10.1371/journal.pone.0050454

Breen, D. P., Vuono, R., Nawarathna, U., Fisher, K., Shneerson, J. M., Reddy, A. B., et al. (2014). Sleep and circadian rhythm regulation in early Parkinson disease. *JAMA Neurol.* 71, 589–595. doi: 10.1001/jamaneurol.2014.65

Cai, Y., Liu, S., Sothern, R. B., Xu, S., and Chan, P. (2010). Expression of clock genes *Per1* and *Bmal1* in total leukocytes in health and Parkinson's disease. *Eur. J. Neurol.* 17, 550–554. doi: 10.1111/j.1468-1331.2009.02848.x

Cermakian, N., Lamont, E. W., Boudreau, P., and Boivin, D. B. (2011). Circadian clock gene expression in brain regions of Alzheimer's disease patients and control subjects. *J. Biol. Rhythms* 26, 160–170. doi: 10.1177/0748730410395732

Chen, H. F., Huang, C. Q., You, C., Wang, Z. R., and Si-Qing, H. (2013a). Polymorphism of CLOCK gene rs 4580704 C > G is associated with susceptibility of Alzheimer's disease in a Chinese population. *Arch. Med. Res.* 44, 203–207. doi: 10.1016/j.arcmed.2013.01.002

Chen, Q., Huang, C. Q., Hu, X. Y., Li, S. B., and Zhang, X. M. (2013b). Functional CLOCK gene rs1554483 G/C polymorphism is associated with susceptibility to Alzheimer's disease in the Chinese population. *J. Int. Med. Res.* 41, 340–346. doi: 10.1177/0300060513476430

Chen, K. F., Possidente, B., Lomas, D. A., and Crowther, D. C. (2014). The central molecular clock is robust in the face of behavioural arrhythmia in a *Drosophila* model of Alzheimer's disease. *Dis. Model. Mech.* 7, 445–458. doi: 10.1242/dmm.014134

Chen, Z., Yoo, S. H., Park, Y. S., Kim, K. H., Wei, S., Buhr, E., et al. (2012). Identification of diverse modulators of central and peripheral circadian clocks by high-throughput chemical screening. *Proc. Natl. Acad. Sci. U.S.A.* 109, 101–106. doi: 10.1073/pnas.1118034108

Cho, K. (2001). Chronic 'jet lag' produces temporal lobe atrophy and spatial cognitive deficits. *Nat. Neurosci.* 4, 567–568. doi: 10.1038/88384

Cho, K., Ennaceur, A., Cole, J. C., and Suh, C. K. (2000). Chronic jet lag produces cognitive deficits. *J. Neurosci.* 20, 1–5.

Coogan, A. N., Schutova, B., Husung, S., Furczyk, K., Baune, B. T., Kropp, P., et al. (2013). The circadian system in Alzheimer's disease: disturbances, mechanisms, and opportunities. *Biol. Psychiatry* 74, 333–339. doi: 10.1016/j.biopsych.2012.11.021

DeBruyne, J. P., Weaver, D. R., and Reppert, S. M. (2007). CLOCK and NPAS2 have overlapping roles in the suprachiasmatic circadian clock. *Nat. Neurosci.* 10, 543–545. doi: 10.1038/nn1884

Duncan, M. J., Smith, J. T., Franklin, K. M., Beckett, T. L., Murphy, M. P., St Clair, D. K., et al. (2012). Effects of aging and genotype on circadian rhythms, sleep, and clock gene expression in APPxPS1 knock-in mice, a model for Alzheimer's disease. *Exp. Neurol.* 236, 249–258. doi: 10.1016/j.expneurol.2012.05.011

Evans, J. A., and Davidson, A. J. (2013). Health consequences of circadian disruption in humans and animal models. *Prog. Mol. Biol. Transl. Sci.* 119, 283–323. doi: 10.1016/B978-0-12-396971-2.00010-5

Fahrenkrug, J., Popovic, N., Georg, B., Brundin, P., and Hannibal, J. (2007). Decreased VIP and VPAC2 receptor expression in the biological clock of the R6/2 Huntington's disease mouse. *J. Mol. Neurosci.* 31, 139–148. doi: 10.1385/JMN/31:02:139

Farajnia, S., Michel, S., Deboer, T., Vanderleest, H. T., Houben, T., Rohling, J. H., et al. (2012). Evidence for neuronal desynchrony in the aged suprachiasmatic nucleus clock. *J. Neurosci.* 32, 5891–5899. doi: 10.1523/JNEUROSCI.0469-12.2012

Gajula Balija, M. B., Griesinger, C., Herzig, A., Zweckstetter, M., and Jackle, H. (2011). Pre-fibrillar α-synuclein mutants cause Parkinson's disease-like non-motor symptoms in *Drosophila*. *PLoS ONE* 6:e24701. doi: 10.1371/journal.pone.0024701

Gibson, E. M., Wang, C., Tjho, S., Khattar, N., and Kriegsfeld, L. J. (2010). Experimental 'jet lag' inhibits adult neurogenesis and produces long-term cognitive deficits in female hamsters. *PLoS ONE* 5:e15267. doi: 10.1371/journal.pone.0015267

Hatfield, C. F., Herbert, J., Van Someren, E. J., Hodges, J. R., and Hastings, M. H. (2004). Disrupted daily activity/rest cycles in relation to daily cortisol rhythms of home-dwelling patients with early Alzheimer's dementia. *Brain* 127, 1061–1074. doi: 10.1093/brain/awh129

Hirota, T., Lee, J. W., Lewis, W. G., Zhang, E. E., Breton, G., Liu, X., et al. (2010). High-throughput chemical screen identifies a novel potent modulator of cellular circadian rhythms and reveals CKIα as a clock regulatory kinase. *PLoS Biol.* 8:e1000559. doi: 10.1371/journal.pbio.1000559

Hu, K., Van Someren, E. J., Shea, S. A., and Scheer, F. A. (2009). Reduction of scale invariance of activity fluctuations with aging and Alzheimer's disease: involvement of the circadian pacemaker. *Proc. Natl. Acad. Sci. U.S.A.* 106, 2490–2494. doi: 10.1073/pnas.0806087106

Huang, Y., Potter, R., Sigurdson, W., Santacruz, A., Shih, S., Ju, Y. E., et al. (2012). Effects of age and amyloid deposition on Aβ dynamics in the human central nervous system. *Arch. Neurol.* 69, 51–58. doi: 10.1001/archneurol.2011.235

Ju, Y. E., Lucey, B. P., and Holtzman, D. M. (2014). Sleep and Alzheimer disease pathology—a bidirectional relationship. *Nat. Rev. Neurol.* 10, 115–119. doi: 10.1038/nrneurol.2013.269

Ju, Y. E., Mcleland, J. S., Toedebusch, C. D., Xiong, C., Fagan, A. M., Duntley, S. P., et al. (2013). Sleep quality and preclinical Alzheimer disease. *JAMA Neurol.* 70, 587–593. doi: 10.1001/jamaneurol.2013.2334

Kang, J. E., Lim, M. M., Bateman, R. J., Lee, J. J., Smyth, L. P., Cirrito, J. R., et al. (2009). Amyloid-β dynamics are regulated by orexin and the sleep–wake cycle. *Science* 326, 1005–1007. doi: 10.1126/science.1180962

Karatsoreos, I. N., Bhagat, S., Bloss, E. B., Morrison, J. H., and Mcewen, B. S. (2011). Disruption of circadian clocks has ramifications for metabolism, brain, and behavior. *Proc. Natl. Acad. Sci. U.S.A.* 108, 1657–1662. doi: 10.1073/pnas.1018375108

Koike, N., Yoo, S. H., Huang, H. C., Kumar, V., Lee, C., Kim, T. K., et al. (2012). Transcriptional architecture and chromatin landscape of the core circadian clock in mammals. *Science* 338, 349–354. doi: 10.1126/science.1226339

Kott, J., Leach, G., and Yan, L. (2012). Direction-dependent effects of chronic "jet-lag" on hippocampal neurogenesis. *Neurosci. Lett.* 515, 177–180. doi: 10.1016/j.neulet.2012.03.048

Krishnan, N., Davis, A. J., and Giebultowicz, J. M. (2008). Circadian regulation of response to oxidative stress in *Drosophila melanogaster*. *Biochem. Biophys. Res. Commun.* 374, 299–303. doi: 10.1016/j.bbrc.2008.07.011

Krishnan, N., Kretzschmar, D., Rakshit, K., Chow, E., and Giebultowicz, J. M. (2009). The circadian clock gene period extends healthspan in aging *Drosophila melanogaster*. *Aging (Albany NY)* 1, 937–948.

Krishnan, N., Rakshit, K., Chow, E. S., Wentzell, J. S., Kretzschmar, D., and Giebultowicz, J. M. (2012). Loss of circadian clock accelerates aging in neurodegeneration-prone mutants. *Neurobiol. Dis.* 45, 1129–1135. doi: 10.1016/j.nbd.2011.12.034

Kudo, T., Loh, D. H., Truong, D., Wu, Y., and Colwell, C. S. (2011a). Circadian dysfunction in a mouse model of Parkinson's disease. *Exp. Neurol.* 232, 66–75. doi: 10.1016/j.expneurol.2011.08.003

Kudo, T., Schroeder, A., Loh, D. H., Kuljis, D., Jordan, M. C., Roos, K. P., et al. (2011b). Dysfunctions in circadian behavior and physiology in mouse models of Huntington's disease. *Exp. Neurol.* 228, 80–90. doi: 10.1016/j.expneurol.2010.12.011

Kudo, T., Tahara, Y., Gamble, K. L., Mcmahon, D. G., Block, G. D., and Colwell, C. S. (2013). Vasoactive intestinal peptide produces long-lasting changes in neural activity in the suprachiasmatic nucleus. *J. Neurophysiol.* 110, 1097–1106. doi: 10.1152/jn.00114.2013

Laposky, A., Easton, A., Dugovic, C., Walisser, J., Bradfield, C., and Turek, F. (2005). Deletion of the mammalian circadian clock gene BMAL1/Mop3 alters baseline sleep architecture and the response to sleep deprivation. *Sleep* 28, 395–409.

Lim, A. S., Kowgier, M., Yu, L., Buchman, A. S., and Bennett, D. A. (2013a). Sleep fragmentation and the risk of incident Alzheimer's disease and cognitive decline in older persons. *Sleep* 36, 1027–1032. doi: 10.5665/sleep.2802

Lim, A. S., Myers, A. J., Yu, L., Buchman, A. S., Duffy, J. F., De Jager, P. L., et al. (2013b). Sex difference in daily rhythms of clock gene expression in the aged human cerebral cortex. *J. Biol. Rhythms* 28, 117–129. doi: 10.1177/0748730413478552

Lim, A. S., Srivastava, G. P., Yu, L., Chibnik, L. B., Xu, J., Buchman, A. S., et al. (2014). 24-hour rhythms of DNA methylation and their relation with rhythms of RNA expression in the human dorsolateral prefrontal cortex. *PLoS Genet.* 10:e1004792. doi: 10.1371/journal.pgen.1004792

Long, D. M., Blake, M. R., Dutta, S., Holbrook, S. D., Kotwica-Rolinska, J., Kretzschmar, D., et al. (2014). Relationships between the circadian system and Alzheimer's disease-like symptoms in *Drosophila*. *PLoS ONE* 9:e106068. doi: 10.1371/journal.pone.0106068

Maywood, E. S., Fraenkel, E., Mcallister, C. J., Wood, N., Reddy, A. B., Hastings, M. H., et al. (2010). Disruption of peripheral circadian timekeeping in a mouse model of Huntington's disease and its restoration by temporally scheduled feeding. *J. Neurosci.* 30, 10199–10204. doi: 10.1523/JNEUROSCI.1694-10.2010

Mohawk, J. A., Green, C. B., and Takahashi, J. S. (2012). Central and peripheral circadian clocks in mammals. *Annu. Rev. Neurosci.* 35, 445–462. doi: 10.1146/annurev-neuro-060909-153128

Mongrain, V., La Spada, F., Curie, T., and Franken, P. (2011). Sleep loss reduces the DNA-binding of BMAL1, CLOCK, and NPAS2 to specific clock genes in the mouse cerebral cortex. *PLoS ONE* 6:e26622. doi: 10.1371/journal.pone.0026622

Morton, A. J., Wood, N. I., Hastings, M. H., Hurelbrink, C., Barker, R. A., and Maywood, E. S. (2005). Disintegration of the sleep–wake cycle and circadian timing in Huntington's disease. *J. Neurosci.* 25, 157–163. doi: 10.1523/JNEUROSCI.3842-04.2005

Musiek, E. S., Lim, M. M., Yang, G., Bauer, A. Q., Qi, L., Lee, Y., et al. (2013). Circadian clock proteins regulate neuronal redox homeostasis and neurodegeneration. *J. Clin. Invest.* 123, 5389–5400. doi: 10.1172/JCI70317

Niwa, F., Kuriyama, N., Nakagawa, M., and Imanishi, J. (2011). Circadian rhythm of rest activity and autonomic nervous system activity at different stages in Parkinson's disease. *Auton. Neurosci.* 165, 195–200. doi: 10.1016/j.autneu.2011.07.010

Pallier, P. N., Maywood, E. S., Zheng, Z., Chesham, J. E., Inyushkin, A. N., Dyball, R., et al. (2007). Pharmacological imposition of sleep slows cognitive decline and reverses dysregulation of circadian gene expression in a transgenic mouse model of Huntington's disease. *J. Neurosci.* 27, 7869–7878. doi: 10.1523/JNEUROSCI.0649-07.2007

Pallier, P. N., and Morton, A. J. (2009). Management of sleep/wake cycles improves cognitive function in a transgenic mouse model of Huntington's disease. *Brain Res.* 1279, 90–98. doi: 10.1016/j.brainres.2009.03.072

Peter-Derex, L., Yammine, P., Bastuji, H., and Croisile, B. (2014). Sleep and Alzheimer's disease. *Sleep Med. Rev.* 19C, 29–38. doi: 10.1016/j.smrv.2014.03.007

Rakshit, K., and Giebultowicz, J. M. (2013). Cryptochrome restores dampened circadian rhythms and promotes healthspan in aging *Drosophila*. *Aging Cell* 12, 752–762. doi: 10.1111/acel.12100

Roh, J. H., Huang, Y., Bero, A. W., Kasten, T., Stewart, F. R., Bateman, R. J., et al. (2012). Disruption of the sleep–wake cycle and diurnal fluctuation of β-amyloid in mice with Alzheimer's disease pathology. *Sci. Transl. Med.* 4, 150ra122. doi: 10.1126/scitranslmed.3004291

Schnell, A., Chappuis, S., Schmutz, I., Brai, E., Ripperger, J. A., Schaad, O., et al. (2014). The nuclear receptor REV-ERBα regulates *Fabp7* and modulates adult hippocampal neurogenesis. *PLoS ONE* 9:e99883. doi: 10.1371/journal.pone.0099883

Skene, D. J., and Swaab, D. F. (2003). Melatonin rhythmicity: effect of age and Alzheimer's disease. *Exp. Gerontol.* 38, 199–206. doi: 10.1016/S0531-5565(02)00198-5

Spira, A. P., Gamaldo, A. A., An, Y., Wu, M. N., Simonsick, E. M., Bilgel, M., et al. (2013). Self-reported sleep and β-amyloid deposition in community-dwelling older adults. *JAMA Neurol.* 70, 1537–1543. doi: 10.1001/jamaneurol.2013.4258

Sterniczuk, R., Dyck, R. H., Laferla, F. M., and Antle, M. C. (2010). Characterization of the 3xTg-AD mouse model of Alzheimer's disease: part 1. Circadian changes. *Brain Res.* 1348, 139–148. doi: 10.1016/j.brainres.2010.05.013

Swaab, D. F., Fliers, E., and Partiman, T. S. (1985). The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia. *Brain Res.* 342, 37–44. doi: 10.1016/0006-8993(85)91350-2

Tranah, G. J., Blackwell, T., Stone, K. L., Ancoli-Israel, S., Paudel, M. L., Ensrud, K. E., et al. (2011). Circadian activity rhythms and risk of incident dementia and mild cognitive impairment in older women. *Ann. Neurol.* 70, 722–732. doi: 10.1002/ana.22468

Valnegri, P., Khelfaoui, M., Dorseuil, O., Bassani, S., Lagneaux, C., Gianfelice, A., et al. (2011). A circadian clock in hippocampus is regulated by interaction between oligophrenin-1 and Rev-erbα. *Nat. Neurosci.* 14, 1293–1301. doi: 10.1038/nn.2911

Videnovic, A., and Golombek, D. (2013). Circadian and sleep disorders in Parkinson's disease. *Exp. Neurol.* 243, 45–56. doi: 10.1016/j.expneurol.2012.08.018

Videnovic, A., Lazar, A. S., Barker, R. A., and Overeem, S. (2014a). 'The clocks that time us'-circadian rhythms in neurodegenerative disorders. *Nat. Rev. Neurol.* 10, 683–693. doi: 10.1038/nrneurol.2014.206

Videnovic, A., Noble, C., Reid, K. J., Peng, J., Turek, F. W., Marconi, A., et al. (2014b). Circadian melatonin rhythm and excessive daytime sleepiness in Parkinson disease. *JAMA Neurol.* 71, 463–469. doi: 10.1001/jamaneurol.2013.6239

Wisor, J. P., Edgar, D. M., Yesavage, J., Ryan, H. S., Mccormick, C. M., Lapustea, N., et al. (2005). Sleep and circadian abnormalities in a transgenic mouse model of

Alzheimer's disease: a role for cholinergic transmission. *Neuroscience* 131, 375–385. doi: 10.1016/j.neuroscience.2004.11.018

Witting, W., Kwa, I. H., Eikelenboom, P., Mirmiran, M., and Swaab, D. F. (1990). Alterations in the circadian rest-activity rhythm in aging and Alzheimer's disease. *Biol. Psychiatry* 27, 563–572. doi: 10.1016/0006-3223(90)90523-5

Wu, Y. H., Fischer, D. F., Kalsbeek, A., Garidou-Boof, M. L., Van Der Vliet, J., Van Heijningen, C., et al. (2006). Pineal clock gene oscillation is disturbed in Alzheimer's disease, due to functional disconnection from the "master clock." *FASEB J.* 20, 1874–1876. doi: 10.1096/fj.05-4446fje

Yamaguchi, Y., Suzuki, T., Mizoro, Y., Kori, H., Okada, K., Chen, Y., et al. (2013). Mice genetically deficient in vasopressin V1a and V1b receptors are resistant to jet lag. *Science* 342, 85–90. doi: 10.1126/science.1238599

Yang, Y. K., Peng, X. D., Li, Y. H., Wang, Z. R., Chang-Quan, H., Hui, W., et al. (2013). The polymorphism of CLOCK gene 3111T/C C>T is associated with susceptibility of Alzheimer disease in Chinese population. *J. Investig. Med.* 61, 1084–1087. doi: 10.231/JIM.0b013e31829f91c0

Zhang, R., Lahens, N. F., Ballance, H. I., Hughes, M. E., and Hogenesch, J. B. (2014). A circadian gene expression atlas in mammals: implications for biology and medicine. *Proc. Natl. Acad. Sci. U.S.A.* 111, 16219–16224. doi: 10.1073/pnas.1408886111

Zhou, J. N., Hofman, M. A., and Swaab, D. F. (1995). VIP neurons in the human SCN in relation to sex, age, and Alzheimer's disease. *Neurobiol. Aging* 16, 571–576. doi: 10.1016/0197-4580(95)00043-E

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 20 December 2014; accepted: 03 February 2015; published online: 27 February 2015.

Citation: Musiek ES (2015) Circadian clock disruption in neurodegenerative diseases: cause and effect? Front. Pharmacol. 6:29. doi: 10.3389/fphar.2015.00029

This article was submitted to Pharmaceutical Medicine and Outcomes Research, a section of the journal Frontiers in Pharmacology.

frontiers in Pharmacology

REVIEW
published: 24 March 2015
doi: 10.3389/fphar.2015.00050

Rodent models to study the metabolic effects of shiftwork in humans

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Edited by:
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University of Pennsylvania, USA

Reviewed by:
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Semmelweis University, Hungary
Frank A. J. L. Scheer,
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Specialty section:
This article was submitted to Pharmaceutical Medicine and Outcomes Research, a section of the journal Frontiers in Pharmacology

Received: 09 December 2014
Accepted: 01 March 2015
Published: 24 March 2015

Citation:
Opperhuizen AL, van Kerkhof LWM, Proper KI, Rodenburg W and Kalsbeek A (2015) Rodent models to study the metabolic effects of shiftwork in humans. Front. Pharmacol. 6:50. doi: 10.3389/fphar.2015.00050

Our current 24-h society requires an increasing number of employees to work nightshifts with millions of people worldwide working during the evening or night. Clear associations have been found between shiftwork and the risk to develop metabolic health problems, such as obesity. An increasing number of studies suggest that the underlying mechanism includes disruption of the rhythmically organized body physiology. Normally, daily 24-h rhythms in physiological processes are controlled by the central clock in the brain in close collaboration with peripheral clocks present throughout the body. Working schedules of shiftworkers greatly interfere with these normal daily rhythms by exposing the individual to contrasting inputs, i.e., at the one hand (dim)light exposure at night, nightly activity and eating and at the other hand daytime sleep and reduced light exposure. Several different animal models are being used to mimic shiftwork and study the mechanism responsible for the observed correlation between shiftwork and metabolic diseases. In this review we aim to provide an overview of the available animal studies with a focus on the four most relevant models that are being used to mimic human shiftwork: altered timing of (1) food intake, (2) activity, (3) sleep, or (4) light exposure. For all studies we scored whether and how relevant metabolic parameters, such as bodyweight, adiposity and plasma glucose were affected by the manipulation. In the discussion, we focus on differences between shiftwork models and animal species (i.e., rat and mouse). In addition, we comment on the complexity of shiftwork as an exposure and the subsequent difficulties when using animal models to investigate this condition. In view of the added value of animal models over human cohorts to study the effects and mechanisms of shiftwork, we conclude with recommendations to improve future research protocols to study the causality between shiftwork and metabolic health problems using animal models.

Keywords: shiftwork, metabolism, animal model, circadian desynchronization, glucose, lipids, activity, obesity

Introduction

Our current 24-h society requires an increasing number of employees to work nightshifts and as a result millions of people worldwide work during the evening or night for a certain period

during their life. In the Netherlands, 16% of the working population works regularly or occasionally during the night, whereas 51% of the population sometimes or regularly works during the evening (Centraal Bureau voor de Statistiek, 2013). Epidemiological studies show correlations between shiftwork and an increased risk of cancer, cardiovascular disease, sleep disturbances, impaired psychosocial health and gastrointestinal problems (Matheson et al., 2014). Moreover, the last two decades, population-based studies have shown that there is also an association between shiftwork and development of metabolic problems, including metabolic syndrome (Van Amelsvoort et al., 1999; Karlsson et al., 2001, 2003; Biggi et al., 2008; Suwazono et al., 2008; Lin et al., 2009; Pietroiusti et al., 2010; Kubo et al., 2011; Li et al., 2011; Tucker et al., 2012; Ye et al., 2013; Kawabe et al., 2014; Kawada and Otsuka, 2014), altered glucose metabolism (De Bacquer et al., 2009; Suwazono et al., 2009; Oyama et al., 2012), altered lipid metabolism (Biggi et al., 2008; De Bacquer et al., 2009; Dochi et al., 2009) and high blood pressure (Morikawa et al., 1999; Sakata et al., 2003; De Bacquer et al., 2009; Lin et al., 2009). Population-based studies are limited in their use for understanding causality and underlying mechanisms to explain the relationship between shiftwork and disease. Using experimental studies in humans is problematic due to the fact that many metabolic outcomes, such as body weight and composition, are long-term effects. Certainly, acute effects of shiftwork conditions on metabolic parameters can be studied in humans, which is currently done (McHill et al., 2014). Therefore, animal studies have been used to gain more insight in these questions. In the current review we provide an overview of the different animal models that are available to investigate the mechanism underlying the negative health consequences of shiftwork.

Daily 24-h rhythms are present throughout the body's physiology and can be observed in, for example, sleep, food consumption, body temperature and numerous hormone levels (Dibner et al., 2010). These rhythms are regulated by the central circadian clock in the suprachiasmatic nucleus (SCN) of the hypothalamus and circadian oscillators in peripheral tissues and organs (the so-called peripheral clocks). The endogenous rhythmicity of the SCN neurons ultimately results from the interaction between a set of rhythmically expressed genes, so-called clock genes, which are expressed in almost every cell of the body. In the SCN, the nearly 24-h (i.e., circadian) rhythms produced by these clock-genes are synchronized to the exact 24-h rhythms in the outer world by their sensitivity to (sun)light (Dibner et al., 2010). The synchronizing stimuli for peripheral clocks in non-SCN tissues are less clear, in addition to nervous and humoral signals from the SCN, behavioral signals such as body temperature, energy metabolism and (feeding) activity also likely play a role (Hastings et al., 1998; Dibner et al., 2010).

In general, the working schedules of shiftworkers profoundly interfere with these normal daily rhythms (Puttonen et al., 2010; Fritschi et al., 2011). Shiftwork leads to a disruption of the circadian rhythms produced by the central and peripheral clocks by confronting them with opposing signals, i.e., light at night and food consumption and activity during the sleep period. Therefore, shiftwork is a challenge that contains many aspects, which might be related to the negative health effects (**Figure 1**): (1) social pattern: shiftwork affects social life due to working hours that conflict with working hours of social contacts; (2) activity: shiftwork affects the timing of people's activity and, as a consequence, possibly affects the amount of activity; (3) sleep: shiftwork affects timing of sleep and possibly duration and quality of sleep; (4) nutrition: shiftwork affects timing of food intake and possibly meal frequency and composition; (5) light exposure: shiftwork affects the timing of light exposure, with possibly different intensity and duration of exposure; (6) sun exposure: shiftwork might affect the duration of sun exposure and as a consequence vitamin D levels. Shiftwork comprises alterations at different levels of the circadian system that each have their own effect, but are interacting as well (**Figure 1**; Puttonen et al., 2010; Fritschi et al., 2011). For example, the altered timing of activity in shiftworkers may result in sleep disturbances (duration, quality,

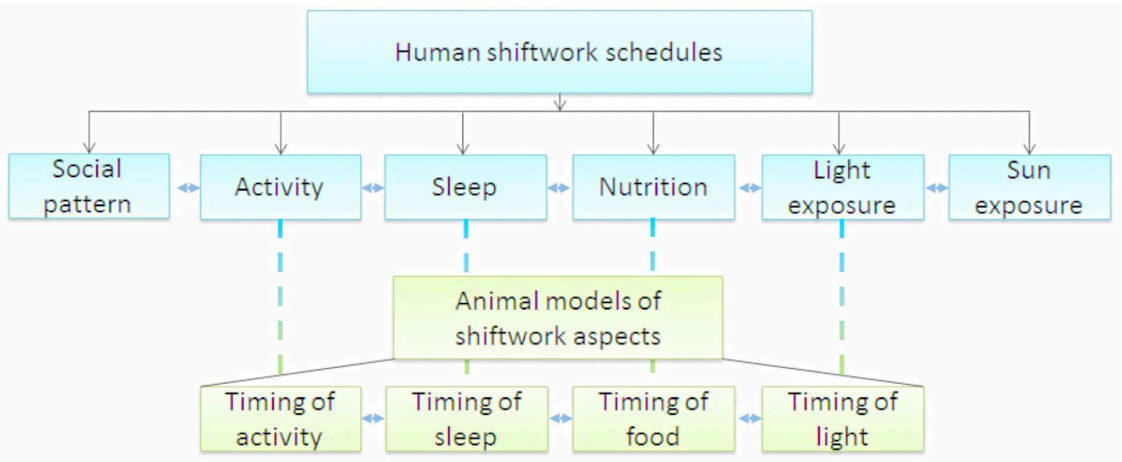

FIGURE 1 | Shiftwork can be disentangled into different aspects (blue blocks), for some of these aspects animal models have been developed (green blocks). Each of these aspects might contribute to health risks associated with shiftwork. However, all aspects strongly interact, making it difficult to separate the effects of each single aspect. In most animal studies only one of the aspects is manipulated, however, it is important to keep in mind that by manipulation of one aspect, other aspects might be affected as well due to this interaction.

and timing), altered nutrition (composition, caloric intake and timing), changed lighting exposure conditions (duration, intensity, and timing), reduced sunlight exposure (possible effect on vitamin D levels) and disturbances in social life. Each of these aspects might, to a greater or lesser extent, contribute to negative health effects. For several aspects, animal models have been developed to examine the metabolic health effects upon manipulation of these aspects of shiftwork individually or in combination (**Figure 1**). To our knowledge, no animal models have so far been developed to study effects of "social life" and "sun exposure."

The aim of the present review is to provide an overview of the available animal studies investigating the mechanism underlying the negative health consequences of shiftwork and their outcome. In addition, we discuss human relevancy of the available animal models for shiftwork to gain insight into animal to human translatability and aid future investigations in choosing optimal animal models. Next to animal models mimicking circadian disruptive shiftwork aspects, consequences of circadian disruption have also been studied in animals using genetic manipulation or SCN lesions. These animal models are not within the scope of the present review, since we do not consider them to represent human shiftwork.

To increase the animal to human translatability the focus of the present review is on animal models investigating the relationship between shiftwork and metabolic risk factors (Haffner, 1998; Carnethon et al., 2004; Esquirol et al., 2011) since these factors are easily translatable from humans to animals and vice versa. In addition, these factors can be measured almost non-invasively in humans and often appear before the full blown disease, allowing for shorter follow-up time and more time for preventive measures or intervention.

Studies were included in the review when they investigated metabolic parameters such as bodyweight, food intake, activity, glucose metabolism (including plasma glucose, insulin and glucagon levels, glucose tolerance, and glycogen levels), leptin levels, and lipid metabolism (including plasma cholesterol and triglyceride levels).

Methods

Search Strategy

A literature search was performed to obtain an overview of the current scientific literature on studies using animal models for shiftwork to investigate the relationship between shiftwork and metabolic function. The search strategy was designed by an information specialist (RIVM) using the MESH-database of Pubmed, to include all MeSH terms and its synonyms and several electronic databases were used (Medline, Embase, BIOSIS Previews en SciSearch). In brief, the search strategy combined keywords related to shiftwork with keywords related to metabolic risk factors. Examples of key words for shiftwork: shift work*, shiftwork*, night work*, night shift*, rotating shift*, jet lag, working rhythm*, "irregular working hours," time restricted, "constant light," "continuous light," "light at night," biological clock*, body clock*, chronobiology*, circadian clock*. Examples of key words for metabolic risk factors: weight, body weight, weight change, metabolic syndrome, obesity, adiposity, glucose, glucose tolerance, lipid metabolism, energy metabolism, insulin, insulin sensitivity, hypertension, leptin. Only papers published after 1993 were included. For the complete search strategy see Supplementary Data.

The search resulted in 1550 publications, but only 44 were included as these met the following criteria:

(1) Using animal models for shiftwork
(2) Investigate effects on at least one of the following metabolic risk factors for disease (Haffner, 1998; Carnethon et al., 2004):

bodyweight and related measures (BMI, fat percentage) or glucose homeostasis: including plasma glucose levels, glucose tolerance, plasma insulin levels, insulin sensitivity or lipid homeostasis: including plasma levels of triglycerides, cholesterol, free fatty acids, HDL or LDL.

The search included papers in English, Dutch, French, and German. However, only papers in English fitted the above mentioned criteria. In addition to this search strategy, the present knowledge of the authors and references from included papers ("snowball method") were used to include additional papers that fitted the above mentioned criteria (including papers published before 1993) or investigated parameters related to metabolic dysfunction.

Categorization of Studies

Included studies were divided in four different categories as presented in **Figure 1**: (1) Models using "timing of food intake," (2) models using "timing of activity," (3) models using "timing of sleep," and (4) models using "timing of light." For each study the outcome parameters were determined, which included the abovementioned metabolic risk factors for disease as well as circadian parameters (e.g., activity, cortisol) and gene expression. For these parameters, results are described in the text and summarized in the table for overview purposes. In the **Tables 1–5**, the left column holds the metabolically relevant parameters which were scored for and were most frequently measured in the studies. The *rat* and *mouse* columns represent the number of studies in which an effect of the manipulation (compared to the control condition) was found in this species against the number of studies in which it was measured. In the *total* column, results are divided in direction of effects and presented as the number of studies observing that direction is summarized. This was not done for gene expression. The most right column shows the studies in which the parameter was measured in this category of models. In the tables, studies are counted twice when multiple experiments are performed in one article, for instance when two types of mice were used. Occasionally, the effect of the manipulation was measured on the total level as well as the rhythm of a parameter within the same study. In this case, both effects are included in the *total* column.

With these tables we aim to provide an overview of the metabolic parameters that are influenced per category and type of animals used. Results of effect on gene expression are described in the text and summarized in the table as "gene expression." The present review was aimed at providing a narrative overview of

TABLE 1 | Summary of animal studies in which timing of food intake was manipulated to mimic human shiftwork.

Food	Rat	Mouse	Total	Studies
Bodyweight	1/3	6/8	+: 3/11 (27, 3%) −: 4/11 (36, 4%) o: 4/11 (36, 4%)	Arble et al., 2009; Bray et al., 2010, 2013; Salgado-Delgado et al., 2010a, 2013; Jang et al., 2012; Sherman et al., 2012; Yoon et al., 2012; Reznick et al., 2013; Oyama et al., 2014; Shamsi et al., 2014
Food intake total	1/3	4/8	+: 0/11 (0%) −: 5/11 (45, 5%) o: 6/11 (54, 5%)	Arble et al., 2009; Bray et al., 2010, 2013; Salgado-Delgado et al., 2010a, 2013; Jang et al., 2012; Sherman et al., 2012; Yoon et al., 2012; Reznick et al., 2013; Oyama et al., 2014; Shamsi et al., 2014
Activity total	1/2	2/5	+: 2/7 (28, 6%) −: 1/7 (14, 3%) o: 4/7 (57, 1%)	Arble et al., 2009; Bray et al., 2010, 2013; Salgado-Delgado et al., 2010a; Sherman et al., 2012; Yoon et al., 2012; Reznick et al., 2013; Shamsi et al., 2014
EE total	1/1	2/2	+: 0/3 (0%) −: 3/3 (100%) o: 0/3 (0%)	Arble et al., 2009; Bray et al., 2010, 2013; Reznick et al., 2013
RER	1/1	2/2	+: 1/3 (33, 3%) −: 1/3 (33, 3%) ∼: 1/3 (33, 3%) o: 0/3 (0%)	Bray et al., 2010, 2013; Reznick et al., 2013
Adiposity	2/3	3/4	+: 3/7 (42, 8%) −: 2/7 (28, 6%) o: 2/7 (28, 6%)	Arble et al., 2009; Bray et al., 2010; Salgado-Delgado et al., 2010a, 2013; Sherman et al., 2012; Reznick et al., 2013; Shamsi et al., 2014
Glucose metabolism	2/3	4/6	+: 1/9 (11, 1%) −: 2/9 (22, 2%) ∼: 3/9 (33, 3%) o: 3/9 (33, 3%)	Bray et al., 2010, 2013; Salgado-Delgado et al., 2010a, 2013; Jang et al., 2012; Sherman et al., 2012; Yoon et al., 2012; Reznick et al., 2013; Shamsi et al., 2014
Lipid metabolism	2/2	3/5	+: 1/7 (14, 3%) −: 2/7 (28, 6%) ∼: 4/7 (57, 1%) o: 2/7 (28, 6%)	Bray et al., 2010, 2013; Salgado-Delgado et al., 2010a; Sherman et al., 2012; Yoon et al., 2012; Reznick et al., 2013; Shamsi et al., 2014
Corticosterone	2/2	2/3	+: 1/5 (20%) −: 0/5 (0%) ∼: 3/5 (60%) o: 1/5 (20%)	Salgado-Delgado et al., 2010a; Sherman et al., 2012; Bray et al., 2013; Reznick et al., 2013; Shamsi et al., 2014
Melatonin				
Leptin	1/1	0/1	+: 1/2 (50%) −: 0/2 (0%) ∼: 1/2 (50%) o: 1/2 (50%)	Bray et al., 2010; Sherman et al., 2012; Reznick et al., 2013
Ghrelin		1/1	+: 0/1 (0%) −: 1/1 (100%) ∼: 0/1 (0%) o: 0/1 (0%)	Sherman et al., 2012
BP/Heart rate		2/2	+: 0/2 (0%) −: 2/2 (100%) ∼: 0/2 (0%) o: 0/2 (0%)	Schroder et al., 2014
Gene expression	2/2	10/10	12/12 (100%)	Damiola et al., 2000; Jang et al., 2012; Sherman et al., 2012; Yoon et al., 2012; Bray et al., 2013; Reznick et al., 2013; Salgado-Delgado et al., 2013; Oyama et al., 2014; Shamsi et al., 2014

For detailed description of the columns, see Methods section.

+, number of studies with increases; −, number of studies with decreases; ∼, number of studies with altered rhythm; o, number of studies with no effect. For glucose metabolism a + indicates increased basal levels of glucose, HbA1c or insulin, increased HOMA index or decreased glucose tolerance.

EE, energy expenditure; RER, respiratory exchange ratio; BP, blood pressure.

TABLE 2 | Summary of animal studies in which timing of activity was manipulated to mimic human shiftwork.

Activity	Rat	Mouse	Total	Studies
Bodyweight	5/6		+: 3/6 (50%) −: 2/6 (33, 3%) o: 1/6 (16, 7%)	Murphy, 2003; Tsai and Tsai, 2007; Salgado-Delgado et al., 2008, 2010a, 2013; Leenaars et al., 2012
Food intake total	2/6		+: 1/6 (16, 7%) −: 1/6 (16, 7%) o: 4/6 (66, 7%)	Murphy, 2003; Tsai and Tsai, 2007; Salgado-Delgado et al., 2008, 2010a,b, 2013
Activity total	4/6		+: 0/6 (0%) −: 4/6 (66, 7%) o: 2/6 (33, 3%)	Salgado-Delgado et al., 2008, 2010a,b, 2013; Leenaars et al., 2012; Hsieh et al., 2014
EE total				
RER				
Adiposity	2/2		+: 2/2 (100%) −: 0/2 (0%) o: 0/2 (0%)	Salgado-Delgado et al., 2010a, 2013
Glucose metabolism	3/3		+: 1/3 (33, 3%) −: 2/3 (66, 7%) ∼: 0/3 (0%) o: 0/3 (0%)	Salgado-Delgado et al., 2008, 2010a, 2013
Lipid metabolism	2/2		+: 0/2 (0%) −: 0/2 (0%) ∼: 2/2 (100%) o: 0/2 (0%)	Salgado-Delgado et al., 2008, 2010a
Corticosterone	2/2		+: 0/2 (0%) −: 0/2 (0%) ∼: 2/2 (100%) o: 0/2 (0%)	Salgado-Delgado et al., 2008, 2010a
Melatonin				
Leptin				
Ghrelin				
BP/Heart rate				
Gene expression	1/1		1/1 (100%)	Salgado-Delgado et al., 2013

For detailed description of the columns, see Methods section.
+, number of studies with increases; −, number of studies with decreases; ∼, number of studies with altered rhythm; o, number of studies with no effect. For glucose metabolism a + indicates increased basal levels of glucose, HbA1c or insulin, increased HOMA index or decreased glucose tolerance.
EE, energy expenditure; RER, respiratory exchange ratio; BP, blood pressure.

available studies and their findings, due to heterogeneity studies were not assessed for quality.

Results

Animal studies that model human shiftwork can be divided into four main categories. The first three categories are based on desynchronization of peripheral clocks from the central clock by the unnatural timing of food intake, sleep or activity. The remaining category consists of studies that manipulate the timing of light exposure, including alterations of duration (i.e., continuous light) and timing of light exposure. Some studies used a shiftwork model that combines multiple categories and those will be mentioned repeatedly in the different categories if appropriate. Below we discuss the main findings of studies using a shiftwork model within the categories where the models fit best.

Category 1: Models Using "Timing of Food Intake"

Shiftwork models using shifted and/or restricted timing of food availability are based on the knowledge that food intake is the most important Zeitgeber for peripheral clocks, in the same

TABLE 3 | Summary of animal studies in which timing of sleep was manipulated to mimic human shiftwork.

Sleep	Rat	Mouse	Total	Studies
Bodyweight	3/3	0/2	+: 1/5 (20%) −: 3/5 (60%) o: 2/5 (20%)	Barf et al., 2010, 2012a,b; Barclay et al., 2012; Husse et al., 2012
Food intake total	1/2	1/2	+: 2/4 (50%) −: 0/4 (0%) o: 2/4 (50%)	Barf et al., 2010, 2012a; Barclay et al., 2012; Husse et al., 2012
Activity total	0/1	0/2	+: 0/3 (0%) −: 0/3 (0%) o: 3/3 (100%)	Barclay et al., 2012; Barf et al., 2012a; Husse et al., 2012
EE total	0/1		+: 0/1 (0%) −: 0/1 (0%) o: 1/1 (100%)	Barf et al., 2012a
RER				
Adiposity				
Glucose metabolism	3/3	2/2	+: 3/5 (60%) −: 2/5 (40%) ∼: 0/5 (0%) o: 0/5 (0%)	Barf et al., 2010, 2012a,b; Barclay et al., 2012; Husse et al., 2012
Lipid metabolism		2/2	+: 2/2 (100%) −: 1/2 (50%) ∼: 0/2 (0%) o: 0/2 (0%)	Barclay et al., 2012; Husse et al., 2012
Corticosterone	1/2	1/2	+: 2/4 (50%) −: 0/4 (0%) ∼: 0/4 (0%) o: 2/4 (50%)	Barclay et al., 2012; Barf et al., 2012a,b; Husse et al., 2012
Melatonin				
Leptin	2/2	2/2	+: 1/4 (25%) −: 3/4 (75%) ∼: 0/4 (0%) o: 0/4 (0%)	Barclay et al., 2012; Barf et al., 2012a,b; Husse et al., 2012
Ghrelin				
BP/heart rate				
Gene expression		2/2	2/2 (100%)	Barclay et al., 2012; Husse et al., 2012

For detailed description of the columns, see Methods section.

+, number of studies with increases; −, number of studies with decreases; ∼, number of studies with altered rhythm; o, number of studies with no effect. For glucose metabolism a + indicates increased basal levels of glucose, HbA1c or insulin, increased HOMA index or decreased glucose tolerance.

EE, energy expenditure; RER, respiratory exchange ratio; BP, blood pressure.

way as light is for the central clock. Shifting timing of food intake disrupts the orchestrated synchrony between peripheral and brain clocks, which might lead to metabolic problems as peripheral organs such as liver and muscle are essential for energy homeostasis. Shifting the timing of food intake is an interesting approach as metabolic disorders such as obesity are also associated with aberrant dietary habits (i.e., quantity, composition and frequency) and shiftworkers also have changed dietary habits. Moreover, more recently several studies have suggested that also the timing of food intake is crucial to maintain energy homeostasis (Gluck et al., 2011; Garaulet et al., 2013; Hibi et al., 2013; Garaulet and Gomez-Abellan, 2014; Wang et al., 2014) and shifting the timing of food intake is another characteristic feature of shiftworkers (Lowden et al., 2010). All in all making this a relevant model for shiftwork.

TABLE 4 | Summary of animal studies in which continuous light (LL) or dimlight (LDim) at night exposure was used to mimic human shiftwork.

LL/LDim	RAT	Mouse	Total	Studies
Bodyweight	1/5	5/6	+: 6/11 (54, 5%) −: 0/11 (0%) o: 5/11 (45, 4%)	Natelson et al., 1993; Dauchy et al., 2010; Fonken et al., 2010; Gale et al., 2011; Coomans et al., 2013a; Aubrecht et al., 2014; Borniger et al., 2014
Food intake total	0/2	2/7	+: 1/9 (11, 1%) −: 1/9 (11, 1%) o: 7/9 (77, 7%)	Dauchy et al., 2010; Fonken et al., 2010; Coomans et al., 2013a; Shi et al., 2013; Aubrecht et al., 2014; Borniger et al., 2014
Activity total		0/5	+: 0/5 (0%) −: 0/5 (0%) o: 5/5 (100%)	Fonken et al., 2010; Shi et al., 2013; Aubrecht et al., 2014; Borniger et al., 2014
EE total		2/2	+: 0/2 (0%) −: 2/2 (100%) o: 0/2 (0%)	Coomans et al., 2013a; Borniger et al., 2014
RER		2/2	+: 2/2 (100%) −: 0/2 (0%) o: 0/2 (0%)	Coomans et al., 2013a; Borniger et al., 2014
Adiposity		1/1	+: 1/1 (100%) −: 0/1 (0%) o: 0/1 (0%)	Shi et al., 2013
Glucose metabolism	3/4	3/3	+: 4/7 (57, 1%) −: 0/7 (0%) ∼: 2/7 (28, 6%) o: 1/7 (14, 3%)	Dauchy et al., 2010; Fonken et al., 2010; Gale et al., 2011; Coomans et al., 2013a
Lipid metabolism	1/2		+: 0/2 (0%) −: 0/2 (0%) ∼: 1/2 (50%) o: 1/2 (50%)	Dauchy et al., 2010
Corticosterone	2/2		+: 1/2 (50%) −: 0/2 (0%) ∼: 2/2 (100%) o: 0/2 (0%)	Dauchy et al., 2010
Melatonin	4/4		+: 0/4 (0%) −: 2/4 (50%) ∼: 2/4 (50%) o: 0/4 (0%)	Dauchy et al., 2010; Gale et al., 2011
Leptin				
Ghrelin				
BP/Heart rate				
Gene expression				

For detailed description of the columns, see Methods section.

+, number of studies with increases; −, number of studies with decreases; ∼, number of studies with altered rhythm; o, number of studies with no effect. For glucose metabolism a + indicates increased basal levels of glucose, HbA1c or insulin, increased HOMA index or decreased glucose tolerance.

EE, energy expenditure; RER, respiratory exchange ratio; BP, blood pressure.

The first evidence for food as a strong entrainment signal for circadian physiology (metabolic and clock gene expression rhythms, hormone secretion rhythms) came from so-called restricted feeding studies. This type of studies usually restricts food availability to a short period (e.g., 2–4 h) during the light phase (which is the resting phase of nocturnal rodents) to study entrainment of peripheral clocks. Clearly these are important studies for chronobiology in general and still are performed frequently to look for and try to understand better the food entrainable oscillator. However, such restricted-feeding models are not

TABLE 5 | Summary of animal studies in which exposure to L/D shifts was used to mimic human shiftwork.

L/D shifts	Rat	Mouse	Total	Studies
Bodyweight	2/5	3/4	+: 4/9 (44, 4%) −: 1/9 (11, 1%) o: 4/9 (44, 4%)	Vilaplana et al., 1995; Tsai et al., 2005; Bartol-Munier et al., 2006; Oishi, 2009; Gale et al., 2011; Karatsoreos et al., 2011; Oishi and Itoh, 2013; Voigt et al., 2014
Food intake total	2/3	1/3	+: 2/6 (33, 3%) −: 1/6 (16, 7%) o: 3/6 (50%)	Vilaplana et al., 1995; Tsai et al., 2005; Bartol-Munier et al., 2006; Oishi, 2009; Karatsoreos et al., 2011; Oishi and Itoh, 2013
Activity total	2/2		+: 0/2 (0%) −: 2/2 (100%) o: 0/2 (0%)	Tsai et al., 2005; Bartol-Munier et al., 2006
EE total				
RER				
Adiposity				
Glucose metabolism	2/3	3/3	+: 4/6 (66, 7%) −: 1/6 (16, 7%) ∼: 0/6 (0%) o: 1/6 (16, 7%)	Bartol-Munier et al., 2006; Oishi, 2009; Gale et al., 2011; Karatsoreos et al., 2011; Oishi and Itoh, 2013
Lipid metabolism	0/1	1/2	+: 1/3 (33, 3%) −: 0/3 (0%) ∼: 0/3 (0%) o: 2/3 (66, 7%)	Bartol-Munier et al., 2006; Oishi, 2009; Oishi and Itoh, 2013
Corticosterone				
Melatonin	2/2		+: 0/2 (0%) −: 0/2 (0%) ∼: 2/2 (100%) o: 0/2 (0%)	Gale et al., 2011
Leptin		1/1	+: 1/1 (100%) −: 0/1 (0%) ∼: 0/1 (0%) o: 0/1 (0%)	Karatsoreos et al., 2011
Ghrelin				
BP/Heart rate	1/1		+: 0/1 (0%) −: 0/1 (0%) ∼: 1/1 (100%) o: 0/1 (0%)	Tsai et al., 2005
Gene expression		1/1	1/1 (100%)	Oishi and Itoh, 2013

For detailed description of the columns, see Methods section.

+, number of studies with increases; −, number of studies with decreases; ∼, number of studies with altered rhythm; o, number of studies with no effect. For glucose metabolism a + indicates increased basal levels of glucose, HbA1c or insulin, increased HOMA index or decreased glucose tolerance.

EE, energy expenditure; RER, respiratory exchange ratio; BP, blood pressure.

an adequate reflection of human food intake behavior during shiftwork as they restrict the duration of food intake to a (very) short period and therefore were not included in this review.

Food restriction studies in which food availability is shifted or restricted to a certain phase of the day (i.e., a large part of or the complete light period or dark period) provide a more suitable approach to mimic human feeding behavior during shift work. One of the first studies with food availability restricted to either the 12-h light or 12-h dark phase was done by Damiola et al. and they showed a strongly disturbed circadian rhythm according to altered daily body temperature rhythms in mice that could eat only during the light (i.e., resting) phase. Several clock genes in liver adjusted their expression to the timing of food intake (Damiola et al., 2000). Although alterations in gene expression cannot

be translated directly into functional changes, it does indicate that food has strong entraining properties even on a molecular level in mice. More recently, Bray et al. performed a short-term experiment and observed metabolic changes within the first 9 days after restricting food intake to the light or dark phase. Whole body energy metabolism was affected within 24 h of food restriction and this was visible in a 5 h phase advance of rhythm in energy expenditure, higher resting energy expenditure (RER) and increased caloric intake. Restricting food to the resting phase caused an increase of bodyweight and blunted plasma glucose and corticosterone rhythm, whereas triglyceride levels were not affected (Bray et al., 2013). A short term experiment by Oyama et al. focused on the effects of food timing on inflammatory response but additionally found reduced food intake and bodyweight in mice fed during the light phase (Oyama et al., 2014). Jang et al. performed the same restriction protocol but studied long-term effects (5–9 weeks). Surprisingly, they observed a protective effect of restricting food to the resting phase with lower bodyweight and food intake when compared to (chow or high-fat diet) *ad libitum* fed animals. Bodyweight did not differ from animals pair-fed during the active phase. Also, alterations in the expression of lipogenic, gluconeogenic and fatty acid oxidation-related genes in liver were found in feeding time-restricted animals (Jang et al., 2012). Shamsi et al. entrained mice to 16L:8D or 8L:16D photoperiods and restricted food availability to light- or dark phase. Neither photoperiod nor food timing affected bodyweight when compared to *ad libitum* feeding. Plasma insulin increased in light phase fed animals despite the photoperiod, whereas plasma glucose tended to be lower and triglycerides significantly decreased when feeding during light was compared to *ad libitum* or dark phase feeding. Rhythms in plasma glucose, insulin and triglyceride secretion shifted by light phase feeding when compared to dark phase feeding and some effects (mostly amplitude) changed over time (i.e., 7 vs. 35 days). Interestingly, long photoperiod caused light phase fed animals to increase glucose tolerance but decrease insulin tolerance compared to dark phase fed animals and *ad libitum* fed animals respectively. Gene expression of metabolic and clock genes in liver was altered by feeding during light phase in both amplitude and phase. Corticosterone rhythm was shifted by light phase feeding after 35 days but not after 7 days (Shamsi et al., 2014). Reznick et al. took a similar approach with a 3-week study performed with Wistar rats instead of mice. No effect was found on bodyweight gain or epididymal white adipose tissue, but animals fed during the resting phase decreased their food intake and total activity levels. Rats fed during the light period showed a 12-h shift in RER and dampening of activity and energy expenditure diurnal variation. The rhythm of plasma insulin altered with higher 24-h levels, corticosterone showed an additional peak and the rhythm of glycogen shifted to an opposite phase in liver but showed increased levels in muscle. Triglyceride levels in liver were reduced whereas muscle content was unaffected in animals fed during the light period. Expression of several other proteins and genes involved in energy metabolism and clock regulation in liver and muscle tissue showed phase changes or altered expression levels. In the same study this experimental design was used for a group of rats fed a high fat diet which aggravated many of the observed effects found in chow day-fed animals with additional disruption of leptin and NEFA (non-esterified fatty acids) levels (Reznick et al., 2013).

A series of studies performed with male Wistar rats used a forced activity protocol as a model for shiftwork (Salgado-Delgado et al., 2008, 2010a,b, 2011, 2013). The effects of forced activity will be described below (see category 2), but the non-working "control" groups of these studies are relevant for the timing of food category. When food was restricted to the resting phase, i.e., chow only available from ZT0 to ZT12, rats displayed a dampening of their core body temperature rhythm, an additional peak in the plasma corticosterone rhythm, and a shift in triglyceride secretion, but no differences were observed for the plasma glucose and activity rhythm or total activity. Total food intake remained the same but bodyweight and peritoneal fat accumulation were increased when compared to *ad libitum* or night fed (food available from ZT12 to ZT24) animals (Salgado-Delgado et al., 2010a). The observed accumulation of abdominal fat was reproduced by the same group in another study where a decreased glucose tolerance in rats fed during the resting phase was observed, in addition to alterations or dampened rhythms in liver clock and metabolic gene expression (Salgado-Delgado et al., 2013).

A couple of other groups used comparable food availability approaches in mice on normal chow diet, however, shorter food restriction periods were used than 12 h during the light period. Yoon et al. enforced a 6-h advance (ZT6-11) or delay (ZT18-23) in food availability for 9 weeks and observed that body temperature, locomotor activity and triglyceride secretion strongly depend on food timing. Cholesterol and HDL levels were moderately increased in both advance and delay groups when compared to *ad libitum* fed animals, and food intake was reduced in the food time advanced group compared to food time delay group and ad-lib controls. Fasting glucose levels increased and poor responses to insulin tolerance test intensified over time in daytime fed animals (advance group) (Yoon et al., 2012). Sherman and colleagues restricted food intake to the light phase, ZT4-8 without caloric restriction, for 18 weeks and performed this with both high- and low-fat (chow) diets. In both diets, time restriction was protective for bodyweight gain, high plasma leptin, insulin, HDL and cholesterol levels. Also the increased epididymal fat observed in *ad libitum* fed animals was diminished in the food time-restricted groups. In low fat diet fed animals triglyceride levels were reduced but corticosterone and adiponectin were increased, as compared to the *ad libitum* low and high-fat and restricted high-fat animals. High-fat diet fed animals also showed improved TNF-alpha and HOMA-IR levels and increased activity levels when food was restricted to the light period, as compared to *ad libitum* fed animals, but were less active than animals on a low-fat diet (Sherman et al., 2012). Schroder et al. focused on the effects on heart rhythm and observed that when food was provided from ZT2-9 only, this negatively affected several aspects of heart rhythm aspects in both wild type and genetically sensitive mice (Schroder et al., 2014).

Most studies mentioned above were performed with normal chow diet which is low on fat derived content. However, many diet-induced obesity studies use high-fat diet *ad libitum* feeding on which animals will develop obesity, diabetes and metabolic

syndrome (Zaragoza and Felber, 1970). For circadian studies it is important to know that feeding rodents a high-fat diet induces a dampening of the amplitude of daily activity and feeding rhythms, and also metabolic markers, hypothalamic neuropeptides and peripherally expressed factors involved in lipid metabolism are affected (Kohsaka et al., 2007). This suggests an interaction between energy metabolism and circadian rhythm control. Some groups, however, combined the restricted-feeding paradigm with a high-fat diet. Restriction of food to one phase of the day may re-induce the entrainment lost on high-fat *ad libitum* feeding. Arble and colleagues made an early attempt and fed mice a high-fat diet solely during the light-phase and observed a significant increase in bodyweight compared to animals fed during the dark phase (Arble et al., 2009). Bray et al. used four different feeding schedules to study in more detail which phase of the L/D-cycle is most detrimental when ingesting a high-fat diet for 12 weeks. They observed higher bodyweight gain and adiposity, decreased glucose tolerance next to high insulin, leptin and triglyceride levels in mice consuming their high-fat meal at the end of the active phase instead of at the beginning. Interestingly, when fat was only available in the light phase no metabolic changes were observed with the exception of slightly decreased energy expenditure and oxygen consumption when compared to animals with fat available in the dark phase (Bray et al., 2010). Several studies experimented with restriction of a nutrient component to a certain phase of the day for the effects on obesity. For instance, providing the fat component or sugar component of a free-choice high-fat-high-sugar diet only during the light phase affects RER, energy expenditure and bodyweight (La Fleur et al., 2014; Oosterman et al., 2014). A slightly different approach was taken by Senador et al. by offering mice a 10% fructose solution additional to their normal chow and water diet. Fructose was either available for 24 h, available for 12 h during light phase, available for 12 h during dark phase or not available. Increased bodyweight, higher fasting glucose levels but decreased plasma triglycerides were observed in both groups with 12 h fructose availability. Fructose restriction to light phase additionally caused glucose intolerance and an attenuated amplitude of blood pressure rhythms. *Ad libitum* availability of fructose only caused glucose intolerance when compared to control animals without fructose (Senador et al., 2012). Another type of timed food restriction is done by dividing food intake into 4 or 6 meals equally divided over the L/D-cycle. For instance, Yamajuku et al. delivered a high cholesterol diet to rats in a 4-meal schedule (every 6 h) without reduction of caloric intake. Those animals developed hypercholesterolemia after 7 days on the protocol and furthermore showed disruption in liver gene expression (Yamajuku et al., 2009). These studies are probably highly relevant as shiftwork models. However, until now it is unclear how shift workers exactly change their dietary habits in timing, composition, frequency and size of meals, making it hard to decide at present what are the best models.

In contrast to studies that restrict food availability to the light phase, Hatori et al. showed that restricting a high-fat diet to the natural main feeding phase (ZT13-21) improved glucose tolerance, insulin sensitivity, adiposity, serum cholesterol levels and leptin levels after fasting or glucose administration, next to prevention of increased liver size and unsaturated fatty acids levels, compared to animals fed *ad libitum* (Hatori et al., 2012).

Summary "Timing of Food Intake" Models

Animal models using a restriction of the timing of food intake affect bodyweight in 64% of the studies, but effects went in different directions with 3 out of 7 studies showing an increase of bodyweight whereas the four other studies found a decrease in bodyweight after timed food intake. Restricting food to the light phase resulted in increased bodyweight compared to animals with food restricted to the dark phase (Arble et al., 2009; Bray et al., 2013) and when compared to *ad libitum* fed (Salgado-Delgado et al., 2010a). However, others observed reduced bodyweight after food restriction to the light phase compared to dark fed animals (Yoon et al., 2012; Oyama et al., 2014) or *ad libitum* fed animals (Sherman et al., 2012; Yoon et al., 2012). Interestingly, in many of these studies food intake was reduced as well. Furthermore, some differences between mouse and rat studies are observed. For example, for bodyweight, mice studies show a significant effect in 6 out of 8 studies (75%), whereas in rats only in 1 out of the 3 studies (33%) a significant effect was observed. Total food intake and glucose metabolism parameters were measured frequently and were affected in 45 and 67% of the studies, respectively. For a complete overview of all parameters see **Table 1**. Together, these results indicate that changing the timing of food intake is effective at influencing several metabolic parameters, although for some parameters results are not consistent. Considering the great variety in types of modulations used it is not possible to pinpoint this to one aspect of the models used.

Category 2: Models Using "Timing of Activity"

"Work" is a difficult concept to model in animal studies and therefore only a very limited number of studies are available using an actual physical shiftwork protocol. Obviously shiftwork requires shifting of phases of sleep and arousal leading, at least partly, to awakening during the usual sleep period and sleep during the usual active period of the day. The few available physical shiftwork models used forced activity by housing animals in slowly rotating wheels. These housing conditions force the animals to be active and prevent them to fall asleep, although the animals can lie down and eat. Salgado-Delgado et al. mimicked human shiftwork protocols by enforcing the working conditions for 8 h per day, either during the active phase or the sleeping phase, 5 days a week for 5 weeks. In addition, they varied the availability of food but always used normal chow diets. A general observation in the groups of animals working during their resting phase was that the animals lost their nocturnal urge to eat and voluntarily consumed their food mostly during their working hours and thereby during their normal resting (i.e., light) phase. Forced activity during the resting phase induced increased bodyweight and abdominal fat, impaired glucose tolerance, altered plasma triglyceride diurnal variation, dampened daily glucose variation and introduced a secondary peak in the corticosterone rhythm. These effects could be prevented when food availability was restricted to the normal active (i.e., dark) phase (Salgado-Delgado et al., 2008, 2010a,b, 2011, 2013).

An early study by Tsai et al., used an extensive design with rats exposed to changes of light schedules (twice a week), forced activity paradigms and combinations of those. They observed higher bodyweight in the first 2 months, but not in the third month, in animals that were subjected to a "shiftwork" schedule of forced activity (i.e., Tue-Thu work from ZT0-ZT12, Fri-Sun work from ZT12-ZT24, Monday free) combined with changes in light schedule which was in synch (i.e., 12 h shift of L:D cycle twice a week) with the work schedule. Animals exposed to forced activity schedules had lower bodyweight and lower levels of cholesterol than animals that only underwent shift of light/dark cycle, indicating that forced activity can reverse some effects on metabolism induced by the regular L/D shifts. Some early effects on bodyweight and food intake disappeared after 2 months on the protocol, whereas other effects were only found after a few months on this protocol (Tsai and Tsai, 2007).

A study by Leenaars et al. using forced activity in rats did not focus on metabolic parameters, but did observe a decrease in total activity levels in animals that had to work either during their active or during their resting phase compared to freely active animals. Animals working shiftwork (i.e., work during the normal resting phase) showed reduced bodyweight gain compared to non-working controls (Leenaars et al., 2012).

A study by Hsieh et al. used 5-week forced activity in rats and closely studied alterations in activity patterns. The animals in this study are from the same study as Salgado-Delgado et al. (2008) but reported more specifically on locomotor activity. A decrease in mean activity levels was observed in shiftwork animals (i.e., working ZT2-10) during weekdays from week 3 onwards and during weekend days from week 1 onwards, but not in animals working during their active phase (i.e., working from ZT14-22). Shiftwork animals showed decreased amplitude of the activity rhythm on weekend days and a differently shaped rhythm of 24-h activity on weekdays. Shiftwork animals showed different activity responses to lights on and off when compared to non-shiftworking rats (Hsieh et al., 2014). The observed disruption of normal activity patterns was reported as an indication for circadian disruption as similar changes have been observed in SCN lesioned animals.

Summary "Timing of Activity" Models

Models using an altered timing of activity have only been carried out with rats and therefore no numbers on mouse studies are available. The limited number of studies and contributing research groups with this paradigm resulted in high numbers of affected studies on all parameters. As shown in **Table 2**, all parameters showed a 100% effectiveness of the different studies, except for bodyweight, total food and total activity levels intake which were affected in 5, 2, and 4 out of 6 studies, respectively. These results suggest that "timing of activity" has metabolic effects, although considering the limited number of studies and research groups these results should be interpreted with some caution.

Category 3: Models Using "Timing of Sleep"

Alterations in timing of activity are directly related to alterations in timing and duration of sleep. However, changes in the timing of sleep are also separately used as a model for shiftwork. These studies are different from the previously described activity models as their first target is to disturb or shorten sleep and affect the timing of sleep, but not necessarily alter activity levels or food intake. However, undoubtedly changes in sleep behavior will also affect activity and feeding patterns. Interestingly, (chronic) sleep restriction is associated with metabolic disorders in both animals and humans (Gangwisch, 2009; Killick et al., 2012). We came across a diversity of methods used to disrupt the normal sleep/wake pattern, including sleep disruption, sleep restriction, sleep fragmentation, sleep perturbation or sleep deprivation. Some of those might be other designations of the same intentions, such as sleep fragmentation and perturbation. These models either use shifting the timing of the normal sleep phase along the light-dark cycle, perturbing sleep in the normal phase, reducing the total number of sleep hours or completely withholding sleep. Most of these sleep studies focused on the effects of sleep perturbation on sleep parameters (such as percentage REM and NREM sleep, EEG recordings), behavioral changes or other non-metabolic factors. In this review only studies using a shift in the timing of sleep to the dark phase and studies using total sleep deprivation for a few hours were included as those were considered most relevant for shiftwork models.

Methods to perturb sleep are diverse and forced activity, as mentioned before, is one of them. Another method involves gentle handling for a few hours, by touching the animal every time it tries to fall asleep. Short term effects of sleep restriction during the first 6 h of the normal sleep phase (ZT0-ZT6) were described in two studies. Barclay et al. observed moderate alterations in the timing of food intake (in the direction of light phase feeding) and locomotor activity (increased levels during light and decreased levels during dark phase), next to major disruptions in liver transcriptome rhythms enriched for lipid and glucose metabolism pathways after 2 weeks of sleep restriction. A decreased response in the pyruvate test, a dampening of the daily rhythms in plasma glycerol, plasma triglyceride, plasma corticosterone and hepatic glycogen levels, and disrupted expression of several clock genes were all rescued by restricting food intake to the dark phase in the sleep restricted groups (Barclay et al., 2012).

Another study (Husse et al., 2012) used the same method but subjected the animals to sleep restriction for only five consecutive days followed by a recovery week during which several parameters were measured. Sleep restriction led to increased food intake despite increased leptin levels, together these changes are indicative for leptin resistance. Blood metabolites such as glucose and triglycerides were increased, but levels improved again during the recovery week. However, a trend toward higher bodyweight gain was observed during the recovery week suggesting long term effects even when the period of sleep restriction has terminated. Analysis of white adipose tissue transcriptome showed that sleep restriction affects many genes involved in lipid metabolism, including increased fatty acid synthesis and triglyceride production and storage.

A series of sleep restriction studies (Barf et al., 2010, 2012a,b) have been done using sleep restriction protocols of different severities: sleep restriction for 20 h each day (sleep ZT0-4; awake ZT4-24) or sleep disruption (14 h sleep—10 h awake in four 2–3 h

episodes). Sleep disturbance and sleep restriction both led to decreased bodyweight when compared to home cage control animals, although food intake was equal and the slight increase in locomotor activity is unlikely to explain the bodyweight differences. Animals exposed to sleep disruption or sleep restriction showed decreased baseline plasma glucose and insulin levels, decreased glucose tolerance and an attenuated insulin response to the glucose infusion within 8 days. A five day recovery period attenuated the sleep restriction-induced decrease of plasma leptin, insulin and glucose levels although bodyweight gain did not recover. Corticosterone levels and food intake were not affected (Barf et al., 2012b). When the same protocol was performed for 4 weeks, but with a work-weekend-schedule (5 work days, 2 non-work days) to resemble human shiftwork conditions, the same authors observed an increase of food intake after the first weekend, possibly to compensate for increased energy expenditure. Bodyweight gain increased during weekends in sleep restricted animals and plasma leptin and insulin levels were decreased when measured during working weeks 1 and 4. Rest during weekend days induced recovery of plasma leptin and insulin levels (Barf et al., 2012a).

Summary "Timing of Sleep" Models

The number of studies using perturbation of sleep as a model for shiftwork with focus on metabolic parameters is limited and therefore it is not yet completely clear if this type of manipulation influences metabolic functioning. Glucose metabolism appears to be affected often (in 5 out of 5 studies), whereas bodyweight (3/5) and food intake (2/4) were not always affected by sleep perturbation. For a complete overview of all parameters see **Table 3**.

Category 4: Models Using "Timing of Light Exposure"

In literature, several "timing of light exposure" models have been reported. These models all use timing of light as a means to disturb the circadian system and as such their main influence is on the SCN, in contrast to the previous 3 categories of models described. One type of these models uses continuous light exposure, i.e., light is present 24 h per day. The continuous light models can be further subdivided in models using constant light (similar amounts of light exposure during 24 h of the day) and models using dim light at night (together with bright light during the day). In addition to models using continuous light, models using changes in light/dark schedules have been used. These models can be further subdivided into models using alterations in period length (e.g., light-dark periods shorter or longer than 24 h) and models using repeated shifts of the light/dark schedule. Studies investigating these types of exposure in relation to metabolic health effects are discussed below per type of model. For overview purposes, models using continuous light are presented together in **Table 4** and models using changes in light/dark schedules are presented together in **Table 5**.

Continuous Light Exposure

Exposure to constant bright light conditions (LL) has been shown to abolish/diminish the circadian rhythmicity of many laboratory animals. This has, for example, been shown for locomotor activity (Depres-Brummer et al., 1995; Gale et al., 2011), melatonin (Wideman and Murphy, 2009; Gale et al., 2011), food intake (Coomans et al., 2013b), and SCN neurons (Coomans et al., 2013b). Hence, constant bright light conditions may be used to severely disrupt circadian rhythms. Since shiftworkers will be exposed to light during most of the day these models might be relevant for shiftwork modeling, although the intensity of all-day light may differ from real-life light exposures.

Disruption of circadian rhythms by constant bright light exposure has also been reported to affect metabolic function. Exposure to continuous light (150–180 lux; 4–8 weeks) led to increased bodyweight gain in two studies in mice (Fonken et al., 2010; Coomans et al., 2013a). This phenotype was apparent in mice fed normal chow (Fonken et al., 2010; Coomans et al., 2013a) as well as a high-fat diet (Coomans et al., 2013a). In addition, a third study reported increased fat mass after exposure to continuous light (Shi et al., 2013). Total food intake was unaltered in these studies, but more food was consumed during the subjective day, indicating changes in the timing of food intake (Fonken et al., 2010; Coomans et al., 2013a). The effect of continuous light exposure on total activity levels is less clear: unaltered activity levels (Fonken et al., 2010), reduced energy expenditure (Coomans et al., 2013a) and a non-significant trend toward a decrease in activity levels (Shi et al., 2013) have been reported. Apart from changes in bodyweight and fat mass, continuous light affected other metabolic parameters, such as increased RER, reduced glucose tolerance and altered rhythmicity of insulin sensitivity (Fonken et al., 2010; Coomans et al., 2013a). However, two studies using relative short-term exposure (6–10 weeks) to continuous bright light in Sprague Dawley rats reported no changes in bodyweight (Dauchy et al., 2010; Gale et al., 2011), indicating a possible difference between rats and mice in this model. Interestingly, long term exposure to bright light for 35 weeks did enhance bodyweight in Rapp-Dahl rats (model for hypertension). In addition, increased systolic blood pressure was observed (Natelson et al., 1993). Short-term exposure to continuous bright light did increase glucose levels and alter the rhythmicity of lipids in the study by Dauchy et al. (2010). In contrast, in the study by Gale et al. changes in glucose metabolism (increased glucose levels and decreased glucose- and arginine- stimulated insulin secretion) were only observed in diabetes-prone HIP rats, but not in wild-type Sprague Dawley rats (Gale et al., 2011). Considering these contradicting results, the differences in duration and strains of rats used, as well as the limited number of studies no firm conclusions can be drawn regarding the effects of continuous bright light exposure on metabolic function in rats. On the other hand, in mice results are more consistent and indicate that disruption of circadian rhythms by continuous bright light exposure increases bodyweight and alters glucose metabolism. This is associated with an altered timing of food intake, but not with change in total amount of food consumed over 24 h (**Table 4**).

Dim Light at Night

Dim light at night (LDim) also affects circadian rhythmicity, but seems less disruptive for circadian rhythms compared to constant bright light. In respect to human circadian disruptions caused

by shiftwork, models using dim light might be more relevant, since human shiftworkers will also experience alterations in the level of light exposure during a day, i.e., dim light at work in the office and bright light when commuting. In addition, models using dim light at night are relevant for studying the possible health consequences of light contamination at home, i.e., evening and nocturnal light is present in an increasing amount in our western society.

In contrast to constant bright light exposure, with dim light exposure at night circadian rhythms remain largely intact. This has for example been observed in rhythms of locomotor activity (Fonken et al., 2010) and corticosterone (Dauchy et al., 2010). Metabolic parameters including plasma levels of glucose and fatty acids also remain intact. Interestingly, dim light at night did affect the circadian pattern of food intake with relatively more food being consumed during the rest phase (Fonken et al., 2010). However, when the brightness of dim light exceeds a certain limit circadian rhythmicity of melatonin and corticosterone will be affected (Dauchy et al., 2010).

In three mice studies, exposure to dim light at night (for 2 and 6 weeks) increased bodyweight (Fonken et al., 2010; Aubrecht et al., 2014; Borniger et al., 2014). This was observed in male (Fonken et al., 2010; Borniger et al., 2014) and female mice (Aubrecht et al., 2014). In female mice, dim light at night resulted in decreased food intake after 4 weeks (Aubrecht et al., 2014). In addition, in dim light exposed animals increases in fat mass and reduced glucose tolerance were observed (Fonken et al., 2010), as well as changes in energy expenditure and RER (reduced whole body expenditure and increased carbohydrate over fat oxidation) (Borniger et al., 2014). In contrast, bodyweight was not affected in a rat study using exposure to dim light at night for 6 weeks (Dauchy et al., 2010). In this study, different intensities of dim light at night were used (0.02–0.08 $\mu W/cm^2$ dim light). The highest intensity of dim light at night disrupted circadian rhythms of plasma corticosterone, melatonin and glucose, but bodyweight was not affected during the 6 weeks of exposure.

Considering the still limited number of studies using dim light, firm conclusions are not possible yet. However, it appears that similar to continuous bright light exposure dim light at night affects bodyweight in mice, but not in rats. The currently available studies suggest that glucose metabolism is affected in mice as well as in rats by dim light at night. Interestingly, the study by Fonken et al., reported that the increases in bodyweight gain and fat mass by dim light at night can be prevented by restricting food access to the dark phase (Fonken et al., 2010). These results suggest an important role for altered timing of food intake in the effects of continuous light on bodyweight, although none of these three dimlight studies clearly quantified the circadian changes in food intake.

Summary "Constant Light" Models

Models using constant light seem to affect bodyweight in 55% of the studies (**Table 4**), all increases in bodyweight. However, there is a clear difference between rat and mice studies, with 1 out of 5 rat studies reporting effects on bodyweight and 5 out of 6 mice studies. Total food intake is not affected in most studies (only affected in 2 out of 9 studies), whereas glucose metabolism is affected in a majority of studies (6/7, 86%). Interestingly, the clear difference observed between rats and mice in the effects of constant light on bodyweight is not that pronounced for glucose metabolism. Thus, these results indicate that models using constant light exposure influence glucose metabolism in both species, while bodyweight is mainly affected in mice. For the other parameters only a limited number of studies are available making firm conclusions difficult. For a complete overview of all parameters see **Table 4**.

Changes in Light/Dark Schedules—Period Length

Under normal conditions, one cycle of light and darkness on the planet earth matches exactly 24 h. Exposure to altered period lengths (<23 h or > than 25 h) usually requires a constant re-entrainment of the circadian system and experiments using such protocols have therefore been used to investigate the effects of circadian disruption. On the other hand, when very short period lengths are used entrainment is not possible, which will result in either free-running rhythms or an abolishment of circadian rhythms. Shorter period lengths have been reported to cause alterations in several circadian parameters, such as locomotor activity (Oishi, 2009; Oishi and Itoh, 2013), drinking pattern (Oishi, 2009) and body temperature (Karatsoreos et al., 2011).

Altered circadian rhythms due to an aberrant period length have been implicated in metabolic disturbances as well. For example, increases in bodyweight have been observed in mice and rats exposed to short period lengths of 6–23 h for 9–10 weeks (Vilaplana et al., 1995; Oishi, 2009; Karatsoreos et al., 2011; Oishi and Itoh, 2013). In addition, changes in glucose homeostasis (increased glucose levels and glucose intolerance), lipid homeostasis (increased cholesterol levels) and expression of liver genes related to glucose metabolism have been reported in one of these models (Oishi and Itoh, 2013). However, human relevancy of these models is poor since period length remains unaltered during shiftwork. Of course, partial shifts in light exposure might occur during shiftwork where light is present during working hours and is avoided during subsequent sleeping hours, but therefore models using shifts in light exposure are more relevant to the human situation than changes in period length.

Changes in Light/Dark Schedules—Shifts

Shifts in light exposure require re-entrainment of the circadian system causing (temporary) disturbance of circadian rhythms. Repeated phase shifts have been investigated using numerous schedules which differ in shift size (1–12 h), frequency (every day—once a week), duration (acute effects—chronic effects), and direction (forwards or backwards), resulting in very heterogeneous study results. For example, a 6 h forward shift every 3 days for 10 weeks abolished locomotor and melatonin rhythmicity (Gale et al., 2011), whereas rhythmicity in locomotor activity and body temperature remained but was disturbed (lengthened period and reduced amplitude) after an 8 h forward shift every 2 days for 10 days (Filipski et al., 2004).

Circadian disruption by shifts in light exposure has also been investigated in relation to metabolic function. To our knowledge, four studies using this type of model have been published. A study by Tsai et al. in rats, observed an increase in bodyweight gain

during exposure to 12 h shifts twice a week (Tsai et al., 2005). This increase was only observed during the first 2 months of exposure, during the third month and a subsequent 10 day recovery period bodyweight gain was unaltered. Interestingly, in this model food intake was increased and locomotor activity was reduced which could both be linked to the observed increase in bodyweight gain. However, in contrast to the bodyweight gain, these changes were present at all time-points of the experiment.

A study by Gale et al. did not observe effects on bodyweight in rats exposed to a 6 h shift every 3 days for 10 weeks. Similar to continuous light exposure, effects of this light shift model on glucose metabolism were only observed in diabetes-prone HIP rats but not in wild type rats (increased glucose levels and decreased glucose- and arginine- stimulated insulin secretion) (Gale et al., 2011). The model used in a third study, by Bartol-Munier et al., was exposure to 10 h shifts twice a week and restriction of food to the dark phase for 5 months. In this study no effects on bodyweight were observed whether animals were on normal chow or on a high-fat diet, but changes in glucose metabolism (lower insulin levels) were present in animals fed normal chow and exposed to the shifts (Bartol-Munier et al., 2006). In the most recent study, mice were exposed to a 12 h shift once a week for 12 weeks on a normal chow diet and an additional 10 weeks on a high-fat and high-sugar diet to investigate effects on the gut microbiome. In this study a small but significant increase in bodyweight was observed in the shifted mice on a normal chow diet. When the diet was changed to a high-fat and high-sugar diet no additional effects by lighting schedule on bodyweight were observed (Voigt et al., 2014).

Summary Models Changes in Light/Dark Schedules

Models using changes in light/dark schedules affect bodyweight in 56% (5 out of 9) of the studies, with studies using mice finding effects more often (3 out of 4) compared to studies using rats (2 out of 5). The total amount of food intake is affected in half of the studies (50%; 3 out of 6). Glucose metabolism is affected in 83% of studies (5 out of 6) with almost an equal number of studies showing an effect when using mouse or rat. These results suggest that changes in the light/dark cycle affect some of the metabolic parameters (bodyweight and glucose metabolism). For other parameters the number of studies is very low making interpretations difficult. For a complete overview of all parameters see **Table 5**.

Discussion

With this review we aim to provide an overview of the available animal studies investigating the relationship between shiftwork and metabolic risk factors. Shiftwork in humans consists of a multi-aspects exposure (**Figure 1**). We focused on the four most relevant manipulations that are being used to mimic human shiftwork conditions in animals: altered timing of food intake, altered timing and/or duration of activity, altered timing and/or duration of sleep, and irregular lighting conditions. The overview provided in this review shows that these types of models are very useful in modeling one aspect of shiftwork and investigating the role of these separate aspects. However, the interaction between the different aspects of shiftwork is an important component of shiftwork as well, which would be beneficial to model in animals. Unfortunately, the heterogeneity of shiftwork in humans as an exposure (i.e., number of subsequent shifts, duration of recovery periods, direction of rotation, etc.) and the variability in behavioral coping responses to shiftwork amongst human individuals (for instance in sleeping and eating strategies) makes modeling shiftwork in animals a very challenging exercise. To develop an animal model that incorporates the interaction between multiple shiftwork aspects requires complete knowledge of human shiftwork behavior (i.e., light exposure, sleep behavior and dietary habits). Although in recent years first attempts have been made to achieve the latter, a complete knowledge has not been reached yet. Clearly, an animal model incorporating multiple shiftwork aspects would have advantages. Firstly, whereas human studies require over 20 years to observe long term health effects of shiftwork, such as development of metabolic disease or cancer, in an animal experiment "long-term" health effects can be studied much faster (∼1 year). Hence, animal studies could accelerate the unraveling of the underlying mechanisms explaining the relationship between shiftwork and health. Secondly, animal models provide opportunities to study parameters and processes that would be impossible or extremely invasive to study in humans. In this discussion we summarize the main findings of this review, differences between models and species and touch upon possible underlying mechanisms.

Main Findings of This Review

All Categories of Models

While selecting articles for this review, we came across a very diverse collection of food, activity, sleep and light manipulations which were all rather different from each other. Although we grouped the studies into four main categories, nearly none of the experimental setups was copied by another research group or was it used in a different species or strain. Furthermore, metabolic parameters were not equally frequent or extensively measured in the different models, which is another factor making it difficult to compare results between models. Five of our selected metabolic parameters (bodyweight, total food intake, total activity, glucose metabolism and lipid metabolism) were described in all four categories, but only three of them were described in both mouse and rat studies in each category. First, we will summarize the main results of all studies, followed by a discussion of the main differences between categories of models and species.

Table 6 represents the percentage of studies reporting effects of the manipulation for the listed parameters in exposed groups compared to the control group. Numerous different parameters were described in the included studies but we concentrated on a few that were measured in most studies. Bodyweight was described in most studies and 62% (26/42) of the studies reported an effect provoked by the manipulated shiftwork aspect. Total food intake and total activity levels were less often affected, in 39% (14/36) and 39% (9/23) of the studies respectively. Other metabolic parameters including energy expenditure [80% (5/6)] glucose metabolism [83% (25/30)], lipid metabolism [69% (11/16)] and adiposity [80% (8/10)] were affected frequently by shiftwork. In most studies, circadian parameters were included

TABLE 6 | A summary of 7 most frequently measured parameters in the 5 categories of shiftwork models (food, activity, sleep, L/D shifts and LL/LDim).

Model type	Food	Activity	Sleep	L/D shift	LL/Dim	All		
	Rat + Mouse	Rat + Mouse	Rat + Mouse	Rat + Mouse	Rat + Mouse	Rat	Mouse	Total
Bodyweight	7/11 (63, 6%)	5/6 (83, 3%)	3/5 (60%)	5/9 (55, 5%)	6/11 (54, 5%)	**12/22 (54, 5%)**	**14/20 (70%)**	**26/42 (61, 9%)**
Total food intake	5/11 (45, 4%)	2/6 (33, 3%)	2/4 (50%)	3/6 (50%)	2/9 (22, 2%)	**6/16 (37, 5%)**	**8/20 (40%)**	**14/36 (38, 9%)**
Total Activity	3/7 (42, 9%)	4/6 (66, 7%)	0/3 (0%)	2/2 (100%)	0/5 (0%)	**7/11 (63, 6%)**	**2/12 (16, 7%)**	**9/23 (39, 1%)**
Total EE	3/3 (100%)		0/1 (0%)		2/2 (100%)	**1/2 (50%)**	**4/4 (100%)**	**5/6 (80%)**
Adiposity	5/7 (71, 4%)	2/2 (100%)			1/1 (100%)	**4/5 (80%)**	**4/5 (80%)**	**8/10 (80%)**
Glucose metabolism	6/9 (66, 7%)	3/3 (100%)	5/5 (100%)	5/6 (83, 3%)	6/7 (85, 7%)	**13/16 (81, 2%)**	**12/14 (85, 7%)**	**25/30 (83, 3%)**
Lipid metabolism	5/7 (71, 4%)	2/2 (100%)	2/2 (100%)	1/3 (33, 3%)	1/2 (50%)	**5/7 (71, 4%)**	**6/9 (66, 7%)**	**11/16 (68, 8%)**

The most right columns represent the results of all studies together. All results are presented as the number of studies in which an effect of the manipulation was found (when compared to the control condition)/the number of studies in which the parameter was measured. Between brackets the number of studies showing an effect is depicted as a percentage. EE, energy expenditure; L/D, light/dark; LL, continuous light; LDim, dimlight at night.

as well and often showed alterations (mainly in rhythm, including changes in amplitude and phase). For example, the circadian rhythm of corticosterone was altered in 54% (7/13) of the studies.

In summary, effects on metabolism are observed in a substantial number of studies, however, results are not completely consistent. Moreover, changes in metabolism did not always translate in changes in bodyweight (gain) or adiposity. Indeed, we have to take into account that there might be a publication bias as perhaps mainly parameters that were affected are described and therefore the actual percentages of studies finding an effect might be lower.

Are There Differences between Categories?

Bodyweight is one of the parameters that was measured in all categories of models and was affected in 64% (7/11) of the food studies, in 83% (5/6) of the activity studies, in 60% (3/5) of the sleep studies, in 56% (5/9) L/D shift-studies and in 55% (6/11) of the continuous light studies. Total food intake showed to be affected in about 45% of the food studies (5/11), in 50% of sleep studies (2/4) and L/D-shift studies (3/6), whereas only 33% (2/6) of the activity-studies and 22% (2/9) of the LL-studies demonstrated an effect. Factors involved in glucose and lipid metabolism were affected in all categories of models, although light models showed low percentages for lipid metabolism (33% (1/3) in L/D shift studies; 50% (1/2) in continuous light studies). The single other parameter which was measured in all five models was total activity levels and this was affected in 43% of food studies (3/7), 67% of activity studies (4/6), 0% of sleep studies (0/3), 100% of L/D studies (2/2) and 0% of LL studies (0/5). These results show that large differences exist between the effects of different categories, however, caution is required when interpreting these results since often only a limited number of studies was available. Another important limitation to draw firm conclusions is the low number of reproducible results for many parameters. On the other hand, remarkable to notice is the 100% score for nearly each parameter measured in the studies that manipulated activity. One possible reason for this might be that 5 out of 8 of these studies came from the same research group and thereby the experimental setup was exactly the same each time, i.e., these authors produced very reproducible results. In conclusion, it is most likely that the variability between the studies (species, type of manipulation, duration of exposure etc.) is important for whether a parameter is affected by the manipulation. This is another representation of the heterogeneity of shiftwork and increases the complexity to model shiftwork. When comparing parameters and categories of models for which multiple studies are available differences are not large. As a consequence, a category with the largest metabolic consequences cannot be appointed. However, when considering human relevance of the models, the use of models using constant light and alterations in period length is least informative.

Are There Differences between Rat and Mouse Studies?

In the articles included for this review we observed that rats and mice are used interchangeably for shiftwork models. Interestingly, however, thus far the observed effects are not identical between species even when exactly the same procedure is carried out (Arble et al., 2009; Reznick et al., 2013). In general, in most categories either mouse (e.g., 0 out of 9 studies in activity-models) or rat studies (e.g., only 3 out of 13 studies in food-models) were underrepresented, thereby making it difficult to compare between the species. When focusing on parameters reported in at least 8 experiments in both species, neglecting the exact model category, total activity [63% (7/11)], and lipid metabolism [71% (5/7)] were more often affected in rat than in mice studies [17% (2/12), 67% (6/9) respectively]. On the other hand, effects were more often observed in mice for bodyweight [70% (14/20)], total food intake [40% (8/20)], glucose metabolism [86% (12/14)] and total energy expenditure [100% (4/4)] than in rats [55% (12/22), 38% (6/16), 81% (13/16), and 50% (1/2) respectively], however, these differences are relatively small. The only parameter showing similar percentages in both species, is adiposity with 80% (4/5) of studies showing an effect of the condition. Generally, choosing a certain type of rodent for an experiment is based on the genetic background of an animal, the similarities between the human situation/disease and the features the animal model displays, the surgical techniques that need to be carried out, the type of behavioral tests that have to be performed or other specific reasons. To this point, shiftwork models have been performed with both species and it is important to keep in mind

that when creating a shiftwork model, behavioral conditions are manipulated. Often we tend to think that behavioral manipulations have similar effects in different species, but we should be aware that mice and rats may respond very differently. Causality of the dissimilar effects between mouse and rat studies is as yet unknown. Hypothetically, the difference in body size and associated metabolic rate could play a role in these differences, but this remains to be investigated.

In our opinion, an important, but lacking, model is exposure of a diurnal species to shiftwork conditions. Day-active animals are considered more similar to human when it comes to circadian research and therefore in principle would be a better model to study the metabolic consequences of shiftwork. Moreover, if similar effects on metabolism are found between nocturnal and diurnal species, this would support the translatability of animal models for human shiftwork simulations.

Possible Mechanism of Health Consequences of Shiftwork

Mimicking human shiftwork conditions in an animal model ultimately aims to study and understand the underlying mechanism of shiftwork leading to health problems. The predominant current theory stresses the process of desynchronization. In general, it is thought that desynchronization leads to a suboptimal functioning of many bodily processes. Observed effects range from shifts in gene expression and altered hormone secretion (i.e., leptin, insulin, melatonin and corticosterone) to modified behavioral output (i.e., food intake rhythm, activity levels and rhythm) and changes in whole body physiology (i.e., bodyweight, food intake, RER, energy expenditure, glucose and lipid metabolism). Metabolic processes within and between important metabolic tissues such as liver and muscle should cooperate in a proper timely manner to control optimally, for instance, glucose and lipid metabolism. If not, this may lead to metabolic problems.

In principal, shiftwork can cause desynchronization at different levels, which in general all result from desynchronization between the environment and the (complete circadian system within an) organism. Within the organism we distinguish 4 separate levels. The first level (1) concerns the desynchronization between the central clock and the peripheral clocks. It is well known that light is the most important Zeitgeber for the SCN, while food and activity are such for the peripheral clocks. During shiftwork these two Zeitgebers present conflicting information resulting in an opposite phase for the central and peripheral oscillators. Question is if and how these disturbances affect downstream processes.

Besides this possible top-down desynchronization between central and peripheral clocks, desynchronization may also occur between anatomically separated organs, the second level (2). Shifting the timing of food intake has been shown to differentially affect liver and muscle clocks (Bray et al., 2013; Reznick et al., 2013). Desynchronization between peripheral clocks supposedly originates from tissue-specific sensitivity to entrainment signals such as activity, energy levels (e.g., periods of fasting/feeding), responses to hormone secretion, input from autonomic nervous system etc. An additional type of desynchronization at level 2 occurs between anatomical parts of the SCN. Clock gene expression and electrical activity resynchronize differently between the dorsal and ventral part of the SCN after 6 h phase shifting (Nagano et al., 2003; Albus et al., 2005). Hypothetically, temporal desynchronization and thereby suboptimal functioning of (parts of) the SCN may lead to a malfunctioning of SCN-mediated downstream mechanisms.

The third level (3) encompasses desynchronization between the molecular clock mechanism and the clock-induced genes. Many genes involved in metabolism display a circadian rhythm in their expression. Several studies have described that a manipulation of SCN output signals, by for instance adrenalectomy or denervation of autonomic inputs, induces a loss of rhythmicity in the expression of clock-induced genes in white adipose tissue, liver and bone, while clock genes remain rhythmic (Cailotto et al., 2005, 2008; Oishi et al., 2005; Fujihara et al., 2014; Su et al., 2014). This suggests that although the molecular clock machinery is still intact, the rhythmic expression of clock-induced genes is disturbed. It is likely that this level of desynchronization indeed also takes place during shiftwork and a first suggestion was made by Salgado-Delgado et al. (2013). They showed that in their forced-activity shiftwork model the effect on the rhythmicity of metabolic genes (NAD+, Nampt, Pparα, Pparγ and Pgc1α) did not resemble the effects on clock genes rhythmicity.

The fourth level (4) of desynchronization concerns desynchronization within the molecular clock itself, i.e., different parts of the molecular clockwork are affected to a different degree within one tissue. Studies in which animals are exposed to phase shifts of the light dark cycle to induce experimental jet lag, a proper method to induce temporal circadian desynchrony, report dissimilar resynchronization speeds of different parts of the molecular clock. For instance, expression of the clock gene Cry1 appeared to resynchronize slower than mPer expression in the SCN after a 6 h phase advance (Reddy et al., 2002). Although this level of desynchronization has not yet been shown in studies using a shiftwork model, the aforementioned jetlag studies resemble studies in category 4 (i.e., shifts in timing of light exposure). Despite the body's ability to adapt to challenging conditions, this obviously becomes metabolically problematic if this occurs every few days or weeks as is the case in most working schedules of employees who are shiftworkers.

However, up to now desynchronization is mostly studied at the level of communication between central and peripheral clocks (level 1). The other three levels of desynchronization were not or only marginally studied in the aforementioned models but potentially may contribute significantly to the causal link between circadian desynchronization and negative health outcomes. Therefore, we encourage future studies to also focus on possible desynchronization at levels 2, 3, and 4.

Interaction of Shiftwork Aspects

Most studies discussed in this review used either one of four manipulations (food, activity, sleep, and light) as a model for shiftwork. Tackling shiftwork conditions by manipulating one aspect is a good approach when studying the effects of that particular aspect of shiftwork. This gives insight in how food, activity, sleep and light manipulations contribute to the associated negative health effects. However, the mentioned aspects of

shiftwork are strongly intertwined and cannot easily be separated. For example, forcing an animal to consume its daily food at an unusual time inevitably also disturbs its activity and sleep pattern, which in itself also affects metabolism. Effects found of a manipulation are rapidly assigned to the main manipulation but it is often not very well considered whether and if so, how the main manipulation affects other aspects and its consequences. For instance, sleep behavior is hardly ever monitored by EEG recordings or high resolution actimetry, thus information about sleep duration and sleep quality is usually missing. In addition, in order to translate results obtained in animal studies properly to humans, also more knowledge regarding these parameters in human shiftwork is required. Thus, we propose more elaborate measurements on the main aspects of shiftwork (**Figure 1**) in animal as well as in human studies.

Conclusion

This review provides an overview of animal models for shiftwork to investigate metabolic health effects. This overview indicates the large variety present in models used as well as a substantial amount of indecisive results. Ideally we would have concluded this review with suggestions for a more standardized model including a number of factors to manipulate and different possible outcome measures. Standardization would reduce the heterogeneity between studies for both methods and outcome parameters. Unfortunately, at this point our mechanistic knowledge on the effects of shiftwork is not sufficient yet to draw firm conclusions and thereby put a certain model forward or eliminate others. Furthermore, human shiftwork conditions are highly variable and not outlined well enough to propose an ideal animal model. For now, we plead for more awareness of the interactions between the aspects of shiftwork which are intentionally and unintentionally manipulated. Shiftwork and the type of manipulations used in animal models are multi-aspects exposures (**Figure 1**). Therefore, it is important to measure additional parameters apart from the ones directly related to the manipulation. For example, measuring sleep behavior when using a model with light shifts. Other examples are circadian parameters, such as gene expression in several organs, hormones, activity, body temperature, sleep behavior and metabolic parameters. More insights into these parameters will be beneficial for comparing different outcomes when different types of manipulations are used.

Where possible these parameters should be measured in human shiftwork studies as well to allow for more insight into translatability of findings. Furthermore, experiments ideally should cover both short- and long-term effects, ranging from days to years, to study details of underlying mechanisms in the development of the unfavorable health outcomes caused by shiftwork. The perfect model is as yet non-existent but ideally combines several aspects of shiftwork to mimic the human situation best (e.g., when manipulating activity and light, changes in food intake will follow and this should be monitored).

Only by properly studying the effects of shiftwork conditions solely and combined, this research eventually will help the general community to learn how to deal with shiftwork conditions best, prevent shiftworkers from becoming disturbed and possibly prevent and treat negative health outcomes.

Acknowledgments

This work was supported by the Technology Foundation STW grant Feeding OnTime (#12195).

Supplementary Material

The Supplementary Material for this article can be found online at: http://www.frontiersin.org/journal/10.3389/fphar.2015.00050/abstract

References

Albus, H., Vansteensel, M. J., Michel, S., Block, G. D., and Meijer, J. H. (2005). A GABAergic mechanism is necessary for coupling dissociable ventral and dorsal regional oscillators within the circadian clock. *Curr. Biol.* 15, 886–893. doi: 10.1016/j.cub.2005.03.051

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. doi: 10.1038/oby.2009.264

Aubrecht, T. G., Jenkins, R., and Nelson, R. J. (2014). Dim light at night increases body mass of female mice. *Chronobiol. Int.* 1–4. doi: 10.3109/07420528.2014.986682. [Epub ahead of print].

Barclay, J. L., Husse, J., Bode, B., Naujokat, N., Meyer-Kovac, J., Schmid, S. M., et al. (2012). Circadian desynchrony promotes metabolic disruption in a mouse model of shiftwork. *PLoS ONE* 7:e37150. doi: 10.1371/journal.pone.0037150

Barf, R. P., Desprez, T., Meerlo, P., and Scheurink, A. J. (2012a). Increased food intake and changes in metabolic hormones in response to chronic sleep restriction alternated with short periods of sleep allowance. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 302, R112–R117. doi: 10.1152/ajpregu.00326.2011

Barf, R. P., Meerlo, P., and Scheurink, A. J. (2010). Chronic sleep disturbance impairs glucose homeostasis in rats. *Int. J. Endocrinol.* 2010:819414. doi: 10.1155/2010/819414

Barf, R. P., Van Dijk, G., Scheurink, A. J., Hoffmann, K., Novati, A., Hulshof, H. J., et al. (2012b). Metabolic consequences of chronic sleep restriction in rats: changes in body weight regulation and energy expenditure. *Physiol. Behav.* 107, 322–328. doi: 10.1016/j.physbeh.2012.09.005

Bartol-Munier, I., Gourmelen, S., Pevet, P., and Challet, E. (2006). Combined effects of high-fat feeding and circadian desynchronization. *Int. J. Obes. (Lond).* 30, 60–67. doi: 10.1038/sj.ijo.0803048

Biggi, N., Consonni, D., Galluzzo, V., Sogliani, M., and Costa, G. (2008). Metabolic syndrome in permanent night workers. *Chronobiol. Int.* 25, 443–454. doi: 10.1080/07420520802114193

Borniger, J. C., Maurya, S. K., Periasamy, M., and Nelson, R. J. (2014). Acute dim light at night increases body mass, alters metabolism, and shifts core body temperature circadian rhythms. *Chronobiol. Int.* 31, 917–925. doi: 10.3109/07420528.2014.926911

Bray, M. S., Ratcliffe, W. F., Grenett, M. H., Brewer, R. A., Gamble, K. L., and Young, M. E. (2013). Quantitative analysis of light-phase restricted feeding reveals metabolic dyssynchrony in mice. *Int. J. Obes. (Lond).* 37, 843–852. doi: 10.1038/ijo.2012.137

Bray, M. S., Tsai, J. Y., Villegas-Montoya, C., Boland, B. B., Blasier, Z., Egbejimi, O., et al. (2010). Time-of-day-dependent dietary fat consumption influences multiple cardiometabolic syndrome parameters in mice. *Int. J. Obes. (Lond).* 34, 1589–1598. doi: 10.1038/ijo.2010.63

Cailotto, C., La Fleur, S. E., Van Heijningen, C., Wortel, J., Kalsbeek, A., Feenstra, M., et al. (2005). The suprachiasmatic nucleus controls the daily variation of plasma glucose via the autonomic output to the liver: are the clock genes involved? *Eur. J. Neurosci.* 22, 2531–2540. doi: 10.1111/j.1460-9568.2005.04439.x

Cailotto, C., Van Heijningen, C., Van Der Vliet, J., Van Der Plasse, G., Habold, C., Kalsbeek, A., et al. (2008). Daily rhythms in metabolic liver enzymes and plasma glucose require a balance in the autonomic output to the liver. *Endocrinology* 149, 1914–1925. doi: 10.1210/en.2007-0816

Carnethon, M. R., Loria, C. M., Hill, J. O., Sidney, S., Savage, P. J., and Liu, K. (2004). Risk factors for the metabolic syndrome: the Coronary Artery Risk Development in Young Adults (CARDIA) study, 1985-2001. *Diabetes Care* 27, 2707–2715. doi: 10.2337/diacare.27.11.2707

Centraal Bureau voor de Statistiek. (2013). *CBS Statline Beroepsbevolking; Regeling Werktijden 2013. Den Haag/Heerlen.* Available online at: http://statline.cbs.nl/StatWeb/publication/?DM=SLNL&PA=71959ned&D1=1-7&D2=0&D3=a&D4=a&VW=T [Accessed 02-12-2014 2014].

Coomans, C. P., Van Den Berg, S. A., Houben, T., Van Klinken, J. B., Van Den Berg, R., Pronk, A. C., et al. (2013a). Detrimental effects of constant light exposure and high-fat diet on circadian energy metabolism and insulin sensitivity. *FASEB J.* 27, 1721–1732. doi: 10.1096/fj.12-210898

Coomans, C. P., Van Den Berg, S. A., Lucassen, E. A., Houben, T., Pronk, A. C., Van Der Spek, R. D., et al. (2013b). The suprachiasmatic nucleus controls circadian energy metabolism and hepatic insulin sensitivity. *Diabetes* 62, 1102–1108. doi: 10.2337/db12-0507

Damiola, F., Le Minh, N., Preitner, N., Kornmann, B., Fleury-Olela, F., and Schibler, U. (2000). Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev.* 14, 2950–2961. doi: 10.1101/gad.183500

Dauchy, R. T., Dauchy, E. M., Tirrell, R. P., Hill, C. R., Davidson, L. K., Greene, M. W., et al. (2010). Dark-phase light contamination disrupts circadian rhythms in plasma measures of endocrine physiology and metabolism in rats. *Comp. Med.* 60, 348–356.

De Bacquer, D., Van Risseghem, M., Clays, E., Kittel, F., De Backer, G., and Braeckman, L. (2009). Rotating shift work and the metabolic syndrome: a prospective study. *Int. J. Epidemiol.* 38, 848–854. doi: 10.1093/ije/dyn360

Depres-Brummer, P., Levi, F., Metzger, G., and Touitou, Y. (1995). Light-induced suppression of the rat circadian system. *Am. J. Physiol.* 268, R1111–R1116.

Dibner, C., Schibler, U., and Albrecht, U. (2010). The mammalian circadian timing system: organization and coordination of central and peripheral clocks. *Annu. Rev. Physiol.* 72, 517–549. doi: 10.1146/annurev-physiol-021909-135821

Dochi, M., Suwazono, Y., Sakata, K., Okubo, Y., Oishi, M., Tanaka, K., et al. (2009). Shift work is a risk factor for increased total cholesterol level: a 14-year prospective cohort study in 6886 male workers. *Occup. Environ. Med.* 66, 592–597. doi: 10.1136/oem.2008.042176

Esquirol, Y., Perret, B., Ruidavets, J. B., Marquie, J. C., Dienne, E., Niezborala, M., et al. (2011). Shift work and cardiovascular risk factors: new knowledge from the past decade. *Arch. Cardiovasc. Dis.* 104, 636–668. doi: 10.1016/j.acvd.2011.09.004

Filipski, E., Delaunay, F., King, V. M., Wu, M. W., Claustrat, B., Grechez-Cassiau, A., et al. (2004). Effects of chronic jet lag on tumor progression in mice. *Cancer Res.* 64, 7879–7885. doi: 10.1158/0008-5472.CAN-04-0674

Fonken, L. K., Workman, J. L., Walton, J. C., Weil, Z. M., Morris, J. S., Haim, A., et al. (2010). Light at night increases body mass by shifting the time of food intake. *Proc. Natl. Acad. Sci. U.S.A.* 107, 18664–18669. doi: 10.1073/pnas.1008734107

Fritschi, L., Glass, D. C., Heyworth, J. S., Aronson, K., Girschik, J., Boyle, T., et al. (2011). Hypotheses for mechanisms linking shiftwork and cancer. *Med. Hypotheses* 77, 430–436. doi: 10.1016/j.mehy.2011.06.002

Fujihara, Y., Kondo, H., Noguchi, T., and Togari, A. (2014). Glucocorticoids mediate circadian timing in peripheral osteoclasts resulting in the circadian expression rhythm of osteoclast-related genes. *Bone* 61, 1–9. doi: 10.1016/j.bone.2013.12.026

Gale, J. E., Cox, H. I., Qian, J., Block, G. D., Colwell, C. S., and Matveyenko, A. V. (2011). Disruption of circadian rhythms accelerates development of diabetes through pancreatic beta-cell loss and dysfunction. *J. Biol. Rhythms* 26, 423–433. doi: 10.1177/0748730411416341

Gangwisch, J. E. (2009). Epidemiological evidence for the links between sleep, circadian rhythms and metabolism. *Obes. Rev.* 10(Suppl. 2), 37–45. doi: 10.1111/j.1467-789X.2009.00663.x

Garaulet, M., and Gomez-Abellan, P. (2014). Timing of food intake and obesity: a novel association. *Physiol. Behav.* 134, 44–50. doi: 10.1016/j.physbeh.2014.01.001

Garaulet, M., Gomez-Abellan, P., Alburquerque-Bejar, J. J., Lee, Y. C., Ordovas, J. M., and Scheer, F. A. (2013). Timing of food intake predicts weight loss effectiveness. *Int. J. Obes. (Lond).* 37, 604–611. doi: 10.1038/ijo.2012.229

Gluck, M. E., Venti, C. A., Salbe, A. D., Votruba, S. B., and Krakoff, J. (2011). Higher 24-h respiratory quotient and higher spontaneous physical activity in nighttime eaters. *Obesity (Silver Spring)* 19, 319–323. doi: 10.1038/oby.2010.206

Haffner, S. M. (1998). Epidemiology of type 2 diabetes: risk factors. *Diabetes Care* 21(Suppl. 3), C3–C6. doi: 10.2337/diacare.21.3.C3

Hastings, M. H., Duffield, G. E., Smith, E. J., Maywood, E. S., and Ebling, F. J. (1998). Entrainment of the circadian system of mammals by nonphotic cues. *Chronobiol. Int.* 15, 425–445. doi: 10.3109/07420529808998700

Hatori, M., Vollmers, C., Zarrinpar, A., Ditacchio, L., Bushong, E. A., Gill, S., 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. doi: 10.1016/j.cmet.2012.04.019

Hibi, M., Masumoto, A., Naito, Y., Kiuchi, K., Yoshimoto, Y., Matsumoto, M., et al. (2013). Nighttime snacking reduces whole body fat oxidation and increases LDL cholesterol in healthy young women. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 304, R94–R101. doi: 10.1152/ajpregu.00115.2012

Hsieh, W. H., Escobar, C., Yugay, T., Lo, M. T., Pittman-Polletta, B., Salgado-Delgado, R., et al. (2014). Simulated shift work in rats perturbs multiscale regulation of locomotor activity. *J. R. Soc. Interface* 11:20140318. doi: 10.1098/rsif.2014.0318

Husse, J., Hintze, S. C., Eichele, G., Lehnert, H., and Oster, H. (2012). Circadian clock genes Per1 and Per2 regulate the response of metabolism-associated transcripts to sleep disruption. *PLoS ONE* 7:e52983. doi: 10.1371/journal.pone.0052983

Jang, H., Lee, G., Kong, J., Choi, G., Park, Y. J., and Kim, J. B. (2012). Feeding period restriction alters the expression of peripheral circadian rhythm genes without changing body weight in mice. *PLoS ONE* 7:49993. doi: 10.1371/journal.pone.0049993

Karatsoreos, I. N., Bhagat, S., Bloss, E. B., Morrison, J. H., and McEwen, B. S. (2011). Disruption of circadian clocks has ramifications for metabolism, brain, and behavior. *Proc. Natl. Acad. Sci. U.S.A.* 108, 1657–1662. doi: 10.1073/pnas.1018375108

Karlsson, B. H., Knutsson, A. K., Lindahl, B. O., and Alfredsson, L. S. (2003). Metabolic disturbances in male workers with rotating three-shift work. Results of the WOLF study. *Int. Arch. Occup. Environ. Health* 76, 424–430. doi: 10.1007/s00420-003-0440-y

Karlsson, B., Knutsson, A., and Lindahl, B. (2001). Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27, 485 people. *Occup. Environ. Med.* 58, 747–752. doi: 10.1136/oem.58.11.747

Kawabe, Y., Nakamura, Y., Kikuchi, S., Murakami, Y., Tanaka, T., Takebayashi, T., et al. (2014). Relationship between shift work and clustering of the metabolic syndrome diagnostic components. *J. Atheroscler. Thromb.* 21, 703–711. doi: 10.5551/jat.19380

Kawada, T., and Otsuka, T. (2014). Effect of shift work on the development of metabolic syndrome after 3 years in Japanese male workers. *Arch. Environ. Occup. Health* 69, 55–61. doi: 10.1080/19338244.2012.732123

Killick, R., Banks, S., and Liu, P. Y. (2012). Implications of sleep restriction and recovery on metabolic outcomes. *J. Clin. Endocrinol. Metab.* 97, 3876–3890. doi: 10.1210/jc.2012-1845

Kohsaka, A., Laposky, A. D., Ramsey, K. M., Estrada, C., Joshu, C., Kobayashi, Y., et al. (2007). High-fat diet disrupts behavioral and molecular circadian rhythms in mice. *Cell Metab.* 6, 414–421. doi: 10.1016/j.cmet.2007.09.006

Kubo, T., Oyama, I., Nakamura, T., Shirane, K., Otsuka, H., Kunimoto, M., et al. (2011). Retrospective cohort study of the risk of obesity among shift workers: findings from the Industry-based Shift Workers' Health study, Japan. *Occup. Environ. Med.* 68, 327–331. doi: 10.1136/oem.2009.054445

La Fleur, S. E., Luijendijk, M. C., Van Der Zwaal, E. M., Brans, M. A., and Adan, R. A. (2014). The snacking rat as model of human obesity: effects of a free-choice

high-fat high-sugar diet on meal patterns. *Int. J. Obes. (Lond).* 38, 643–649. doi: 10.1038/ijo.2013.159

Leenaars, C. H., Kalsbeek, A., Hanegraaf, M. A., Foppen, E., Joosten, R. N., Post, G., et al. (2012). Unaltered instrumental learning and attenuated body-weight gain in rats during non-rotating simulated shiftwork. *Chronobiol. Int.* 29, 344–355. doi: 10.3109/07420528.2011.654018

Li, Y., Sato, Y., and Yamaguchi, N. (2011). Shift work and the risk of metabolic syndrome: a nested case-control study. *Int. J. Occup. Environ. Health* 17, 154–160. doi: 10.1179/oeh.2011.17.2.154

Lin, Y. C., Hsiao, T. J., and Chen, P. C. (2009). Persistent rotating shift-work exposure accelerates development of metabolic syndrome among middle-aged female employees: a five-year follow-up. *Chronobiol. Int.* 26, 740–755. doi: 10.1080/07420520902929029

Lowden, A., Moreno, C., Holmback, U., Lennernas, M., and Tucker, P. (2010). Eating and shift work - effects on habits, metabolism and performance. *Scand. J. Work Environ. Health* 36, 150–162. doi: 10.5271/sjweh.2898

Matheson, A., O'Brien, L., and Reid, J. A. (2014). The impact of shiftwork on health: a literature review. *J. Clin. Nurs.* 23, 3309–3320. doi: 10.1111/jocn.12524

McHill, A. W., Melanson, E. L., Higgins, J., Connick, E., Moehlman, T. M., Stothard, E. R., et al. (2014). Impact of circadian misalignment on energy metabolism during simulated nightshift work. *Proc. Natl. Acad. Sci. U.S.A.* 111, 17302–17307. doi: 10.1073/pnas.1412021111

Morikawa, Y., Nakagawa, H., Miura, K., Ishizaki, M., Tabata, M., Nishijo, M., et al. (1999). Relationship between shift work and onset of hypertension in a cohort of manual workers. *Scand. J. Work Environ. Health* 25, 100–104. doi: 10.5271/sjweh.411

Murphy. (2003). A laboratory animal model of human shift work. *Integr. Physiol. Behav. Sci.* 38, 316–328. doi: 10.1007/BF02688860

Nagano, M., Adachi, A., Nakahama, K., Nakamura, T., Tamada, M., Meyer-Bernstein, E., et al. (2003). An abrupt shift in the day/night cycle causes desynchrony in the mammalian circadian center. *J. Neurosci.* 23, 6141–6151.

Natelson, B. H., Servatius, R. J., Tapp, W. N., Gross, J. L., and Ottenweller, J. E. (1993). Effect of life in a constant light environment on the course of hypertension in Dahl rats. *Physiol. Behav.* 53, 1219–1222. doi: 10.1016/0031-9384(93)90383-Q

Oishi, K. (2009). Disrupted light-dark cycle induces obesity with hyperglycemia in genetically intact animals. *Neuro Endocrinol. Lett.* 30, 458–461.

Oishi, K., Amagai, N., Shirai, H., Kadota, K., Ohkura, N., and Ishida, N. (2005). Genome-wide expression analysis reveals 100 adrenal gland-dependent circadian genes in the mouse liver. *DNA Res.* 12, 191–202. doi: 10.1093/dnares/dsi003

Oishi, K., and Itoh, N. (2013). Disrupted daily light-dark cycle induces the expression of hepatic gluconeogenic regulatory genes and hyperglycemia with glucose intolerance in mice. *Biochem. Biophys. Res. Commun.* 432, 111–115. doi: 10.1016/j.bbrc.2013.01.076

Oosterman, J. E., Foppen, E., Van Der Spek, R., Fliers, E., Kalsbeek, A., and La Fleur, S. E. (2014). Timing of fat and liquid sugar intake alters substrate oxidation and food efficiency in male Wistar rats. *Chronobiol. Int.* 32, 289–298. doi: 10.3109/07420528.2014.971177

Oyama, I., Kubo, T., Fujino, Y., Kadowaki, K., Kunimoto, M., Shirane, K., et al. (2012). Retrospective cohort study of the risk of impaired glucose tolerance among shift workers. *Scand. J. Work Environ. Health* 38, 337–342. doi: 10.5271/sjweh.3297

Oyama, Y., Iwasaka, H., Koga, H., Shingu, C., Matsumoto, S., and Noguchi, T. (2014). Uncoupling of peripheral and master clock gene rhythms by reversed feeding leads to an exacerbated inflammatory response after polymicrobial sepsis in mice. *Shock* 41, 214–221. doi: 10.1097/SHK.0000000000000094

Pietroiusti, A., Neri, A., Somma, G., Coppeta, L., Iavicoli, I., Bergamaschi, A., et al. (2010). Incidence of metabolic syndrome among night-shift healthcare workers. *Occup. Environ. Med.* 67, 54–57. doi: 10.1136/oem.2009.046797

Puttonen, S., Harma, M., and Hublin, C. (2010). Shift work and cardiovascular disease - pathways from circadian stress to morbidity. *Scand. J. Work Environ. Health* 36, 96–108. doi: 10.5271/sjweh.2894

Reddy, A. B., Field, M. D., Maywood, E. S., and Hastings, M. H. (2002). Differential resynchronisation of circadian clock gene expression within the suprachiasmatic nuclei of mice subjected to experimental jet lag. *J. Neurosci.* 22, 7326–7330.

Reznick, J., Preston, E., Wilks, D. L., Beale, S. M., Turner, N., and Cooney, G. J. (2013). Altered feeding differentially regulates circadian rhythms and energy metabolism in liver and muscle of rats. *Biochim. Biophys. Acta* 1832, 228–238. doi: 10.1016/j.bbadis.2012.08.010

Sakata, K., Suwazono, Y., Harada, H., Okubo, Y., Kobayashi, E., and Nogawa, K. (2003). The relationship between shift work and the onset of hypertension in male Japanese workers. *J. Occup. Environ. Med.* 45, 1002–1006. doi: 10.1097/01.jom.0000085893.98441.96

Salgado-Delgado, R., Angeles-Castellanos, M., Buijs, M. R., and Escobar, C. (2008). Internal desynchronization in a model of night-work by forced activity in rats. *Neuroscience* 154, 922–931. doi: 10.1016/j.neuroscience.2008.03.066

Salgado-Delgado, R., Angeles-Castellanos, M., Saderi, N., Buijs, R. M., and Escobar, C. (2010a). Food intake during the normal activity phase prevents obesity and circadian desynchrony in a rat model of night work. *Endocrinology* 151, 1019–1029. doi: 10.1210/en.2009-0864

Salgado-Delgado, R. C., Saderi, N., Basualdo Mdel, C., Guerrero-Vargas, N. N., Escobar, C., and Buijs, R. M. (2013). Shift work or food intake during the rest phase promotes metabolic disruption and desynchrony of liver genes in male rats. *PLoS ONE* 8:e60052. doi: 10.1371/journal.pone.0060052

Salgado-Delgado, R., Nadia, S., Angeles-Castellanos, M., Buijs, R. M., and Escobar, C. (2010b). In a rat model of night work, activity during the normal resting phase produces desynchrony in the hypothalamus. *J. Biol. Rhythms* 25, 421–431. doi: 10.1177/0748730410383403

Salgado-Delgado, R., Tapia Osorio, A., Saderi, N., and Escobar, C. (2011). Disruption of circadian rhythms: a crucial factor in the etiology of depression. *Depress. Res. Treat.* 2011:839743. doi: 10.1155/2011/839743

Schroder, E. A., Burgess, D. E., Manning, C. L., Zhao, Y., Moss, A. J., Patwardhan, A. R., et al. (2014). Light-phase restricted feeding slows basal heart rate to exaggerate the type 3 long QT syndrome phenotype in mice. *Am. J. Physiol. Heart Circ. Physiol. Ajpheart.* 341:2014. doi: 10.1152/ajpheart.00341.2014

Senador, D., Shewale, S., Irigoyen, M. C., Elased, K. M., and Morris, M. (2012). Effects of restricted fructose access on body weight and blood pressure circadian rhythms. *Exp. Diabetes Res.* 2012:459087. doi: 10.1155/2012/459087

Shamsi, N. A., Salkeld, M. D., Rattanatray, L., Voultsios, A., Varcoe, T. J., Boden, M. J., et al. (2014). Metabolic consequences of timed feeding in mice. *Physiol. Behav.* 128, 188–201. doi: 10.1016/j.physbeh.2014.02.021

Sherman, H., Genzer, Y., Cohen, R., Chapnik, N., Madar, Z., and Froy, O. (2012). Timed high-fat diet resets circadian metabolism and prevents obesity. *FASEB J.* 26, 3493–3502. doi: 10.1096/fj.12-208868

Shi, S. Q., Ansari, T. S., McGuinness, O. P., Wasserman, D. H., and Johnson, C. H. (2013). Circadian disruption leads to insulin resistance and obesity. *Curr. Biol.* 23, 372–381. doi: 10.1016/j.cub.2013.01.048

Su, Y., Van Der Spek, R., Foppen, E., Kwakkel, J., Fliers, E., and Kalsbeek, A. (2014). Effects of adrenalectomy on daily gene expression rhythms in the rat suprachiasmatic and paraventricular hypothalamic nuclei and in white adipose tissue. *Chronobiol. Int.* 32, 211–224. doi: 10.3109/07420528.2014.963198

Suwazono, Y., Dochi, M., Oishi, M., Tanaka, K., Kobayashi, E., and Sakata, K. (2009). Shiftwork and impaired glucose metabolism: a 14-year cohort study on 7104 male workers. *Chronobiol. Int.* 26, 926–941. doi: 10.1080/07420520903044422

Suwazono, Y., Dochi, M., Sakata, K., Okubo, Y., Oishi, M., Tanaka, K., et al. (2008). A longitudinal study on the effect of shift work on weight gain in male Japanese workers. *Obesity (Silver Spring)* 16, 1887–1893. doi: 10.1038/oby.2008.298

Tsai, L. L., and Tsai, Y. C. (2007). The effect of scheduled forced wheel activity on body weight in male F344 rats undergoing chronic circadian desynchronization. *Int. J. Obes. (Lond).* 31, 1368–1377. doi: 10.1038/sj.ijo.0803607

Tsai, L. L., Tsai, Y. C., Hwang, K., Huang, Y. W., and Tzeng, J. E. (2005). Repeated light-dark shifts speed up body weight gain in male F344 rats. *Am. J. Physiol. Endocrinol. Metab.* 289, E212–E217. doi: 10.1152/ajpendo.00603.2004

Tucker, P., Marquie, J. C., Folkard, S., Ansiau, D., and Esquirol, Y. (2012). Shiftwork and metabolic dysfunction. *Chronobiol. Int.* 29, 549–555. doi: 10.3109/07420528.2012.675259

Van Amelsvoort, L. G., Schouten, E. G., and Kok, F. J. (1999). Duration of shiftwork related to body mass index and waist to hip ratio. *Int. J. Obes. Relat. Metab. Disord.* 23, 973–978. doi: 10.1038/sj.ijo.0801028

Vilaplana, J., Madrid, J. A., Sanchez-Vazquez, J., Campuzano, A., Cambras, T., and Diez-Noguera, A. (1995). Influence of period length of light/dark cycles on

the body weight and food intake of young rats. *Physiol. Behav.* 58, 9–13. doi: 10.1016/0031-9384(95)00021-A

Voigt, R. M., Forsyth, C. B., Green, S. J., Mutlu, E., Engen, P., Vitaterna, M. H., et al. (2014). Circadian disorganization alters intestinal microbiota. *PLoS ONE* 9:e97500. doi: 10.1371/journal.pone.0097500

Wang, J. B., Patterson, R. E., Ang, A., Emond, J. A., Shetty, N., and Arab, L. (2014). Timing of energy intake during the day is associated with the risk of obesity in adults. *J. Hum. Nutr. Diet.* 27(Suppl. 2), 255–262. doi: 10.1111/jhn.12141

Wideman, C. H., and Murphy, H. M. (2009). Constant light induces alterations in melatonin levels, food intake, feed efficiency, visceral adiposity, and circadian rhythms in rats. *Nutr. Neurosci.* 12, 233–240. doi: 10.1179/147683009X423436

Yamajuku, D., Okubo, S., Haruma, T., Inagaki, T., Okuda, Y., Kojima, T., et al. (2009). Regular feeding plays an important role in cholesterol homeostasis through the liver circadian clock. *Circ. Res.* 105, 545–548. doi: 10.1161/CIRCRESAHA.109.199034

Ye, H. H., Jeong, J. U., Jeon, M. J., and Sakong, J. (2013). The Association between Shift Work and the Metabolic Syndrome in Female Workers. *Ann. Occup. Environ. Med.* 25:33. doi: 10.1186/2052-4374-25-33

Yoon, J. A., Han, D. H., Noh, J. Y., Kim, M. H., Son, G. H., Kim, K., et al. (2012). Meal time shift disturbs circadian rhythmicity along with metabolic and behavioral alterations in mice. *PLoS ONE* 7:e44053. doi: 10.1371/journal.pone.0044053

Zaragoza, N., and Felber, J. P. (1970). Studies on the metabolic effects induced in the rat by a high fat diet. I. Carbohydrate metabolism *in vivo*. *Horm. Metab. Res.* 2, 323–329. doi: 10.1055/s-0028-1095057

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

MINI REVIEW
published: 01 April 2015
doi: 10.3389/fphar.2015.00071

Recent advances in circadian rhythms in cardiovascular system

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Growing evidence shows that intrinsic circadian clocks are tightly related to cardiovascular functions. The diurnal changes in blood pressure and heart rate are well known circadian rhythms. Endothelial function, platelet aggregation and thrombus formation exhibit circadian changes as well. The onset of many cardiovascular diseases (CVDs) or events, such as myocardial infarction, stroke, arrhythmia, and sudden cardiac death, also exhibits temporal trends. Furthermore, there is strong evidence from animal models and epidemiological studies showing that disruption of circadian rhythms is a significant risk factor for many CVDs, and the intervention of CVDs may have a time dependent effect. In this mini review, we summarized recent advances in our understanding of the relationship between circadian rhythm and cardiovascular physiology and diseases including blood pressure regulation and myocardial infarction.

Keywords: circadian rhythm, circadian clock, CVDs, blood pressure, myocardial infarction

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Pharmaceutical Medicine and Outcomes Research, a section of the journal Frontiers in Pharmacology

Received: *20 January 2015*
Accepted: *16 March 2015*
Published: *01 April 2015*

Citation:
Chen L and Yang G (2015) Recent advances in circadian rhythms in cardiovascular system. Front. Pharmacol. 6:71. doi: 10.3389/fphar.2015.00071

Introduction

Circadian rhythms are biological processes displaying endogenous oscillations of about 24-h. These rhythms are widely observed in animals, plants, bacteria, and even cultured cells (Harmer et al., 2001). They are driven by a group of genes called clock genes. In mammals, the core clock genes consist of Bmal1 (Brain and muscle aryl-hydrocarbon receptor nuclear translocator-like 1), CLOCK (Circadian Locomotor Output Cycles Kaput), Per (Period), and Cry (Cryptochrome). They form a tightly regulated system with interlocking feedback and feed-forward loops (**Figure 1**) (Yang et al., 2013). BMAL1 and CLOCK proteins, or its paralog NPAS2 (neuronal PAS domain protein 2), form a heterodimer, bind to E-box elements in Per and Cry promoter regions and activate their transcription. Upon accumulation in the cytoplasm, PER and CRY proteins translocate to the nucleus where they repress the BMAL1:CLOCK/NPAS2 regulatory complex, thereby shutting down their own transcription. This core loop is interconnected with additional positive and negative regulatory loops involving nuclear receptors, such as RORα (RAR-related orphan receptor alpha), REV-ERBα (NR1D1, nuclear receptor subfamily 1, group D, member 1), and PPARs (Peroxisome proliferator-activated receptors). Additionally, these clock genes control numerous target genes (termed clock controlled genes, CCGs), thus regulating the circadian rhythms of various biochemical and physiological processes (Chen and Yang, 2014).

The circadian clock exists as the central clock in the suprachiasmatic nucleus (SCN) in the hypothalamus, and its peripheral tissues serve as the peripheral clock. The SCN receives light input from the retina, and then conveys the photic information into neural and/or humoral signals that orchestrate multifarious behavioral and biological rhythms, such as sleep-wake, hunger, body temperature, and hormone secretion cycles (Kohsaka et al., 2012). Although

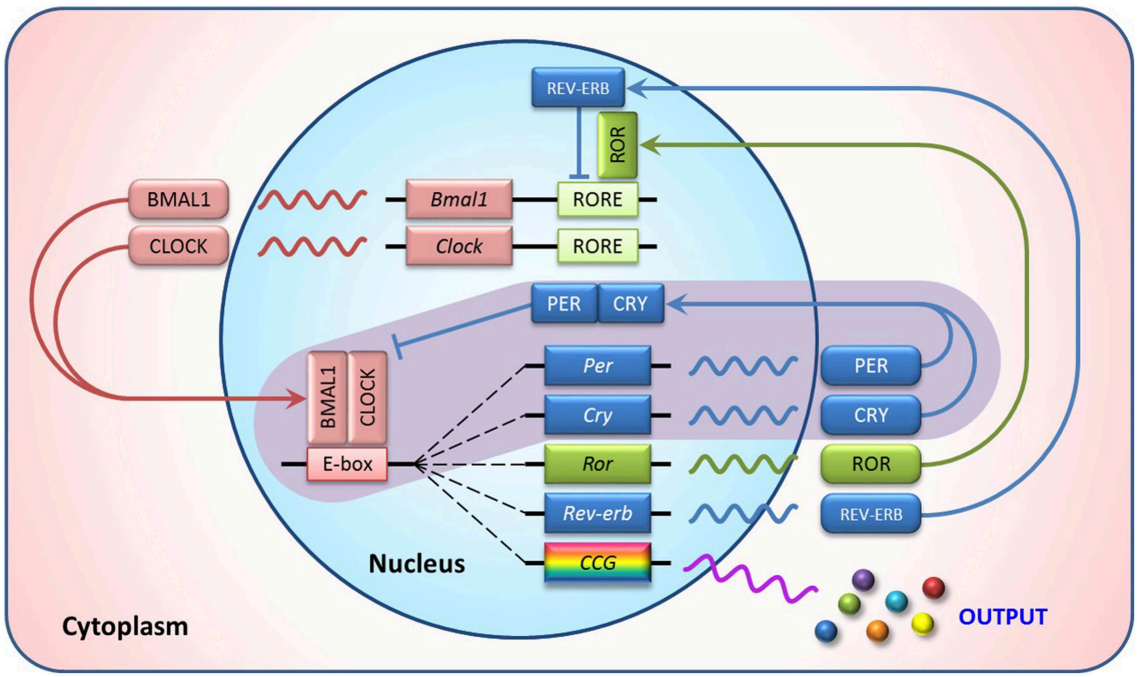

FIGURE 1 | Transcriptional feedback loops of the mammalian circadian clock. In the core loop (purple background), BMAL1/CLOCK heterodimer activates transcription of the Per and Cry genes via binding to the E-box elements in their promoter regions. The resulting PER and CRY proteins heterodimerize, translocate to the nucleus and interact with the BMAL1/CLOCK complex to inhibit their own transcription. In addition, ROR activates and REV-ERB represses RORE-mediated transcription, forming the secondary autoregulatory feedback loops. This clock mechanism also controls rhythmic expression of numerous genes, called clock controlled genes (CCG), to perform biochemical or physiological roles in a circadian manner.

the SCN synchronizes internal time in various tissues, growing evidence from *in vitro* and *ex vivo* experiments has proved that the peripheral clock can function autonomously without central or systemic cues (Kowalska and Brown, 2007; Takeda and Maemura, 2011).

Circadian Clock in Cardiovascular System

Circadian expression of clock genes in mouse heart (Young et al., 2001) and aorta (McNamara et al., 2001) were first described in 2001. Recently, Zhang et al. (2014) used a high temporal resolution of RNA-seq data and found that 6 and 4% of protein coding genes showed circadian rhythms in transcription in mouse heart and aorta, respectively. *Ex vivo* experiments displayed varied functions of mouse heart (Durgan et al., 2007) and aorta (Keskil et al., 1996; Prasai et al., 2013) that depended on the time the tissues were collected. In addition, human hearts were found to express clock genes in a time sensitive manner as well (Leibetseder et al., 2009). Furthermore, the observations of gene cycling were extended to cultured cells. In rat cardiomyocytes, the presence of 2.5% of fetal calf serum in culture medium is sufficient to maintain rhythmic expression of core clock genes Bmal1, Rev-erbα, and Per2 and energy metabolic genes pyruvate dehydrogenase kinase 4 and uncoupling protein 3 (Durgan et al., 2005). Functional clocks are also expressed in cultured endothelial cells (Takeda et al., 2007) and vascular smooth muscle cells (Nonaka et al., 2001). To study the role of circadian clocks in cardiovascular system, several tissue specific clock gene deletion mouse models were recently generated. For instance, cardiomyocyte deletion of Bmal1 results in abnormal electrocardiography with prolonged RR and QRS intervals (Schroder et al., 2013). The hearts from knockout mice were more susceptible to arrhythmia. Bmal1 deletion in endothelial cells (Westgate et al., 2008) or vascular smooth muscle cells (Xie et al., 2015) compromised the diurnal variation of blood pressure. These findings are consistent with the presence and importance of intrinsic clocks in cardiovascular system.

On the other hand, although all cell types in the cardiovascular system have intact molecular clocks, these peripheral clocks need to coordinate with the central clock to synchronize responsiveness of the heart and blood vessels to diurnal variations in their environment. The disruption of normal day-night cycles, such as jet lag, leads to desynchronization between central and peripheral clocks, heterogeneity of entrainment kinetics between different organs, and dysregulation of clock genes (Kiessling et al., 2010). Because circadian clocks control a large number of tissue specific CCGs (Zhang et al., 2014), the disruption of this mechanism will initiate a chain reaction to result in perturbation of a wide range of biochemical and physiological outputs, potentially contributing to the incidence of cardiovascular diseases (CVDs). For example, using a mouse model of pressure overload–induced cardiac hypertrophy, Martino et al. found that rhythm disturbance

by housing mice under 10-h light: 10-h dark conditions adversely affected cardiac structure and function as well as altered expression of clock genes and cardiac remodeling genes (Martino et al., 2007). Interestingly and importantly, restoration of a normal 24-h diurnal rhythm could rescue these changes, suggesting that maintaining a normal rhythm is crucial to cardiovascular health.

Circadian Regulation of Blood Pressure

Day-night variations in blood pressure (BP) and heart rate (HR) are among the best known circadian rhythms of physiology. In humans, there is a 24-h variation in BP with a sharp rise before awakening, the highest BP value is around midmorning (Millar-Craig et al., 1978). Concomitantly, many cardiovascular events, such as sudden cardiac death, myocardial infarction and stroke, display diurnal variations with an increased incidence in the morning (Muller et al., 1985, 1987; Elliott, 1998; Reavey et al., 2013). These events, as well as kidney albuminuria and progression to end-stage renal diseases, are relatively common in patients whose blood pressure fails to decline during the night, so-called non-dippers (Takeda and Maemura, 2010). Inverse dippers—BP rises instead of decreases at night—showed even higher cardiovascular mortality (Kario et al., 2001). These time-dependent effects are not just consequences of the sleep/wakefulness cycle or the rhythms in neuroendocrine constituents, but are also believed to be attributed to the intrinsic properties of the hearts and blood vessels whose functions show significant fluctuations during the course of the day (Durgan and Young, 2010; Paschos and Fitzgerald, 2010).

Studies in genetic manipulated mice have suggested the involvement of intrinsic circadian clock in BP rhythm regulation. One of the most interesting findings is the dissociation between behavior and BP regulation (**Figure 2**). As the closest phylogenetic neighbor of ROR and REV-ERB, nuclear receptor PPARγ regulates the circadian rhythms of BP and heart rate via direct interaction with Bmal1 gene (Wang et al., 2008b; Yang et al., 2012). Although both vascular and global PPARγ knockout mice responded to light well and displayed rhythmic behavior pattern under regular light/dark conditions, the diurnal variations of BP was dampened or even abolished in these knockout mice. This striking dissociation between physiology and behavior strongly suggests that intrinsic clocks inside the blood vessels contribute to their functions that fluctuate in a 24-h cycle. Several other core clock genes were also reported to regulate BP in various ways. Global deletion of Bmal1 in mice abolishes the circadian rhythm of BP, which is accompanied by hypotension likely due to the reduced production of catecholamines (Curtis et al., 2007) or the lack of Bmal1 in vascular smooth muscle cells (Xie et al., 2015). By contrast, double deletion of Cry1/2 genes in mice give rise to salt-sensitive hypertension (Masuki et al., 2005).

It is worthwhile to note that the intrinsic circadian regulation of BP in humans remains to be determined. Kerkhof et al. failed to detect a significant 24-h variation of blood pressure in human when individuals were subjected to a 26-h constant light condition, while the heart rate exhibited a significant circadian pattern (Kerkhof et al., 1998). On the contrary, Scheer et al. found that, independent of environmental and behavioral changes, the endogenous circadian system modulates diurnal BP variation in

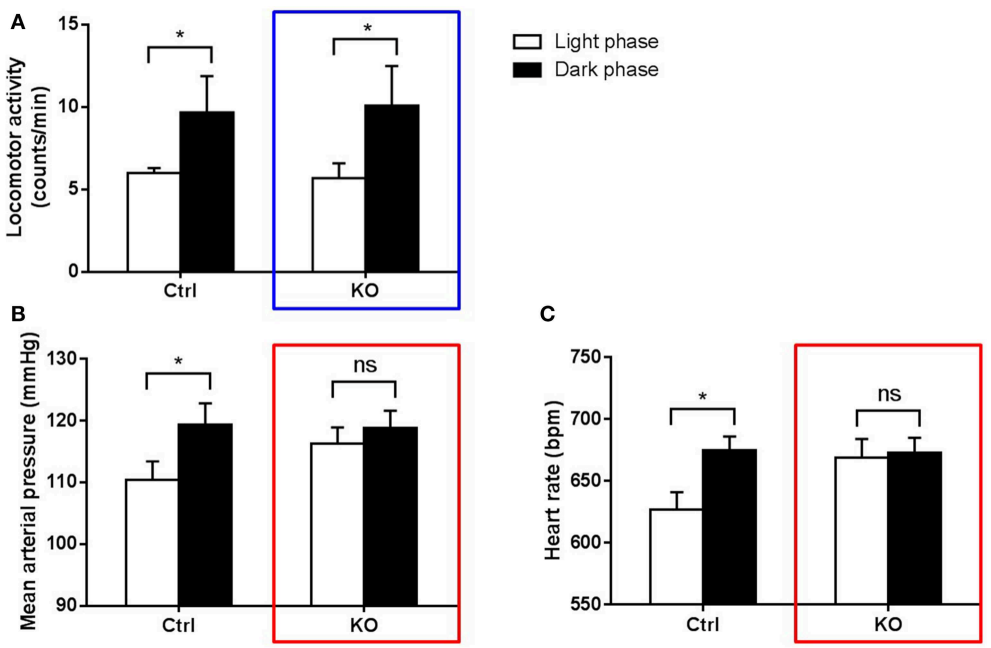

FIGURE 2 | Dissociation between behavior and BP regulation in circadian-disrupted mice (Yang et al., 2012). PPARγ knockout mice (KO) and their littermate controls (Ctrl) were kept under regular light/dark cycles. Locomotor activity **(A)**, mean arterial pressure **(B)** and heart rate **(C)** were recorded using radiotelemetry. Both KO (blue box) and control mice display obvious day/night variation in locomotor activity. However, KO mice cannot maintain normal variations in BP and heart rate (red boxes) as control mice. *$p < 0.05$; ns, not significant.

humans (Scheer et al., 2010), while circadian misalignment by scheduling a recurring 28-h "day" for 8 days will induce hypertension and other adverse cardio-metabolic implications (Scheer et al., 2009).

Conventionally, most of the hypertensive patients were treated with anti-hypertensive medications in the mornings. Based on the consideration of the day-night variation of BP and the previous studies showing BP lowering effect of low dose of aspirin at bedtime (Hermida et al., 2003, 2009), Hermida et al., compared the potential differential reduction of CVD morbidity and mortality risk by a bedtime vs. upon-awakening hypertension treatment schedule in a large-scale (2156 untreated hypertensive subjects) and long-term (median follow-up of 5.6 years) study. They found that the patients who took at least one regular antihypertensive medication at bedtime gained better BP control and exhibited a significant reduction in CVD risk (Hermida et al., 2010). Although their results were impressive, independent antihypertension studies and intensive studies on hypertension-related complications are required to confirm the time-dependent effects of antihypertensive drugs and to establish chronotherapy to manage in hypertension.

Circadian Rhythms and Myocardial Infarction

Circadian rhythms in timing of onset and tolerance to myocardial infarction (MI) have been well established. It has been reported that the occurrence of MI is two to three times more frequent in the morning than at night (Muller et al., 1985; Culic, 2014). In the early morning, the increased systolic BP and HR results in an increased energy and oxygen demand by the heart, while the vascular tone of the coronary artery rises in the morning, resulting in a decreased coronary blood flow and oxygen supply. This mismatch between supply and demand elicits the high frequency of the onset of MI. In addition, plasminogen activator inhibitor-1 (Kurnik, 1995) and many platelet surface activation markers such as GPIb and P-selectin (Scheer et al., 2011) displayed a circadian pattern with high levels in the morning, which is coincident with the morning peak of thrombus formation and platelet aggregation (Tofler et al., 1987; Scheer and Shea, 2014). The resulting hypercoagulability partially underlies the morning onset of MI. Disruption of circadian rhythm like shiftwork and jetlag has been well established to be a risk factor for many CVDs, including MI (Knutsson et al., 1999). Even a 1 h shift, such as the transition from regular time to daylight saving time, can significantly increase the chances of MI occurring (Janszky and Ljung, 2008).

A series of cardiac functions related to the heart remodeling after MI are also known to have circadian variation. The early healing after MI relies on coordinated removal of necrotic tissues through an early inflammatory phase (Frangogiannis, 2012), followed by replacement and remodeling of the myocardium and extracellular matrix deposition (Liehn et al., 2011). As remodeling progresses toward the maturation phase, the heart undergoes size, shape and structure changes, which lead to ventricular dilation, dysfunction, and ultimately failure (Liehn et al., 2011). Most recently, Alibhai et al. (2014) demonstrated that short-term disruption of diurnal rhythms after myocardial infarction adversely affected the early inflammatory phase of left ventricular remodeling, altered the innate immune infiltration and scar formation, and eventually led to exacerbated maladaptive cardiac remodeling in mice. In contrast, maintaining normal rhythms throughout the course of the disease better preserved cardiac structure and function. Although no animal model can completely reflect patient experience, maintenance of normal diurnal rhythm during the recovery phase after MI should still aid in a coordinated and effective infarct healing response and improve patient outcome.

Moreover, clock genes may also exert non-clock roles in the cardiovascular system, which should be taken into account when interpreting the effect of circadian disruption. For instance, activation of an adenosine receptor Adora2b acts via Per2, but not other clock genes, to induce an energy utilization switch from fatty acid to glucose in cardiomyocytes, which promotes glycolysis and protects against cardiac ischemic injury (Eckle et al., 2012; Yang and Fitzgerald, 2012).

Circadian Rhythms and other Cardiovascular Diseases

Numerous animal models and human epidemiological studies also proved the adverse effects of circadian disruption in other CVDs. Mouse hearts in rhythm-disruptive environments are prone to malfunctions with altered clock gene cycling and reduced contractility (Martino et al., 2007). Clock gene deletion or mutation in mice dampened cardiovascular circadian rhythms accompanied by dilated cardiomyopathy (Lefta et al., 2012), arterial stiffness (Anea et al., 2010), or endothelial dysfunction (Viswambharan et al., 2007; Wang et al., 2008a; Anea et al., 2009). Impaired cholesterol metabolism and increased development of atherosclerosis was also verified in CLOCK mutant mice on a western as well as a normal diet (Pan et al., 2013). Aortic grafts from Bmal1 knockout mice transplanted into wild type mice developed robust arteriosclerosis without affecting systemic hemodynamics (Cheng et al., 2011). This data suggests that the intrinsic circadian clocks in blood vessels exert significant roles as an autonomous influence in arteriosclerotic diseases.

On the other hand, CVDs affect clock gene expression as well. For example, in salt sensitive rats, high salt diet induced cardiac hypertrophy is associated with attenuated rhythmic expression of core clock genes (Mohri et al., 2003). Aortic constriction induced pressure overload, which decreased the amplitude of circadian expression of clock genes in the rat heart (Young et al., 2001; Durgan et al., 2005). In a type 2 diabetic rat model, cardiac clock genes exhibited a phase shift with a 3 h delay, suggesting a loss of normal synchronization in diabetic hearts (Young et al., 2002). However, in high fat diet induced obese mice, vascular tissues are less sensitive to pathological disruption of circadian clocks than adipose tissue (Prasai et al., 2013). This evidence raises the possibility that although all cardiovascular cell types possess functional circadian clocks, this mechanism may be regulated in a cell-type specific manner. Desynchronization between different organs (e.g., heart and aorta) or cell types (e.g., VSMCs and ECs) could occur during specific physiological/pathological situations and may give an increased chance of CVDs.

Moreover, the day-night variations of blood pressure, heart rate and baroreflex sensitivity (a homeostatic mechanism for maintaining blood pressure) also coincide with diurnal variability in many other CVDs or events, such as cardiac arrhythmias, atherosclerosis and sudden death (Portaluppi et al., 2012; Yang et al., 2013). The timing of sudden cardiac death displayed circadian variability. It has a circadian pattern prominent in the early morning similar to that described in patients with coronary artery disease (Muller et al., 1987). Both atrial and ventricular arrhythmias appear to exhibit circadian patterning as well, with a higher frequency during the day than at night (Portaluppi et al., 2012). In hospital, many arrhythmias are observed as a consequence of MI. More complicatedly, circadian disruption not only impairs cardiovascular functions, but has also been linked to other diseases such as obesity, diabetes, immune disorders, mental illness that may affect each other (Harrington, 2010). Therefore, controlling or prevent the diseases that are related both to circadian rhythm and to cardiovascular functions becomes very important.

Conclusion

Cardiovascular disease is the leading cause of death in many industrialized countries. Intensive effort has been made to understand the basic mechanisms. One field of investigation in recent years is the study of circadian rhythms. Increasing evidence has shown adverse effects of circadian disruption in the cardiovascular system. It becomes more and more evident and important in the modern age, particularly in developed countries, due to frequent disruptions to normal rhythms caused by shift work, artificial light, transmeridian air flight, and social activities (Boggild and Knutsson, 1999; Knutsson and Boggild, 2000).

The circadian rhythms not only affect health, but also drug efficiency. It's not surprising that some drugs for treating CVDs have been reported to exhibit time dependent effects since there's eminent circadian function of heart and blood vessels driven by both systemic and intrinsic clocks. Although several other mechanisms outside the cardiovascular system, such as chronopharmacokinetics (Musiek and Fitzgerald, 2013), have been suggested, the circadian clock within the heart and blood vessels should not be overlooked. Time dependent effects should be investigated when developing new drugs for CVDs.

Acknowledgments

The authors are grateful to Lindsay Herman for reading, editing, and commenting on this paper.

References

Alibhai, F. J., Tsimakouridze, E. V., Chinnappareddy, N., Wright, D. C., Billia, F., O'Sullivan, M. L., et al. (2014). Short-term disruption of diurnal rhythms after murine myocardial infarction adversely affects long-term myocardial structure and function. *Circ. Res.* 114, 1713–1722. doi: 10.1161/CIRCRESAHA.114.302995

Anea, C. B., Ali, M. I., Osmond, J. M., Sullivan, J. C., Stepp, D. W., Merloiu, A. M., et al. (2010). Matrix metalloproteinase 2 and 9 dysfunction underlie vascular stiffness in circadian clock mutant mice. *Arterioscler. Thromb. Vasc. Biol.* 30, 2535–2543. doi: 10.1161/ATVBAHA.110.214379

Anea, C. B., Zhang, M., Stepp, D. W., Simkins, G. B., Reed, G., Fulton, D. J., et al. (2009). Vascular disease in mice with a dysfunctional circadian clock. *Circulation* 119, 1510–1517. doi: 10.1161/CIRCULATIONAHA.108.827477

Boggild, H., and Knutsson, A. (1999). Shift work, risk factors and cardiovascular disease. *Scand. J. Work Environ. Health* 25, 85–99. doi: 10.5271/sjweh.410

Chen, L., and Yang, G. (2014). PPARs integrate the mammalian clock and energy metabolism. *PPAR Res.* 2014:653017. doi: 10.1155/2014/653017

Cheng, B., Anea, C. B., Yao, L., Chen, F., Patel, V., Merloiu, A., et al. (2011). Tissue-intrinsic dysfunction of circadian clock confers transplant arteriosclerosis. *Proc. Natl. Acad. Sci. U.S.A.* 108, 17147–17152. doi: 10.1073/pnas.1112998108

Culic, V. (2014). Chronobiological rhythms of acute cardiovascular events and underlying mechanisms. *Int. J. Cardiol.* 174, 417–419. doi: 10.1016/j.ijcard.2014.04.043

Curtis, A. M., Cheng, Y., Kapoor, S., Reilly, D., Price, T. S., and Fitzgerald, G. A. (2007). Circadian variation of blood pressure and the vascular response to asynchronous stress. *Proc. Natl. Acad. Sci. U.S.A.* 104, 3450–3455. doi: 10.1073/pnas.0611680104

Durgan, D. J., Hotze, M. A., Tomlin, T. M., Egbejimi, O., Graveleau, C., Abel, E. D., et al. (2005). The intrinsic circadian clock within the cardiomyocyte. *Am. J. Physiolog.* 289, H1530–H1541. doi: 10.1152/ajpheart.00406.2005

Durgan, D. J., Moore, M. W., Ha, N. P., Egbejimi, O., Fields, A., Mbawuike, U., et al. (2007). Circadian rhythms in myocardial metabolism and contractile function: influence of workload and oleate. *Am. J. Physiol. Heart Circ. Physiol.* 293, H2385–H2393. doi: 10.1152/ajpheart.01361.2006

Durgan, D. J., and Young, M. E. (2010). The cardiomyocyte circadian clock: emerging roles in health and disease. *Circ. Res.* 106, 647–658. doi: 10.1161/CIRCRESAHA.109.209957

Eckle, T., Hartmann, K., Bonney, S., Reithel, S., Mittelbronn, M., Walker, L. A., et al. (2012). Adora2b-elicited Per2 stabilization promotes a HIF-dependent metabolic switch crucial for myocardial adaptation to ischemia. *Nat. Med.* 18, 774–782. doi: 10.1038/nm.2728

Elliott, W. J. (1998). Circadian variation in the timing of stroke onset: a meta-analysis. *Stroke* 29, 992–996. doi: 10.1161/01.STR.29.5.992

Frangogiannis, N. G. (2012). Regulation of the inflammatory response in cardiac repair. *Circ. Res.* 110, 159–173. doi: 10.1161/CIRCRESAHA.111.243162

Harmer, S. L., Panda, S., and Kay, S. A. (2001). Molecular bases of circadian rhythms. *Annu. Rev. Cell Dev. Biol.* 17, 215–253. doi: 10.1146/annurev.cellbio.17.1.215

Harrington, M. (2010). Location, location, location: important for jet-lagged circadian loops. *J. Clin. Invest.* 120, 2265–2267. doi: 10.1172/JCI43632

Hermida, R. C., Ayala, D. E., Calvo, C., Lopez, J. E., Fernandez, J. R., Mojon, A., et al. (2003). Administration time-dependent effects of aspirin on blood pressure in untreated hypertensive patients. *Hypertension* 41, 1259–1267. doi: 10.1161/01.HYP.0000072335.73748.0D

Hermida, R. C., Ayala, D. E., Mojon, A., and Fernandez, J. R. (2009). Ambulatory blood pressure control with bedtime aspirin administration in subjects with prehypertension. *Am. J. Hypertens.* 22, 896–903. doi: 10.1038/ajh.2009.83

Hermida, R. C., Ayala, D. E., Mojon, A., and Fernandez, J. R. (2010). Influence of circadian time of hypertension treatment on cardiovascular risk: results of the MAPEC study. *Chronobiol. Int.* 27, 1629–1651. doi: 10.3109/07420528.2010.510230

Janszky, I., and Ljung, R. (2008). Shifts to and from daylight saving time and incidence of myocardial infarction. *N. Engl. J. Med.* 359, 1966–1968. doi: 10.1056/NEJMc0807104

Kario, K., Pickering, T. G., Matsuo, T., Hoshide, S., Schwartz, J. E., and Shimada, K. (2001). Stroke prognosis and abnormal nocturnal blood pressure falls in older hypertensives. *Hypertension* 38, 852–857. doi: 10.1161/hy1001.092640

Kerkhof, G. A., Van Dongen, H. P., and Bobbert, A. C. (1998). Absence of endogenous circadian rhythmicity in blood pressure? *Am. J. Hypertens.* 11, 373–377. doi: 10.1016/S0895-7061(97)00461-5

Keskil, Z., Gorgun, C. Z., Hodoglugil, U., and Zengil, H. (1996). Twenty-four-hour variations in the sensitivity of rat aorta to vasoactive agents. *Chronobiol. Int.* 13, 465–475. doi: 10.3109/07420529609020917

Kiessling, S., Eichele, G., and Oster, H. (2010). Adrenal glucocorticoids have a key role in circadian resynchronization in a mouse model of jet lag. *J. Clin. Invest.* 120, 2600–2609. doi: 10.1172/JCI41192

Knutsson, A., and Boggild, H. (2000). Shiftwork and cardiovascular disease: review of disease mechanisms. *Rev. Environ. Health* 15, 359–372. doi: 10.1515/REVEH.2000.15.4.359

Knutsson, A., Hallquist, J., Reuterwall, C., Theorell, T., and Akerstedt, T. (1999). Shiftwork and myocardial infarction: a case-control study. *Occup. Environ. Med.* 56, 46–50. doi: 10.1136/oem.56.1.46

Kohsaka, A., Waki, H., Cui, H., Gouraud, S. S., and Maeda, M. (2012). Integration of metabolic and cardiovascular diurnal rhythms by circadian clock. *Endocr. J.* 59, 447–456. doi: 10.1507/endocrj.EJ12-0057

Kowalska, E., and Brown, S. A. (2007). Peripheral clocks: keeping up with the master clock. *Cold Spring Harb. Symp. Quant. Biol.* 72, 301–305. doi: 10.1101/sqb.2007.72.014

Kurnik, P. B. (1995). Circadian variation in the efficacy of tissue-type plasminogen activator. *Circulation* 91, 1341–1346. doi: 10.1161/01.CIR.91.5.1341

Lefta, M., Campbell, K. S., Feng, H. Z., Jin, J. P., and Esser, K. A. (2012). Development of dilated cardiomyopathy in Bmal1-deficient mice. *Am. J. Physiol. Heart Circ. Physiol.* 303, H475–H485. doi: 10.1152/ajpheart.00238.2012

Leibetseder, V., Humpeler, S., Svoboda, M., Schmid, D., Thalhammer, T., Zuckermann, A., et al. (2009). Clock genes display rhythmic expression in human hearts. *Chronobiol. Int.* 26, 621–636. doi: 10.1080/07420520902924939

Liehn, E. A., Postea, O., Curaj, A., and Marx, N. (2011). Repair after myocardial infarction, between fantasy and reality: the role of chemokines. *J. Am. Coll. Cardiol.* 58, 2357–2362. doi: 10.1016/j.jacc.2011.08.034

Martino, T. A., Tata, N., Belsham, D. D., Chalmers, J., Straume, M., Lee, P., et al. (2007). Disturbed diurnal rhythm alters gene expression and exacerbates cardiovascular disease with rescue by resynchronization. *Hypertension* 49, 1104–1113. doi: 10.1161/HYPERTENSIONAHA.106.083568

Masuki, S., Todo, T., Nakano, Y., Okamura, H., and Nose, H. (2005). Reduced alpha-adrenoceptor responsiveness and enhanced baroreflex sensitivity in Cry-deficient mice lacking a biological clock. *J. Physiol.* 566, 213–224. doi: 10.1113/jphysiol.2005.086728

McNamara, P., Seo, S. B., Rudic, R. D., Sehgal, A., Chakravarti, D., and FitzGerald, G. A. (2001). Regulation of CLOCK and MOP4 by nuclear hormone receptors in the vasculature: a humoral mechanism to reset a peripheral clock. *Cell* 105, 877–889. doi: 10.1016/S0092-8674(01)00401-9

Millar-Craig, M. W., Bishop, C. N., and Raftery, E. B. (1978). Circadian variation of blood-pressure. *Lancet* 1, 795–797. doi: 10.1016/S0140-6736(78)92998-7

Mohri, T., Emoto, N., Nonaka, H., Fukuya, H., Yagita, K., Okamura, H., et al. (2003). Alterations of circadian expressions of clock genes in Dahl salt-sensitive rats fed a high-salt diet. *Hypertension* 42, 189–194. doi: 10.1161/01.HYP.0000082766.63952.49

Muller, J. E., Ludmer, P. L., Willich, S. N., Tofler, G. H., Aylmer, G., Klangos, I., et al. (1987). Circadian variation in the frequency of sudden cardiac death. *Circulation* 75, 131–138. doi: 10.1161/01.CIR.75.1.131

Muller, J. E., Stone, P. H., Turi, Z. G., Rutherford, J. D., Czeisler, C. A., Parker, C., et al. (1985). Circadian variation in the frequency of onset of acute myocardial infarction. *N. Engl. J. Med.* 313, 1315–1322. doi: 10.1056/NEJM198511213132103

Musiek, E. S., and Fitzgerald, G. A. (2013). Molecular clocks in pharmacology. *Handb. Exp. Pharmacol.* 217, 243–260. doi: 10.1007/978-3-642-25950-0_10

Nonaka, H., Emoto, N., Ikeda, K., Fukuya, H., Rohman, M. S., Raharjo, S. B., et al. (2001). Angiotensin II induces circadian gene expression of clock genes in cultured vascular smooth muscle cells. *Circulation* 104, 1746–1748. doi: 10.1161/hc4001.098048

Pan, X., Jiang, X. C., and Hussain, M. M. (2013). Impaired cholesterol metabolism and enhanced atherosclerosis in clock mutant mice. *Circulation* 128, 1758–1769. doi: 10.1161/CIRCULATIONAHA.113.002885

Paschos, G. K., and Fitzgerald, G. A. (2010). Circadian clocks and vascular function. *Circ. Res.* 106, 833–841. doi: 10.1161/CIRCRESAHA.109.211706

Portaluppi, F., Tiseo, R., Smolensky, M. H., Hermida, R. C., Ayala, D. E., and Fabbian, F. (2012). Circadian rhythms and cardiovascular health. *Sleep Med. Rev.* 16, 151–166. doi: 10.1016/j.smrv.2011.04.003

Prasai, M. J., Mughal, R. S., Wheatcroft, S. B., Kearney, M. T., Grant, P. J., and Scott, E. M. (2013). Diurnal variation in vascular and metabolic function in diet-induced obesity: divergence of insulin resistance and loss of clock rhythm. *Diabetes* 62, 1981–1989. doi: 10.2337/db11-1740

Reavey, M., Saner, H., Paccaud, F., and Marques-Vidal, P. (2013). Exploring the periodicity of cardiovascular events in Switzerland: variation in deaths and hospitalizations across seasons, day of the week and hour of the day. *Int. J. Cardiol.* 168, 2195–2200. doi: 10.1016/j.ijcard.2013.01.224

Scheer, F. A., Hilton, M. F., Mantzoros, C. S., and Shea, S. A. (2009). Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc. Natl. Acad. Sci. U.S.A.* 106, 4453–4458. doi: 10.1073/pnas.0808180106

Scheer, F. A., Hu, K., Evoniuk, H., Kelly, E. E., Malhotra, A., Hilton, M. F., et al. (2010). Impact of the human circadian system, exercise, and their interaction on cardiovascular function. *Proc. Natl. Acad. Sci. U.S.A.* 107, 20541–20546. doi: 10.1073/pnas.1006749107

Scheer, F. A., Michelson, A. D., Frelinger, A. L. III., Evoniuk, H., Kelly, E. E., McCarthy, M., et al. (2011). The human endogenous circadian system causes greatest platelet activation during the biological morning independent of behaviors. *PLoS ONE* 6:e24549. doi: 10.1371/journal.pone.0024549

Scheer, F. A., and Shea, S. A. (2014). Human circadian system causes a morning peak in prothrombotic plasminogen activator inhibitor-1 (PAI-1) independent of the sleep/wake cycle. *Blood* 123, 590–593. doi: 10.1182/blood-2013-07-517060

Schroder, E. A., Lefta, M., Zhang, X., Bartos, D. C., Feng, H. Z., Zhao, Y., et al. (2013). The cardiomyocyte molecular clock, regulation of Scn5a, and arrhythmia susceptibility. American journal of physiology. *Cell Physiol.* 304, C954–C965. doi: 10.1152/ajpcell.00383.2012

Takeda, N., and Maemura, K. (2010). Circadian clock and vascular disease. *Hypert. Res.* 33, 645–651. doi: 10.1038/hr.2010.68

Takeda, N., and Maemura, K. (2011). Circadian clock and cardiovascular disease. *J. Cardiol.* 57, 249–256. doi: 10.1016/j.jjcc.2011.02.006

Takeda, N., Maemura, K., Horie, S., Oishi, K., Imai, Y., Harada, T., et al. (2007). Thrombomodulin is a clock-controlled gene in vascular endothelial cells. *J. Biol. Chem.* 282, 32561–32567. doi: 10.1074/jbc.M705692200

Tofler, G. H., Brezinski, D., Schafer, A. I., Czeisler, C. A., Rutherford, J. D., Willich, S. N., et al. (1987). Concurrent morning increase in platelet aggregability and the risk of myocardial infarction and sudden cardiac death. *N. Engl. J. Med.* 316, 1514–1518. doi: 10.1056/NEJM198706113162405

Viswambharan, H., Carvas, J. M., Antic, V., Marecic, A., Jud, C., Zaugg, C. E., et al. (2007). Mutation of the circadian clock gene Per2 alters vascular endothelial function. *Circulation* 115, 2188–2195. doi: 10.1161/CIRCULATIONAHA.106.653303

Wang, C. Y., Wen, M. S., Wang, H. W., Hsieh, I. C., Li, Y., Liu, P. Y., et al. (2008a). Increased vascular senescence and impaired endothelial progenitor cell function mediated by mutation of circadian gene Per2. *Circulation* 118, 2166–2173. doi: 10.1161/CIRCULATIONAHA.108.790469

Wang, N., Yang, G., Jia, Z., Zhang, H., Aoyagi, T., Soodvilai, S., et al. (2008b). Vascular PPARgamma controls circadian variation in blood pressure and heart rate through Bmal1. *Cell Metab.* 8, 482–491. doi: 10.1016/j.cmet.2008.10.009

Westgate, E. J., Cheng, Y., Reilly, D. F., Price, T. S., Walisser, J. A., Bradfield, C. A., et al. (2008). Genetic components of the circadian clock regulate thrombogenesis *in vivo*. *Circulation* 117, 2087–2095. doi: 10.1161/CIRCULATIONAHA.107.739227

Xie, Z., Su, W., Liu, S., Zhao, G., Esser, K., Schroder, E. A., et al. (2015). Smooth-muscle BMAL1 participates in blood pressure circadian rhythm regulation. *J. Clin. Invest.* 125, 324–336. doi: 10.1172/JCI76881

Yang, G., and Fitzgerald, G. A. (2012). (Almost) Everything is illuminated: adenosine shines a light on cardioprotection. *Circ. Res.* 111, 965–966. doi: 10.1161/CIRCRESAHA.112.279752

Yang, G., Jia, Z., Aoyagi, T., McClain, D., Mortensen, R. M., and Yang, T. (2012). Systemic PPARgamma deletion impairs circadian rhythms of behavior and metabolism. *PLoS ONE* 7:e38117. doi: 10.1371/journal.pone.0038117

Yang, G., Paschos, G., Curtis, A. M., Musiek, E. S., McLoughlin, S. C., and Fitzgerald, G. A. (2013). Knitting up the raveled sleave of care. *Sci. Transl. Med.* 5, 212rv213. doi: 10.1126/scitranslmed.3007225

Young, M. E., Razeghi, P., and Taegtmeyer, H. (2001). Clock genes in the heart: characterization and attenuation with hypertrophy. *Circ. Res.* 88, 1142–1150. doi: 10.1161/hh1101.091190

Young, M. E., Wilson, C. R., Razeghi, P., Guthrie, P. H., and Taegtmeyer, H. (2002). Alterations of the circadian clock in the heart by streptozotocin-induced diabetes. *J. Mol. Cell. Cardiol.* 34, 223–231. doi: 10.1006/jmcc.2001.1504

Zhang, R., Lahens, N. F., Ballance, H. I., Hughes, M. E., and Hogenesch, J. B. (2014). A circadian gene expression atlas in mammals: implications for biology and medicine. *Proc. Natl. Acad. Sci. U.S.A.* 111, 16219–16224. doi: 10.1073/pnas.1408886111

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

MINI REVIEW
published: 17 April 2015
doi: 10.3389/fphar.2015.00077

Therapeutic applications of circadian rhythms for the cardiovascular system

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The cardiovascular system exhibits dramatic time-of-day dependent rhythms, for example the diurnal variation of heart rate, blood pressure, and timing of onset of adverse cardiovascular events such as heart attack and sudden cardiac death. Over the past decade, the circadian clock mechanism has emerged as a crucial factor regulating these daily fluctuations. Most recently, these studies have led to a growing clinical appreciation that targeting circadian biology offers a novel therapeutic approach toward cardiovascular (and other) diseases. Here we describe leading-edge therapeutic applications of circadian biology including (1) timing of therapy to maximize efficacy in treating heart disease (chronotherapy); (2) novel biomarkers discovered by testing for genomic, proteomic, metabolomic, or other factors at different times of day and night (chronobiomarkers); and (3) novel pharmacologic compounds that target the circadian mechanism with potential clinical applications (new chronobiology drugs). Cardiovascular disease remains a leading cause of death worldwide and new approaches in the management and treatment of heart disease are clearly warranted and can benefit patients clinically.

Keywords: chronotherapy, circadian, diurnal, biomarkers, cardiovascular disease

OPEN ACCESS

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Specialty section:
This article was submitted to Pharmaceutical Medicine and Outcomes Research, a section of the journal Frontiers in Pharmacology

Received: *26 January 2015*
Accepted: *26 March 2015*
Published: *17 April 2015*

Citation:
Tsimakouridze EV, Alibhai FJ and Martino TA (2015) Therapeutic applications of circadian rhythms for the cardiovascular system. Front. Pharmacol. 6:77. doi: 10.3389/fphar.2015.00077

Introduction

Cardiovascular disease is the leading cause of death worldwide (Public Health Agency of Canada, 2009; World Health Organization [WHO], 2011; Mozaffarian et al., 2014; Townsend et al., 2014). Available therapies have had only limited success improving long-term survival of patients. In recent years there have been a flurry of studies demonstrating time-of-day variations in drug toxicity and efficacy (reviewed in Smolensky and D'Alonzo, 1988; Smolensky and Peppas, 2007), daily cardiovascular gene and protein expression (reviewed in Martino and Sole, 2009; Durgan and Young, 2010; Paschos and FitzGerald, 2010), and there are reports of new pharmacological compounds targeting the circadian mechanism (reviewed in Chen et al., 2013; Kojetin and Burris, 2014). These have led to novel opportunities to investigate and apply the important field of chronobiology on clinical cardiology, and medicine in general.

The underlying foundation for cardiovascular chronotherapy stems from observations that biological processes in humans (and other mammals) exhibit 24-h daily rhythms, and these are controlled by molecular circadian clocks in the brain, heart, and other organs (**Figures 1A,B**). There are many excellent reviews on the circadian system (reviewed in Hastings et al., 2003; Roenneberg and Merrow, 2005; Dardente and Cermakian, 2007; Mohawk et al., 2012). Cardiovascular physiology appears to follow a rhythm as well; heart rate (HR), blood pressure (BP), and cardiac contractility

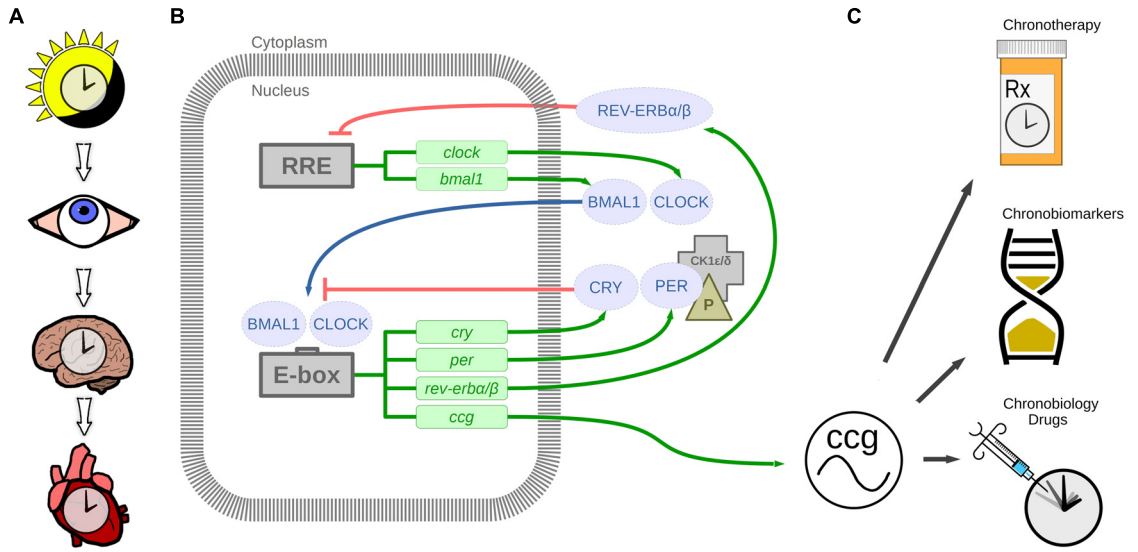

FIGURE 1 | The circadian timing system. (A) Light stimulus is relayed by the eye to the suprachiasmatic nucleus in the brain, which in turn synchronizes the heart and other organ clocks to the day and night environment. **(B)** These signals entrain the molecular clock mechanism, which keeps 24-h time in tissues and cells via transcription-translation feedback loops. BMAL1 and CLOCK are transcribed and translated. BMAL1 and CLOCK heterodimers bind to E-box enhancer elements to promote transcription of *cryptochrome* (CRY), *period* (PER), nuclear receptor subfamily 1, group D, member 1/2 (rev-erbα/β; nr1d1/2), and other clock controlled genes (ccg). Proteins CRY and PER are phosphorylated by casein kinase 1δ/ε (CK1δ/ε) in the cytoplasm, which translocate to the nucleus to repress CLOCK and BMAL1 mediated transcription. Additional loops exist whereby REV-ERBα/β negatively regulates *bmal1* transcription by binding to RRE (REV-ERB/retinoic acid receptor-related orphan receptor (ROR) response element). This mechanism regulates 24-h transcription of clock controlled genes which in play a crucial role in diurnal cardiovascular physiology. **(C)** Therapeutic applications of circadian rhythms include chronotherapy by timing treatment to daily rhythmic processes, chronobiomarkers of differing rhythmic profiles between health and disease, and new chronobiology drugs targeting the circadian clock mechanism.

all peak in the wake hours and reach a nadir during sleep (reviewed in Martino and Sole, 2009; Durgan and Young, 2010; Paschos and FitzGerald, 2010). Indeed, many cardiovascular functions that oscillate over the 24-h period are influenced by the circadian clock mechanism as well as daily fluctuations in the neurohormonal milieu (reviewed in Bray and Young, 2008; Sole and Martino, 2009; Gamble et al., 2014). Timing of onset of cardiac pathologies also follows a rhythm (e.g., onset of myocardial infarction [MI, or heart attack; Muller et al., 1985), and sudden cardiac death (Muller et al., 1987)]. These time-of-day variations in cardiovascular physiology and pathophysiology have led to a growing clinical appreciation that endogenous circadian rhythms may be an important factor to consider in treating disease. Here, we review the current knowledge regarding therapeutic applications of circadian rhythms for the cardiovascular system (**Figure 1C**), specifically (1) timing of therapy (chronotherapy), (2) circadian biomarkers (chronobiomarkers), and (3) how modifiers of the circadian clock mechanism may be useful in the treatment of heart disease.

Chronotherapy

Rationale

Chronotherapy is an important therapeutic application of circadian rhythms for the cardiovascular system. The rationale for chronotherapy is that it offers translational benefit by considering factors such as the underlying circadian rhythms in drug pharmacology, specifically pharmacokinetics (i.e., drug absorption, distribution, metabolism, and excretion) and pharmacodynamics (i.e., affinity and specificity for target receptor binding, downstream intracellular signaling). Chronotherapy also takes into account the patients' underlying physiology and disease pathology (reviewed in Labrecque and Belanger, 1991; Reinberg, 1991; Paschos et al., 2010; Musiek and Fitzgerald, 2013). That the majority of the best-selling drugs and World Health Organization essential medicines target the products of circadian genes provides a mechanistic basis for understanding chronotherapy (Zhang et al., 2014), and provides further support for the clinical application of chronotherapy. Specific examples applied to the treatment of cardiovascular disease are discussed in further detail below. We also created a blog featuring published chronotherapy studies for cardiovascular and other diseases[1].

Chronotherapy Decreases Adverse Cardiovascular Remodeling

In our recent pre-clinical study in mice, we showed that chronotherapy can have direct benefits on the heart in cardiovascular disease models (Martino et al., 2011). Mice with pressure-overload induced cardiac hypertrophy were administered the short-acting angiotensin converting enzyme inhibitor

[1]http://chronobioapp.blogspot.ca/

(ACEi) captopril at either sleep-time or wake-time. We found that only sleep-time administration improves cardiac function, and reduces cardiac remodeling, as compared to wake-time captopril and placebo-treated animals. Mechanistically, captopril given at sleep-time appears to target the peak in the renin-angiotensin-system gene profiles in the heart (Martino et al., 2011). Thus this study demonstrates the direct beneficial effects of chronotherapy for cardiac hypertrophy in the murine model. The important clinical implications are that ACEis given at bedtime can benefit myocardial remodeling in hypertensive patients, or after MI, or in congestive heart failure. Indeed, clinically, ACEis are one of the most commonly prescribed drugs given to hypertensive patients and also for ischemic heart disease (Pfeffer et al., 1992; AIRE, 1993; Ambrosioni et al., 1995; Kober et al., 1995; Yusuf et al., 2000; Fox and EURopean trial On reduction of cardiac events with Perindopril in stable coronary Artery disease Investigators, 2003; Nissen et al., 2004).

Chronotherapy Benefits Daily BP and HR Rhythms

Diurnal BP rhythms are an important part of healthy cardiovascular physiology, and thus are also a key target for chronotherapeutic strategies. Indeed, it is well-known that daily BP profiles are characterized by a dramatic BP surge that occurs around the time of wakening, followed by a progressive fall (∼10%) to reach a nadir during sleep (Floras et al., 1978; Millar-Craig et al., 1978). Conversely, loss of the nocturnal BP fall (non-dipper profile) adversely affects the heart (Verdecchia et al., 1990; Ohkubo et al., 2002; Dolan et al., 2005; Fagard et al., 2009), and chronotherapy to improve the nocturnal BP profile is beneficial. There are many studies that take a chronotherapeutic approach to regulate 24-h BP profiles in hypertensive patients. This includes treatment with ACEis, angiotensin receptor blockers (ARBs), β-blockers, acetylsalicylic acid (aspirin), and combination therapies at specific times of day or night. These studies are summarized in **Table 1**.

Intriguingly, HR also exhibits a rhythm that peaks in the day and is lowest at night (Clarke et al., 1976). The effects of chronotherapy on HR are not as well investigated as with BP profiles, however, several studies have indicated a time-of-day influence of β-blockers on HR. (1) In healthy subjects, the β-blocker propanolol exhibits a significantly faster time to peak effect on HR if taken in the morning (8 A.M.) as compared to late at night (2 A.M; Langner and Lemmer, 1988). (2) The suppressive effect of propranolol on the rise in HR during exercise is significantly greater if the drug is taken in the morning versus at night (Fujimura et al., 1990). (3) In patients with stable coronary disease, myocardial ischemic episodes associated with HR increases are more likely to occur during the day time than at night; propranolol reduces the proportion of these daily HR-related episodes (Andrews et al., 1993). (4) In hypertensive patients, the β-blocker bisoprolol reduces the 24-h ambulatory HR if the drug is taken in the morning (Mengden et al., 1992). (5) Lastly, experimental studies in rodents help confirm that HR is differentially influenced by some β-blockers depending on the time of drug application; propanolol causes a near maximum decrease in HR when given in the light period (rodent sleep time) as compared to the dark period (rodent wake time; Lemmer et al., 1985). Collectively these findings illustrate the importance of maintaining daily BP and HR profiles, and the clinical applicability of chronotherapy to benefit cardiovascular physiology.

Aspirin Chronotherapy and Timing of Acute Cardiovascular Events

In an exciting recent chronotherapy study, it was found that evening administration of low-dose aspirin reduces morning platelet reactivity, via COX-1 dependent pathways, as compared with taking aspirin upon awakening (Bonten et al., 2014). This finding is consistent with earlier reports of a circadian rhythm in platelet surface markers (Scheer et al., 2011), and in platelet aggregability (Andrews et al., 1996). Collectively these studies are clinically important because acute cardiovascular events (e.g., MI) are most likely to occur in the early morning hours vs. other times of day or night (Muller et al., 1985), and platelet reactivity likely contributes to this early morning peak. Thus it is postulated that aspirin chronotherapy taken at bedtime instead of on awakening, as a preventative measure in healthy subjects and by patients with cardiovascular disease, can reduce the incidence of adverse cardiac events during the high-risk morning hours (Bonten et al., 2014). That daily low-dose aspirin reduces the peak frequency of MIs in the morning and overall risk across the 24-h cycle (Ridker et al., 1990), provides further support for this notion.

It is worth noting that several factors important for thrombosis and fibrinolysis in MI, in addition to platelet reactivity and cycling, also exhibit daily rhythms and could provide additional targets for chronotherapy for treatment of acute cardiovascular events. These factors include plasminogen activator inhibitor-1 (PAI-1 a key inhibitor of fibrinolysis; Angleton et al., 1989; Scheer and Shea, 2013), tissue factor pathway inhibitor and factor VII (Pinotti et al., 2005), and plasma fibrinogen (Bremner et al., 2000). Moreover, several experimental rodent studies mechanistically link these coagulation pathways directly to the circadian clock mechanism. That is, transcription of the anti-coagulant factor thrombomodulin is regulated by the mechanism factors CLOCK and BMAL2 heterodimers (Takeda et al., 2007), and PAI-1 transcription is regulated by CLOCK and BMAL proteins (Schoenhard et al., 2003). Endothelial responses to vascular injury also appear to be regulated by the clock mechanism (Westgate et al., 2008). In terms of clinical translation, time-of-day variation in the efficacy of thrombolytic therapy in MI has been reported, which shows a marked early morning resistance and significantly better results later in the day (Reisin et al., 2004). Taken together, these and earlier studies provide support for cardiovascular chronotherapy to limit the pathogenesis and improve treatment following the onset of acute cardiovascular events.

Nocturnal Hemodialysis (NHD) Benefits Cardiovascular Disease

Cardiovascular disease is a significant cause of death in patients with end-stage renal disease (Harnett et al., 1995; Collins et al., 2007), and left ventricular hypertrophy contributes to the high

TABLE 1 | The benefits of chronotherapy for blood pressure (BP) in patients with mild to moderate hypertension.

Drug (dose)	Study design (n)	Chronotherapeutic benefit	Reference
Angiotensin converting enzyme inhibitor (ACEi)			
Quinapril (20 mg/day for 4 weeks)	Double-blind cross-over (18)	Evening quinapril more effectively decreased nighttime BP and 24 h BP profile compared to morning treatment	Palatini (1992)
Enalapril (10 mg/day for 3 weeks)	Randomized cross-over (10)	Evening enalapril caused a greater reduction in nocturnal BP as compared to morning administration	Witte et al. (1993)
Lisinopril (20 mg/day for 2 months)	Randomized cross-over (40)	Evening lisinopril resulted in a largest reduction in morning BP (6 AM–11 AM) as compared to morning and afternoon treatment	Macchiarulo et al. (1999)
Ramipril (5 mg/day for 6 weeks)	PROBE[a] multicenter (115)	Bedtime ramipril led to the largest decrease in sleep-time BP and increased the number of patients with controlled 24 h ambulatory BP	Hermida and Ayala (2009)
Spirapril (6 mg/day for 12 weeks)	Randomized, open-label, parallel group, blinded endpoint (165)	Bedtime spirapril more effectively decreased the nocturnal BP and increased the proportion of patients with controlled 24 h ambulatory BP	Hermida et al. (2010a)
Angiotensin receptor blocker (ARB)			
Valsartan (160 mg/day for 3 months)	PROBE[a] non-dipper (148)	Bedtime valsartan further decreased nocturnal BP mean and led to a greater proportion of patients with dipper profiles and controlled BP over 24 h as compared to treatment upon awakening	Hermida et al. (2005b)
Telmisartan (80 mg/day for 12 weeks)	PROBE[a] (215)	Bedtime telmisartan further decreased sleep-time BP and increased the number of patients with dipper profiles as compared to morning administration	Hermida et al. (2007)
Olmesartan (20 mg/day for 3 months)	PROBE[a] (123)	Bedtime olmesartan resulted in the largest reduction in nocturnal BP mean and decreased prevalence of non-dipping from baseline as compared to the morning dose	Hermida et al. (2009)
β-Blocker			
Nebivolol (5 mg/day for 1 week; titrated to 10 mg/day for 2 weeks)	Single-center, prospective, randomized, double-blind, placebo controlled, cross-over (38)	Evening but not morning, nebivolol significantly decreased morning preawakening systolic BP from baseline	Acelajado et al. (2012)
Non-steroidal anti-inflammatory drugs			
Acetylsalicylic acid (100 mg/day for 3 months)	PROBE[a] (328)	Only bedtime administration reduced the 24 h BP mean, but not treatment upon awakening	Hermida et al. (2005a)
Combination therapy			
Amlodipine (2.5–10 mg) **plus Olmesartan** (20–40 mg) (for 8 weeks)	Randomized, open-label, crossover (31)	Evening treatment significantly decreased the morning BP surge and decreased nocturnal BP in non-dippers as compared with morning treatment	Hoshino et al. (2010)
Valsartan (160 mg/day) **plus Hydrochlo-rothiazide** (12 mg/day) (for 12 weeks)	PROBE,[a] (204)	Bedtime dose more effectively reduced sleep-time systolic BP mean and increased the proportion of patients with controlled sleep-time BP as compared to treatment upon awakening	Hermida et al. (2011)
Valsartan (160 mg/day) **plus Amlodipine** (5 mg/day) (for 12 weeks)	PROBE[a] (203)	Bedtime administration more efficiently decreased the 48h BP mean, lowered sleep-time BP, and had the largest percentage of patients with controlled BP over 24 h compared to morning administration	Hermida et al. (2010b)

[a]*Prospective, randomized, open-label, parallel group, blinded endpoint (PROBE).*

mortality rates in patients given conventional daytime hemodialysis (CHD) treatment (Harnett et al., 1994). Intriguingly, NHD, renal replacement therapy during sleep) offers better BP control (Pierratos et al., 1998; Raj et al., 1999), and is accompanied by regression of left ventricular hypertrophy (Chan et al., 2002), as compared to patients given conventional daytime therapy. In addition to decreasing the nighttime BP, NHD also decreases 24-h mean arterial BP compared to CHD (Chan et al., 2003). These findings of a chronotherapeutic benefit are further corroborated by a randomized controlled clinical trial demonstrating that frequent NHD improves systemic BP and reduces left ventricular mass compared with CHD (Culleton et al., 2007). Mechanistically, the beneficial effects of NHD are associated with changes in myocardial mechanics in patients, and experimentally correlated with unique cardiac gene expression signatures in rodent studies *in vivo* (Chan et al., 2012). These studies demonstrate chronotherapeutic benefit for the heart, in patients with end-stage renal disease, by chronotherapeutically converting from CHD to NHD treatment.

Nocturnal Therapy for Obstructive Sleep Apnea Benefits the Heart

Obstructive sleep apnea (OSA) is a common sleep disorder, with cardiovascular consequences (e.g., through increased sympathetic activation, etc. as has been well reviewed in Bradley and Floras, 2003; Somers et al., 2008; Bradley and Floras, 2009; Kasai and Bradley, 2011; Ayas et al., 2014; Floras, 2014). OSA is a target for chronotherapy, as several studies have revealed that sleep time treatment with continuous positive airway pressure (CPAP) attenuates some of the adverse effects on the cardiovascular system. For example, CPAP therapy decreases the risk of non-fatal and fatal adverse cardiovascular events in severe OSA patients (apnea-hypopnea index >30 h) as compared to untreated patients, as demonstrated in a 10 years long term follow-up study (Marin et al., 2005). In another study, it was shown that CPAP therapy improves ejection fraction, lowers systolic BP, and reduces HR in heart failure patients with OSA (Kaneko et al., 2003). Also, CPAP treatment decreases cardiovascular-related deaths in OSA patients, as compared to an untreated OSA group, as was demonstrated over a follow-up period of 7.5 years (Doherty et al., 2005). Thus these studies underscore the notion that time-of-day therapies, such as nocturnal CPAP treatment, benefits cardiovascular physiology, and reduces pathophysiology in patients with OSA.

Chronobiomarkers

Definition

A second area for therapeutic application of circadian rhythms is in the development of time-of-day biomarkers for heart disease. The National Institutes of Health defines biomarkers as *"a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention"* (Biomarker Definitions Working Group, 2001). Classic biomarkers of cardiovascular disease relate to patient state (e.g., lifestyle risk factor profiles such as diet, exercise, and smoking) or biological processes (e.g., molecular gene and protein levels; reviewed in Jaffe et al., 2006; Maisel et al., 2006; Pletcher and Pignone, 2011). However, in contrast to these classic biomarkers which are measured during the daytime, chronobiomarkers provide a novel approach because clinical sampling is done at different times of day or night. Thus chronobiomarkers (unlike classic biomarkers) take into consideration the time-of-day rhythms important for body physiology and molecular processes. It is worth noting that timing of sampling is also relevant to translational research, since experiments on rodents are routinely performed during the working day when the animals are in their sleep period (rodents are nocturnal) with the intent of comparison to the human daytime. Sampling tissues and detecting biomarkers at different times across the day and night cycle can allow for better correlation with humans. New frontiers investigating molecular chronobiomarkers, with application to the clinical setting, are described below.

Genomic Chronobiomarkers

Genomic chronobiomarkers are the most identifiable type of biomarker because the circadian clock mechanism is transcriptional in nature. That is, many labs have shown that the circadian mechanism underlies gene expression in the heart (and other) organs, and thus investigating how these gene patterns change in heart disease could lead to *de novo* chronobiomarker discoveries. The first large scale study examining rhythmic gene expression in the heart was by Storch et al. (2002), and revealed that ∼8% of genes (mRNA) in the murine heart exhibit circadian variations by microarray and bioinformatics analyses. Of note, this study was done under circadian (constant dark) conditions to elucidate clock controlled genes. However, since humans (and clinical medicine) exist in a 24-h light and dark and not circadian environment, we also demonstrated that ∼13% of murine cardiac genes (mRNA) exhibit rhythmic expression under normal day and night cycles, by microarray and COSOPT bioinformatics analyses (Martino et al., 2004). Most recently rhythmic mRNA profiles have also been shown in human heart tissue for the core clock genes (per1, per2, and bmal1; Leibetseder et al., 2009).

Interestingly, chromatin remodelers play a role in orchestrating time-of-day gene expression, by regulating rhythms in the epigenome (reviewed in Aguilar-Arnal and Sassone-Corsi, 2014), such as the histone deactylases termed silent information regulator 1 (SIRT1; Nakahata et al., 2008), and histone deacetylase 3 (HDAC3; Alenghat et al., 2008), and the histone methyltransferase termed mixed lineage leukemia 1 (MLL1; Katada and Sassone-Corsi, 2010). These are recruited to the promoters of clock controlled genes in a circadian manner, and rhythmic expression of clock controlled genes is altered in the absence of these chromatin modifiers (Alenghat et al., 2008; Nakahata et al., 2008; Katada and Sassone-Corsi, 2010). Moreover, the epigenetic markers of histone acetylation and methylation also exhibit rhythmic oscillations over 24 h (Etchegaray et al., 2003; Vollmers

et al., 2012). In terms of therapeutic potential, pharmacological modulation with SIRT1 activators reduces histone acetylation and decreases the amplitude of circadian gene expression in mice (Bellet et al., 2013).

Since rhythmic gene expression underlies the vital cardiac processes, we also investigated whether time-of-day gene expression signatures could be utilized as *de novo* biomarkers of heart disease (i.e., chronobiomarkers). In a proof-of-concept study, we identified 300 mRNA chronobiomarkers, using a murine model of cardiac hypertrophy (transaortic constriction, TAC), microarrays, and a novel bioinformatics algorithm termed Delta Gene (Tsimakouridze et al., 2012). For example, the mitochondrial metabolism genes uncoupling protein 3 (Ucp3) and pyruvate dehydrogenase kinase 4 (Pdk4) exhibit significantly increased expression in TAC hearts in the light period (animals asleep) but not dark period (animals awake). Conversely, the apoptosis pathway gene BCL2/adenovirus E1B interacting protein 3 (Bnip3) exhibits increased expression in the dark. Moreover, we further demonstrated that day/night gene rhythms change over the course of the disease, and that later profiles can be predictive of heart failure. For example, decreased sleep-time expression of Ucp3 and increased wake-time expression of Bnip3 are simultaneously observed with progression to heart failure. (Tsimakouridze et al., 2012). Further optimization for clinical translation in heart disease would of course need to be considered, such as blood sampling instead of tissue, and the development of gene chips targeting specific disease profiles. Nevertheless, these early studies demonstrate the novelty and feasibility of such an approach, for genomic chronobiomarkers with application to clinical molecular diagnostics.

Proteomic Chronobiomarkers

A second approach is to characterize the proteomic chronobiomarkers instead of the genetic markers. This is important because it is the proteins, and not the mRNA, that underlie many crucial biological processes in health and disease. In support of this approach, we demonstrated that ∼8% of the murine cardiac proteome exhibits significant changes in abundance over the 24-h day and night cycle, by using 2-dimensional difference in gel electrophoresis and liquid chromatography mass spectrometry (Podobed et al., 2012, 2014). Moreover, a role for the circadian clock mechanism is indicated in regulating time-of-day protein abundance, as differences in protein profiles are observed in the hearts of cardiomyocyte-specific clock mutant mice (Podobed et al., 2014). This includes many rate limiting enzymes important for key metabolic pathways in the heart (Podobed et al., 2014). As a proof-of-concept for application to heart disease, we demonstrated that protein chronobiomarkers have characteristic disease signatures in our murine model of TAC-induced cardiac hypertrophy (Podobed et al., 2012, 2014; Tsimakouridze et al., 2012). It is worth noting that although our studies report day/night protein signatures of heart disease, these studies rely on sampling directly from the heart tissue. For routine biomarker testing a more minimally invasive technique would need to be developed, such as detecting time-of-day protein biomarker signatures in the blood. To demonstrate the feasibility of less invasive testing, we showed time-of-day *de novo* chronobiomarkers in murine blood plasma samples, using surface-enhanced laser desorption/ionization mass spectrometry (Martino et al., 2007). In terms of translation, one interesting example illustrating the clinical potential of time-of-day biomarkers in heart disease comes from studies by Dominguez-Rodriguez et al. (2006), who show that nighttime serum melatonin levels are predictive of a subsequent adverse cardiovascular event in patients with ST-segment elevation MI. Thus taken together, these studies demonstrate significant clinical potential for protein chronobiomarkers for the diagnosis, prognosis, and personalized treatment of heart disease.

Metabolomic Chronobiomarkers

The circadian clock regulates metabolism in the body (Turek et al., 2005; Paschos et al., 2012) and in the heart (reviewed in Young, 2006; Durgan and Young, 2010) and thus there is significant opportunity to investigate the circadian metabolome for chronobiomarkers of health and disease. For example, the liver metabolome exhibits rhythmic oscillations and disrupting the circadian clock mechanism alters these profiles (Eckel-Mahan et al., 2012). In another study in humans, it was demonstrated that ∼15% of metabolites in plasma and saliva samples are rhythmic and under circadian control (Dallmann et al., 2012). One clinical application is in the measurement of internal body time-of-day, which may be exploited to maximize efficacy and minimize toxicity of drugs therapies (e.g., for chronotherapy; Ueda et al., 2004). In this regard, the Ueda group designed a molecular-timetable of the murine blood metabolome, quantifying hundreds of clock controlled metabolites, using a liquid chromatography mass spectrometry approach (Minami et al., 2009). This same group subsequently applied their molecular metabolite timetable concept to successfully estimate internal body time in humans (Kasukawa et al., 2012). The CircadiOmics website provides a consolidated model that integrates these metabolomic data with genomics and proteomics, to better understand time-of-day coordination of physiology/pathophysiology (Patel et al., 2012). Indeed, taken together these data reveal the convenience and feasibility of adopting time-of-day testing for clinical use. It is tempting to speculate that additional "-omics" approaches, such as lipidomics or breathomics, could also be developed in the future as valuable clinical tools for personalized medicine.

New Frontiers for Chronobiology Drugs

Recently, there has been a new focus on the creation of pharmacological compounds designed to target the REV-ERB and ROR nuclear receptors in the circadian mechanism, with clinical applications (reviewed in Kojetin and Burris, 2014). For example, administering REV-ERB agonists to mice alters their circadian behavior and hypothalamic gene expression, leading to

the notion that these drugs may be useful in the treatment of metabolic disorders (Solt et al., 2012). Since REV-ERB also plays a key role in regulating mitochondrial content and the oxidative capacity of skeletal muscle, it is postulated that pharmacologic activation of REV-ERB may also be used to treat skeletal muscle diseases (Woldt et al., 2013). Moreover, it was recently shown that REV-ERB agonists can regulate sleep architecture and emotion in mice, and thus they may be useful in the treatment of sleep disorders and anxiety (Banerjee et al., 2014). There are new pharmacological agents that modulate other components of the circadian clock mechanism as well (e.g., reviewed in Chen et al., 2013); some of these hold considerable promise for offsetting the adverse effects of shift work (e.g., Walton et al., 2009; Meng et al., 2010; Pilorz et al., 2014). Most recently it was demonstrated that human peripheral blood mononuclear cell clocks are entrained by glucocorticoids, and that pharmacologic treatment directed at these peripheral targets could also help counteract the deleterious effects of shift work (Cuesta et al., 2014). Although the new chronobiology drugs have not yet been examined in heart disease, it is tempting to speculate that they may be useful, especially in light of their influences on muscle metabolism, on sleep, and on circadian phase, that they may benefit cardiovascular physiology and pathophysiology.

Conclusions and future directions

In terms of future directions in basic science, use of murine transgenic models and pharmacologic approaches will undoubtedly provide new pre-clinical insights into how targeting the circadian mechanism can contribute to the diagnosis and management of heart disease. In terms of clinical chronotherapy, the US public clinical trials database (ClinicalTrials.gov., 2015) already lists seven studies when the search term "cardiovascular chronotherapy" is used, and 18 studies for "chronotherapy" in general, attesting to the clinical promise that chronotherapeutic treatments may hold. There are also significant opportunities to discover *de novo* chronobiomarker tests, for product development by biotechnology sectors, and for establishing routine applications in chronobiology, and sleep clinics. Thus therapeutic consideration of circadian rhythms for the cardiovascular system is an exciting new area with significant clinical potential.

Acknowledgment

This work was supported by funding from CIHR to TM.

References

Acelajado, M. C., Pisoni, R., Dudenbostel, T., Oparil, S., Calhoun, D. A., and Glasser, S. P. (2012). Both morning and evening dosing of nebivolol reduces trough mean blood pressure surge in hypertensive patients. *J. Am. Soc. Hypertens.* 6, 66–72. doi: 10.1016/j.jash.2011.09.001

Aguilar-Arnal, L., and Sassone-Corsi, P. (2014). Chromatin landscape and circadian dynamics: spatial and temporal organization of clock transcription. *Proc. Natl. Acad. Sci. U.S.A.* doi: 10.1073/pnas.1411264111 [Epub ahead of print].

AIRE. (1993). Effect of ramipril on mortality and morbidity of survivors of acute myocardial infarction with clinical evidence of heart failure. The acute infarction ramipril efficacy (AIRE) study Investigators. *Lancet* 342, 821–828.

Alenghat, T., Meyers, K., Mullican, S. E., Leitner, K., Adeniji-Adele, A., Avila, J., et al. (2008). Nuclear receptor corepressor and histone deacetylase 3 govern circadian metabolic physiology. *Nature* 456, 997–1000. doi: 10.1038/nature07541

Ambrosioni, E., Borghi, C., and Magnani, B. (1995). The effect of the angiotensin-converting-enzyme inhibitor zofenopril on mortality and morbidity after anterior myocardial infarction. The survival of myocardial infarction long-term evaluation (SMILE) study investigators. *N. Engl. J. Med.* 332, 80–85. doi: 10.1056/NEJM199501123320203

Andrews, N. P., Gralnick, H. R., Merryman, P., Vail, M., and Quyyumi, A. A. (1996). Mechanisms underlying the morning increase in platelet aggregation: a flow cytometry study. *J. Am. Coll. Cardiol.* 28, 1789–1795. doi: 10.1016/S0735-1097(96)00398-1

Andrews, T. C., Fenton, T., Toyosaki, N., Glasser, S. P., Young, P. M., Maccallum, G., et al. (1993). Subsets of ambulatory myocardial ischemia based on heart rate activity. Circadian distribution and response to anti-ischemic medication. The angina and silent ischemia study group (ASIS). *Circulation* 88, 92–100. doi: 10.1161/01.CIR.88.1.92

Angleton, P., Chandler, W. L., and Schmer, G. (1989). Diurnal variation of tissue-type plasminogen activator and its rapid inhibitor (PAI-1). *Circulation* 79, 101–106. doi: 10.1161/01.CIR.79.1.101

Ayas, N. T., Hirsch, A. A., Laher, I., Bradley, T. D., Malhotra, A., Polotsky, V. Y., et al. (2014). New frontiers in obstructive sleep apnoea. *Clin. Sci.* (*Lond.*) 127, 209–216. doi: 10.1042/CS20140070

Banerjee, S., Wang, Y., Solt, L. A., Griffett, K., Kazantzis, M., Amador, A., et al. (2014). Pharmacological targeting of the mammalian clock regulates sleep architecture and emotional behaviour. *Nat. Commun.* 5, 5759. doi: 10.1038/ncomms6759

Bellet, M. M., Nakahata, Y., Boudjelal, M., Watts, E., Mossakowska, D. E., Edwards, K. A., et al. (2013). Pharmacological modulation of circadian rhythms by synthetic activators of the deacetylase SIRT1. *Proc. Natl. Acad. Sci. U.S.A.* 110, 3333–3338. doi: 10.1073/pnas.1214266110

Biomarker Definitions Working Group, N. I. O. H. (2001). Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Biomarkers definitions working group. *Clin. Pharmacol. Ther.* 69, 89–95. doi: 10.1067/mcp.2001.113989

Bonten, T. N., Saris, A., Van Oostrom, M. J., Snoep, J. D., Rosendaal, F. R., Zwaginga, J., et al. (2014). Effect of aspirin intake at bedtime versus on awakening on circadian rhythm of platelet reactivity. A randomised cross-over trial. *Thromb. Haemost.* 112, 1209–1218. doi: 10.1160/TH14-05-0453

Bradley, T. D., and Floras, J. S. (2003). Sleep apnea and heart failure: Part I: obstructive sleep apnea. *Circulation* 107, 1671–1678. doi: 10.1161/01.CIR.0000061757.12581.15

Bradley, T. D., and Floras, J. S. (2009). Obstructive sleep apnoea and its cardiovascular consequences. *Lancet* 373, 82–93. doi: 10.1016/S0140-6736(08)61622-0

Bray, M. S., and Young, M. E. (2008). Diurnal variations in myocardial metabolism. *Cardiovasc. Res.* 79, 228–237. doi: 10.1093/cvr/cvn054

Bremner, W. F., Sothern, R. B., Kanabrocki, E. L., Ryan, M., Mccormick, J. B., Dawson, S., et al. (2000). Relation between circadian patterns in levels of circulating lipoprotein(a), fibrinogen, platelets, and related lipid variables in men. *Am. Heart. J.* 139, 164–173. doi: 10.1016/S0002-8703(00)90324-7

Chan, C. T., Arab, S., Carasso, S., Moravsky, G., Li, G. H., Liu, P. P., et al. (2012). Impact of frequent nocturnal hemodialysis on myocardial mechanics and cardiomyocyte gene expression. *Circ. Cardiovasc. Imaging* 5, 474–480. doi: 10.1161/CIRCIMAGING.111.971606

Chan, C. T., Floras, J. S., Miller, J. A., Richardson, R. M., and Pierratos, A. (2002). Regression of left ventricular hypertrophy after conversion to nocturnal hemodialysis. *Kidney Int.* 61, 2235–2239. doi: 10.1046/j.1523-1755.2002.00362.x

Chan, C. T., Harvey, P. J., Picton, P., Pierratos, A., Miller, J. A., and Floras, J. S. (2003). Short-term blood pressure, noradrenergic, and vascular effects of nocturnal home hemodialysis. *Hypertension* 42, 925–931. doi: 10.1161/01.HYP.0000097605.35343.64

Chen, Z., Yoo, S. H., and Takahashi, J. S. (2013). Small molecule modifiers of circadian clocks. *Cell Mol. Life. Sci.* 70, 2985–2998. doi: 10.1007/s00018-012-1207-y

Clarke, J. M., Hamer, J., Shelton, J. R., Taylor, S., and Venning, G. R. (1976). The rhythm of the normal human heart. *Lancet* 1, 508–512. doi: 10.1016/S0140-6736(76)90801-1

ClinicalTrials.gov. (2015). *National Institutes of Health (US)*. Available at: https://clinicaltrials.gov/ct2/home (accessed January 24, 2015).

Collins, A. J., Kasiske, B., Herzog, C., Chavers, B., Foley, R., Gilbertson, D., et al. (2007). Excerpts from the United States renal data system 2006 annual data report. *Am. J. Kidney Dis.* 49(1 Suppl. 1), A6–A7, S1–S296. doi: 10.1053/j.ajkd.2006.11.019

Cuesta, M., Cermakian, N., and Boivin, D. B. (2014). Glucocorticoids entrain molecular clock components in human peripheral cells. *FASEB J.* doi: 10.1096/fj.14-265686 [Epub ahead of print].

Culleton, B. F., Walsh, M., Klarenbach, S. W., Mortis, G., Scott-Douglas, N., Quinn, R. R., et al. (2007). Effect of frequent nocturnal hemodialysis vs conventional hemodialysis on left ventricular mass and quality of life: a randomized controlled trial. *JAMA* 298, 1291–1299. doi: 10.1001/jama.298.11.1291

Dallmann, R., Viola, A. U., Tarokh, L., Cajochen, C., and Brown, S. A. (2012). The human circadian metabolome. *Proc. Natl. Acad. Sci. U.S.A.* 109, 2625–2629. doi: 10.1073/pnas.1114410109

Dardente, H., and Cermakian, N. (2007). Molecular circadian rhythms in central and peripheral clocks in mammals. *Chronobiol. Int.* 24, 195–213. doi: 10.1080/07420520701283693

Doherty, L. S., Kiely, J. L., Swan, V., and Mcnicholas, W. T. (2005). Long-term effects of nasal continuous positive airway pressure therapy on cardiovascular outcomes in sleep apnea syndrome. *Chest* 127, 2076–2084. doi: 10.1378/chest.127.6.2076

Dolan, E., Stanton, A., Thijs, L., Hinedi, K., Atkins, N., Mcclory, S., et al. (2005). Superiority of ambulatory over clinic blood pressure measurement in predicting mortality: the dublin outcome study. *Hypertension* 46, 156–161. doi: 10.1161/01.HYP.0000170138.56903.7a

Dominguez-Rodriguez, A., Abreu-Gonzalez, P., Garcia-Gonzalez, M., and Reiter, R. J. (2006). Prognostic value of nocturnal melatonin levels as a novel marker in patients with ST-segment elevation myocardial infarction. *Am. J. Cardiol.* 97, 1162–1164. doi: 10.1016/j.amjcard.2005.11.033

Durgan, D. J., and Young, M. E. (2010). The cardiomyocyte circadian clock: emerging roles in health and disease. *Circ. Res.* 106, 647–658. doi: 10.1161/CIRCRESAHA.109.209957

Eckel-Mahan, K. L., Patel, V. R., Mohney, R. P., Vignola, K. S., Baldi, P., and Sassone-Corsi, P. (2012). Coordination of the transcriptome and metabolome by the circadian clock. *Proc. Natl. Acad. Sci. U.S.A.* 109, 5541–5546. doi: 10.1073/pnas.1118726109

Etchegaray, J. P., Lee, C., Wade, P. A., and Reppert, S. M. (2003). Rhythmic histone acetylation underlies transcription in the mammalian circadian clock. *Nature* 421, 177–182. doi: 10.1038/nature01314

Fagard, R. H., Thijs, L., Staessen, J. A., Clement, D. L., De Buyzere, M. L., and De Bacquer, D. A. (2009). Night-day blood pressure ratio and dipping pattern as predictors of death and cardiovascular events in hypertension. *J. Hum. Hypertens.* 23, 645–653. doi: 10.1038/jhh.2009.9

Floras, J. S. (2014). Sleep apnea and cardiovascular risk. *J. Cardiol.* 63, 3–8. doi: 10.1016/j.jjcc.2013.08.009

Floras, J. S., Jones, J. V., Johnston, J. A., Brooks, D. E., Hassan, M. O., and Sleight, P. (1978). Arousal and the circadian rhythm of blood pressure. *Clin. Sci. Mol. Med. Suppl.* 4, 395s–397s. doi: 10.1016/j.cjca.2014.02.008ů3.12

Fox, K. M., and EURopean trial On reduction of cardiac events with Perindopril in stable coronary Artery disease Investigators. (2003). Efficacy of perindopril in reduction of cardiovascular events among patients with stable coronary artery disease: randomised, double-blind, placebo-controlled, multicentre trial (the EUROPA study). *Lancet* 362, 782–788. doi: 10.1016/S0140-6736(03)14286-9

Fujimura, A., Kumagai, Y., Sugimoto, K., Nakashima, H., Kajiyama, H., Ebihara, A., et al. (1990). Circadian influence on effect of propranolol on exercise-induced tachycardia in healthy subjects. *Eur. J. Clin. Pharmacol.* 38, 133–137. doi: 10.1007/BF00265971

Gamble, K. L., Berry, R., Frank, S. J., and Young, M. E. (2014). Circadian clock control of endocrine factors. *Nat. Rev. Endocrinol.* 10, 466–475. doi: 10.1038/nrendo.2014.78

Harnett, J. D., Foley, R. N., Kent, G. M., Barre, P. E., Murray, D., and Parfrey, P. S. (1995). Congestive heart failure in dialysis patients: prevalence, incidence, prognosis and risk factors. *Kidney Int.* 47, 884–890. doi: 10.1038/ki.1995.132

Harnett, J. D., Kent, G. M., Barre, P. E., Taylor, R., and Parfrey, P. S. (1994). Risk factors for the development of left ventricular hypertrophy in a prospectively followed cohort of dialysis patients. *J. Am. Soc. Nephrol.* 4, 1486–1490.

Hastings, M. H., Reddy, A. B., and Maywood, E. S. (2003). A clockwork web: circadian timing in brain and periphery, in health and disease. *Nat. Rev. Neurosci.* 4, 649–661. doi: 10.1038/nrn1177

Hermida, R. C., and Ayala, D. E. (2009). Chronotherapy with the angiotensin-converting enzyme inhibitor ramipril in essential hypertension: improved blood pressure control with bedtime dosing. *Hypertension* 54, 40–46. doi: 10.1161/HYPERTENSIONAHA.109.130203

Hermida, R. C., Ayala, D. E., Calvo, C., and Lopez, J. E. (2005a). Aspirin administered at bedtime, but not on awakening, has an effect on ambulatory blood pressure in hypertensive patients. *J. Am. Coll. Cardiol.* 46, 975–983. doi: 10.1016/j.jacc.2004.08.071

Hermida, R. C., Calvo, C., Ayala, D. E., Fernandez, J. R., Covelo, M., Mojon, A., et al. (2005b). Treatment of non-dipper hypertension with bedtime administration of valsartan. *J. Hypertens.* 23, 1913–1922. doi: 10.1097/01.hjh.0000182522.21569.c5

Hermida, R. C., Ayala, D. E., Chayan, L., Mojon, A., and Fernandez, J. R. (2009). Administration-time-dependent effects of olmesartan on the ambulatory blood pressure of essential hypertension patients. *Chronobiol. Int.* 26, 61–79. doi: 10.1080/07420520802548135

Hermida, R. C., Ayala, D. E., Fernandez, J. R., and Calvo, C. (2007). Comparison of the efficacy of morning versus evening administration of telmisartan in essential hypertension. *Hypertension* 50, 715–722. doi: 10.1161/HYPERTENSIONAHA.107.094235

Hermida, R. C., Ayala, D. E., Fontao, M. J., Mojon, A., Alonso, I., and Fernandez, J. R. (2010a). Administration-time-dependent effects of spirapril on ambulatory blood pressure in uncomplicated essential hypertension. *Chronobiol. Int.* 27, 560–574. doi: 10.3109/07420528.2010.485411

Hermida, R. C., Ayala, D. E., Fontao, M. J., Mojon, A., and Fernandez, J. R. (2010b). Chronotherapy with valsartan/amlodipine fixed combination: improved blood pressure control of essential hypertension with bedtime dosing. *Chronobiol. Int.* 27, 1287–1303. doi: 10.3109/07420528.2010.489167

Hermida, R. C., Ayala, D. E., Mojon, A., Fontao, M. J., and Fernandez, J. R. (2011). Chronotherapy with valsartan/hydrochlorothiazide combination in essential hypertension: improved sleep-time blood pressure control with bedtime dosing. *Chronobiol. Int.* 28, 601–610. doi: 10.3109/07420528.2011.589935

Hoshino, A., Nakamura, T., and Matsubara, H. (2010). The bedtime administration ameliorates blood pressure variability and reduces urinary albumin excretion in amlodipine-olmesartan combination therapy. *Clin. Exp. Hypertens.* 32, 416–422. doi: 10.3109/10641961003667948

Jaffe, A. S., Babuin, L., and Apple, F. S. (2006). Biomarkers in acute cardiac disease: the present and the future. *J. Am. Coll. Cardiol.* 48, 1–11. doi: 10.1016/j.jacc.2006.02.056

Kaneko, Y., Floras, J. S., Usui, K., Plante, J., Tkacova, R., Kubo, T., et al. (2003). Cardiovascular effects of continuous positive airway pressure in patients with heart failure and obstructive sleep apnea. *N. Engl. J. Med.* 348, 1233–1241. doi: 10.1056/NEJMoa022479

Kasai, T., and Bradley, T. D. (2011). Obstructive sleep apnea and heart failure: pathophysiologic and therapeutic implications. *J. Am. Coll. Cardiol.* 57, 119–127. doi: 10.1016/j.jacc.2010.08.627

Kasukawa, T., Sugimoto, M., Hida, A., Minami, Y., Mori, M., Honma, S., et al. (2012). Human blood metabolite timetable indicates internal body time. *Proc. Natl. Acad. Sci. U.S.A.* 109, 15036–15041. doi: 10.1073/pnas.1207768109

Katada, S., and Sassone-Corsi, P. (2010). The histone methyltransferase MLL1 permits the oscillation of circadian gene expression. *Nat. Struct. Mol. Biol.* 17, 1414–1421. doi: 10.1038/nsmb.1961

Kober, L., Torp-Pedersen, C., Carlsen, J. E., Bagger, H., Eliasen, P., Lyngborg, K., et al. (1995). A clinical trial of the angiotensin-converting-enzyme inhibitor trandolapril in patients with left ventricular dysfunction after myocardial infarction. Trandolapril cardiac evaluation (TRACE) study group. *N. Engl. J. Med.* 333, 1670–1676. doi: 10.1056/NEJM199512213332503

Kojetin, D. J., and Burris, T. P. (2014). REV-ERB and ROR nuclear receptors as drug targets. *Nat. Rev. Drug Discov.* 13, 197–216. doi: 10.1038/nrd4100

Labrecque, G., and Belanger, P. M. (1991). Biological rhythms in the absorption, distribution, metabolism and excretion of drugs. *Pharmacol. Ther.* 52, 95–107. doi: 10.1016/0163-7258(91)90088-4

Langner, B., and Lemmer, B. (1988). Circadian changes in the pharmacokinetics and cardiovascular effects of oral propranolol in healthy subjects. *Eur. J. Clin. Pharmacol.* 33, 619–624. doi: 10.1007/BF00542498

Leibetseder, V., Humpeler, S., Svoboda, M., Schmid, D., Thalhammer, T., Zuckermann, A., et al. (2009). Clock genes display rhythmic expression in human hearts. *Chronobiol. Int.* 26, 621–636. doi: 10.1080/07420520902924939

Lemmer, B., Winkler, H., Ohm, T., and Fink, M. (1985). Chronopharmacokinetics of β-receptor blocking drugs of different lipophilicity (propranolol, metoprolol, sotalol, atenolol) in plasma and tissues after single and multiple dosing in the rat. *Naunyn Schmiedebergs Arch. Pharmacol.* 330, 42–49. doi: 10.1007/BF00586708

Macchiarulo, C., Pieri, R., Mitolo, D. C., and Pirrelli, A. (1999). Management of antihypertensive treatment with Lisinopril: a chronotherapeutic approach. *Eur. Rev. Med. Pharmacol. Sci.* 3, 269–275.

Maisel, A. S., Bhalla, V., and Braunwald, E. (2006). Cardiac biomarkers: a contemporary status report. *Nat. Clin. Pract. Cardiovasc. Med.* 3, 24–34. doi: 10.1038/ncpcardio0405

Marin, J. M., Carrizo, S. J., Vicente, E., and Agusti, A. G. (2005). Long-term cardiovascular outcomes in men with obstructive sleep apnoea-hypopnoea with or without treatment with continuous positive airway pressure: an observational study. *Lancet* 365, 1046–1053. doi: 10.1016/S0140-6736(05)71141-7

Martino, T., Arab, S., Straume, M., Belsham, D. D., Tata, N., Cai, F., et al. (2004). Day/night rhythms in gene expression of the normal murine heart. *J. Mol. Med.* 82, 256–264. doi: 10.1007/s00109-003-0520-1

Martino, T. A., and Sole, M. J. (2009). Molecular time: an often overlooked dimension to cardiovascular disease. *Circ. Res.* 105, 1047–1061. doi: 10.1161/CIRCRESAHA.109.206201

Martino, T. A., Tata, N., Bjarnason, G. A., Straume, M., and Sole, M. J. (2007). Diurnal protein expression in blood revealed by high throughput mass spectrometry proteomics and implications for translational medicine and body time of day. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 293, R1430–R1437. doi: 10.1152/ajpregu.00183.2007

Martino, T. A., Tata, N., Simpson, J. A., Vanderlaan, R., Dawood, F., Kabir, M. G., et al. (2011). The primary benefits of angiotensin-converting enzyme inhibition on cardiac remodeling occur during sleep time in murine pressure overload hypertrophy. *J. Am. Coll. Cardiol.* 57, 2020–2028. doi: 10.1016/j.jacc.2010.11.022

Meng, Q. J., Maywood, E. S., Bechtold, D. A., Lu, W. Q., Li, J., Gibbs, J. E., et al. (2010). Entrainment of disrupted circadian behavior through inhibition of casein kinase 1 (CK1) enzymes. *Proc. Natl. Acad. Sci. U.S.A.* 107, 15240–15245. doi: 10.1073/pnas.1005101107

Mengden, T., Battig, B., Schubert, M., Jeck, T., Weisser, B., Buddeberg, C., et al. (1992). Comparison of casual, ambulatory and self-measured blood pressure in a study of nitrendipine vs bisoprolol. *Eur. J. Clin. Pharmacol.* 42, 569–575. doi: 10.1007/BF00265917

Millar-Craig, M. W., Bishop, C. N., and Raftery, E. B. (1978). Circadian variation of blood-pressure. *Lancet* 1, 795–797. doi: 10.1016/S0140-6736(78)92998-7

Minami, Y., Kasukawa, T., Kakazu, Y., Iigo, M., Sugimoto, M., Ikeda, S., et al. (2009). Measurement of internal body time by blood metabolomics. *Proc. Natl. Acad. Sci. U.S.A.* 106, 9890–9895. doi: 10.1073/pnas.0900617106

Mohawk, J. A., Green, C. B., and Takahashi, J. S. (2012). Central and peripheral circadian clocks in mammals. *Annu. Rev. Neurosci.* 35, 445–462. doi: 10.1146/annurev-neuro-060909-153128

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., et al. (2014). Heart disease and stroke statistics-2015 update: a report from the American heart association. *Circulation* 131, e29–e322. doi: 10.1161/CIR.0000000000000152

Muller, J. E., Ludmer, P. L., Willich, S. N., Tofler, G. H., Aylmer, G., Klangos, I., et al. (1987). Circadian variation in the frequency of sudden cardiac death. *Circulation* 75, 131–138. doi: 10.1161/01.CIR.75.1.131

Muller, J. E., Stone, P. H., Turi, Z. G., Rutherford, J. D., Czeisler, C. A., Parker, C., et al. (1985). Circadian variation in the frequency of onset of acute myocardial infarction. *N. Engl. J. Med.* 313, 1315–1322. doi: 10.1056/NEJM198511213132103

Musiek, E. S., and Fitzgerald, G. A. (2013). Molecular clocks in pharmacology. *Handb. Exp. Pharmacol.* 217, 243–260. doi: 10.1007/978-3-642-25950-0_10

Nakahata, Y., Kaluzova, M., Grimaldi, B., Sahar, S., Hirayama, J., Chen, D., et al. (2008). The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. *Cell* 134, 329–340. doi: 10.1016/j.cell.2008.07.002

Nissen, S. E., Tuzcu, E. M., Libby, P., Thompson, P. D., Ghali, M., Garza, D., et al. (2004). Effect of antihypertensive agents on cardiovascular events in patients with coronary disease and normal blood pressure: the CAMELOT study: a randomized controlled trial. *JAMA* 292, 2217–2225. doi: 10.1001/jama.292.18.2217

Ohkubo, T., Hozawa, A., Yamaguchi, J., Kikuya, M., Ohmori, K., Michimata, M., et al. (2002). Prognostic significance of the nocturnal decline in blood pressure in individuals with and without high 24-h blood pressure: the Ohasama study. *J. Hypertens.* 20, 2183–2189. doi: 10.1097/00004872-200211000-00017

Palatini, P. (1992). Can an angiotensin-converting enzyme inhibitor with a short half-life effectively lower blood pressure for 24 hours? *Am. Heart J.* 123, 1421–1425. doi: 10.1016/0002-8703(92)91064-8

Paschos, G. K., Baggs, J. E., Hogenesch, J. B., and Fitzgerald, G. A. (2010). The role of clock genes in pharmacology. *Annu. Rev. Pharmacol. Toxicol.* 50, 187–214. doi: 10.1146/annurev.pharmtox.010909.105621

Paschos, G. K., and FitzGerald, G. A. (2010). Circadian clocks and vascular function. *Circ. Res.* 106, 833–841. doi: 10.1161/CIRCRESAHA.109.211706

Paschos, G. K., Ibrahim, S., Song, W. L., Kunieda, T., Grant, G., Reyes, T. M., et al. (2012). Obesity in mice with adipocyte-specific deletion of clock component Arntl. *Nat. Med.* 18, 1768–1777. doi: 10.1038/nm.2979

Patel, V. R., Eckel-Mahan, K., Sassone-Corsi, P., and Baldi, P. (2012). CircadiOmics: integrating circadian genomics, transcriptomics, proteomics and metabolomics. *Nat. Methods* 9, 772–773. doi: 10.1038/nmeth.2111

Pfeffer, M. A., Braunwald, E., Moye, L. A., Basta, L., Brown, E. J. Jr., Cuddy, T. E., et al. (1992). Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. Results of the survival and ventricular enlargement trial. The SAVE investigators. *N. Engl. J. Med.* 327, 669–677. doi: 10.1056/NEJM199209033271001

Pierratos, A., Ouwendyk, M., Francoeur, R., Vas, S., Raj, D. S., Ecclestone, A. M., et al. (1998). Nocturnal hemodialysis: three-year experience. *J. Am. Soc. Nephrol.* 9, 859–868.

Pilorz, V., Cunningham, P. S., Jackson, A., West, A. C., Wager, T. T., Loudon, A. S., et al. (2014). A novel mechanism controlling resetting speed of the circadian clock to environmental stimuli. *Curr. Biol.* 24, 766–773. doi: 10.1016/j.cub.2014.02.027

Pinotti, M., Bertolucci, C., Portaluppi, F., Colognesi, I., Frigato, E., Foa, A., et al. (2005). Daily and circadian rhythms of tissue factor pathway inhibitor and factor VII activity. *Arterioscler. Thromb. Vasc. Biol.* 25, 646–649. doi: 10.1161/01.ATV.0000153140.13148.e0

Pletcher, M. J., and Pignone, M. (2011). Evaluating the clinical utility of a biomarker: a review of methods for estimating health impact. *Circulation* 123, 1116–1124. doi: 10.1161/CIRCULATIONAHA.110.943860

Podobed, P. S., Kirby, G. M., and Martino, T. A. (2012). "Circadian proteomics and Its unique advantage for discovery of biomarkers of heart disease," in *Proteomics. Human Diseases and Protein Functions*, eds T. K. Man and R. J. Flores (Rijeka: InTech), 65–88.

Podobed, P., Pyle, W. G., Ackloo, S., Alibhai, F. J., Tsimakouridze, E. V., Ratcliffe, W. F., et al. (2014). The day/night proteome in the murine heart. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* R121–R137. doi: 10.1152/ajpregu.00011.2014

Public Health Agency of Canada. (2009). *Tracking Heart Disease and Stroke in Canada, 2009*. Ottawa: Public Health Agency of Canada.

Raj, D. S., Charra, B., Pierratos, A., and Work, J. (1999). In search of ideal hemodialysis: is prolonged frequent dialysis the answer? *Am. J. Kidney Dis.* 34, 597–610. doi: 10.1016/S0272-6386(99)70382-3

Reinberg, A. E. (1991). Concepts of circadian chronopharmacology. *Ann. N. Y. Acad. Sci.* 618, 102–115. doi: 10.1111/j.1749-6632.1991.tb27239.x

Reisin, L. H., Pancheva, N., Berman, M., Khalameizer, V., Jafary, J., Yosefy, C., et al. (2004). Circadian variation of the efficacy of thrombolytic therapy in acute

myocardial infarction-isn't the time ripe for cardiovascular chronotherapy? *Angiology* 55, 257–263. doi: 10.1177/000331970405500304

Ridker, P. M., Manson, J. E., Buring, J. E., Muller, J. E., and Hennekens, C. H. (1990). Circadian variation of acute myocardial infarction and the effect of low-dose aspirin in a randomized trial of physicians. *Circulation* 82, 897–902. doi: 10.1161/01.CIR.82.3.897

Roenneberg, T., and Merrow, M. (2005). Circadian clocks - the fall and rise of physiology. *Nat. Rev. Mol. Cell Biol.* 6, 965–971. doi: 10.1038/nrm1766

Scheer, F. A., Michelson, A. D., Frelinger, A. L. III, Evoniuk, H., Kelly, E. E., Mccarthy, M., et al. (2011). The human endogenous circadian system causes greatest platelet activation during the biological morning independent of behaviors. *PLoS ONE* 6:e24549. doi: 10.1371/journal.pone.0024549

Scheer, F. A., and Shea, S. A. (2013). Human circadian system causes morning peak in pro-thrombotic plasminogen activator inhibitor-1 (PAI-1) independent of sleep/wake cycle. *Blood* 123, 590–593. doi: 10.1182/blood-2013-07-517060

Schoenhard, J. A., Smith, L. H., Painter, C. A., Eren, M., Johnson, C. H., and Vaughan, D. E. (2003). Regulation of the PAI-1 promoter by circadian clock components: differential activation by BMAL1 and BMAL2. *J. Mol. Cell Cardiol.* 35, 473–481. doi: 10.1016/S0022-2828(03)00051-8

Smolensky, M. H., and D'Alonzo, G. E. (1988). Biologic rhythms and medicine. *Am. J. Med.* 85, 34–46. doi: 10.1016/0002-9343(88)90240-9

Smolensky, M. H., and Peppas, N. A. (2007). Chronobiology, drug delivery, and chronotherapeutics. *Adv. Drug Deliv. Rev.* 59, 828–851. doi: 10.1016/j.addr.2007.07.001

Sole, M. J., and Martino, T. A. (2009). Diurnal physiology: core principles with application to the pathogenesis, diagnosis, prevention, and treatment of myocardial hypertrophy and failure. *J. Appl. Physiol.* 107, 1318–1327. doi: 10.1152/japplphysiol.00426.2009

Solt, L. A., Wang, Y., Banerjee, S., Hughes, T., Kojetin, D. J., Lundasen, T., et al. (2012). Regulation of circadian behaviour and metabolism by synthetic REV-ERB agonists. *Nature* 485, 62–68. doi: 10.1038/nature11030

Somers, V. K., White, D. P., Amin, R., Abraham, W. T., Costa, F., Culebras, A., et al. (2008). Sleep apnea and cardiovascular disease: an American Heart Association/American College of Cardiology Foundation Scientific Statement from the American Heart Association Council for High Blood Pressure Research Professional Education Committee, Council on Clinical Cardiology, Stroke Council, and Council on Cardiovascular Nursing. *J. Am. Coll. Cardiol.* 52, 686–717. doi: 10.1016/j.jacc.2008.05.002

Storch, K. F., Lipan, O., Leykin, I., Viswanathan, N., Davis, F. C., Wong, W. H., et al. (2002). Extensive and divergent circadian gene expression in liver and heart. *Nature* 417, 78–83. doi: 10.1038/nature744

Takeda, N., Maemura, K., Horie, S., Oishi, K., Imai, Y., Harada, T., et al. (2007). Thrombomodulin is a clock-controlled gene in vascular endothelial cells. *J. Biol. Chem.* 282, 32561–32567. doi: 10.1074/jbc.M705692200

Townsend, N., Williams, J., Bhatnagar, P., Wickramasinghe, K., and Rayner, M. S. (2014). *Cardiovascular Disease Statistics*, *2014*. Greater London House: British Heart Foundation.

Tsimakouridze, E. V., Straume, M., Podobed, P. S., Chin, H., Lamarre, J., Johnson, R., et al. (2012). Chronomics of pressure overload-induced cardiac hypertrophy in mice reveals altered day/night gene expression and biomarkers of heart disease. *Chronobiol. Int.* 29, 810–821. doi: 10.3109/07420528.2012.691145

Turek, F. W., Joshu, C., Kohsaka, A., Lin, E., Ivanova, G., Mcdearmon, E., et al. (2005). Obesity and metabolic syndrome in circadian Clock mutant mice. *Science* 308, 1043–1045. doi: 10.1126/science.1108750

Ueda, H. R., Chen, W., Minami, Y., Honma, S., Honma, K., Iino, M., et al. (2004). Molecular-timetable methods for detection of body time and rhythm disorders from single-time-point genome-wide expression profiles. *Proc. Natl. Acad. Sci. U.S.A.* 101, 11227–11232. doi: 10.1073/pnas.0401882101

Verdecchia, P., Schillaci, G., Guerrieri, M., Gatteschi, C., Benemio, G., Boldrini, F., et al. (1990). Circadian blood pressure changes and left ventricular hypertrophy in essential hypertension. *Circulation* 81, 528–536. doi: 10.1161/01.CIR.81.2.528

Vollmers, C., Schmitz, R. J., Nathanson, J., Yeo, G., Ecker, J. R., and Panda, S. (2012). Circadian oscillations of protein-coding and regulatory RNAs in a highly dynamic mammalian liver epigenome. *Cell Metab.* 16, 833–845. doi: 10.1016/j.cmet.2012.11.004

Walton, K. M., Fisher, K., Rubitski, D., Marconi, M., Meng, Q. J., Sladek, M., et al. (2009). Selective inhibition of casein kinase 1 epsilon minimally alters circadian clock period. *J. Pharmacol. Exp. Ther.* 330, 430–439. doi: 10.1124/jpet.109.151415

Westgate, E. J., Cheng, Y., Reilly, D. F., Price, T. S., Walisser, J. A., Bradfield, C. A., et al. (2008). Genetic components of the circadian clock regulate thrombogenesis in vivo. *Circulation* 117, 2087–2095. doi: 10.1161/CIRCULATIONAHA.107.739227

Witte, K., Weisser, K., Neubeck, M., Mutschler, E., Lehmann, K., Hopf, R., et al. (1993). Cardiovascular effects, pharmacokinetics, and converting enzyme inhibition of enalapril after morning versus evening administration. *Clin. Pharmacol. Ther.* 54, 177–186. doi: 10.1038/clpt.1993.129

Woldt, E., Sebti, Y., Solt, L. A., Duhem, C., Lancel, S., Eeckhoute, J., et al. (2013). Rev-erb-alpha modulates skeletal muscle oxidative capacity by regulating mitochondrial biogenesis and autophagy. *Nat. Med.* 19, 1039–1046. doi: 10.1038/nm.3213

World Health Organization [WHO] (2011). "Description of the global burden of NCDs, their risk factors and determinants," in *Global Status Report on Non-communicable Diseases 2010*, Geneva. Available at: http://www.who.int/nmh/publications/ncd_report2010/en/

Young, M. E. (2006). The circadian clock within the heart: potential influence on myocardial gene expression, metabolism, and function. *Am. J. Physiol. Heart Circ. Physiol.* 290, H1–H16. doi: 10.1152/ajpheart.00582.2005

Yusuf, S., Sleight, P., Pogue, J., Bosch, J., Davies, R., and Dagenais, G. (2000). Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The heart outcomes prevention evaluation study investigators. *N. Engl. J. Med.* 342, 145–153. doi: 10.1056/NEJM200001203420301

Zhang, R., Lahens, N. F., Ballance, H. I., Hughes, M. E., and Hogenesch, J. B. (2014). A circadian gene expression atlas in mammals: implications for biology and medicine. *Proc. Natl. Acad. Sci. U.S.A.* 111, 16219–16224. doi: 10.1073/pnas.1408886111

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

MINI REVIEW
published: 27 May 2015
doi: 10.3389/fphar.2015.00112

Circadian clocks, feeding time, and metabolic homeostasis

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Metabolic processes exhibit diurnal variation from cyanobacteria to humans. The circadian clock is thought to have evolved as a time keeping system for the cell to optimize the timing of metabolic events according to physiological needs and environmental conditions. Circadian rhythms temporally separate incompatible cellular processes and optimize cellular and organismal fitness. A modern 24 h lifestyle can run at odds with the circadian rhythm dictated by our molecular clocks and create desynchrony between internal and external timing. It has been suggested that this desynchrony compromises metabolic homeostasis and may promote the development of obesity (Morris et al., 2012). Here we review the evidence supporting the association between circadian misalignment and metabolic homeostasis and discuss the role of feeding time.

Keywords: circadian clock, metabolic homeostasis, circadian misalignment, feeding time, circadian rhythms

OPEN ACCESS

Edited by:
Erquan Zhang, National Institute of Biological Sciences, China

Reviewed by:
Domenico Criscuolo, Genovax, Italy
Frank A. J. L. Scheer, Brigham and Women's Hospital, USA
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Specialty section:
This article was submitted to Pharmaceutical Medicine and Outcomes Research, a section of the journal Frontiers in Pharmacology

Received: *10 March 2015*
Accepted: *12 May 2015*
Published: *27 May 2015*

Citation:
Paschos GK (2015) Circadian clocks, feeding time, and metabolic homeostasis. Front. Pharmacol. 6:112. doi: 10.3389/fphar.2015.00112

Life on earth has adapted to our world of days and nights by evolving molecular mechanisms anticipating the most advantageous time of day for biological processes. In mammals, these daily rhythms are maintained by autoregulatory transcriptional and translational feedback loops involving the basic helix loop helix PER-ARNT-SIM (bHLH/PAS) transcription factors BMAL1, CLOCK, and NPAS2. BMAL1 heterodimerizes with either CLOCK or NPAS2 and drive transcription through E-boxes located within the promoters of numerous target genes. Among the target genes are Period homolog (Per1-3), Cryptochrome (Cry1-2) and Rev-erbα that encode repressors of the BMAL1: CLOCK/NPAS2 transcriptional activity. After a delay, the translated PER and CRY proteins heterodimerize, translocate to the nucleus, and repress BMAL1: CLOCK/NPAS2 heterodimers. The PER and CRY heterodimers are progressively degraded, allowing the circuit to start again. This negative feedback leads to a cycle in gene expression that takes approximately 24 h to complete (Ukai and Ueda, 2010). Post-translational modifications of the proteins of the circuit generate the essential time delay that maintains the period of the cycle at approximately 24 h (Crane and Young, 2014). As a result, BMAL1: CLOCK/NPAS2 bind to DNA in a rhythmic manner leading to rhythmic expression of target genes (Koike et al., 2012). Additional feedback pathways by nuclear receptors retinoid-related orphan receptor alpha (RORα) (Sato et al., 2004), peroxisome proliferator–activated receptor gamma (PPARγ) (Yang et al., 2012) and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) (Liu et al., 2007) provide further robustness to the circuit. The circadian system is organized in a hierarchical manner with a master clock located at the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN receives photic input through direct retinal innervation that initiates gene expression in the SCN (Hastings and Herzog, 2004). In this way, light exposure entrains the SCN clock to solar time, adjusting the oscillator to a precise 24 h cycle (Khalsa et al., 2003). The master clock of the SCN communicates day-night information to the rest of the body. Through neuronal and humoral signals, the SCN sends this information to peripheral circadian clocks that exist in almost all cells of the rest of the

body and synchronize them to the same phase (Mohawk et al., 2012). Whereas light is the dominant timing cue for the SCN oscillator, the clocks of the periphery respond to other environmental cues such as temperature (Glaser and Stanewsky, 2007) and food intake (Damiola et al., 2000) and alter their phase accordingly.

The notion that running at odds with the timing imposed by the master pacemaker (the term "circadian clock" will be used for the rest of the manuscript) results in inefficiency in energy expenditure and obesity has been supported by epidemiological studies. Circadian misalignment has been associated with an increased prevalence of obesity and diabetes. The prevalence of obesity is higher among night-shift workers compared to day workers, and chronic shift work is positively associated with body mass index (BMI) (Karlsson et al., 2001; Parkes, 2002; Di Lorenzo et al., 2003; Ostry et al., 2006; Pan et al., 2011). Prospective studies of healthy volunteers undergoing a 6-day simulated shiftwork protocol show a reduction of energy expenditure in response to the shiftwork (Mchill et al., 2014). Certain sleep disorders also generate misalignment between the rhythms imposed by the circadian clock and behavioral rhythms. Patients with sleep disorders have a higher risk for developing obesity (Phillips et al., 2000; Liu et al., 2013), and the duration of sleep is inversely correlated with body weight in healthy men and women (Patel et al., 2006, 2008; Cappuccio et al., 2008; Chen et al., 2008; Mozaffarian et al., 2011). Prospective study of sleep deprivation shows an increase in body weight after 5 days of insufficient sleep, characterized by an increase in food intake at night (Markwald et al., 2013). A 12-h shift of the sleep/wake and fasting/feeding cycle compared with the central circadian system, while maintaining an isocaloric diet, reduces glucose tolerance, increases blood pressure, and decreases the satiety hormone leptin (Scheer et al., 2009). Exposure of human volunteers to a 28 h day as a mean for circadian disruption in combination with sleep deprivation results in reduced resting metabolic rate and increased post-prandial glycemia as a result of reduced pancreatic insulin secretion (Buxton et al., 2012).

The metabolic impact of circadian misalignment has been studied in animals. The link between the circadian clock and metabolism first emerged from transcriptome analysis of mouse suprachiasmatic nuclei and liver (Panda et al., 2002). Panda et al. showed rhythmically expressed genes encoding regulators and enzymes from multiple metabolic pathways, especially cholesterol synthesis and gluconeogenesis, and suggested that the expression of these genes is under the control of the circadian clock (Panda et al., 2002). Since that study, amino acids and fatty acids were found to oscillate in both mouse liver (Eckel-Mahan et al., 2012) and human plasma (Dallmann et al., 2012). Studies in animal models of circadian clock disruption provide evidence for the requirement of circadian rhythms for metabolic fitness. Early studies showed that gluconeogenesis is impaired in Bmal1 knockout mice and Clock Δ19 mutants, resulting in loss of the circadian variation in the recovery of blood glucose in response to insulin (Rudic et al., 2004). Zhang et al. showed that Cry1 inhibits hepatic gluconeogenesis by blocking adenyl cyclase signaling in response to glucagon (Zhang et al., 2010). Hepatic overexpression of Cry1 improves sensitivity to insulin in db/db pro-diabetic mice (Zhang et al., 2010). On the other hand, deletion of Cry1 and Cry2 results in impaired glucocorticoid-receptor-mediated repression of glucocorticoid synthesis (Lamia et al., 2011). This in turn results in increased gluconeogenesis in the Cry1, Cry2 double knockout animals and increased levels of blood glucose in response to both feeding and fasting (Lamia et al., 2011). Deletion of Bmal1 in the liver results in reduced blood glucose levels during the rest period of the daily cycle and increased glucose clearance from the circulation (Lamia et al., 2008). Pancreas-specific deletion of Bmal1 leads to reduced ability of the pancreas to secrete insulin in response to glucose during the active period of the daily cycle (Marcheva et al., 2010). As a result, mice with a dysfunctional pancreatic clock showed impaired glucose tolerance and increased *ad libitum* plasma glucose levels (Marcheva et al., 2010).

The circadian clock has a profound effect on overall energy homeostasis. Exposure of mice to constant light disrupts their rhythms in locomotor activity and leads to obesity without an increase in total food intake (Shi et al., 2013). ClockΔ19 mutant mice on the C57BL/6J background are obese due to hyperphagia and an attenuation of the regular diurnal feeding rhythm (Turek et al., 2005). Mice deficient in Per2 have no glucocorticoid rhythm, lose diurnal feeding rhythm and develop obesity when fed a high fat diet (Yang et al., 2009). Mutation of the core clock gene Per1 that alters the phosphorylation site of PER1 results in a phase advance of food intake by several hours into the rest/sleep period and in obesity (Liu et al., 2014). Further to support the findings in mice with mutations of clock genes, SCN lesions in mice leads to increased body weight and hepatic insulin resistance (Coomans et al., 2013). This suggests that the increased body weight found in mice carrying mutations of clock genes is due to the disruption of the circadian clock and not because of developmental defects. However, the possible developmental effects of mutations/deletions of clock genes have to be formally tested experimentally with the use of post-natal genetic manipulations. A common parameter in all the above animal models of clock disruption that develop obesity is the increase in food intake during the rest/sleep phase, a phase of the daily cycle when mice normally consume little food. Adding further support to the role of food intake timing, disruption of the circadian clock specifically in adipocytes results in obesity also due to attenuation of the normal feeding rhythm (Paschos et al., 2012). Mice with no functional adipocyte clocks eat more than normal during the rest period of the 24 h cycle, without an increase in total daily food intake. Adipocyte clock controls de novo fatty acid synthesis and release to the circulation, which serves as a signal to the hypothalamus to regulate feeding activity (Paschos et al., 2012). Taken together, the studies in clock deficient mice suggest involvement of the circadian clock in the regulation of feeding. Several studies provide support for the role of the time of food intake in body weight homeostasis (Masaki et al., 2004; Fonken et al., 2010; Salgado-Delgado et al., 2010; Hatori et al., 2012; Stucchi et al., 2012; Chaix et al., 2014). Rats forced to eat opposite to their normal eating time develop obesity (Salgado-Delgado et al., 2010). Similarly, a shift of feeding time to the rest phase in a genetic model of irregular feeding behavior (Masaki et al., 2004) or by exposure to light during nighttime

increases body weight (Fonken et al., 2010). An increase in the amount of calories consumed during the rest/sleep phase of the daily cycle is causal for the development of obesity during high fat diet feeding (Stucchi et al., 2012; Hatori et al., 2012; Chaix et al., 2014).

Time of day of food consumption appears to be important for energy homeostasis however the mechanisms under which feeding at inappropriate time leads to obesity are not yet understood. Feeding rhythms drive rhythms in liver triglycerides and proteins independent of the circadian clock (Adamovich et al., 2014; Mauvoisin et al., 2014). Feeding at "inappropriate" time entrains those rhythms into a phase opposite to the phase of other physiological rhythms dictated by the master clock. This circadian misalignment may result to inefficiency in energy expenditure and obesity (Mattson et al., 2014). In support of this hypothesis, correction of the feeding time in mice fed a high fat diet rescues the onset of obesity and restores the phase of rhythms in serum metabolites (Chaix et al., 2014). The clinical relevance of the findings in animal studies is highlighted by the increased prevalence of obesity in the human Night Eating Syndrome (Gallant et al., 2012), characterized by a delayed pattern of food intake such that more than 25% of the total daily intake takes place after dinner and into the rest/sleep period (Allison et al., 2010). Some first evidence in humans show that volunteers on a weight loss diet lost 25 percent more weight when they consumed their largest meal earlier in the day (Garaulet et al., 2013). In another study, consuming half of the total daily calories during breakfast as part of a weight loss diet led to greater weight loss compared to high caloric intake during dinner time (Jakubowicz et al., 2013). Further studies are required to elucidate the therapeutic implications of feeding time on energy homeostasis and body weight regulation.

Acknowledgments

This work was supported by NIH Grant RO1 HL097800.

References

Adamovich, Y., Rousso-Noori, L., Zwighaft, Z., Neufeld-Cohen, A., Golik, M., Kraut-Cohen, J., et al. (2014). Circadian clocks and feeding time regulate the oscillations and levels of hepatic triglycerides. *Cell Metab.* 19, 319–330. doi: 10.1016/j.cmet.2013.12.016

Allison, K. C., Lundgren, J. D., O'reardon, J. P., Geliebter, A., Gluck, M. E., Vinai, P., et al. (2010). Proposed diagnostic criteria for night eating syndrome. *Int. J. Eat. Disord.* 43, 241–247. doi: 10.1002/eat.20693

Buxton, O. M., Cain, S. W., O'connor, S. P., Porter, J. H., Duffy, J. F., Wang, W., et al. (2012). Adverse metabolic consequences in humans of prolonged sleep restriction combined with circadian disruption. *Sci. Transl. Med.* 4, 129ra143. doi: 10.1126/scitranslmed.3003200

Cappuccio, F. P., Taggart, F. M., Kandala, N. B., Currie, A., Peile, E., Stranges, S., et al. (2008). Meta-analysis of short sleep duration and obesity in children and adults. *Sleep* 31, 619–626.

Chaix, A., Zarrinpar, A., Miu, P., and Panda, S. (2014). Time-restricted feeding is a preventative and therapeutic intervention against diverse nutritional challenges. *Cell Metab.* 20, 991–1005. doi: 10.1016/j.cmet.2014.11.001

Chen, X., Beydoun, M. A., and Wang, Y. (2008). Is sleep duration associated with childhood obesity? A systematic review and meta-analysis. *Obesity (Silver Spring)* 16, 265–274. doi: 10.1038/oby.2007.63

Coomans, C. P., Van Den Berg, S. A., Lucassen, E. A., Houben, T., Pronk, A. C., Van Der Spek, R. D., et al. (2013). The suprachiasmatic nucleus controls circadian energy metabolism and hepatic insulin sensitivity. *Diabetes* 62, 1102–1108. doi: 10.2337/db12-0507

Crane, B. R., and Young, M. W. (2014). Interactive features of proteins composing eukaryotic circadian clocks. *Annu. Rev. Biochem.* 83, 191–219. doi: 10.1146/annurev-biochem-060713-035644

Dallmann, R., Viola, A. U., Tarokh, L., Cajochen, C., and Brown, S. A. (2012). The human circadian metabolome. *Proc. Natl. Acad. Sci. U.S.A.* 109, 2625–2629. doi: 10.1073/pnas.1114410109

Damiola, F., Le Minh, N., Preitner, N., Kornmann, B., Fleury-Olela, F., and Schibler, U. (2000). Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev.* 14, 2950–2961. doi: 10.1101/gad.183500

Di Lorenzo, L., De Pergola, G., Zocchetti, C., L'abbate, N., Basso, A., Pannacciulli, N., et al. (2003). Effect of shift work on body mass index: results of a study performed in 319 glucose-tolerant men working in a Southern Italian industry. *Int. J. Obes. Relat. Metab. Disord.* 27, 1353–1358. doi: 10.1038/sj.ijo.0802419

Eckel-Mahan, K. L., Patel, V. R., Mohney, R. P., Vignola, K. S., Baldi, P., and Sassone-Corsi, P. (2012). Coordination of the transcriptome and metabolome by the circadian clock. *Proc. Natl. Acad. Sci. U.S.A.* 109, 5541–5546. doi: 10.1073/pnas.1118726109

Fonken, L. K., Workman, J. L., Walton, J. C., Weil, Z. M., Morris, J. S., Haim, A., et al. (2010). Light at night increases body mass by shifting the time of food intake. *Proc. Natl. Acad. Sci. USA.* 107, 18664–18669. doi: 10.1073/pnas.1008734107

Gallant, A. R., Lundgren, J., and Drapeau, V. (2012). The night-eating syndrome and obesity. *Obes. Rev.* 13, 528–536. doi: 10.1111/j.1467-789X.2011.00975.x

Garaulet, M., Gomez-Abellan, P., Alburquerque-Bejar, J. J., Lee, Y. C., Ordovas, J. M., and Scheer, F. A. (2013). Timing of food intake predicts weight loss effectiveness. *Int. J. Obes. (Lond.)* 37, 604–611. doi: 10.1038/ijo.2013.18

Glaser, F. T., and Stanewsky, R. (2007). Synchronization of the Drosophila circadian clock by temperature cycles. *Cold Spring Harb. Symp. Quant. Biol.* 72, 233–242. doi: 10.1101/sqb.2007.72.046

Hastings, M. H., and Herzog, E. D. (2004). Clock genes, oscillators, and cellular networks in the suprachiasmatic nuclei. *J. Biol. Rhythms* 19, 400–413. doi: 10.1177/0748730404268786

Hatori, M., Vollmers, C., Zarrinpar, A., Ditacchio, L., Bushong, E. A., Gill, S., 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. doi: 10.1016/j.cmet.2012.04.019

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. doi: 10.1002/oby.20460

Karlsson, B., Knutsson, A., and Lindahl, B. (2001). Is there an association between shift work and having a metabolic syndrome? Results from a population based study of 27,485 people. *Occup. Environ. Med.* 58, 747–752. doi: 10.1136/oem.58.11.747

Khalsa, S. B., Jewett, M. E., Cajochen, C., and Czeisler, C. A. (2003). A phase response curve to single bright light pulses in human subjects. *J. Physiol.* 549, 945–952. doi: 10.1113/jphysiol.2003.040477

Koike, N., Yoo, S. H., Huang, H. C., Kumar, V., Lee, C., Kim, T. K., et al. (2012). Transcriptional architecture and chromatin landscape of the core circadian clock in mammals. *Science* 338, 349–354. doi: 10.1126/science.1226339

Lamia, K. A., Papp, S. J., Yu, R. T., Barish, G. D., Uhlenhaut, N. H., Jonker, J. W., et al. (2011). Cryptochromes mediate rhythmic repression of the glucocorticoid receptor. *Nature* 480, 552–556. doi: 10.1038/nature10700

Lamia, K. A., Storch, K. F., and Weitz, C. J. (2008). Physiological significance of a peripheral tissue circadian clock. *Proc. Natl. Acad. Sci. U.S.A.* 105, 15172–15177. doi: 10.1073/pnas.0806717105

Liu, C., Li, S., Liu, T., Borjigin, J., and Lin, J. D. (2007). Transcriptional coactivator PGC-1alpha integrates the mammalian clock and energy metabolism. *Nature* 447, 477–481. doi: 10.1038/nature05767

Liu, J., Hay, J., and Faught, B. E. (2013). The association of sleep disorder, obesity status, and diabetes mellitus among US adults-the NHANES 2009-2010 survey results. *Int. J. Endocrinol.* 2013:234129. doi: 10.1155/2013/234129

Liu, Z., Huang, M., Wu, X., Shi, G., Xing, L., Dong, Z., et al. (2014). PER1 phosphorylation specifies feeding rhythm in mice. *Cell Rep.* 7, 1509–1520. doi: 10.1016/j.celrep.2014.04.032

Marcheva, B., Ramsey, K. M., Buhr, E. D., Kobayashi, Y., Su, H., Ko, C. H., et al. (2010). Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. *Nature* 466, 627–631. doi: 10.1038/nature09253

Markwald, R. R., Melanson, E. L., Smith, M. R., Higgins, J., Perreault, L., Eckel, R. H., et al. (2013). Impact of insufficient sleep on total daily energy expenditure, food intake, and weight gain. *Proc. Natl. Acad. Sci. U.S.A.* 110, 5695–5700. doi: 10.1073/pnas.1216951110

Masaki, T., Chiba, S., Yasuda, T., Noguchi, H., Kakuma, T., Watanabe, T., et al. (2004). Involvement of hypothalamic histamine H1 receptor in the regulation of feeding rhythm and obesity. *Diabetes* 53, 2250–2260. doi: 10.2337/diabetes.53.9.2250

Mattson, M. P., Allison, D. B., Fontana, L., Harvie, M., Longo, V. D., Malaisse, W. J., et al. (2014). Meal frequency and timing in health and disease. *Proc. Natl. Acad. Sci. U.S.A.* 111, 16647–16653. doi: 10.1073/pnas.1413965111

Mauvoisin, D., Wang, J., Jouffe, C., Martin, E., Atger, F., Waridel, P., et al. (2014). Circadian clock-dependent and -independent rhythmic proteomes implement distinct diurnal functions in mouse liver. *Proc. Natl. Acad. Sci. U.S.A.* 111, 167–172. doi: 10.1073/pnas.1314066111

Mchill, A. W., Melanson, E. L., Higgins, J., Connick, E., Moehlman, T. M., Stothard, E. R., et al. (2014). Impact of circadian misalignment on energy metabolism during simulated nightshift work. *Proc. Natl. Acad. Sci. U.S.A.* 111, 17302–17307. doi: 10.1073/pnas.1412021111

Mohawk, J. A., Green, C. B., and Takahashi, J. S. (2012). Central and peripheral circadian clocks in mammals. *Annu. Rev. Neurosci.* 35, 445–462. doi: 10.1146/annurev-neuro-060909-153128

Morris, C. J., Yang, J. N., and Scheer, F. A. (2012). The impact of the circadian timing system on cardiovascular and metabolic function. *Prog. Brain Res.* 199, 337–358. doi: 10.1016/B978-0-444-59427-3.00019-8

Mozaffarian, D., Hao, T., Rimm, E. B., Willett, W. C., and Hu, F. B. (2011). Changes in diet and lifestyle and long-term weight gain in women and men. *N. Engl. J. Med.* 364, 2392–2404. doi: 10.1056/NEJMoa1014296

Ostry, A. S., Radi, S., Louie, A. M., and Lamontagne, A. D. (2006). Psychosocial and other working conditions in relation to body mass index in a representative sample of Australian workers. *BMC Public Health* 6:53. doi: 10.1186/1471-2458-6-53

Pan, A., Schernhammer, E. S., Sun, Q., and Hu, F. B. (2011). Rotating night shift work and risk of type 2 diabetes: two prospective cohort studies in women. *PLoS Med.* 8:e1001141. doi: 10.1371/journal.pmed.1001141

Panda, S., Antoch, M. P., Miller, B. H., Su, A. I., Schook, A. B., Straume, M., et al. (2002). Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* 109, 307–320. doi: 10.1016/S0092-8674(02)00722-5

Parkes, K. R. (2002). Shift work and age as interactive predictors of body mass index among offshore workers. *Scand. J. Work Environ. Health* 28, 64–71. doi: 10.5271/sjweh.648

Paschos, G. K., Ibrahim, S., Song, W. L., Kunieda, T., Grant, G., Reyes, T. M., et al. (2012). Obesity in mice with adipocyte-specific deletion of clock component Arntl. *Nat. Med.* 18, 1768–1777. doi: 10.1038/nm.2979

Patel, S. R., Blackwell, T., Redline, S., Ancoli-Israel, S., Cauley, J. A., Hillier, T. A., et al. (2008). The association between sleep duration and obesity in older adults. *Int. J. Obes. (Lond.)* 32, 1825–1834. doi: 10.1038/ijo.2008.198

Patel, S. R., Malhotra, A., White, D. P., Gottlieb, D. J., and Hu, F. B. (2006). Association between reduced sleep and weight gain in women. *Am. J. Epidemiol.* 164, 947–954. doi: 10.1093/aje/kwj280

Phillips, B. G., Kato, M., Narkiewicz, K., Choe, I., and Somers, V. K. (2000). Increases in leptin levels, sympathetic drive, and weight gain in obstructive sleep apnea. *Am. J. Physiol. Heart Circ. Physiol.* 279, H234–H237.

Rudic, R. D., Mcnamara, P., Curtis, A. M., Boston, R. C., Panda, S., Hogenesch, J. B., et al. (2004). BMAL1 and CLOCK, two essential components of the circadian clock, are involved in glucose homeostasis. *PLoS Biol.* 2:e377. doi: 10.1371/journal.pbio.0020377

Salgado-Delgado, R., Angeles-Castellanos, M., Saderi, N., Buijs, R. M., and Escobar, C. (2010). Food intake during the normal activity phase prevents obesity and circadian desynchrony in a rat model of night work. *Endocrinology* 151, 1019–1029. doi: 10.1210/en.2009-0864

Sato, T. K., Panda, S., Miraglia, L. J., Reyes, T. M., Rudic, R. D., Mcnamara, P., et al. (2004). A functional genomics strategy reveals rora as a component of the mammalian circadian clock. *Neuron* 43, 527–537. doi: 10.1016/j.neuron.2004.07.018

Scheer, F. A., Hilton, M. F., Mantzoros, C. S., and Shea, S. A. (2009). Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc. Natl. Acad. Sci. U.S.A.* 106, 4453–4458. doi: 10.1073/pnas.0808180106

Shi, S. Q., Ansari, T. S., Mcguinness, O. P., Wasserman, D. H., and Johnson, C. H. (2013). Circadian disruption leads to insulin resistance and obesity. *Curr. Biol.* 23, 372–381. doi: 10.1016/j.cub.2013.01.048

Stucchi, P., Gil-Ortega, M., Merino, B., Guzman-Ruiz, R., Cano, V., Valladolid-Acebes, I., et al. (2012). Circadian feeding drive of metabolic activity in adipose tissue and not hyperphagia triggers overweight in mice: is there a role of the pentose-phosphate pathway? *Endocrinology* 153, 690–699. doi: 10.1210/en.2011-1023

Turek, F. W., Joshu, C., Kohsaka, A., Lin, E., Ivanova, G., Mcdearmon, E., et al. (2005). Obesity and metabolic syndrome in circadian Clock mutant mice. *Science* 308, 1043–1045. doi: 10.1126/science.1108750

Ukai, H., and Ueda, H. R. (2010). Systems biology of mammalian circadian clocks. *Annu. Rev. Physiol.* 72, 579–603. doi: 10.1146/annurev-physiol-073109-130051

Yang, G., Jia, Z., Aoyagi, T., Mcclain, D., Mortensen, R. M., and Yang, T. (2012). Systemic PPARgamma deletion impairs circadian rhythms of behavior and metabolism. *PLoS ONE* 7:e38117. doi: 10.1371/journal.pone.0038117

Yang, S., Liu, A., Weidenhammer, A., Cooksey, R. C., Mcclain, D., Kim, M. K., et al. (2009). The role of mPer2 clock gene in glucocorticoid and feeding rhythms. *Endocrinology* 150, 2153–2160. doi: 10.1210/en.2008-0705

Zhang, E. E., Liu, Y., Dentin, R., Pongsawakul, P. Y., Liu, A. C., Hirota, T., et al. (2010). Cryptochrome mediates circadian regulation of cAMP signaling and hepatic gluconeogenesis. *Nat. Med.* 16, 1152–1156. doi: 10.1038/nm.2214

Conflict of Interest Statement: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 05 June 2015
doi: 10.3389/fphar.2015.00111

Inositols affect the mating circadian rhythm of *Drosophila melanogaster*

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Accumulating evidence indicates that the molecular circadian clock underlies the mating behavior of *Drosophila melanogaster.* However, information about which food components affect circadian mating behavior is scant. The ice plant, *Mesembryanthemum crystallinum* has recently become a popular functional food. Here, we showed that the close-proximity (CP) rhythm of *D. melanogaster* courtship behavior was damped under low-nutrient conditions, but significantly enhanced by feeding the flies with powdered ice plant. Among various components of ice plants, we found that *myo*-inositol increased the amplitude and slightly shortened the period of the CP rhythm. Real-time reporter assays showed that *myo*-inositol and D-pinitol shortened the period of the circadian reporter gene *Per2-luc* in NIH 3T3 cells. These data suggest that the ice plant is a useful functional food and that the ability of inositols to shorten rhythms is a general phenomenon in insects as well as mammals.

Keywords: *Drosophila melanogaster*, circadian rhythm, ice plant, *myo*-inositol, mating succession

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Pharmaceutical Medicine and Outcomes Research, a section of the journal Frontiers in Pharmacology

Received: *14 February 2015*
Accepted: *11 May 2015*
Published: *05 June 2015*

Citation:
Sakata K, Kawasaki H, Suzuki T, Ito K, Negishi O, Tsuno T, Tsuno H, Yamazaki Y and Ishida N (2015) Inositols affect the mating circadian rhythm of Drosophila melanogaster. Front. Pharmacol. 6:111. doi: 10.3389/fphar.2015.00111

Introduction

The physiology and behavior of many organisms can adapt to daily and seasonal environmental changes via circadian clocks that comprise an endogenous self-sustained timekeeping system (Dunlap, 1999). Furthermore, the molecular mechanisms of circadian clock genes that consist of transcriptional–translational feedback loops are conserved from flies to humans (Kako and Ishida, 1998). A core oscillator mechanism of circadian rhythm and feedback loops involving several clock genes such as including *period* (*per*) control locomotor activity and eclosion of the fruit fly, *Drosophila melanogaster* (Dunlap, 1999). The relationships between behavioral rhythms and circadian clock genes have been studied in mutants of this fly with defective feedback loops.

Accumulating evidence indicates that the circadian clock underlies the reproductive behavior of *D. melanogaster* (Beaver and Giebultowicz, 2004; Kadener et al., 2006). The circadian rhythm of mating succession is controlled by the clock genes, *per* and *tim* in *Drosophila* (Sakai and Ishida, 2001). Heterosexual fly couples exhibit significantly different circadian activity from individual flies, having a brief rest phase around dusk followed by activity throughout the night and early morning (Fujii et al., 2007); this is referred to as the close-proximity (CP) rhythm. Analyses of CP rhythms have shown that circadian clocks regulate male courtship behavior in a circadian manner and that a core component of circadian clock, *per*, is regulated to generate CP rhythms. We previously identified the brain clock neurons that

are responsible for the circadian rhythms of the CP behavior that reflects male courtship motivation under normal nutrient conditions (Hamasaka et al., 2010). However, how low-nutrient foods (LNFs) affect *Drosophila* circadian CP behavioral rhythms remains unknown.

A recent study found that inositol synthesis is involved in maintaining the period of circadian behavior in mice (Ohnishi et al., 2014), suggesting that dietary inositol affects the circadian rhythm of CP behavior. Furthermore, inositol is useful against depression (Mukai et al., 2014; Zhao et al., 2015). The African ice plant, *Mesembryanthemum crystallinum*, is abundant in inositols that are known to promote health (Lee et al., 2014). Here, we found that powdered ice plant gradually increased the CP behavior of *D. melanogaster* under low-nutrient conditions. Furthermore, adding inositol to the diet slightly shortened the period of the *Drosophila* CP rhythm. We also found that inositols concentration-dependently shortened the circadian rhythms of clock gene expression in mammalian NIH3T3 cells. These findings when taken together indicate that the ability of inositols to shorten these rhythms is a general phenomenon in animals regardless of species.

Materials and Methods

Food Composition

Boiled standard medium consisting of 8% corn meal, 5% glucose, 5% dry yeast extract, 0.64% agar was supplemented with 0.5% propionic acid and 0.5% butyl p-hydroxybenzoate (standard food, SF). Designated LNF comprising 5% glucose, 1.5% agar, 0.5% butyl *p*-hydroxybenzoate was supplemented without (LNF) or with (LNFI) 0.5% ice plant powder (Nihon Advanced Agri Corporation, Nagahama, Shiga, Japan).

Separation of Inositols in Ice Plant

Myo-inositol and pinitol that have similar structures were separated from ice plant powder by high-performance anion exchange chromatography (HPAE-PAD) using a column containing Dinox CarboPac MA1 (Negishi et al., 2015).

Fly Strains

The wild-type strain, Oregon-R and the clock mutant per^0 were raised under a 12-h light/12-h dark cycle at 25°C on SF.

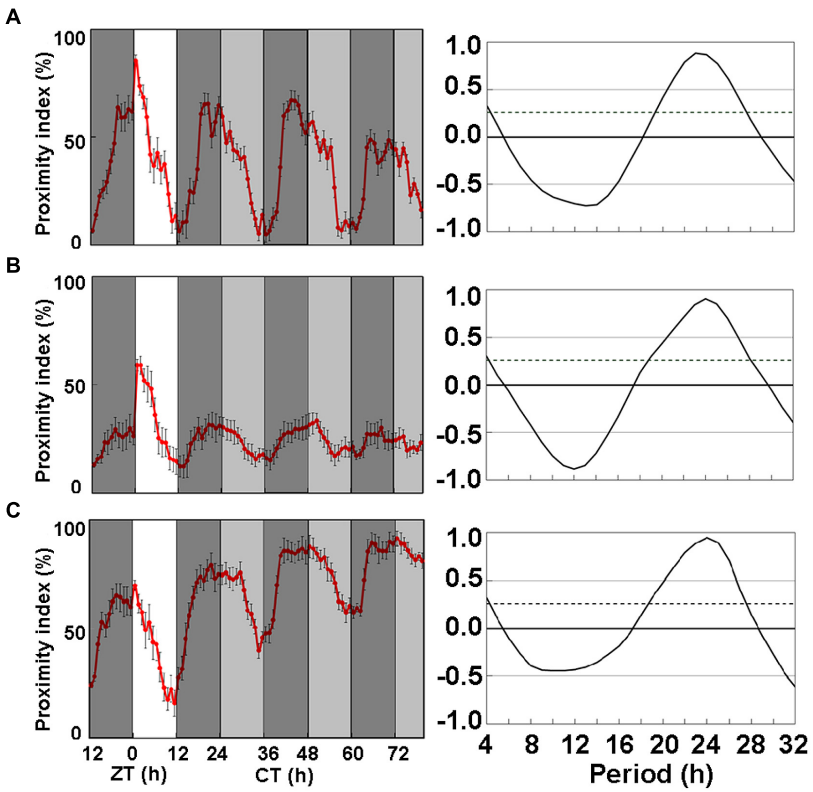

FIGURE 1 | Close-proximity (CP) rhythms of wild-type *Drosophila melanogaster* strain, Oregon-R on three types of medium. (A) Proximity index shows obvious circadian rhythms in Oregon-R. Flies were paired at dusk during LD 12:12 cycle. Data were obtained under constant darkness (DD) after 24 h under LD 12:12. Pairs of Oregon-R flies exhibited daily CP behavior under LD 12:12. Rhythmic CP behavior persisted under DD on **(A)** Standard food (SF; $n = 7$), **(B)** LNF ($n = 27$), and **(C)** LNF containing 0.5% ice plant powder (LNFI; $n = 21$). All CP rhythms were statistically tested by autocorrelation (CORREL function) analysis (right panels), resulting in significant circadian rhythmicity (95% significance indicated by dotted line). The amplitude of CP rhythm decreased on flies fed with low-nutrient food (LNF). White area on graph indicates day; black and gray bars indicate subjective night and subjective day, respectively. Data from 7 to 27 pairs were averaged for each panel. Black error bars indicate SEM.

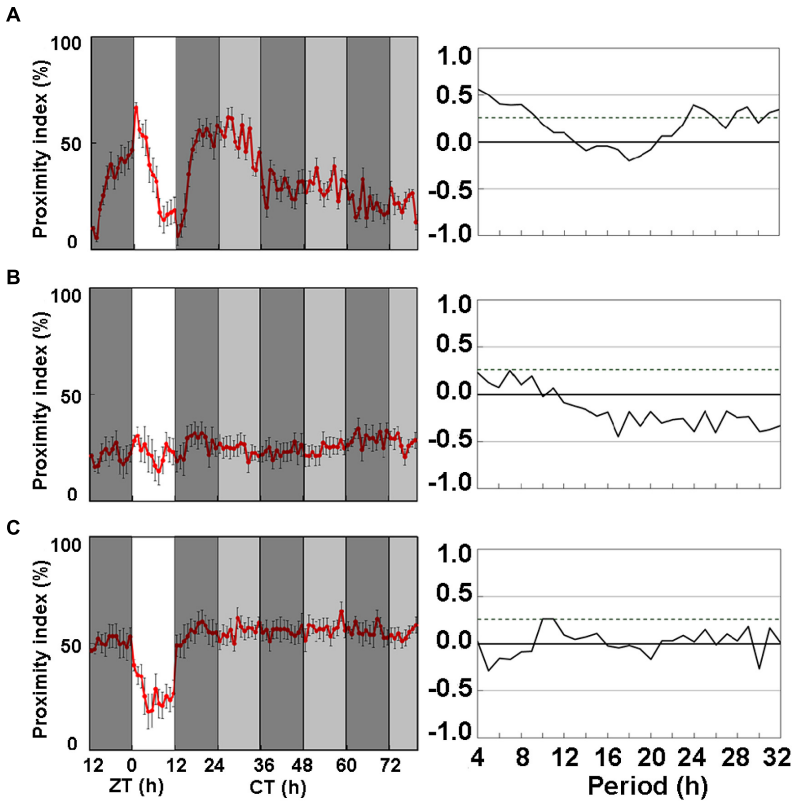

FIGURE 2 | Close-proximity rhythms of *D. melanogaster* mutant, *per*^{0}, on three types of medium. Proximity index shows arrhythmia in mutant strain *per*^{0} under constant darkness (DD). Data were obtained under DD after 24 h under LD 12:12. Pairs of *per*^{0} flies exhibited daily CP behavior under LD 12:12. Arrhythmic CP behavior persisted under DD on **(A)** SF (n = 8), **(B)** low nutrient food (LNF; n = 10), and **(C)** LNF containing 0.5% ice plant powder (LNFI; n = 10). All CP findings were statistically tested by autocorrelation (CORREL function) analysis (right panels), resulting in non-circadian rhythmicity (95% significance indicated by dotted line). White area on graph indicates day; black and gray bars indicate subjective night and subjective day, respectively. Data from 8 to 10 pairs are averaged for each panel. Black error bars indicate SEM.

Close-Proximity Assays

About 40 male and female flies were maintained in vials with SF for 3 days starting from the third day after eclosion. One male and one female from the same genotype were lightly anesthetized with CO_2 and rapidly placed in 35-mm-diameter dishes containing SF or LNF. The dishes were then mounted under a CCD camera, (Watec Co. Ltd., Yamagata, Japan) which is sensitive to light at the near infra-red range and a recording system was established as described (Fujii et al., 2007; Hamasaka et al., 2010). A fluorescent lamp provided illumination at 100 lux and a red LED provided constant dim light <1 lux. Time-lapse images (one frame per 10 s) were sent to a personal computer. The locations of the flies on the X and Y-axes of the images were determined using ImageJ Plugin (http://rsb.info.nih.gov/ij/). The CP index of each pair was calculated from the X–Y value with a threshold (<5 mm) between them. Male flies moving to within 5 mm of a female and those remaining >5 mm from a female were scored as 1 or 0, respectively, in the algorithm of the CP index program. All CP assays proceeded with flies of the same genotypes and the data were averaged for each genotype. The circadian rhythmicity of CP was determined using autocorrelation (CORREL function) analysis (Levine et al., 2002). The free-running period and the power of rhythmicity in each genotype were calculated as the average of the free-running period and the maximum correlation between each pair evaluated by autocorrelation as being rhythmic (CORREL function; Hamasaka et al., 2010).

Statistical Analysis

All data are expressed as means ± SEM and were statistically evaluated using Student's t-test for single comparisons and one-way ANOVA. $P < 0.05$ was considered to indicate a statistically significant difference.

Cell Culture

NIH3T3 cells were incubated in Dulbecco's modified Eagle's medium (D-MEM) supplemented with 10% fetal bovine serum and a mixture of penicillin and streptomycin at 37°C under a humidified 5% CO_2 atmosphere.

Real-Time Luciferase Assays

The *Per2* promoter regions were cloned into pGL3-dLuc (Ohno et al., 2007), and then reporter plasmids (2 μg) were transfected into NIH3T3 cells (35-mm collagen type I-coated dishes)

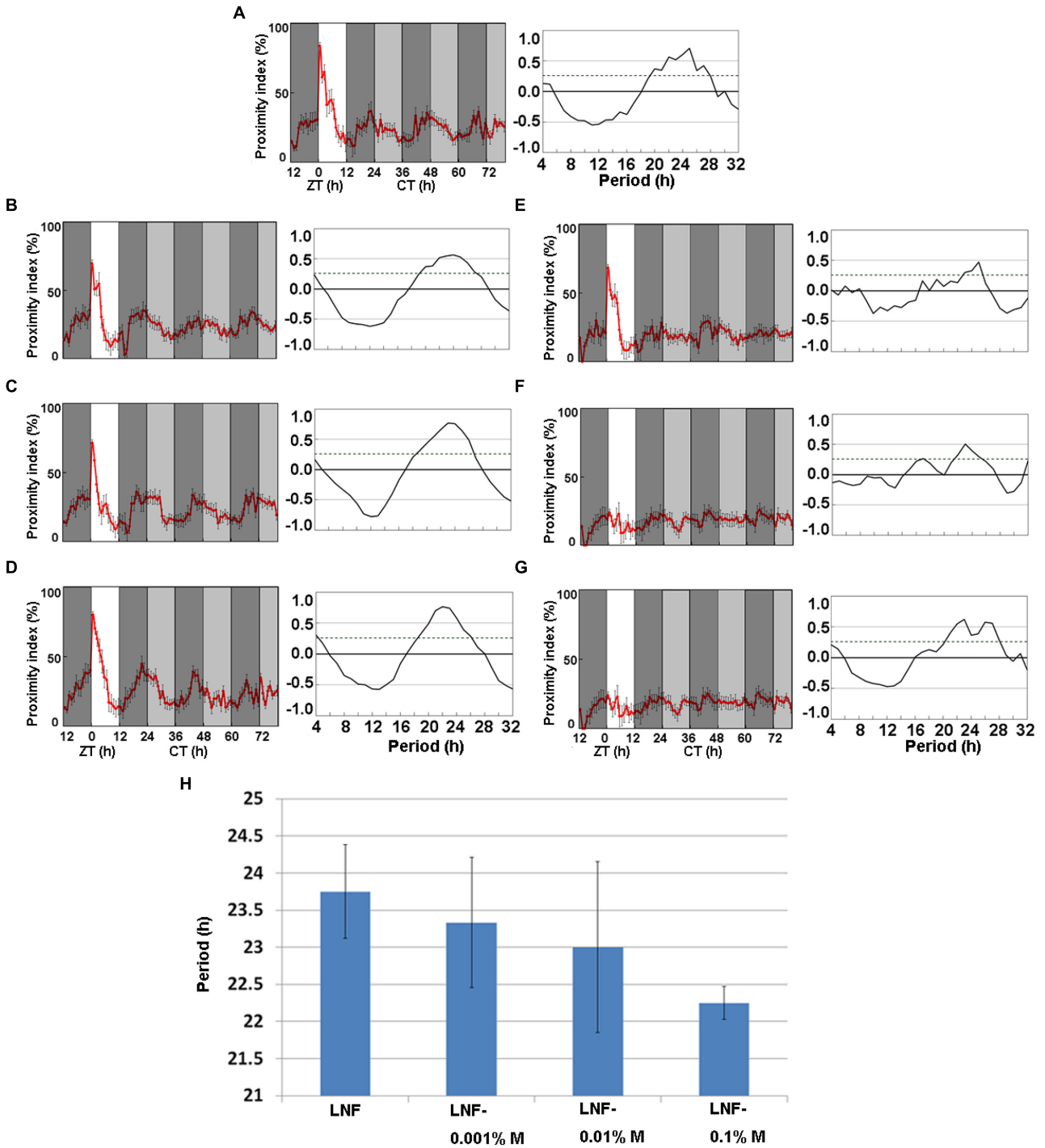

FIGURE 3 | Close-proximity rhythms of wild-type *D. melanogaster* strain, Oregon-R on (A) LNF and LNF including (B) 0.001%, (C) 0.01%, and (D) 0.1% *myo*-inositol and (E) 0.001%, (F) 0.01 (G) and 0.1% D-pinitol. (H) Period of CP rhythms at different *myo*-inositol concentrations in LNF. Proximity index shows obvious circadian rhythms on *myo*-inositol mixed low nutrient food. Flies were paired at dusk during LD 12:12 cycle. Data were obtained under constant darkness (DD) after 24 h under LD 12:12. Pairs of Oregon-R flies exhibited daily CP behavior under LD 12:12. Rhythmic CP behavior persisted under DD on **(A)** LNF, **(B)** LNF-0.001% M, **(C)** LNF-0.01% M, **(D)** LNF-0.1% M (all $n = 5$). Arrhythmic CP behavior persisted under DD on **(E)** LNF including D-pinitol 0.001% ($n = 4$), **(F)** 0.01% ($n = 5$), and **(G)** 0.1% ($n = 5$). All CP data were statistically tested using autocorrelation (CORREL function) analysis (right panels), resulting in significant circadian rhythmicity (95% significance indicated by dotted line). White area on graphs indicates day; black and gray bars indicate subjective night and subjective day, respectively. Data from five pairs were averaged for each panel. Black error bars indicate SEM. **(H)** Period length of CP rhythms of wild-type *D. melanogaster* strain, Oregon-R on **(A)** LNF (23.75 h), LNF containing **(B)** 0.001% (23.33 h), **(C)** 0.01% (23 h), and **(D)** 0.1% (22.25 h) *myo*-inositol. Differences among these period lengths were not significant (Student's *t*-test). LNF, low-nutrient food; M, *myo*-inositol.

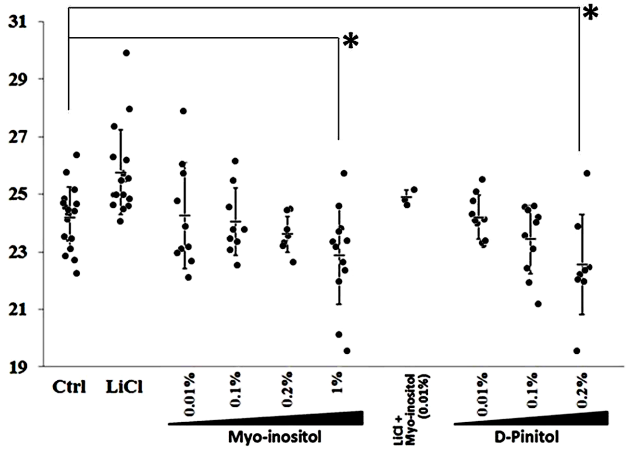

FIGURE 4 | Inositols at various concentrations shorten period of Per2-luc reporter gene oscillation in mammalian cultured cells. Expression of Per2-luc reporter gene introduced into NIH 3T3 cells determined using real-time reporter assays. Mean estimated expression period was 24.2 h (Control). Adding lithium chloride (LiCl; positive control) lengthened period of Per2-luc expression. Data are shown as mean ± SEM. Significant difference between control and treated cells (*$p < 0.05$, *t*-test).

using HilyMax (Dojindo Laboratories, Kumamoto, Japan). The cells were stimulated with 100 nM dexamethasone (Sigma–Aldrich) for 2 h in serum-free Dulbecco's MEM and then the medium was replaced with fresh Dulbecco's MEM containing 100 μM luciferin (Wako Pure Chemical Industries), 25 mM HEPES (pH 7.2), and 10% fetal bovine serum. Bioluminescence was measured and integrated for 1 min at intervals of 10 min using an LM-2400 photon detection unit (Hamamatsu Photonics, Hamamatsu, Japan). The cells were cultured in a luminometer for 3 days to evaluate bioluminescence. Reporter gene expression was detrended by subtracting an average of 12 h from the raw data. Peaks and troughs were measured on detrended charts using a scale to calculate the phase of reporter-gene expression. The average period (hours) between peaks was calculated from detrended data accumulated for >5 days.

Results

Feeding with Ice Plant Powder Enhanced CP Rhythm of *Drosophila* Courtship Behavior

The CP rhythms of heterosexual pairs of Oregon-R flies dipped at dusk under LD12:12 as described (Fujii et al., 2007; Hamasaka et al., 2010) and persisted under DD with a dip at subjective lights-off (CT12; Hamasaka et al., 2010). The circadian CP rhythm of Oregon-R fed with SF was obvious (**Figure 1A**) and the amplitude and period were very similar to those previously reported (Hamasaka et al., 2010). To understand the effect of LNF on CP rhythms, we examined the CP rhythms of Oregon-R flies on LNF without cornmeal and yeast extract (**Figure 1B**). The amplitude of the CP rhythm declined under DD after a light and dark (LD12:12) cycle. Among several compounds that we screened for the ability to recover the amplitude of the CP rhythm under LNF, that LNFI sequentially promoted the activity of the rhythm (**Figure 1C**). Thus, ice plant powder contains candidate substances that might recover the amplitude of the CP rhythm under LNF.

Rhythmicity of *Drosophila* Courtship Behavior Requires the Clock Gene *Period*

Since ice plant powder promoted the activity and amplitude of CP rhythms in wild-type flies, we investigated the effects of LNFI on *period* mutant, per^0 flies. **Figures 2A–C** shows the CP rhythms of per^0 mutants fed with SF, LNF, and LNFI, respectively. The CP rhythms of per^0 heterosexual pairs of flies dipped at dusk under LD, but became arrhythmic under DD for 2 days (**Figure 2A**) and in flies fed with LNF (**Figure 2B**). However, LNFI significantly enhanced the activity of the CP rhythm (**Figure 2C**). These data indicate that rhythmicity of CP behavior requires the *period* gene and that ice plant powder includes a promoter of CP rhythmic activity.

Myo-Inositol Shortens Circadian Period of CP Rhythms and Activates the Amplitude of CP Behavior

Figure 1 indicates that ice plant powder contains substances that promote the activity of CP rhythm. We therefore separated low-molecular weight substances in ice plant powder using HPLC and found 4.5 and 51.4 mg of *myo*-inositol and D-pinitol/g of fresh weight, respectively. We examined the effects of LNF containing either *myo*-inositol (**Figures 3B–D**) or D-pinitol (**Figures 3E–G**) at concentrations of 0.001, 0.01, and 0.1% each on the wild-type strain, Oregon-R to determine whether they are involved in promoting the activity of CP rhythms. **Figure 3A** shows the CP rhythms of heterosexual pairs of flies fed with LNF and **Figures 3B–D** shows that 0.001, 0.01, and 0.1% *myo*-inositol slightly promoted the amplitude of the CP rhythm, whereas the effects of the respective tested concentrations of D-pinitol did not significantly differ (**Figures 3E–G**). *Myo*-inositol seemed to dose-dependently shorten the period of CP rhythms (**Figure 3H**). These data suggest that *myo*-inositol not only increases the amplitude, but also shortens the phase of CP rhythms.

Myo-Inositol Shortened Per2-luc Oscillation Period in Mammalian Cultured Cells

We investigated the effects of *myo*-inositol on the period of reporter gene expression driven by Per2 in NIH 3T3 cells to determine whether it affects the phase of CP rhythms in mammals (**Figure 4**). Increasing *myo*-inositol concentrations tended to shorten the period of the CP rhythm, and 1% *myo*-inositol significantly shortened the period. D-pinitol (0.2%) also shortened the period of Per2-luc oscillation in cultured NIH 3T3 cells. Thus, inositols not only shortened the period of *Drosophila* behavior but also the period of mammalian cells.

Discussion

We showed that the amplitude of CP rhythms was significantly reduced in wild-type flies fed with LNF. In contrast, LNF containing 0.5% ice plant powder (LNFI) recovered the amplitude of CP rhythm in these flies and the rhythm gradually became robust and high at tough. These findings suggested that ice plant powder contains substances that promote CP activity.

We analyzed inositol contents in ice plants using HPLC. The ice plants (100 g) grown in plant factory contained 51.4 mg of D-pinitol and 4.5 mg of *myo*-inositol and we analyzed the effects of these inositols upon the amplitude of CP rhythm. *Myo*-inositol increased the amplitude of CP rhythm in *Drosophila*, whereas D-pinitol did not. Therefore, we postulated that nutrients missing from LNF such as yeast extract and corn meal might contain *myo*-inositol. In fact, 5% dry yeast extract in the SF contained 1.07-mM *myo*-inositol and 758-nM D-pinitol, and 0.5% ice plant powder contained 991-μM *myo*-inositol and 14.2-μM D-pinitol. Thus, yeast extract in SF contains a sufficient amount of *myo*-inositol to promote the CP rhythm.

Myo-inositol slightly reduced the period of the CP rhythm, but it did not affect either the amplitude or the period of the locomotor rhythm (**Supplementary Figure S1**). This suggests that the CP rhythm might be one output for the circadian clock or that *myo*-inositol is involved in mating biochemistry (Papaleo et al., 2009; Colone et al., 2010).

The CP behavior under DD in per^0 circadian clock mutant was arrhythmic, indicating that this rhythm required the molecular circadian clock. However, ice plant powder constantly enhanced the CP rhythm in this mutant, suggesting that it contains unknown factors that promote the CP behavior without affecting circadian rhythms in both per^0 mutant and wild-type Oregon-R flies.

We studied the CP rhythms of Oregon-R flies in LNF containing 0.001, 0.01, and 0.1% *myo*-inositol (55.5 μM, 555 μM, and 5.55 mM, respectively) or D-pinitol (51.5 μM, 515 μM, and 5.15 mM, respectively). *Myo*-inositol at 0.01 and 0.1% promoted the amplitude, and dose-dependently shortened the period of the CP rhythm. Considering the recent suggestion that *myo*-inositol is required to maintain the period of circadian behavior in mice (Ohnishi et al., 2014), it appears to be common circadian regulator among various species. Otherwise, promoting the amplitude and the period of CP rhythms did not significantly differ among flies fed with LNF containing different ratios of D-pinitol. Although *myo*-inositol increased the amplitude of the CP rhythm, D-pinitol had no effect despite having a similar chemical structure to that of *myo*-inositol. However, both *myo*-inositol and D-pinitol shortened the period of mammalian cells, indicating that D-pinitol exerts different effects upon *Drosophila* and mammals. *Myo*-inositol shortened the male CP rhythm and increased the amplitude of the rhythm. Considering with that inositols are used to treat depression(Mukai et al., 2014; Zhao et al., 2015), the CP rhythm assay of *Drosophila* might be useful for screening drugs to treat depressive disorders in future.

Conclusion

The CP behavior under DD in the per^0 mutant was arrhythmic, indicating a requirement for the molecular circadian clock gene *period*. Ice plant powder enhanced the CP activity of the per^0 mutant without recovering rhythmicity. These data suggest that ice plant powder has unknown factors that promote the activity of CP behavior without affecting the circadian rhythm in *Drosophila*. The ice plant component *myo*-inositol increased the proximity index but also slightly shortened the period of CP rhythm in *Drosophila*. Exogenous inositols concentration-dependently shortened the period of the circadian oscillation rhythm of the *mPer2-luc* reporter in cultured mammalian NIH3T3 cells. The ability of inositols to shorten rhythms might be a general feature of insects as well as mammals.

Acknowledgment

We thank A. Tsuji (Nihon Advanced Agri, Co. Ltd., Japan) for providing the ice plant powder.

Supplementary Material

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fphar.2015.00111/abstract

Figure S1 | Myo-inositol did not affect the locomotor activity rhythm of Drosophila fed with low-nutrient food. Locomotor activity was analyzed as described (Inoue et al., 2002). Data are shown as means ± SEM. No significant difference among these period lengths (Student's *t*-test).

References

Beaver, L. M., and Giebultowicz, J. M. (2004). Regulation of copulation duration by period and timeless in *Drosophila melanogaster*. *Curr. Biol.*14, 1492–1497. doi: 10.1016/j.cub.2004.08.022

Colone, M., Marelli, G., Unfer, V., Bozzuto, G., Molinari, A., and Stringaro, A. (2010). Inositol activity in oligoasthenoteratospermia-an in vitro study. *Eur. Rev. Med. Pharmacol. Sci.* 14, 891–896.

Dunlap, J. C. (1999). Molecular bases for circadian clocks. *Cell* 96, 271–290. doi: 10.1016/S0092-8674(00)80566-8

Fujii, S., Krishnan, P., Hardin, P., and Amrein, H. (2007). Nocturnal male sex drive in *Drosophila*. *Curr. Biol.* 17, 244–251. doi: 10.1016/j.cub.2006.11.049

Hamasaka, Y., Suzuki, T., Hanai, S., and Ishida, N. (2010). Evening circadian oscillator as the primary determinant of rhythmic motivation for *Drosophila* courtship behavior. *Genes Cells* 15, 1240–1248. doi: 10.1111/j.1365-2443.2010.01456.x

Inoue, S., Shimoda, M., Nishinokubi, I., Siomi, M. C., Okamura, M., Nakamura, A., et al. (2002). A role for the *Drosophila* fragile X-related gene in circadian output. *Curr. Biol.* 12, 1331–1335. doi: 10.1016/S0960-9822(02)01036-9

Kadener, S., Villella, A., Kula, E., Palm, K., Pyza, E., Botas, J., et al. (2006). Neurotoxic protein expression reveals connections between the circadian clock and mating behavior in *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* 103, 13537–13542. doi: 10.1073/pnas.0605962103

Kako, K., and Ishida, N. (1998). The role of transcription factors in circadian gene expression. *Neurosci. Res.* 31, 257–264. doi: 10. 1016/S0168-0102(98) 00054-6

Lee, B. H., Lee, C. C., and Wu, S. C. (2014). Ice plant (*Mesembryanthemum crystallinum*) improves hyperglycaemia and memory impairments in a Wistar rat model of streptozotocin-induced diabetes. *J. Sci. Food Agric.* 94, 2266–2273. doi: 10.1002/jsfa.6552

Levine, J. D., Funes, P., Dowse, H. B., and Hall, J. C. (2002). Signal analysis of behavioral and molecular cycles. *BMC Neurosci.* 3:1. doi: 10.1186/1471-2202-3-1

Mukai, T., Kishi, T., Matsuda, Y., and Iwata, N. (2014). A meta-analysis of inositol for depression and anxiety disorders. *Hum. Psychopharmacol.* 29, 55–63. doi: 10.1002/hup.2369

Negishi, O., Mun'im, A., and Negishi, Y. (2015). Content of methylated inositols in familiar edible plants. *J. Agric. Food Chem.* 63, 2683–2688. doi: 10.1021/jf5041367

Ohnishi, T., Murata, T., Watanabe, A., Hida, A., Ohba, H., Iwayama, Y., et al. (2014). Defective craniofacial development and brain function in a mouse model for depletion of intracellular inositol synthesis. *J. Biol. Chem.* 289, 10785–10796. doi: 10.1074/jbc.M113.536706

Ohno, T., Onishi, Y., and Ishida, N. (2007). A novel E4BP4 element drives circadian expression of mPeriod2. *Nucleic Acids Res.* 35, 648–655. doi: 10.1093/nar/gkl868

Papaleo, E., Unfer, V., Baillargeon, J. P., and Chiu, T. T. (2009). Contribution of myo-inositol to reproduction. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 147, 120–123. doi: 10.1016/j.ejogrb.2009.09.008

Sakai, T., and Ishida, N. (2001). Circadian rhythms of female activity governed by clock genes in *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* 98, 9221–9225. doi: 10.1073/pnas.151443298

Zhao, J., Jung, Y. H., Jang, C. G., Chun, K. H., Kwon, S. W., and Lee, J. (2015). Metabolomic identification of biochemical changes induced by fluoxetine and imipramine in a chronic mild stress mouse model of depression. *Sci. Rep.* 5:8890. doi: 10.1038/srep08890

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
published: 19 June 2015
doi: 10.3389/fphar.2015.00125

Personalized medicine for pathological circadian dysfunctions

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The recent approval of a therapeutic for a circadian disorder has increased interest in developing additional medicines for disorders characterized by circadian disruption. However, previous experience demonstrates that drug development for central nervous system (CNS) disorders has a high failure rate. Personalized medicine, or the approach to identifying the right treatment for the right patient, has recently become the standard for drug development in the oncology field. In addition to utilizing Companion Diagnostics (CDx) that identify specific genetic biomarkers to prescribe certain targeted therapies, patient profiling is regularly used to enrich for a responsive patient population during clinical trials, resulting in fewer patients required for statistical significance and a higher rate of success for demonstrating efficacy and hence receiving approval for the drug. This personalized medicine approach may be one mechanism that could reduce the high clinical trial failure rate in the development of CNS drugs. This review will discuss current circadian trials, the history of personalized medicine in oncology, lessons learned from a recently approved circadian therapeutic, and how personalized medicine can be tailored for use in future clinical trials for circadian disorders to ultimately lead to the approval of more therapeutics for patients suffering from circadian abnormalities.

Keywords: personalized medicine, circadian disorders, circadian therapeutics, patient enrollment, companion diagnostics, targeted therapeutics

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Specialty section:
This article was submitted to Pharmaceutical Medicine and Outcomes Research, a section of the journal Frontiers in Pharmacology

Received: *27 January 2015*
Accepted: *02 June 2015*
Published: *19 June 2015*

Citation:
Skelton RL, Kornhauser JM and Tate BA (2015) Personalized medicine for pathological circadian dysfunctions. Front. Pharmacol. 6:125. doi: 10.3389/fphar.2015.00125

Introduction

The approval of HETLIOZ® (tasimelteon) for Non-24 disorder has reignited an interest in developing therapeutics for circadian abnormalities. However, as the history of tasimelteon demonstrates, the path to approval of a drug for a circadian dysfunction is not an easy one. One component of the challenge is the ability to match the therapeutic approach to the patient most likely to benefit from the intervention, a model often called personalized medicine. Patient selection or optimization of therapy is guided in some indications by genetic or biochemical markers which point to the underlying molecular mechanisms causing disease. In central nervous system (CNS) disorders, genetic markers have been difficult to pinpoint, possibly because the clinical symptoms actually represent multiple diseases with different molecular drivers. However, treating circadian dysfunction has an advantage over other CNS disorders, in that the phenotype of the disorder is relatively easy to measure, is highly translatable from animal models to humans, and modulation of the phenotype can be quantified, providing an accessible proof of mechanism biomarker. Here we review the recent history of clinical trials for circadian disorders; conduct a comparison to oncology, where personalized approaches have greatly improved success for therapeutics; and

discuss the challenges that remain in circadian medicine to incorporate personalized approaches to improve the approval rate of medicines for circadian disorders.

Background

Circadian clocks regulate a plethora of biological processes. Cellular processes such as gene expression and physiological processes such as body temperature, hormone and neurotransmitter release, as well as behaviors including activity, sleep, learning and memory are under circadian control. Recent advances in understanding the molecular components of the clock, together with global transcriptome approaches, provided insight into the vast extent of the genetic landscape regulated by the clock, helping to explain at the molecular level how such a variety of physiological systems are circadian-regulated. The cycling clock proteins (BMAL1, CLOCK, PER-1 and -2, CRY-1 and -2) directly bind to gene regulatory elements and regulate thousands of transcripts in multiple tissues in a temporally coordinated manner (Koike et al., 2012). Recent genome-wide studies have shown that an astonishing 43% of genes are transcribed with a rhythmic pattern in a largely tissue-specific manner (Zhang et al., 2014). Finally, epigenetic mechanisms of gene regulation are also clock-controlled; a large number of non-coding RNAs are rhythmically expressed. Among these cycling mRNAs, only about half are regulated by *de novo* transcription; post-transcriptional regulation is also modulated by circadian input.

This recent understanding of the enormous impact of the clock on the human organism and the elucidation of the molecular components of the clock provide the pharmaceutical industry with potential targets that should prove fruitful for the discovery and development of new therapeutics. As of late 2014, there were 34 open clinical trials listed at https://clinicaltrials.gov/ that included the keyword "circadian." The purpose of several of these trials is to phenotype circadian rhythms in specific patient populations, for example the NIAAA study on the circadian dopamine rhythm in cocaine addicts (**Table 1**, NCT02233829) and the UCSF study on sleep disruption and delirium (**Table 1**, NCT01280097). Other trials are studying behavioral interventions to alter circadian rhythms, for example a study at Rhode Island Hospital using chronobiological interventions to treat post-partum depression (**Table 1**, NCT02053649). Several studies are testing the efficacy of melatonin in various patient populations or the efficacy of the approved drugs Modafinil, Circadin, and tasimelteon for new indications. Disappointingly, no trials listed novel agents for primary circadian disorders, such as advanced- or delayed-phase syndromes, or for circadian disorders secondary to other diseases, such as sundowning in Alzheimer's disease patients, despite the prevalence of these disorders. Sundowning, for instance, has been reported in 2.4–25% patients diagnosed with Alzheimer's disease (AD). Thus, it appears that drug development for circadian dysfunction remains an under-invested area, although the growing understanding of the profound impact of circadian patterns of expression of the majority of human genes in both healthy and sick individuals may stimulate research into potential therapies for circadian disorders. Several major pharmaceutical companies appear to be invested in programs aimed at molecular targets associated with circadian biology. These including programs on inhibitors of casein kinase 1 (CK1, Pfizer, and Amgen) (Sprouse et al., 2010; Long et al., 2012), which is also a target of interest in oncology. In addition, the growing appreciation of the intimate role of clock proteins in metabolism is reflected in research programs in major pharmaceutical and several biotech companies, including projects examining Cry1 [(Griebel et al., 2014); Reset Therapeutics (http://resettherapeutics.com/programs/)] and REV-ERB (Kojetin and Burris, 2014).

This review will discuss why trials for circadian disorders risk a high failure rate, what has been learned from the more recent success with the approval of tasimelteon (HETLIOZ®), and what can be done in the future to reduce the risk of clinical trial failures and improve the chances of delivering medications to patients suffering from circadian disorders.

Human Circadian Clocks

Humans, in common with most (if not all) living organisms, have biological clocks that organize the timing of physiological events and keep them synchronized with the external 24-h day. This coordination with the 24-h day led to the term "circadian." The master circadian clock in mammals resides in the suprachiasmatic nucleus (SCN) of the hypothalamus (Meijer and Rietveld, 1989). Rhythms generated by the SCN clock are autonomous (i.e., they do not depend upon environmental cues), but under normal conditions and in healthy individuals the timing of the internal oscillator is synchronized with the external 24-h day by environmental stimuli, principally light. In addition to the master SCN pacemaker, other tissues and organs also possess cellular clocks that are synchronized by signals from the SCN. These peripheral circadian clocks regulate the timing of many metabolic and cellular processes that are specific to the function of the particular cell type and tissue (Mohawk et al., 2012).

The human clock cycles with a period (*tau*) of nearly, but not exactly, 24 h. When an individual is placed into an environment removed from any time cues (free-running conditions), the inherent period of the internal clock is revealed; *tau* has been measured at 24 h 11 ± 16 min (Czeisler et al., 1999). Environmental light normally keeps the internal clock synchronized or entrained to the external day-night cycle. This synchronization is accomplished by an adjustment in the phase of the oscillation, or phase shift, whenever a disparity exists between the internal "time of day" and external lighting conditions. For example, if the internal clock is slow, or delayed, morning light occurring before the internal clock "anticipates" it will advance the phase of the clock; whereas, in a clock running fast, light in the evening will delay the phase of the clock. Light occurring coincident with the internal "daytime" will not shift the clock. The differential effects of light at different times relative to the internal phase can be measured and plotted as a phase-response curve (PRC). The PRCs of humans and other diurnal mammals compared to nocturnal mammals are generally similar—light in the early subjective night lead to a phase delay, while light in the late subjective night lead to an advance, keeping the

TABLE 1 | Open drug intervention clinical trials in clinicaltrials.gov that include "circadian," "clock," or "chronobiology" as a keyword.

Sponsor/Collaborators	Interventions	Conditions	Phase	NCT Number
University of Chicago	Drug: exenatide and Placebo	Type 2 diabetes; sleep disordered breathing	NP	NCT01136798
Xinhua Hospital; Shanghai Jiao Tong University School of Medicine	Drug: etomidate; midazolam; propofol	Congenital hydronephrosis; congenital choledochal cyst; fracture	Phase 4	NCT02013986
Universitätsklinikum Hamburg-Eppendorf	Drug: Melatonin 2 mg and Placebo	Healthy night shift workers, sleep disorders	Phase 3	NCT02108353
Mount Sinai School of Medicine; National Institute of Mental Health (NIMH)	Drug: Modafinil and Placebo	Bipolar disorder	Phase 4	NCT01965925
Stanford University; Patient Centered Outcome Research Institute	Other: CONV care for the diagnosis and treatment of sleep disorders Other: PCCM for the diagnosis and treatment of sleep disorders	Obstructive sleep apnea of adult; insomnia; circadian rhythm sleep disorder, unspecified type; restless legs syndrome; narcolepsy and hypersomnia	NP	NCT02037438
National Institute on Alcohol Abuse and Alcoholism (NIAAA); National Institutes of Health Clinical Center (CC)	Drug: brain dopamine reactivity methylphenidate; brain dopamine receptor C-11 raclopride	Cocaine abuse	Phase 0	NCT02233829
Rhode Island Hospital; The Depressive and Bipolar Disorder Alternative Treatment Foundation	Behavioral: triple chronotherapy; usual care	Depression; major depressive disorder; post-partum depression	NP	NCT02053649
Hopital Foch	Drug: propofol; remifentanil	Anesthesia, general	Phase 3	NCT00896714
University of California, San Francisco; Masimo Labs	NP	Delirium; sleep disorders, circadian rhythm	NP	NCT01280097
National Human Genome Research Institute (NHGRI); National Institutes of Health Clinical Center (CC)	Drug: dTR Melatonin (NIH CC PDS); melatonin CR Device: phototherapy (bright light)	Developmental delay disorders; chromosome deletion; mental retardation; sleep disorders, circadian rhythm; self-injurious behavior	Phase 1	NCT00506259
Oregon Health and Science University	Drug: melatonin Behavioral: regular sleep schedule; light	Insomnia; blindness; daytime sleepiness	NP	NCT00911053
Hospital de Clinicas de Porto Alegre	Drug: melatonin and Placebo; amitriptyline and Placebo; melatonin and amitriptylin	Fibromyalgia	Phase 2 Phase 3	NCT02041455
Paracelsus Medical University; Technische Universität München	Drug: testosterone supplementation	Circadian; exercise; testosterone	NP	NCT02134470
Sogo Rinsho Médéfi Co., Ltd.; Takeda	Drug: azilsartan; amlodipine	Hypertension	NP	NCT01762501
Ann & Robert H Lurie Children's Hospital of Chicago; Children's Research Institute	Drug: prednisone and Placebo	Duchenne Muscular Dystrophy (DMD)	Phase 2	NCT02036463
Brigham and Women's Hospital	Biological: melatonin and Placebo	Delayed sleep phase disorder; jet-lag; shift-work disorder	NP	NCT00950885
Oregon Health and Science University; Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)	Dietary supplement: melatonin Biological: melatonin	Blindness	NP	NCT00691444
Prince of Songkla University	Device: selective laser trabeculoplasty Drug: travoprost	Intraocular pressure	Phase 4	NCT02105311
Neurim Pharmaceuticals Ltd.	Drug: circadin 2/5/10 mg and Placebo	Sleep disorders	Phase 3	NCT01906866

(Continued)

TABLE 1 | Continued

Sponsor/Collaborators	Interventions	Conditions	Phase	NCT Number
Hospices Civils de Lyon	Drug: melatonin and Placebo	Sleep disorders	Phase 2	NCT01993251
University of Vigo	Drug: aspirin	Type 2 diabetes	Phase 4	NCT00725127
University of Bergen	Drug: paracetamol and buprenorphine; paracetamol and Placebo; buprenorphine and Placebo	Depression; pain; dementia	Phase 4	NCT02267057
University of Pittsburgh; National Heart, Lung, and Blood Institute (NHLBI)	Behavioral: modified ME intervention; education only	Sleep apnea, obstructive	Phase 1	NCT01377584
Herlev Hospital	Drug: melatonin, N-acetyl-5-methoxytryptamine; isotonic saline, natrium chloride	Acute myocardial infarction; ischemia-reperfusion injury	Phase 2	NCT01172171
Vanda Pharmaceuticals	Drug: tasimelteon	Smith-Magenis syndrome; circadian	Phase 2	NCT02231008
University of British Columbia	Drug: melatonin and Placebo	Delirium	Phase 4	NCT02282241
University of Michigan; University of Pennsylvania; Washington University Early Recognition Center	Drug: ISOFLURANE- experimental arm Other: control group: cognitive testing	Post-operative cognitive dysfunction	NP	NCT01911195
Haukeland University Hospital	Drug: Solu-Cortef; Cortef	Addison disease	Phase 1 Phase 2	NCT02096510
Charite University; Technische Universität München; University of Erlangen-Nürnberg Medical School; Praxis für Neurologie und Psychiatrie am Prinzregentenplatz, München; Technische Universität Berlin	Behavioral: patient centered structured support program	Mini-stroke	NP	NCT01586702
Teva Pharmaceutical Industries; United BioSource Corporation	Drug: modafinil; armodafinil	Narcolepsy; obstructive sleep apnea; shift work sleep disorder	NP	NCT01792583
Vanda Pharmaceuticals	Drug: tasimelteon	Non-24-H sleep-wake disorder	Phase 3	NCT01429116
Vanda Pharmaceuticals	Drug: tasimelteon	Non 24 H sleep wake disorder	Phase 3	NCT01218789
Aretaieion University Hospital; Baxter Healthcare Corporation	Procedure: maintenance with desflurane Procedure: maintenance with propofol	Anesthesia; surgery; sleep disorders	NP	NCT02061514
Endo Pharmaceuticals	Drug: morphine Sulfate 30 mg; Oxycodone 20 mg; Morphine 45 mg; Oxycodone 30 mg; Morphine sulfate 15 mg; Oxycodone 10 mg; morphine Sulfate 30 mg; Oxycodone 15 mg	Chronic around the clock opioid users	Phase 2	NCT01871285
Mundipharma Research GmbH & Co KG	Drug: Oxycodone/Naloxone prolonged release (OXN PR) tablets; oxycodone prolonged release (OxyPR) tablets	Pain|Constipation	Phase 3	NCT01438567
Brigham and Women's Hospital|National Center for Complementary and Integrative Health (NCCIH)	Drug: vitamin B12	Sleep disorders, circadian rhythm		NCT00120484
Endo Pharmaceuticals|BioDelivery Sciences International	Drug: EN3409	Low back pain|Osteoarthritis|Neuropathic pain	Phase 3	NCT01755546

(Continued)

TABLE 1 | Continued

Sponsor/Collaborators	Interventions	Conditions	Phase	NCT Number
Mundipharma Research GmbH & Co KG	Drug: Oxycodone; Naloxone	Malignant pain|Non-malignant pain	Phase 2|Phase 3	NCT02321397
Purdue Pharma LP	Drug: Oxycodone/Naloxone controlled-release; Placebo	Low back pain	Phase 3	NCT01358526
Janssen Pharmaceutical K.K.	Drug: tapentadol ER; morphine SR	Neoplasms	Phase 3	NCT01309386
Purdue Pharma LP	Drug: Oxycodone/Naloxone controlled-release; Oxycodone HCl controlled-release; Placebo	Low back pain	Phase 3	NCT01427270
Purdue Pharma LP	Drug: oxycodone/naloxone controlled-release; oxycodone HCl controlled-release; Placebo	Low back pain	Phase 3	NCT01427283
Oregon Health and Science University|Forest Laboratories	Drug: Placebo/escitalopram	Depression		NCT01214044
Technische Universität München|Cephalon	Drug: modafinil (Vigil); Placebo	Depression	Phase 2	NCT00670813
University of Copenhagen| Rigshospitalet, Denmark	Drug: erythropoietin (Epoetin-beta, NeoRecormon); erythropoietin (Epoetin-beta, NeoRecormon); Placebo	Renal effects	Phase 1	NCT01584921
Norwegian University of Science and Technology|St. Olavs Hospital|Fondazione IRCCS Istituto Nazionale dei Tumori, Milano|L'Hospitalet de Llobregat|University Hospital, Bonn|Cantonal Hospital of St. Gallen|Maastricht University Medical Center|Flinders University	Drug: intranasal fentanyl spray; slow release morphine	Cancer|Pain	Phase 3	NCT01906073
Endo Pharmaceuticals	Drug: oxymorphone IR	Chronic pain	Phase 3	NCT01206907
Mundipharma Research GmbH & Co KG	Drug: laxative	Opioid induced constipation	Phase 4	NCT01957046
Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran	Drug: haloperidol; Placebo; non-pharmacologic measures	Hypoactive delirium	Phase 3	NCT02345902
University of Oklahoma	Drug: memory XL; Placebo	Mild cognitive impairment	Phase 2	NCT00903695
University of Illinois at Chicago|Genentech, Inc.	Drug: ranibizumab (lucentis)	Glaucoma|New onset Glaucoma|Neovascular Glaucoma|New onset neovascular glaucoma	Phase 1|Phase 2	NCT00727038
Universitätsklinikum Hamburg-Eppendorf	Drug: melatonin 2 mg; Placebo	Healthy night shift workers, sleep disorders	Phase 3	NCT02108353
Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)|National Institutes of Health Clinical Center (CC)	Drug: hydrocortisone; Placebo; hydrocortisone and melatonin; melatonin	Jet lag syndrome	Phase 2	NCT00097474
University of California, San Diego|California Breast Cancer Research Program	Device: light box (litebook)	Breast cancer		NCT00478257
St. Olavs Hospital	Drug: fentanyl	Chronic pain|Cancer	Phase 1|Phase 2	NCT01248611

(Continued)

TABLE 1 | Continued

Sponsor/Collaborators	Interventions	Conditions	Phase	NCT Number
National Heart, Lung, and Blood Institute (NHLBI)	Drug: melatonin; methylxanthine Device: light therapy	Sleep disorders, circadian rhythm		NCT00387179
Takeda	Drug: ramelteon; Placebo	Circadian dysregulation	Phase 4	NCT00492011
AstraZeneca	Drug: AZD1386; Placebo	Pain|Esophageal sensitivity	Phase 1	NCT00711048
Delray Medical Center	Drug: IV Ibuprofen	Pain	Phase 4	NCT02152163
Attikon Hospital	Drug: sugammadex; neostigmine/atropine	Post-operative cognitive dysfunction		NCT02419352
Mundipharma Research GmbH & Co KG	Drug: OXN PR followed by OxyPR tablets; OxyPR followed by OXN PR tablets	Severe chronic pain	Phase 2	NCT01915147
Greater Houston Retina Research	Drug: ranibizumab (lucentis)	Ischemic central retinal vein occlusion	Phase 1	NCT00406471
Pfizer	Drug: donepezil	Dementia, vascular|Dementia, mixed	Phase 3	NCT00174382
Pfizer	Drug: morphine sulfate extended release capsules	Pain	Phase 4	NCT00640042
Meander Medical Center|Dutch Kidney Foundation	Drug: melatonin tablet 3 mg once daily; Placebo comparator	Sleep Problems|Haemodialysis	Phase 3	NCT00388661
Meander Medical Center|Dutch Kidney Foundation	Drug: melatonin	Hemodialysis|Peritoneal dialysis|Sleep problems	Phase 3	NCT00404456
Ever Neuro Pharma GmbH|acromion GmbH|Geny Research Corp.	Drug: cerebrolysin; 0.9% saline solution	Vascular dementia	Phase 4	NCT00947531
Neovii Biotech	Drug: catumaxomab; prednisolone	Cancer|Neoplasms|Carcinoma|Malignant ascites	Phase 3	NCT00822809
Singapore General Hospital|Novartis|National Neuroscience Institute	Drug: exelon (rivastigmine); placebo	Cognitive impairment	Phase 4	NCT00669344
University of Toledo Health Science Campus	Drug: continuous release dopamine agonists	Parkinson disease	Phase 3	NCT00465452
Novartis	Drug: rivastigmine capsule; rivastigmine transdermal patch	Parkinson's disease dementia	Phase 3	NCT00623103
University of Rochester|Forest Laboratories	Drug: namenda	Delirium|Post-operative states	Phase 4	NCT00303433
INSYS Therapeutics Inc	Drug: fentanyl sublingual spray	Cancer|Pain	Phase 3	NCT00538863
Endo Pharmaceuticals|BioDelivery Sciences International	Drug: EN3409; Placebo	Low back pain	Phase 3	NCT01633944
Endo Pharmaceuticals|BioDelivery Sciences International	Drug: EN3409	Low back pain	Phase 3	NCT01675167
Memorial Sloan Kettering Cancer Center	Drug: d-Methadone; Placebo	Pain|Bladder Cancer|Breast Cancer|CNS Cancer|Colon Cancer|Esophageal Cancer|Pancreatic Cancer|Prostate Cancer|Uterine Cancer|Head and neck Cancer|Eye Cancer|Otorhinolaryngologic neoplasms	Phase 1|Phase 2	NCT00588640

(Continued)

TABLE 1 | Continued

Sponsor/Collaborators	Interventions	Conditions	Phase	NCT Number
Neurim Pharmaceuticals Ltd.	Drug: melatonin (circadin); Placebo	Non-24 H sleep-wake disorder|Blindness	Phase 2	NCT00972075
National Eye Institute (NEI)	Drug: melatonin	Blindness		NCT00686907
Collegium Pharmaceutical, Inc.	Drug: oxycodone DETERx; Placebo	Chronic low back pain	Phase 3	NCT01685684
Cephalon|Teva Pharmaceutical Industries	Drug: ACTIQ (Oral Transmucosal Fentanyl Citrate [OTFC])	Pain|Cancer|Sickle cell Anemia|Severe burns	Phase 2	NCT00236093
Shaare Zedek Medical Center	Drug: extended-release tramadol; paracetamol	Post-operative pain		NCT01024348
Cephalon|Teva Pharmaceutical Industries	Drug: ACTIQ	Cancer|Breakthrough pain	Phase 2	NCT00236041
Accera, Inc.	Drug: AC-1204; Placebo	Alzheimer's disease	Phase 2|Phase 3	NCT01741194
Mundipharma Pharmaceuticals B.V.	Drug: oxycodone hydrochloride and naloxone hydrochloride combination, prolonged release	Pain	Phase 3	NCT01167127
Melissa Voigt Hansen|University of Copenhagen|Rigshospitalet, Denmark|Pharma Nord|Herlev Hospital	Drug: melatonin (N-acetyl-5-methoxytryptamine); Placebo	Breast cancer|Depression	Phase 2|Phase 3	NCT01355523
Mundipharma Pharmaceuticals B.V.	Drug: oxycodone and naloxone	Pain	Phase 3	NCT01167699
James Graham Brown Cancer Center|University of Louisville	Drug: Fentanyl Citrate Nasal Spray (FCNS)	Pain	Phase 4	NCT01839552
Kaplan Medical Center	Drug: oxycodone 10 mg	Elective laproscopic bilateral inguinal Hernia|Elective laproscopic cholecystectomy	Phase 4	NCT00480142
Brigham and Women's Hospital|Takeda	Drug: ramelteon; Placebo	Healthy		NCT00595075
Loma Linda University	Drug: morphine PCA started at the end of surgery, 1 Percocet 1/325 mg every 4 h; may receive a second Percocet if needed. For the 30 ml ropivacaine the intervention would be the subject can request extra pain medication which would be Percocet and/or morphine PCA	Post-op pain		NCT01939379
Vanda Pharmaceuticals	Drug: tasimelteon 20 mg capsule; tasimelteon 2 mg I.V.	Non-24-H-sleep-wake disorder	Phase 4	NCT02130999
Oregon Health and Science University	Drug: melatonin	Insomnia|Blindness|Daytime sleepiness		NCT00911053
Erasmus Medical Center|ZonMw: The Netherlands Organization for Health Research and Development	Drug: enalapril/hydrochlorothiazide; Placebo	Essential hypertension	Phase 4	NCT02214498
National Institute on Alcohol Abuse and Alcoholism (NIAAA)|National Institutes of Health Clinical Center (CC)	Drug: brain dopamine reactivity; brain dopamine receptor	Cocaine abuse	Phase 0	NCT02233829
Boehringer Ingelheim	Drug: telmisartan; ramipril	Hypertension	Phase 4	NCT00274612
Ottawa Heart Institute Research Corporation|Schering-Plow|Medtronic	Drug: eptifibatide facilitated PCI	Myocardial infarction	Phase 3	NCT00251823

(Continued)

TABLE 1 | Continued

Sponsor/Collaborators	Interventions	Conditions	Phase	NCT Number
Boehringer Ingelheim	Drug: telmisartan combined with hydrochlorothiazide (80/12.5 mg); valsartan combined with hydrochlorothiazide (160/12.5 mg)	Hypertension|Diabetes mellitus, Type 2	Phase 4	NCT00239538
US WorldMeds LLC|National Institute on Drug Abuse (NIDA)	Drug: lofexidine HCl	Renally impaired subjects	Phase 1	NCT02313103
University of Washington|Paul G. Allen Family Foundation	Drug: botox; normal saline	Painful bladder syndrome|Interstitial cystitis	Phase 4	NCT00194610
Children's Hospital of Philadelphia|Bayer|University of Pennsylvania	Drug: paracervical nerve block; sham paracervical block	Pain	Phase 4	NCT02352714
University of Alabama at Birmingham|National Heart, Lung, and Blood Institute (NHLBI)	Drug: losartan	Anemia, sickle cell|Sickle cell disease|Kidney disease|Hypertension|Proteinuria	Phase 2	NCT02373241
University of Alberta|Vancouver Coastal Health Research Institute	Drug: warfarin	Atrial fibrillation|Thrombus due to heart valve prosthesis|Deep venous thrombosis|Thromboembolism|DVT	Phase 4	NCT02376803
National Heart, Lung, and Blood Institute (NHLBI)	Drug: melatonin; methylxanthine| Procedure: light therapy	Sleep disorders, circadian rhythm		NCT00387179
Orphan Medical	Drug: sodium oxybate	Narcolepsy	Phase 3	NCT00049803
National Institute of Mental Health (NIMH)	Drug: low-dose sodium oxybate; high-dose sodium oxybate; low-dose zolpidem; high-dose zolpidem; Placebo	Sleep		NCT00777829
Massachusetts General Hospital	Drug: ramelteon; Placebo	Huntington's disease|Parkinson's disease|Dementia with lewy bodies|Sleep disorders|Circadian dysregulation		NCT00907595
Takeda	Drug: ramelteon; Placebo	Circadian dysregulation	Phase 4	NCT00492011
Massachusetts General Hospital	Drug: zolpidem CR; Placebo	Dementia|Alzheimer disease|Dementia, vascular|Sleep disorders|Circadian dysregulation		NCT00814502
Vanda Pharmaceuticals	Drug: tasimelteon; Placebo	Non-24-H sleep-wake disorder	Phase 3	NCT01163032
Vanda Pharmaceuticals	Drug: tasimelteon; Placebo	Non-24-H sleep-wake disorder	Phase 3	NCT01430754
Cephalon|Teva Pharmaceutical Industries	Drug: PROVIGIL 200 mg; armodafinil 250 mg; armodafinil 200 mg; armodafinil 150 mg; Placebo	Chronic shift work sleep disorder	Phase 3	NCT00236080
Child Psychopharmacology Institute	Drug: risperidone	Sleep disorders, circadian rhythm|Insomnia|psychomotor agitation		NCT00723580
Cephalon|Teva Pharmaceutical Industries	Drug: CEP-10953 (Armodafinil)	Narcolepsy|Sleep apnea, Obstructive|Sleep apnea Syndromes|Shift-work sleep disorder	Phase 3	NCT00078312
Vanda Pharmaceuticals	Drug: tasimelteon	Smith-Magenis syndrome|Circadian	Phase 2	NCT02231008
Vanda Pharmaceuticals	Drug: tasimelteon	Non-24-H sleep-wake disorder	Phase 3	NCT01429116
Takeda	Drug: ramelteon; Placebo	Sleep disorders, circadian rhythm	Phase 2	NCT00593736

(Continued)

TABLE 1 | Continued

Sponsor/Collaborators	Interventions	Conditions	Phase	NCT Number
Boehringer Ingelheim	Drug: pharmaton caplets; Placebo	Sleep disorders, circadian rhythm	Phase 2	NCT02199847
Sheba Medical Center	Drug: melatonin	Delayed sleep phase syndrome	Phase 1	NCT00282061
Cephalon|Teva Pharmaceutical Industries	Drug: armodafinil 150 mg/day; Placebo	Excessive sleepiness|Shift work sleep disorder	Phase 3	NCT00080288
National Human Genome Research Institute (NHGRI)|National Institutes of Health Clinical Center (CC)	Drug: dTR melatonin (NIH CC PDS); melatonin CR Device: phototherapy (bright light)	Developmental delay disorders|Chromosome deletion|Mental retardation|Sleep disorders, circadian rhythm|Self injurious behavior	Phase 1	NCT00506259
Brigham and Women's Hospital|Sunovion|Massachusetts General Hospital	Drug: eszopiclone; matching Placebo	Shift-work sleep disorder		NCT00900159
Vanda Pharmaceuticals	Drug: tasimelteon 20 mg capsule; tasimelteon 2 mg I.V.	Non-24-H-sleep-wake disorder	Phase 4	NCT02130999
Neurim Pharmaceuticals Ltd.	Drug: melatonin (circadin); Placebo	Non-24 H sleep-wake disorder|blindness	Phase 2	NCT00972075
Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)|National Institutes of Health Clinical Center (CC)	Drug: hydrocortisone; Placebo; hydrocortisone and melatonin; melatonin	Jet lag syndrome	Phase 2	NCT00097474
Teva Pharmaceutical Industries|United BioSource Corporation	Drug: modafinil/armodafinil	Narcolepsy|Obstructive sleep apnea|Shift work sleep disorder		NCT01792583
Vanda Pharmaceuticals	Drug: VEC-162	Circadian rhythm sleep disorders	Phase 2	NCT00490945
Vanda Pharmaceuticals	Drug: tasimelteon	Non 24 H sleep wake disorder	Phase 3	NCT01218789
Cephalon|Teva Pharmaceutical Industries	Drug: armodafinil 100–250 mg/day	Excessive daytime sleepiness|Narcolepsy|Obstructive sleep apnea/hypopnea syndrome|Chronic shift work sleep disorder	Phase 3	NCT00228553
Brigham and Women's Hospital|National Center for Complementary and Integrative Health (NCCIH)	Drug: vitamin B12	Sleep disorders, circadian rhythm		NCT00120484

internal clock synchronized to sunrise and sunset (Johnson, 1990).

While light is the predominant stimulus that entrains the phase of the clock, a variety of other stimuli also affect entrainment. Of particular relevance in terms of human health and disease, inputs including food consumption, exercise, and social interactions can shift clock phase. These non-photic stimuli generally shift the clock when they occur during the circadian inactive phase (i.e., during the night in humans) (Rosenwasser and Dwyer, 2001).

In recent years, the molecular "gears" of the circadian clock have been elucidated. A series of interlocking negative feedback loops involving transcription, translation, and post-translational phosphorylation form the molecular clockwork. At its core, the transcriptional activators BMAL1, CLOCK and NPAS2 activate the *Period* (Per1 and Per2) and *Cryptochrome* (Cry1 and Cry2) genes (Mohawk et al., 2012). PER and CRY transcripts and proteins gradually accumulate during the daytime, associate with one another and translocate into the nucleus during the evening, and interact with the CLOCK/NPAS2:BMAL1 complex to repress their own transcription. The PER and CRY proteins are progressively phosphorylated by a CK1 kinase during the night, targeting them for ubiquitylation and eventual degradation by the proteasome, relieving their transcriptional autorepression and beginning the cycle again. The timing of this feedback loop takes about 24 h to complete. Because CK1 phosphorylation of PER and CRY regulates the timing of degradation of these protein and the link to a specific human circadian phenotype, CK1 is a target under investigation for its therapeutic potential. Other clock components represent potential drug targets, although little is available in the public domain confirming pharmaceutical investment in these targets.

A secondary or modulatory protein loop modifies the core clock loop. REV-ERBα transcription is also activated by

the BMAL1/CLOCK and repressed by CRY/PER, resulting in circadian oscillations of REV-ERBα. In turn, REV-ERBα represses BMAL1 and CLOCK transcription. This REV-ERBα/RORα feedback loop modulates the core circadian clock (Bugge et al., 2012). REV-ERBα has also generated interested as a drug target.

Circadian Rhythms and Disease

Underlying genetic mutations have been discovered which lead to abnormal circadian rhythms. In this review we refer to these genetic-driven circadian abnormalities as Primary Circadian Disorders. For example, some cases of advanced sleep phase disorder (ASPD) and delayed sleep phase disorder (DSPD), characterized by a circadian phase that is either "fast" or "slow," respectively, are due to mutant forms of key clock proteins. Familial forms of ASPD arise from mutations in either CK1 delta or in a phosphorylation site in the PER2 protein targeted by CK1. Specific CK1 variants are also associated with DSPD, and 75% of DSPD patients are homozygous for a shorter allele of PER3 that affects phosphorylation by CK1. These human circadian phenotypes are predicted by the spontaneous mutations in CK1 that result in the circadian period mutants found in hamster, *Tau*, and in Drosophila, *double-time*. In general, it appears that increasing CK1 activity leads to a shortened circadian period. Thus, CK1 activity is an important regulator of circadian timing and a potential target for therapeutic intervention. Clearly, it is critical to take into account the circadian phenotype and phase of an individual to predict clinical outcome in trials of drugs that modulate CK1. As in clinical trials for oncology treatments, companion diagnostic (CDx) tests that genotype for specific clock gene mutations would allow selection of the target patient population. Therefore, as in oncology, these genetic disorders provide the opportunity to demonstrate, as a proof of principle, the efficacy of the drug mechanism in a targeted population, with the goal of expanding therapeutics into Secondary Circadian Disorders (see below).

In addition to mutations in core clock genes, genetic variations that affect the timing of the circadian cycle of humans may also exist. For example, polymorphisms in *Clock* are associated with morning vs. evening preference in humans (Katzenberg et al., 1998). A recent paper describes an association of a Per3 polymorphism with bipolar disorder (Karthikeyan et al., 2014). Diagnostics that detect these kinds of genetic markers would provide a mechanism to enrich enrollment in clinical trials with patients most likely to benefit from specific circadian therapies.

Beyond the genetic disorders discussed above that are the direct result of mutations in clock genes or disrupted entrainment of the circadian system, there is a growing appreciation that circadian abnormalities may be a key core symptom of a variety of diseases including metabolic disorders, mood disorders, and dementia. In this review we refer to circadian abnormalities that are closely associated with another disease as Secondary Circadian Disorders. In Secondary Circadian Disorders, circadian disorganization may present both as a symptom of the disease, and as a potential risk factor contributing to disease pathogenesis (Golombek et al., 2013; Smolensky et al., 2014a,b; Zelinski et al., 2014). An instructive example is the occurrence of circadian abnormalities in patients with metabolic disorder. The growing appreciation of the role of clock proteins in metabolism suggests several potential molecular targets for therapeutics aimed at treating obesity. A feedback between the central circadian clock and peripheral oscillators in liver, skeletal muscle and other tissues helps to coordinate the complex processes of food intake, activity, and lipid homeostasis (Feng and Lazar, 2012). Disruption of these highly regulated interacting rhythms by rotational shift work, for example, is a risk factor for developing metabolic syndrome, obesity, and diabetes mellitus (Feng and Lazar, 2012; Bailey et al., 2014; Maury et al., 2014). While not all obese patients may suffer from circadian disruption, phenotyping, and appropriately regulating the sleep-wake cycles of patients in therapeutic trials for obesity may be critical to uncovering the full potential of a drug. Indeed, individualizing the timing of drug administration may be an unappreciated factor to improve efficacy, especially in a population where sleep-wake disruption is overrepresented.

Abnormal circadian rhythms are also common in patients with mood disorders. Bipolar disorder patients have been reported to have unstable and lower amplitude circadian rhythms (McCarthy and Welsh, 2012; Seleem et al., 2014) while those suffering from major depressive disorder appear to be phase delayed. It has been shown that circadian programs of gene expression are distinctly altered in depressive patients (McCarthy and Welsh, 2012; Karatsoreos, 2014). While the long-standing hypothesis has been that circadian disruption may be a causal factor in these disorders, circadian-based treatments have not always shown pronounced efficacy. There is an ongoing debate regarding the efficacy of agomelatine, an approved treatment of major depressive disorder in Europe and Australia although the drug is not approved for this indication in the US (Gahr, 2014). A recent prospective study suggested that treatment responsiveness was related to circadian phenotype. Patients with major depressive disorder that scored as a morning type were more likely to respond to agomelatine treatment than those that scored as an evening type (Corruble et al., 2013). Thus, treatment regimes informed by an individual patient's circadian phenotype and/or administered at a specific circadian phase might enhance the therapeutic benefit for this chronobiotic.

Seasonal affective disorder (SAD) represents a sub-type of mood disorder closely linked to the circadian system. SAD affects individuals who become depressed during the short daylight periods of winter. One leading hypothesis of the cause of SAD suggests it a results from circadian misalignment (Lewy et al., 2007), or difficulty entraining due to the absence of bright light in morning or evening. Therapy with bright light and melatonin is effective for some SAD patients, and is thought to act by advancing or delaying phase to re-synchronize the clock. Especially in this disorder, treatment aligned with the individual patient's circadian phase is likely to improve outcomes.

In dementia patients, especially those suffering from AD, up to 25% experience sundowning, a disturbing syndrome characterized by agitation, worsening cognitive function, pacing and wandering in the evening/night, and daytime sleepiness. A patient's reduced internal distinction between night and day, caused by the low amplitude oscillation of their circadian

clock, appears to contribute to sundowning (Khachiyants et al., 2011). Therapeutics that enhance the amplitude of the circadian oscillator could benefit this patient population.

Finally, the recently approved drug, tasimelteon, is prescribed for patients with Non-24-h sleep-wake disorder (Non-24). Non-24 is a consequence of the failure of the circadian system to entrain to the external 24-h day. A majority of Non-24 patients are blind; and up to 70% of totally blind individuals may suffer from Non-24. The absence of light perception leads to the lack of clock entrainment, and as a result the sleep-wake cycle is free-running. Non-24 is the first circadian disorder for which a pharmaceutical intervention, tasimelteon, has been approved. Because Non-24 patients express a range of free-running *tau* (from 23.7 to 25.3 h) (Dressman et al., 2012), consideration of their circadian phenotypes proved to be key to successful clinical trials (see further discussion of the clinical trial, below).

Considering that circadian rhythmicity is ubiquitous and closely intertwined with both normal physiological processes and disease states, any therapeutic approach targeting a disease (either a "circadian" disorder, or one with rhythmic components) must take into account the timing of the intervention relative to the circadian system of the individual. The experience in drug development for oncology demonstrates the value of a personalized medicine approach to discovering and testing therapeutics.

Definition of Personalized Medicine

Personalized medicine can be defined as a targeted prevention and treatment regimen that is developed for an individual using data gathered from medical records and diagnostic analysis of "biomarkers," or specific biological markers that distinguish one individual from another. Most often, biomarkers are genetic; however, biomarkers can also be phenotypic, such as circadian subtypes (e.g., advance phase or delayed phase syndrome). Ultimately, the goal of personalized medicine is to give the right patient the right treatment at the right time.

Following the completion of the Human Genome Project over 10 years ago, the cost and time for genomic sequencing has decreased exponentially. On average, sequencing of a human genome decreased from $1 billion in 2003 to between $3–5000 today (Personalized Medicine Coalition, 2014), with a time to result from ∼7 years decreased to ∼2 days. In addition to influencing all of biological research, mapping of the genome has also translated to clinical chemistry, with many therapeutics now designed to target specific proteins, protein classes, or even mutated forms of a protein. In contrast to traditional chemotherapies, which function against any actively dividing cells, targeted therapeutics act on a molecule in the signaling pathway driving a specific tumor (http://www.cancer.gov/cancertopics/factsheet/Therapy/targeted).

The following section will examine the growing field of personalized medicine in oncology, which has focused on targeting the genetic abnormalities driving cancer with targeted therapies. As patients with circadian disorders continue to be profiled both at the phenotypic and molecular levels, the ability to subtype patient populations in order to identify the predicted responsive cohort is critical in ensuring more efficient and more successful circadian clinical trials.

Personalized Medicine in Oncology

The oncology field has led to the use of personalized medicine in healthcare, mainly due to the effectiveness of targeted therapeutics specifically developed to inhibit oncogenic driver proteins. Currently, targeted therapeutics are available for patients with melanoma, chronic myeloid leukemia, colon, breast, and lung cancer. In non-small cell lung cancer (NSCLC), mutations in two oncogenic driver genes, EGFR and ALK, make up ∼25–40% of all NSCLC cases (Kwak et al., 2010; Melosky, 2014). Two FDA-approved EGFR inhibitors and two FDA-approved ALK inhibitors are commercially available (EGFR: Gilotrif™, Boehringer Ingelheim and Tarceva®, Roche; ALK: Xalkori®, Pfizer and Zykadia™, Novartis). Each of these inhibitors requires testing of patients with a unique FDA-approved diagnostic test (aka CDx) prior to prescription. CDx assays are FDA regulated as Class III Medical Devices (Olsen and Jorgensen, 2014) that were demonstrated during the pivotal trial to show clinical utility in selecting for the responding patient population, were contemporaneously FDA-approved alongside the corresponding therapeutic, and are specified in the drug labeling to be required for use in identifying the target patient population. Therefore, a CDx is the mechanism to identify the right patient for the right drug.

Personalized Medicine Growing Pains

Use of CDx in the oncology space was initially met with resistance; the thought of narrowing the market to a select patient population does not inherently make financial sense. However, as drugs in development functioned through a more selective mechanism of action, it was clear that identification of responders carrying the genetic target was paramount, as dictated by the FDA. For example, the small molecule Iressa™ (gefitinib, AstraZeneca) was initially approved in Japan in July 2002 and in May 2003 through the FDA Accelerated Approval path for NSCLC. Under the Accelerated Approval guidelines, approved therapeutics are required to complete a post-approval Phase III study. Completion of the obligatory Phase III Iressa Survival Evaluation in Lung Cancer (ISEL) trial in 2004 revealed no improvement in overall survival in patients taking Iressa vs. placebo (Thatcher et al., 2005). However, an academic study published that same year suggested that the subset of Iressa responders correlated to mutations in the tyrosine kinase Epidermal Growth Factor Receptor (EGFR) gene. Retrospective analysis of the ISEL data with genetic testing for mutations in EGFR clearly indicated that patients with mutations in EGFR responded significantly better to Iressa than patients without EGFR mutations; interestingly, the patient cohorts carrying the EGFR mutations were predominantly Asian women with no smoking history (Lynch et al., 2004). In 2005 FDA responded to the Iressa-EGFR findings by allowing for use of Iressa only in patients currently taking the drug and showing a response or via new clinical trials. The increased control required over distribution of the drug resulted in a near-complete drop in

revenue generated in the US. With a much more limited patient population [EGFR mutations contribute to roughly 20% of NSCLC (Agarwal, 2012)], AstraZeneca forged ahead to change their commercialization strategy in countries outside the US. Interestingly, despite the clear link between EGFR mutation status and response, restrictions for use were not altered in Japan and Iressa was approved for use in China, mostly likely based on its efficacy in Asian populations. To expand to approval in Western countries, AstraZeneca positioned Iressa as both a second-line therapy to Taxotere (docetaxel) and partnered with the diagnostics company, DxS (which was subsequently bought by QIAGEN), to develop a molecular based assay for genotyping of EGFR. In 2009, Iressa was approved by the European Union with the CDx therascreen EGFR RGQ RT-PCR Kit. As of 2011, Iressa sales in Europe were ∼$150 million, with a total of ∼$520 million globally (Agarwal, 2012).

The "sea change" toward the utilization of CDx for FDA submission that resulted from the Iressa story, and others not discussed in this review, was ultimately driven by risk mitigation - mainly mitigation of the possibility of FDA rejecting approval of a therapeutic due to the lack of use of a CDx. FDA took a formal stance on CDx with the release of a draft guidance in 2011, which was subsequently finalized in August 2014 (http://www.fda.gov/downloads/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/UCM262327.pdf). This guidance defines an *In Vitro* CDx (IVD), indicates that in most instances a drug and the CDx should be FDA approved contemporaneously, outlines the FDA's regulatory enforcement policy and regulatory approval pathways for CDx, and discusses the implementation of labeling requirements upon co-approval of a drug and IVD assay. It goes without saying that the FDA oversight is not uniquely positioned to regulate only oncology therapeutics. Therefore, as part of each and every drug development program, from oncology to CNS, there could be questions about whether the drug can be shown to be safe and effective without enrichment of a particular patient population.

Study Outcomes as a Result of Personalized Medicine Approaches

There are significant financial benefits to utilizing a CDx strategy throughout drug development. Following the discovery of ALK-driven NSCLC in ∼5–10% of the patient population (Soda et al., 2007), Pfizer proceeded with utilizing a fluorescence *in situ* hybridization (FISH) assay to detect the ALK chromosomal translocation as an eligibility requirement for patient enrollment. What would have taken years, hundreds millions of dollars, and thousands of patients to complete, took less than 3 years and less than 350 patients to achieve statistical power for regulatory submission and approval (Kwak et al., 2010). The short timeline and small patient enrollment is not unique to the ALK story. As shown in **Table 2** multiple therapeutics have rapidly progressed from Phase I to market in less than 5 years through the use of patient enrichment with CDx.

Although there are less than 20 FDA Approved (Pre-Market Approval) CDx on the market to date (http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm301431.htm), there are over 100 drugs with pharmacogenomics information within the label (Personalized Medicine Coalition, 2014) and a projected 30% of drugs in late clinical development rely on biomarkers for patient enrollment (http://www.personalizedmedicinecoalition.org/Userfiles/PMC-Corporate/file/pmc_personalized_medicine_by_the_numbers.pdf). One group has examined the effectiveness of utilizing a CDx during 676 clinical trials with 199 compounds. Utilizing a biomarker-driven approach to Phase III trials, the success rate increased from 28% (no biomarker) to 62% (Falconi et al., 2014; Olsen and Jorgensen, 2014). Therefore, utilizing a biomarker-driven clinical trial increased the success rate of Phase III trials, and subsequent approval, almost 2.5-fold.

TABLE 2 | Examples of rapidly-progressing therapeutics.

Company	Therapeutic	Phase I	Approval
Roche/Genentech	Vemurafenib (Zelboraf™)	November 2006	August 2011
Pfizer	Crizotinib (Xalkori®)	December 2007	August 2011
GSK	Trametinib (Mekinist™)	May 2008	May 2013
Roche/Genentech	Ado-trastuzumab emtansine (Kadcyla®)	June 2009	February 2013
GSK	Dabrafenib (Tafinlar®)	April 2009	May 2013

Reimbursement Pressure

As discussed earlier, initially the narrowing of oncology patient populations appeared to translate to a loss in revenue potential for the therapeutic. However, with the evolution of regulatory agencies requiring patient enrichment to show safety and efficacy, and the increasing amount of data supporting faster and more successful routes to drug approval using CDx, the thinking has changed from assays being "burdensome" to assays being "useful" toward successful drug development. In addition, pressure is increasingly being put on pharmaceutical companies by insurance providers. Generally, targeted therapies are significantly more costly to a patient than generalized therapies; one report approximates the average cost per month for a branded oncology drug has doubled in the U.S. from $5000 to $10,000 in the past decade (http://www.imshealth.com/deployedfiles/imshealth/Global/Content/Corporate/IMS%20Health%20Institute/Reports/Secure/IMSH_Oncology_Trend_Report.pdf). In order to justify reimbursement of such expensive targeted therapies, insurance companies are requiring diagnostic tests be performed prior to prescription of a therapeutic. In European countries, a high level of scrutiny has been placed on newly developed therapeutics in comparison to existing treatments. For example, the UK National Institute for Health and Care Excellence (NICE) does not recommend the ALK inhibitor, Xalkori® for use in ALK-driven NSCLC, based on the ruling that the drug does not offer value for money.

In order to align with the fast-paced personalized medicine trend in healthcare and to ensure that each patient receives the most appropriate treatment for their disease, payers should support the use of CDx. Promotion of standardization of coverage and value-based reimbursements; reimbursement strategies that cover research-based innovations, such as Next

Generation Sequencing; and flexibility in medical coding and billing facilitate the use of personalized medicine approaches to ensure the right patient receives the right treatment at the right time.

Personalizing Therapeutic Approaches for Circadian Disorders

As discussed above, several genetic mutations have been identified that lead to a primary circadian disorder. Many more diseases have concomitant circadian abnormalities, which we refer to as secondary circadian disorders. For both types of disorders, phenotyping of the circadian disruption prior to initiation of therapy is critical to success—timing is everything when it comes to treatment of circadian dysfunction. In fact, measuring multiple rhythms within an individual is essential, as some disorders result from a misalignment of endogenous rhythms (Lewy et al., 2007). Traditional methods for monitoring the circadian phase and period in ambulatory humans include activity and body position monitoring via wearable devices (Bonmati-Carrion et al., 2015) and body temperature via wrist skin temperature (Kolodyazhniy et al., 2012). In a laboratory setting, multiple blood or saliva sampling to assess melatonin or other hormone patterns has been employed (Keijzer et al., 2014). In addition, the recent description of the rhythmic expression pattern of nearly half of the genome (Zhang et al., 2014) provides the opportunity to develop more and potentially better biomarkers for assessing the phase and period of individuals. Finally, mobile smartphones provide a unique and potentially highly effective method to collect robust data on circadian rhythms from an individual (Roenneberg, 2013). Thus, collecting robust data on a patient's circadian phenotype is both technically feasible and critical to the successful treatment of a circadian abnormality.

Tasimelteon, a Circadian Success Story

Approved on January 31st, 2014, HETLIOZ® (tasimelteon) is the first FDA approved treatment for adults with Non-24-H Sleep-Wake Disorder (Non-24), which is a rare circadian disorder occurring mostly in the totally blind. Vanda reports HETLIOZ® U.S. sales grew to $5.2 million in the first full quarter after launch. However, the road to approval was winding, with several failed clinical trials in other indications before the drug successfully demonstrated efficacy in Non-24.

Tasimelteon is a melatonin receptor MT1/2 agonist that was originally discovered by BMS (previously known as BMS-214,778) and licensed to Vanda Pharmaceuticals. Vanda opened an IND in 2004 for Shift Work Disorder, Jet Lag Disorder (due to eastward travel) and DSPD, however to date Vanda has not sought approval for any of these indications. Following on the success of another melatonin agonist, ramelteon (Rozerem®), which is approved in the US for treatment of insomnia, tasimelteon was tested in clinical trials for sleep disorders, including a Phase II trial on circadian rhythm sleep disorders and several phase III primary insomnia trials. However, the drug failed to show significant efficacy in these clinical trials. Additionally, the drug was also considered as a treatment for depression.

After several years of frustratingly weak results in other indications, Vanda demonstrated that tasimelteon produced phase shifts in healthy adults (Neubauer, 2015). Vanda then began Phase III clinical trials of the drug in a population with Non-24. There are ∼1,300,000 blind people in the United States and ∼10% of these individuals have no light perception. Without light input these totally blind individuals free run, drifting in and out of phase with the environment, impairing their ability to work, disrupting family life and impacting their overall health. Tasimelteon treatment entrained a higher number of totally blind to a 24-h cycle vs. placebo and the drug was approved in the US and available to appropriate patients via specialty pharmacy (Neubauer, 2015).

Vanda faced several significant challenges in the clinical trials testing the efficacy of tasimelteon for Non-24. The first task was setting the appropriate enrollment criteria and developing a screening protocol to capture the desired subjects. The number of enrollment failures in the SET trial was high; 391 subjects were screened; yet only 84 were enrolled in the randomized trial (Lockley et al., 2013). Vanda did not study subjects with a *tau* shorter than 24 h even though it is reasonable to expect that subjects with a short *tau* would eventually respond to treatment once the timing of dosing coincided with a sensitive phase in the circadian rhythm. Subjects with *tau* shorter than 24 h were excluded from the study because the dosing regime was fixed to administer the drug at 1 h prior to bedtime, based on concerns about drug-induced somnolence. Even though Vanda has not done a PRC for the drug, they were concerned that they would be unable to show efficacy in patients with a short *tau* if the drug was always to be taken at 1 h prior to bedtime.

Timing of treatment was not always aligned to the sensitive circadian phase of the treated subject's rhythm. The drug was always taken at 1 h prior to bedtime and although Vanda had tried to initiate dosing when subjects were in phase, 16% of subjects were out of phase when dosing was started. Subjects that were out of phase at the start of trial took a longer time on treatment to see efficacy, which contributed to the length of trial (Lockley et al., 2013). Circadian *tau* at was measured at 1 month and 7 months of treatment in the RESET trial (Lockley et al., 2013). RESET took almost 2 years to complete.

A final challenge in the tasimelteon trial was the use of entrainment as an outcome measure; an endpoint that is more correctly considered a proof of mechanism for the drug, not a proof that the drug is a treatment for the disorder. Only clinical benefit, defined by an improvement in how patients feel, function or survive, constitutes an acceptable primary endpoint for registration of a drug. An improvement in sleep did constitute a positive clinical outcome. The tasimelteon-treated group had significant improvement in the duration and timing of nighttime sleep and a significant decrease in daytime napping. Thus, tasimelteon is now available to provide treatment for blind patients suffering from Non-24.

Final Perspective

Circadian biologists have been collecting human subject circadian data in the research setting for decades, but few circadian drug treatment trials have been attempted. The general population has a growing familiarity with circadian biology in general and with their own unique rhythms, thanks to smart phone apps and wearable personal activity trackers. Nevertheless, drug developers have not yet linked the profound impact of circadian dysfunction on health to an influx of circadian drugs into pharma pipelines. The growing awareness of the link between disrupted circadian rhythms and obesity necessitates developing clinical trial strategies to effectively demonstrate the therapeutic benefit of drugs that alter circadian rhythms (chronotherapies). Better education of clinical researchers in the science of circadian biology is essential to developing enrollment criteria that will effectively capture the appropriate patient population. It should be apparent that circadian therapies must also be appropriately timed; treatment must be adjusted to be consistent with the patient's circadian phenotype, requiring a high degree of physician expertise and skill in interpreting circadian rhythms and possibly even daily input on scheduling dosing. Technological advances in remote data collection and the growing acceptance of sharing personal data will aid in personalizing circadian therapies to the appropriate patient. As data collection is improved, it may become obvious that not all patients will benefit from a specific therapy, as occurred in oncology. Biomarker strategies must be incorporated early in development to reduce screening failures and improve the potential to see efficacy, thus reducing the number of failed clinical trials. Biomarkers in this sense do not have to be genetic markers, but could be a clear circadian phenotype based on activity or other rhythm data.

Finally, circadian biologists have a deep appreciation of the negative impact on overall health of misaligned and disrupted rhythms, but simply demonstrating correction of the rhythm will not be sufficient for approval of a drug. Circadian drugs must also demonstrate that clinically relevant endpoints are improved by circadian adjustments.

References

Agarwal, A. (2012). Do companion diagnostics make economic sense for drug developers? *N. Biotechnol.* 29, 695–708. doi: 10.1016/j.nbt.2012.04.004

Bailey, S. M., Udoh, U. S., and Young, M. E. (2014). Circadian regulation of metabolism. *J. Endocrinol.* 222, R75–R96. doi: 10.1530/JOE-14-0200

Bonmati-Carrion, M. A., Middleton, B., Revell, V. L., Skene, D. J., Rol, M. A., and Madrid, J. A. (2015). Validation of an innovative method, based on tilt sensing, for the assessment of activity and body position. *Chronobiol. Int.* 3, 1–10. doi: 10.3109/07420528.2015.1016613

Bugge, A., Feng, D., Everett, L. J., Briggs, E. R., Mullican, S. E., Wang, F., et al. (2012). Rev-erbalpha and Rev-erbbeta coordinately protect the circadian clock and normal metabolic function. *Genes Dev.* 26, 657–667. doi: 10.1101/gad.186858.112

Corruble, E., De Bodinat, C., Belaidi, C., and Goodwin, G. M. (2013). Efficacy of Agomelatine and Escitalopram on depression, subjective sleep and emotional experiences in patients with major depressive disorder: a 24-wk randomized, controlled, doubleblind trial. *Int. J. Neuropsychopharmacology* 16, 2219–2234. doi: 10.1017/S1461145713000679

Czeisler, C. A., Duffy, J. F., Shanahan, T. L., Brown, E. N., Mitchell, J. F., Rimmer, D. W., et al. (1999). Stability, precision, and near-24-hour period of the human circadian pacemaker. *Science* 284, 2177–2181. doi: 10.1126/science.284.5423.2177

Dressman, M. A., Licamele, L., Feeney, J., and Polymeropoulos, M. H. (2012). "Seventy percent of totally blind people with sleep complaints are not entrained to the 24 hour clock," in *26th Annual Meeting of the Associated Professional Sleep Societies* (Boston, MA: Associated Professional Sleep Societies, LLC).

Falconi, A., Lopes, G., and Parker, J. L. (2014). Biomarkers and receptor targeted therapies reduce clinical trial risk in non-small-cell lung cancer. *J. Thorac. Oncol.* 9, 163–169. doi: 10.1097/JTO.0000000000000075

Feng, D., and Lazar, M. A. (2012). Clocks, metabolism, and the epigenome. *Mol. Cell* 47, 158–167. doi: 10.1016/j.molcel.2012.06.026

Gahr, M. (2014). Agomelatine in the treatment of major depressive disorder: an assessment of benefits and risks. *Curr. Neuropharmacol.* 12, 287–398. doi: 10.2174/1570159X12999140619122914

Golombek, D. A., Casiraghi, L. P., Agostino, P. V., Paladino, N., Duhart, J. M., Plano, S. A., et al. (2013). The times they're a-changing: effects of circadian desynchronization on physiology and disease. *J. Physiol. Paris* 107, 310–322. doi: 10.1016/j.jphysparis.2013.03.007

Griebel, G., Ravinet-Trillou, C., Beeske, S., Avenet, P., and Pichat, P. (2014). Mice deficient in cryptochrome 1 (cry1 (-/-)) exhibit resistance to obesity induced by a high-fat diet. *Front. Endocrinol. (Lausanne).* 5:49. doi: 10.3389/fendo.2014.00049

Johnson, C. H. (1990). *An Atlas of Phase Response Curves for Circadian and Circatidal Rhythms*. Available online at: http://as.vanderbilt.edu/johnsonlab/prcatlas/.

Karatsoreos, I. N. (2014). Links between circadian rhythms and psychiatric disease. *Front. Behav. Neurosci.* 8:162. doi: 10.3389/fnbeh.2014.00162

Karthikeyan, R., Marimuthu, G., Ramasubramanian, C., Arunachal, G., Bahammam, A. S., Spence, D. W., et al. (2014). Association of Per3 length polymorphism with bipolar I disorder and schizophrenia. *Neuropsychiatr. Dis. Treat.* 10, 2325–2330. doi: 10.2147/NDT.S73765

Katzenberg, D., Young, T., Finn, L., Lin, L., King, D. P., Takahashi, J. S., et al. (1998). A CLOCK polymorphism associated with human diurnal preference. *Sleep* 21, 569–576.

Keijzer, H., Smits, M. G., Duffy, J. F., and Curfs, L. M. G. (2014). Why the dim light melatonin onset (DLMO) should be measured before treatment of patients with circadian rhythm sleep disorders. *Sleep Med. Rev.* 18, 333–339. doi: 10.1016/j.smrv.2013.12.001

Khachiyants, N., Trinkle, D., Son, S. J., and Kim, K. Y. (2011). Sundown syndrome in persons with dementia: an update. *Psychiatry Investig.* 8, 275–287. doi: 10.4306/pi.2011.8.4.275

Koike, N., Yoo, S. H., Huang, H. C., Kumar, V., Lee, C., Kim, T. K., et al. (2012). Transcriptional architecture and chromatin landscape of the core circadian clock in mammals. *Science* 338, 349–354. doi: 10.1126/science.1226339

Kojetin, D. J., and Burris, T. P. (2014). REV-ERB and ROR nuclear receptors as drug targets. *Nat. Rev. Drug Discov.* 13, 197–216. doi: 10.1038/nrd4100

Kolodyazhniy, V., Späti, J., Frey, S., Götz, T., Wirz-Justice, A., Kräuchi, K., et al. (2012). An improved method for estimating human circadian phase derived from multichannel ambulatory monitoring and artificial neural networks. *Chronobiol. Int.* 28, 1078–1097. doi: 10.3109/07420528.2012.700669

Kwak, E. L., Bang, Y. J., Camidge, D. R., Shaw, A. T., Solomon, B., Maki, R. G., et al. (2010). Anaplastic lymphoma kinase inhibition in non-small-cell lung cancer. *N. Engl. J. Med.* 363, 1693–1703. doi: 10.1056/NEJMoa1006448

Lewy, A. J., Rough, J. N., Songer, J. B., Mishra, N., Yuhas, K., and Emens, J. S. (2007). The phase shift hypothesis for the circadian component of winter depression. *Dialogues Clin. Neurosci.* 9, 291–300.

Lockley, S. W., Dressman, M. A., Xiao, C., Licamele, L., and Polymeropoulos, M. H. (2013). "RESET study demonstrates that tasimelteon maintains entrainment of melatonin and cortisol in totally blind individuals with non-24-hour circadian rhythms," in The *Endocrine Society's 95th Annual Meeting and Expo* (*ENDO*) (San Francisco, CA: Endocrine Society).

Long, A. M., Zhao, H., and Huang, X. (2012). Structural basis for the potent and selective inhibition of casein kinase 1 epsilon. *J. Med. Chem.* 55, 10307–10311. doi: 10.1021/jm301336n

Lynch, T. J., Bell, D. W., Sordella, R., Gurubhagavatula, S., Okimoto, R. A., Brannigan, B. W., et al. (2004). Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N. Engl. J. Med.* 350, 2129–2139. doi: 10.1056/NEJMoa040938

Maury, E., Hong, H. K., and Bass, J. (2014). Circadian disruption in the pathogenesis of metabolic syndrome. *Diabetes Metab.* 40, 338–346. doi: 10.1016/j.diabet.2013.12.005

McCarthy, M. J., and Welsh, D. K. (2012). Cellular circadian clocks in mood disorders. *J. Biol. Rhythms* 27, 339–352. doi: 10.1177/0748730412456367

Meijer, J. H., and Rietveld, W. J. (1989). Neurophysiology of the suprachiasmatic circadian pacemaker in rodents. *Physiol. Rev.* 69, 671–707.

Melosky, B. (2014). Review of EGFR TKIs in metastatic NSCLC, including ongoing trials. *Front. Oncol.* 4:244. doi: 10.3389/fonc.2014.00244

Mohawk, J. A., Green, C. B., and Takahashi, J. S. (2012). Central and peripheral circadian clocks in mammals. *Annu. Rev. Neurosci.* 35, 445–462. doi: 10.1146/annurev-neuro-060909-153128

Neubauer, D. N. (2015). Tasimelteon for the treatment of non-24-hour sleep-wake disorder. *Drugs Today* 51, 29–35. doi: 10.1358/dot.2015.51.1.2258364

Olsen, D., and Jorgensen, J. T. (2014). Companion diagnostics for targeted cancer drugs - clinical and regulatory aspects. *Front. Oncol.* 4:105. doi: 10.3389/fonc.2014.00105

Personalized Medicine Coalition. (2014). *The Case for Personalized Medicine, 4th Edn* (Washington, DC).

Roenneberg, T. (2013). Chronobiology: the human sleep project. *Nature* 498, 427–428. doi: 10.1038/498427a

Rosenwasser, A. M., and Dwyer, S. M. (2001). Circadian phase shifting: Relationships between photic and nonphotic phase-response curves. *Physiol. Behav.* 73, 175–183. doi: 10.1016/S0031-9384(01)00466-8

Seleem, M. A., Merranko, J. A., Goldstein, T. R., Goldstein, B. I., Axelson, D. A., Brent, D. A., et al. (2014). The longitudinal course of sleep timing and circadian preferences in adults with bipolar disorder. *Bipolar Disord.* 17, 392–402. doi: 10.1111/bdi.12286

Smolensky, M. H., Portaluppi, F., Manfredini, R., Hermida, R. C., Tiseo, R., Sackett-Lundeen, L. L., et al. (2014a). Diurnal and twenty-four hour patterning of human diseases: acute and chronic common and uncommon medical conditions. *Sleep Med. Rev.* 21, 12–22. doi: 10.1016/j.smrv.2014.06.005

Smolensky, M. H., Portaluppi, F., Manfredini, R., Hermida, R. C., Tiseo, R., Sackett-Lundeen, L. L., et al. (2014b). Diurnal and twenty-four hour patterning of human diseases: cardiac, vascular, and respiratory diseases, conditions, and syndromes. *Sleep Med. Rev.* 21, 3–11. doi: 10.1016/j.smrv.2014.07.001

Soda, M., Choi, Y. L., Enomoto, M., Takada, S., Yamashita, Y., Ishikawa, S., et al. (2007). Identification of the transforming EML4-ALK fusion gene in non-small-cell lung cancer. *Nature* 448, 561–566. doi: 10.1038/nature05945

Sprouse, J., Reynolds, L., Kleiman, R., Tate, B., Swanson, T. A., and Pickard, G. E. (2010). Chronic treatment with a selective inhibitor of casein kinase I delta/epsilon yields cumulative phase delays in circadian rhythms. *Psychopharmacology (Berl).* 210, 569–576. doi: 10.1007/s00213-010-1860-5

Thatcher, N., Chang, A., Parikh, P., Rodrigues Pereira, J., Ciuleanu, T., Von Pawel, J., et al. (2005). Gefitinib plus best supportive care in previously treated patients with refractory advanced non-small-cell lung cancer: results from a randomised, placebo-controlled, multicentre study (Iressa Survival Evaluation in Lung Cancer). *Lancet* 366, 1527–1537. doi: 10.1016/S0140-6736(05)67625-8

Zelinski, E. L., Deibel, S. H., and McDonald, R. J. (2014). The trouble with circadian clock dysfunction: multiple deleterious effects on the brain and body. *Neurosci. Biobehav. Rev.* 40, 80–101. doi: 10.1016/j.neubiorev.2014.01.007

Zhang, R., Lahens, N. F., Ballance, H. I., Hughes, M. E., and Hogenesch, J. B. (2014). A circadian gene expression atlas in mammals: Implications for biology and medicine. *Proc. Natl. Acad. Sci. U.S.A.* 111, 16219–16224. doi: 10.1073/pnas.1408886111

Conflict of Interest Statement: Barbara A. Tate is the acting CEO of Armada Therapeutics. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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