J Appl Physiol 107: 1647–1654, 2009.
First published August 20, 2009; doi:10.1152/japplphysiol.00725.2009.

HIGHLIGHTED TOPIC | *The Role of Clock Genes in Cardiometabolic Disease*

Working around the clock: circadian rhythms and skeletal muscle

Xiping Zhang, Thomas J. Dube, and Karyn A. Esser

Center for Muscle Biology, Department of Physiology, University of Kentucky, Lexington, Kentucky

Submitted 6 July 2009; accepted in final form 19 August 2009

Zhang X, Dube TJ, Esser KA. Working around the clock: circadian rhythms and skeletal muscle. *J Appl Physiol* 107: 1647–1654, 2009. First published August 20, 2009; doi:10.1152/japplphysiol.00725.2009.—The study of the circadian molecular clock in skeletal muscle is in the very early stages. Initial research has demonstrated the presence of the molecular clock in skeletal muscle and that skeletal muscle of a clock-compromised mouse, Clock mutant, exhibits significant disruption in normal expression of many genes required for adult muscle structure and metabolism. In light of the growing association between the molecular clock, metabolism, and metabolic disease, it will also be important to understand the contribution of circadian factors to normal metabolism, metabolic responses to muscle training, and contribution of the molecular clock in muscle-to-muscle disease (e.g., insulin resistance). Consistent with the potential for the skeletal muscle molecular clock modulating skeletal muscle physiology, there are findings in the literature that there is significant time-of-day effects for strength and metabolism. Additionally, there is some recent evidence that temporal specificity is important for optimizing training for muscular performance. While these studies do not prove that the molecular clock in skeletal muscle is important, they are suggestive of a circadian contribution to skeletal muscle function. The application of well-established models of skeletal muscle research in function and metabolism with available genetic models of molecular clock disruption will allow for more mechanistic understanding of potential relationships.

molecular clock; *Bmal1*; *MyoD*; peroxisome proliferator-activated receptor-γ coactivator-1

CIRCADIAN RHYTHMS

THE TERM CIRCADIAN COMES FROM the Latin circa, "around", and diem, "day", meaning "about a day." Almost all organisms, ranging from single-cell bacteria to humans, exhibit a variety of behavioral, physiological, and biochemical circadian rhythms (4, 34, 59). The presence of a molecular clock within a cell and/or organism provides the necessary timekeeping for anticipation of daily changes in environmental/external conditions (3, 28, 35, 41, 79, 89). Synchronizing the molecular clock and intracellular physiology with external day-night cycles represents an evolutionary survival advantage for organisms (4, 41, 68). While much has been learned about the components of the molecular clock, there is still very little known about its regulation and function in skeletal muscle. Thus the goal of this review is to present the latest findings regarding the molecular clock in skeletal muscle and present what is known regarding circadian changes in skeletal muscle physiology and metabolism. This is a very new and open field of research, so much is yet to be done to mechanistically link the function of the molecular clock in skeletal muscle to the known functional and metabolic oscillations in the intact organism.

Address for reprint requests and other correspondence: K. A. Esser, Center for Muscle Biology, Dept. of Physiology, Chandler College of Medicine, Univ. of Kentucky, 800 Rose St., Lexington, KY 40536 (e-mail: kaesse2@uky.edu).

CENTRAL AND PERIPHERAL CLOCKS IN MAMMALS

To date, it has been established that cells in all tissues/organs of mammals have an endogenous molecular clock (2, 40, 50, 54, 57, 60, 69, 71, 77, 86, 99, 100). The synchronization of all of the molecular clocks in the body is largely under the control of the central clock, the suprachiasmatic nucleus (SCN) of the hypothalamus. The central clock is considered the "master", based on its role in controlling the daily sleep-wake cycle, body temperature, and feeding and activity behaviors of the animal (52, 55, 80). As the "master" clock, the SCN has historically been thought to have a master-slave relationship to peripheral clocks by coordinating their respective circadian rhythms via neural-humoral signals (58, 76). However, more recent studies have demonstrated that the molecular clock in the liver, skeletal muscle, and other peripheral tissues can be phase dissociated from the molecular clock in the SCN by restricting feeding to a time period inverse to normal feeding behavior (18, 85). While not as clearly defined, Zambon et al. (98) report that there is an interaction between time of day and contraction on gene expression and suggest that contractile activity might be an external time cue, or zeitgeber, for the clock in skeletal muscle. These results are significant in that they indicate that feeding, and maybe contractile activity, can act as a dominant zeitgeber for setting clocks in peripheral tissues, and, second, that the circadian rhythms of the periph-

eral tissues can be dissociated from control by the SCN through nonphotic environmental signals.

The genes encoding the components of the mammalian molecular clock have only been identified in the last 10+ yr, and the oscillation of these genes in peripheral tissues was believed to be the result of cues from the SCN (13, 35, 89, 92). Work by Balsalobre et al. (9) and Yamazaki et al. (94) provided the first evidence that the molecular clocks in peripheral tissues were cell autonomous. In 2005, Yoo and coworkers (97) used a genetic mouse model to definitively confirm that peripheral tissues possess endogenous, self-sustaining rhythms that oscillate over a 24-h period. This finding was based on the use of a circadian reporter mouse, in which the luciferase cDNA was knocked in to the *Per2* coding region, giving rise to a chimeric protein, PER2:LUC, that maintained normal PER2 circadian function, but also contained luciferase activity. Tissue explants from the SCN and peripheral organs of these PER2:LUC mice were put into culture with luciferin, and luminescent emissions were obtained for up to 2 wk. The results of these studies demonstrated that the molecular clock in peripheral tissues was self-sustaining in the absence of systemic neural or humoral cues (93, 96, 97). In addition, these findings opened the door to new lines of research on topics such as defining what the time cues are for different peripheral tissues, determining mechanisms of synchronization of cellular clocks within a tissue and the potential for disruption of the peripheral clocks and their contribution to disease.

THE MOLECULAR CLOCK

The molecular mechanism responsible for generating circadian rhythms is a highly conserved gene regulatory network composed of transcriptional-translational feedback loops, and this topic has been reviewed in greater detail by others (35, 41, 47, 56, 79, 89). The molecular components of the circadian time-keeping mechanism are referred to as the core clock genes (Table 1). The positive loop of the core clock is formed by two members of the PAS-bHLH family of transcription factors, *Clock* (circadian locomotor output control kaput) and *Bmal1* (brain muscle arnt-like 1) (7, 13, 39, 46, 92). The CLOCK:BMAL1 heterodimer activates transcription of the negative arm of the molecular clock comprised of core clock genes *Period* (*Per1*, *Per2* and *Per3*) and *Cryptochrome* (*Cry1* and *Cry2*) (3, 28, 47, 56, 79). The CRY and PER proteins constitute the negative loop of the core clock by forming a multimeric complex that inhibits CLOCK:BMAL1 transcriptional activity upon translocation to the nucleus. Additions to the core clock family of genes are the orphan nuclear receptors *Rev-erb* α/β and *Rora* (RAR-related orphan receptor-α) (1, 21, 72, 78, 95). These genes are part of the core clock, as they link the feedback loops by repressing *Bmal1* expression (Rev-erbs) or activating *Bmal1* expression (Rora). Like other cell types, the circadian expression of these core clock genes described in this section has been demonstrated in mammalian skeletal muscle (6, 57, 60, 98).

In the last few years, new molecules have been identified that can modulate the core clock mechanism. Specifically, Liu et al. (49) have found that peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α can modulate the core clock components, *Bmal1* and *Rev-erb*-α, via coactivation of the ROR family of orphan nuclear receptors. They found that expression of PGC-1α mRNA was circadian in liver and muscle and that mice lacking *PGC-1*α show abnormal rhythms of activity, body temperature, and metabolic rate. The disruption of physiological rhythms in these animals is correlated with aberrant expression of clock genes and those involved in energy metabolism (49). Circadian expression of *PGC-1*β has also been shown to be circadian in skeletal muscle of mice (57). These studies implicate the contribution of the *PGC-1* coactivator family in the regulation of the core clock mechanism.

In addition to the transcription factors that regulate the molecular clock, recent studies have demonstrated that acetylation/deacetylation contributes to the regulation of the clock mechanism (5, 20, 23, 30, 61, 62, 75, 95). These studies have found that regions of the genome are more or less acetylated in a time-of-day dependent manner. This level of genomic control is intriguing, as it suggests that a cell's response to environmental triggers will be sensitive to time-of-day-dependent genomic modifications. A molecular link between the acetylation state of the genome and circadian rhythms was established when Doi et al. (20) showed that CLOCK possesses intrinsic histone acetyltransferase activity, and this enzymatic function contributes to chromatin-remodeling events implicated in circadian control of gene expression (20). CLOCK has also been shown to acetylate a nonhistone substrate: its own binding partner, BMAL1. BMAL1 is specifically acetylated by CLOCK on a unique, highly conserved Lys 537 residue. Hirayama et al. (37) found that BMAL1 undergoes rhythmic acetylation in mouse liver, with a timing that parallels the downregulation of circadian transcription of clock-controlled genes. The acetylation of BMAL1 facilitates recruitment of CRY1 to the CLOCK-BMAL1 heterodimer promoting transcriptional repression (37).

In contrast, the histone acetyltransferase activity of CLOCK is counterbalanced by the role of histone deacetylases. Alenghat et al. (5) found that disruption of the interaction between nuclear receptor corepressor 1 (*Ncor1*) and histone deacetylase 3 (*Hdac3*) can alter core clock gene expression. *Ncor1* functions as an activating subunit for the chromatin modifying enzyme *Hdac3*. Alenghat et al. created a mouse in which the site on Ncor1 for Hdac3 interaction was mutated. This disrupted NCOR1:HDAC3 interaction and led to increased expression of Bmal1 and modified circadian behavior (5). The other deacetylase that has most recently been linked to the

Table 1.

	Genes	Ref. No.
Core clock genes	*Bmal1, Clock, Per1, Per2, Cry1, Cry2, Rora, Rev-erba, Csnk1d, Fbxl3*	79, 89
Clock-controlled genes	*Dbp, Tef, Nampt, p21, Id1, Dec1*	3, 22, 47, 59, 66, 77
Circadian genes in muscle	*UCP3, PDK4, Atrogin, MuRF1, Pank1, Dgat2, MHC IIX, Thra, Tbc1d4*	6, 57, 60

molecular clock and circadian function is SIRT1, the nicotinamide adenine dinucleotide (NAD+)-dependent histone deacetylase (8, 62, 63, 75). Mammalian SIRT1 (Sir2α) is a member of the Sir2 (silent information regulator 2) family. *Sir2* is an NAD-dependent deacetylase that is broadly conserved from bacteria to humans. Sir2 is most commonly known to be important in the regulation of aging and is required for calorie restriction-induced lifespan extension in yeast and flies (12, 15, 43, 45, 51).

Asher et al. (8) showed that SIRT1 activity oscillates over a 24-h period and is required for high-level circadian expression through interactions with CLOCK and BMAL1. In particular, experiments showed that SIRT1 targets PER2 for deacetylation and subsequent degradation, and this contributed to the fidelity of the molecular clock. More recently, studies by Ramsey et al. (75) and Nakahata et al. (63) presented data establishing SIRT1 as part of a new negative-feedback loop to the molecular clock involving the metabolite NAD+ (63, 75). These studies showed that intracellular levels of NAD+ oscillate in mouse embryonic fibroblasts, liver, and white adipose tissue, and this contributes to circadian regulation of SIRT1 function. They went on to show that SIRT1 interacts with CLOCK:BMAL1 on the promoter of the gene, *nampt* (nicotinamide phosphoribosyltransferase; also known as PBEF or visfatin), the rate-limiting enzyme in the NAD+ salvage pathway. Thus this provides a new negative feedback loop to the circadian pathway through CLOCK:BMAL1 regulating expression of *nampt*, leading to synthesis of the coenzyme NAD+, which enhances SIRT1 deacetylase activity and represses CLOCK:BMAL1 transcriptional targets. In addition to the effects on the molecular clock, the circadian changes in NAD+ levels and SIRT1 activity suggest that circadian factors could lead to an intrinsic fluctuation in cellular metabolism in liver. This new area of research is exciting; however, implications for CLOCK:BMAL1 regulation of *nampt* and NAD+ feedback to the molecular clock in skeletal muscle are unclear at this time. Based on expression profiling data, *nampt* is not expressed in a circadian manner in skeletal muscle; however, levels of *nampt* mRNA are significantly downregulated in the muscle of the Clock mutant mouse (57). A detailed temporal analysis of NAD+ levels in skeletal muscle has not been performed to date, but it is likely that levels are dynamic in metabolically active skeletal muscle (25). One challenge in this field will be to delineate how much of the control of NAD+ levels in skeletal muscle come from metabolic activity (AMPK activation of *nampt*) vs. circadian regulated events (25).

SKELETAL MUSCLE CIRCADIAN TRANSCRIPTOME

Several groups have performed expression profiling studies on the SCN and different peripheral tissues [e.g., liver, fat, heart, smooth muscle, skeletal muscle] to identify the genes that are expressed in a circadian manner (2, 40, 54, 57, 60, 69, 77, 86, 99, 100). To analyze tissue gene expression for circadian patterns requires tissue samples to be collected at set intervals, most often every 4 h, and for at least 24 h, but preferably for 48 h. Different statistical tests, such as COSOPT, have been applied to expression profiling data sets to identify genes whose expression oscillates over a 24-h period (42, 57, 87). The results of such studies have found that, in addition to the known core clock genes, ~2–10% of the genes expressed in a tissue have a circadian pattern of expression. This means that there are many genes in a tissue, >100, that have an expression that is in a sinusoidal pattern with a 24-h period length, and these genes are either the direct transcriptional output of the core clock transcriptional mechanism (clock-controlled genes) or are circadian genes based on their oscillating expression due to neural/humoral/biochemical factors. What is most intriguing, however, is the fact that <10% of the circadian genes in any one tissue are commonly expressed in another tissue (2, 40, 54, 57, 60, 69, 77, 86, 99, 100). This remarkable tissue specificity of circadian genes is thought to reflect the unique physiological characteristics of a particular tissue. How such a high degree of specificity is achieved for each tissue, even though the core clock mechanism is the same across tissues, is still unclear.

The skeletal muscle circadian transcriptome was first reported by Miller et al. (60). Data for this study came from analysis of gene expression from muscle collected every 4 h over two circadian cycles (48 h) and supported the diurnal results of gene expression published by Zambon et al. in 2003 (98). Further bioinformatic analysis by McCarthy et al. (57) described ~215 mRNAs that were expressed in a circadian pattern in skeletal muscle, including known core clock genes, *Bmal1*, *Per2*, and *Cry2* (57). The identification of core clock gene expression in skeletal muscle was also reported from a study of rat skeletal muscle published by Almon et al. (6). While they defined fewer genes as circadian ($n = 109$), there was still significant overlap in the gene list, including the core clock genes, *Bmal1* and *Per2*, and other genes, such as *Pdk4*, *Dgat2*, and *Asb2*. McCarthy et al. (57) reported that the circadian genes expressed in skeletal muscle could be classified by a range of cellular processes, including transcription, lipid metabolism, protein degradation, ion transport, and vesicular trafficking. The tissue specificity of the skeletal muscle circadian transcriptome was highlighted by the presence of known muscle-specific genes, such as *Myod1*, *Ucp3*, *Atrogin1* (*Fbxo32*), and *Myh1* (myosin heavy chain IIX). These genes all exhibit a circadian pattern of expression only in skeletal muscle. The observation of circadian expression of *Myod1* was intriguing because it is a well-known transcription factor with a central role in the determination of the skeletal muscle lineage. From a metabolic perspective, it was exciting to find that PGC-1β was oscillating in skeletal muscle. As discussed in the section above on the molecular clock, there is growing evidence that the PGC-1 coactivator family is an integral molecular link between cell metabolism and the molecular clock. In summary, the results of the expression profiling of circadian genes in skeletal muscle provides exciting clues as to the potential output of the molecular clock for the regulation of skeletal muscle gene expression and metabolism. However, there it is still much to be learned regarding which circadian genes in skeletal muscle are due to direct transcriptional regulation by CLOCK:BMAL1 vs. genes that are regulated in a circadian manner due to behavior (such as feeding or activity) of the animal. In addition, gene array data and real-time PCR only provide information about mRNA levels. For understanding whether any of these circadian mRNA changes induce functional changes in the cell will require more detailed protein analysis and cell biological studies.

McCarthy et al. (57) also compared the expression of the circadian genes in skeletal muscle of the *Clock* mutant mouse to those in muscle of wild-type mice. The *Clock* mutant mouse

was originally described by Vitaterna et al. (92) and has a mutation that leads to deletion of *exon 19* of CLOCK (7, 46, 92). The mouse exhibits a lengthened free run period, and this mouse was recently shown to develop metabolic disease (91). McCarthy and colleagues found many core clock (e.g., *Bmal1*, *Per2*) and direct clock-controlled genes (e.g., *Dbp*, D site albumin promoter binding protein: *Tef*, thyrotroph embryonic factor) did not oscillate in the muscle of the Clock mutant mouse (57). Specifically, expression of the core clock genes, *Bmal1* and *Per2*, and known direct clock-controlled gene [target for CLOCK:BMAL1 transcription], *Dbp* and *Tef*, were flat over 24 h in the muscle of Clock mutant mice. Interestingly, expression of *Myod1* and *PGC1β* did not oscillate over 24 h, suggesting that these genes might be considered as a target for direct CLOCK:BMAL1 transcriptional regulation (i.e., direct clock-controlled gene). In contrast, other genes that had exhibited a circadian pattern of expression in muscle, such as *Ucp3* and *Pdk4*, still continued to oscillate in the muscle of the CLOCK mutant mouse, but the peak of expression was shifted ~12 h. These results suggest that CLOCK:BMAL1 do not directly control the circadian transcription of *Ucp3* or *Pdk4*, but that some behavioral, humoral, or neural signal(s) is altered in the Clock mutant mouse, leading to a complete phase shift in expression. It is interesting to contemplate whether the phase shift in *Ucp3* and *Pdk4* mRNA expression leads to any alteration in metabolism or changes in response to muscle use/training. It is also important to keep in mind that skeletal muscle comprises a large percentage of body mass, so how much of the metabolic phenotype seen at the systems level in the Clock mutant mouse is due to altered metabolic function in the skeletal muscle needs to be considered. Generation of genetically modified mice in which the core clock genes are selectively altered in skeletal muscle will be necessary to address this important issue.

McCarthy et al. (57) also compared the expression of non-circadian genes between muscle of wild-type and *Clock* mutant mice and found that 35% of the genes (3,146 out of 9,002 genes expressed) were significantly altered in expression (57). This indicates that the Clock mutation has a significant impact beyond the core clock mechanism and leads to disrupted expression of a number of genes in skeletal muscle. There were many common muscle-specific genes that were altered in the muscle of Clock mutant mice, with decreased expression seen in skeletal actin, troponins, titin, dystrophin, and myosin heavy chain mRNAs. Metabolic genes were also affected with decreased expression of muscle creatine kinase, phosphofructokinase, PGC-1α, and several subunits of the mitrochondrial ATP synthase. Since expression of *Myod1* was altered in the muscle of these mice, McCarthy et al. also compared the changes in gene expression to other data sets of *Myod1*-regulated genes (57). Consistent with the concept of *Myod1* being an output of the molecular clock, they found that 125 *Myod1*-regulated genes were significantly changed in *Clock* mutant skeletal muscle. The majority (84%, 105/125) of these *Myod1*-regulated genes were significantly downregulated in the *Clock* mutant compared with control levels, with an average decrease of ~40% (−15 to −90%). These results from the expression profiling study suggest that the molecular clock may regulate important downstream targets, such as *MyoD* and *PGC1* coactivators, and this contributes to maintenance of expression of critical structural and metabolic gene expression in skeletal muscle.

In summary, our understanding of the role of the molecular clock in skeletal muscle is in very early stages. Studies have demonstrated that the molecular clock genes are expressed in skeletal muscle, and that they oscillate in a similar manner to that seen in the central clock (SCN) and other peripheral clocks, such as the liver, adiopose tissue, and heart (2, 6, 40, 54, 57, 60, 77, 99). Neural or humoral cues from the SCN are important for synchronizing the molecular clock in muscle, but recent work has shown that restricted feeding to the light phase leads to phase disruption of the molecular clock in muscle (18, 49, 80). The similarities or differences in amplitude and phase of the molecular clocks across all of the skeletal muscles from hindlimb to forelimb to diaphragm to eye muscles are not yet understood. In addition, little is known about the molecular mechanisms that link the molecular clock in skeletal muscle to the physiological and metabolic parameters that change over the time of day, as discussed in the next section. We view this as an open and exciting area for research in the next years.

SKELETAL MUSCLE PHYSIOLOGY AND CIRCADIAN RHYTHMS

There is a significant amount of research that has been performed analyzing the daily variations in muscular strength and power in humans. The work by Gauthier et al. (26) investigated the influence of time of day on the torque and electromyographic (EMG) activity of the elbow flexor muscles during maximum and submaximum isometric contractions. Measurements of torque and oral temperature were made at seven time points across a 24-h period in male subjects. The greatest torque was measured in the late afternoon (4–6 PM) compared with torque measurements made in the morning between 8 and 10 AM. The diurnal rhythm in the elbow flexor torque was also found to be in phase with the diurnal rhythm of oral temperature. To determine whether this change in torque was due to muscle vs. neurological changes, the authors also determined EMG activity and calculated the torque-to-EMG ratio. The results from these experiments found no change in EMG signal, resulting in a higher torque-to-EMG ratio in the evening compared with the morning (26). These findings suggested that the greater torque in the afternoon was the result of peripheral/muscle changes rather than central effects.

Several studies since the initial work by Gauthier et al. (26) provide support that skeletal muscle torque, strength, and power are higher in the late afternoon compared with the morning (10, 19, 26, 27, 31–33, 38, 44, 53, 64, 65, 67, 70, 73, 74, 81, 82, 88). Martin et al. (53) used the adductor pollicis muscle in humans to evaluate voluntary and electrically induced contractions to test central vs. peripheral factors for the time-of-day force variations. In this paper, they reported greater force in the evening, as well as significant changes in both rate of tension development and one-half relaxation time (53). Several other groups have also reported greater muscle strength, torque, and power in the afternoon compared with the morning (10, 14, 19, 27, 33, 64, 67, 70, 81). These studies were all performed in human subjects, but included analysis of different muscle groups, such as the triceps surae (gastrocnemius, plantaris, soleus muscle group) and knee extensors, analysis of

tendon compliance, as well as including female subjects at a defined stage of their menstrual cycle. The diaphragm is the only skeletal muscle that may exhibit greater strength in the morning compared with the evening, but this study was performed on patients with sleep apnea, so it is not known if the clinical conditions may have modified the functional outcomes (88). The majority of studies done to date provide evidence that greater muscle strength is seen in the afternoon, and this is due to a peripheral or muscle-related variable rather than central/neurological factors. However, there are a few studies that suggest that both central and peripheral/muscle factors contribute to the circadian variations in strength (14, 29, 73). Since this work has only been performed in humans, it will be important to pursue this line of question with more defined in vitro measures of muscle function using either rodent muscle or single fibers from humans and/or rodents to unequivocally determine the site of circadian variations in muscle strength and power.

In parallel with the observations of circadian variations in muscle strength, some groups have investigated muscle strength training and temporal specificity (36, 82, 83). Temporal specificity refers to the potential that muscular strength will adapt and be highest when tested at the time of day when training occurred. Souissi et al. (83) found that training at a specific hour increases the peak torque and the peak anaerobic power specifically at that hour. Peak torque, measured by an isokinetic test, and peak anaerobic power, measured by the Wingate test, were lower when tested at a time different from when training occurred (83). Similarly, Hill et al. (36) conducted an anaerobic threshold test using the Wingate test and found that the value for ventilatory anaerobic threshold was higher when tested at the same time as training. The results suggest that there is temporal specificity in training to increase work capacity and that greater improvements in performance seem to occur at the time of day at which high-intensity training is regularly performed. More recently, Sedliak et al. (82) combined EMG studies with strength measures to test potential sites of temporal specificity following training. The results of their work suggested that peripheral/muscle adaptations rather than neural adaptations are the main source of temporal specificity in strength training. As noted earlier, these human studies suggest that muscle function and adaptation to training are influenced by circadian factors. However, this work does not say that training-induced increases in strength are better at a specific time of day. There is much need for research in this area to identify the molecular and cellular sites regulating the functional and training specificity effects seen in humans.

SKELETAL MUSCLE METABOLISM AND CIRCADIAN RHYTHMS

Early studies by Conlee et al. (17) and Clark and Conlee (16) demonstrated that glycogen levels in rodent skeletal muscles exhibited circadian variation (16, 17). The first study in 1976 measured glycogen content in hindlimb muscles of rats every 4 h over 24 h and found that glycogen levels peaked at 8 AM and were lowest 12 h later at 8 PM (17). Experiments reported by Clark and Conlee (16) confirmed the original observation and found that animals swam 60% longer when muscle glycogen levels were highest, providing a link between the circadian variation in muscle glycogen and physiological performance. Work from Newsholme's group (48) used established in vitro muscle incubation methods to study insulin sensitivity, glycolysis, and glycogen synthesis limb skeletal muscle (collected at 3 AM, 9 AM, 4 PM, and 8 PM) of rats, which had been entrained to a 12:12-h light-dark cycle (48). In this study, lights were off (active period) at 8 PM, and lights on (sleep period) at 8 AM, and they measured insulin sensitivity, glucose metabolism, and glycogen content in limb skeletal muscles of rats at 3 AM, 9 AM, 4 PM, and 8 PM. The results showed that glycogen content in all skeletal muscles examined (soleus, extensor digitorum longus, and gastrocnemius/plantaris muscles) peaked at 3 AM and was lowest at 8 PM. The glycogen content varied over time of day by 30–200%, depending on the muscle studied, and the changes in total glycogen were consistent with changes in glycogen synthesis rates across the four time points. In contrast to these changes, they reported no difference over 24 h in the maximal insulin-stimulated rates of glycolysis (used as an indicator of glucose uptake). However, the concentration of insulin required to stimulate glycolysis to 50% of maximum was bimodal, with greatest insulin sensitivity at 9 AM and 10 PM and lowest sensitivity at 3 PM and 3 AM. Altered insulin sensitivity of skeletal muscle has also been reported by others (24). Reid et al. (75a) studied glycogen content of the diaphragm muscle of hamsters at six time points from 3 AM to 11 PM. While the variation in glycogen levels was much smaller in the diaphragm (~10%) compared with limb muscles, they also found that the lowest levels occurred mid-active phase, between 7 and 11 PM.

There is much less known about potential variations in fat uptake or oxidative metabolism over time of day in skeletal muscle. Benavides et al. (11) studied lipoprotein lipase (LPL) activities every 3 h (8 time points) over a 24-h period in plasma, skeletal muscle, white adipose tissue, heart, and liver. They found that LPL activity oscillated in all tissues studied, with the highest level of activity in the skeletal muscle during the light phase. In 2002, Tsutsumi et al. (90) correlated the circadian changes in LPL activity in skeletal muscle with whole body measures of respiratory quotient (RQ). RQ is the steady-state ratio of carbon dioxide produced to oxygen consumed for the whole body. High RQ values (≈1) reflect higher use of carbohydrates as fuel, while lower RQ values (<0.8) indicate greater use of fat oxidation. They found that the circadian variation in LPL in skeletal muscle was completely antiphase with the circadian variation in RQ. For example, during the light phase in rats, RQ is low and muscle LPL activity is high, suggesting that muscle fat oxidation is contributing significantly to total body metabolic activity at that time. To study the sensitivity of skeletal muscle to circulating fats, Stavinoha et al. (84) used three different models, high-fat feeding, fasting, and streptozotocin-induced diabetes, to modulate circulating fatty acid levels in rats. The read out for muscle responsiveness for their study was to measure changes in expression of *Pdk4* mRNA, a representative peroxisome proliferator-activated receptor-α-regulated gene. In this study, they found that there was a significant diurnal variation in the skeletal muscle response to high circulating levels of fatty acids, with the greatest response occurring in the dark/active phase. These studies provide data indicating that circadian rhythms of skeletal muscle will likely be a factor contributing to the regulation of fat metabolism.

The circadian rhythms and skeletal muscle carbohydrate and fat metabolism studies reviewed in this section provide evidence that circadian rhythms are potentially one intrinsic component contributing to variations in metabolic activity in skeletal muscle. As noted in more detail in the other highlighted topic review papers, there is significant molecular evidence in other cell types that demonstrate interactions between circadian rhythms, the molecular clock, and metabolism. However, this is an area in which there is very little known in skeletal muscle under normal conditions or in response to training or disease. One of the challenges in this area will be to sort out the contribution of the internal clock mechanism vs. fiber type, muscle use, body temperature, and circulating hormones as they contribute to metabolic activity and sensitivity to metabolic hormones.

SUMMARY

The study of the circadian molecular clock in skeletal muscle is in the very early stages. Initial research has demonstrated the presence of the molecular clock in skeletal muscle and that skeletal muscle of a clock-compromised mouse, *Clock* mutant, exhibits significant disruption in normal expression of many genes required for adult muscle structure and metabolism. In light of the growing association between the molecular clock, metabolism, and metabolic disease, it will also be important to understand the contribution of circadian factors to normal metabolism, metabolic responses to muscle training, and contribution of the molecular clock in muscle to muscle disease (e.g., insulin resistance). In addition, the observation that 215 mRNAs oscillate over the time of day also highlights the importance for time-dependent controls in experimental design. Consistent with the potential for the skeletal muscle molecular clock modulating skeletal muscle physiology, there are findings in the literature that there is significant time-of-day effects for strength and metabolism. Additionally, there is some recent evidence that temporal specificity is important for optimizing training for muscular performance. While these studies do not prove that the molecular clock in skeletal muscle is important, they are suggestive of a circadian contribution to skeletal muscle function. The application of well-established models of skeletal muscle research in function and metabolism with available genetic models of molecular clock disruption will allow for more mechanistic understanding of potential relationships.

ACKNOWLEDGMENTS

We thank Dr. John J. McCarthy for valuable discussions.

GRANTS

This work was supported by National Institute of Arthritis and Musculoskeletal and Skin Diseases R01AR55246.

REFERENCES

1. **Akashi M, Takumi T.** The orphan nuclear receptor RORalpha regulates circadian transcription of the mammalian core-clock Bmal1. *Nat Struct Mol Biol* 12: 441–448, 2005.
2. **Akhtar RA, Reddy AB, Maywood ES, Clayton JD, King VM, Smith AG, Gant TW, Hastings MH, Kyriacou CP.** Circadian cycling of the mouse liver transcriptome, as revealed by cDNA microarray, is driven by the suprachiasmatic nucleus. *Curr Biol* 12: 540–550, 2002.
3. **Albrecht U.** Invited review: Regulation of mammalian circadian clock genes. *J Appl Physiol* 92: 1348–1355, 2002.
4. **Albrecht U, Oster H.** The circadian clock and behavior. *Behav Brain Res* 125: 89–91, 2001.
5. **Alenghat T, Meyers K, Mullican SE, Leitner K, Adeniji-Adele A, Avila J, Bucan M, Ahima RS, Kaestner KH, Lazar MA.** Nuclear receptor corepressor and histone deacetylase 3 govern circadian metabolic physiology. *Nature* 456: 997–1000, 2008.
6. **Almon RR, Yang E, Lai W, Androulakis IP, Ghimbovschi S, Hoffman EP, Jusko WJ, Dubois DC.** Relationships between circadian rhythms and modulation of gene expression by glucocorticoids in skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* 295: R1031–R1047, 2008.
7. **Antoch MP, Song EJ, Chang AM, Vitaterna MH, Zhao Y, Wilsbacher LD, Sangoram AM, King DP, Pinto LH, and Takahashi JS.** Functional identification of the mouse circadian Clock gene by transgenic BAC rescue. *Cell* 89: 655–667, 1997.
8. **Asher G, Gatfield D, Stratmann M, Reinke H, Dibner C, Kreppel F, Mostoslavsky R, Alt FW, and Schibler U.** SIRT1 regulates circadian clock gene expression through PER2 deacetylation. *Cell* 134: 317–328, 2008.
9. **Balsalobre A, Damiola F, and Schibler U.** A serum shock induces circadian gene expression in mammalian tissue culture cells. *Cell* 93: 929–937, 1998.
10. **Bambaeichi E, Reilly T, Cable NT, Giacomoni M.** Influence of time of day and partial sleep loss on muscle strength in eumenorrheic females. *Ergonomics* 48: 1499–1511, 2005.
11. **Benavides A, Siches M, Llobera M.** Circadian rhythms of lipoprotein lipase and hepatic lipase activities in intermediate metabolism of adult rat. *Am J Physiol Regul Integr Comp Physiol* 275: R811–R817, 1998.
12. **Braunstein M, Rose AB, Holmes SG, Allis CD, Broach JR.** Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. *Genes Dev* 7: 592–604, 1993.
13. **Bunger MK, Wilsbacher LD, Moran SM, Clendenin C, Radcliffe LA, Hogenesch JB, Simon MC, Takahashi JS, Bradfield CA.** Mop3 is an essential component of the master circadian pacemaker in mammals. *Cell* 103: 1009–1017, 2000.
14. **Castaingts V, Martin A, Van Hoecke J, Perot C.** Neuromuscular efficiency of the triceps surae in induced and voluntary contractions: morning and evening evaluations. *Chronobiol Int* 21: 631–643, 2004.
15. **Chen D, Guarente L.** SIR2: a potential target for calorie restriction mimetics. *Trends Mol Med* 13: 64–71, 2007.
16. **Clark JH, Conlee RK.** Muscle and liver glycogen content: diurnal variation and endurance. *J Appl Physiol* 47: 425–428, 1979.
17. **Conlee RK, Rennie MJ, Winder WW.** Skeletal muscle glycogen content: diurnal variation and effects of fasting. *Am J Physiol* 231: 614–618, 1976.
18. **Damiola F, Le Minh N, Preitner N, Kornmann B, Fleury-Olela F, Schibler U.** Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev* 14: 2950–2961, 2000.
19. **Deschenes MR, Kraemer WJ, Bush JA, Doughty TA, Kim D, Mullen KM, Ramsey K.** Biorhythmic influences on functional capacity of human muscle and physiological responses. *Med Sci Sports Exerc* 30: 1399–1407, 1998.
20. **Doi M, Hirayama J, Sassone-Corsi P.** Circadian regulator CLOCK is a histone acetyltransferase. *Cell* 125: 497–508, 2006.
21. **Duez H, Staels B.** The nuclear receptors Rev-erbs and RORs integrate circadian rhythms and metabolism. *Diab Vasc Dis Res* 5: 82–88, 2008.
22. **Duffield GE, Watson NP, Mantani A, Peirson SN, Robles-Murguia M, Loros JJ, Israel MA, Dunlap JC.** A role for Id2 in regulating photic entrainment of the mammalian circadian system. *Curr Biol* 19: 297–304, 2009.
23. **Etchegaray JP, Lee C, Wade PA, Reppert SM.** Rhythmic histone acetylation underlies transcription in the mammalian circadian clock. *Nature* 421: 177–182, 2003.
24. **Feneberg R, Lemmer B.** Circadian rhythm of glucose uptake in cultures of skeletal muscle cells and adipocytes in Wistar-Kyoto, Wistar, Goto-Kakizaki, and spontaneously hypertensive rats. *Chronobiol Int* 21: 521–538, 2004.
25. **Fulco M, Sartorelli V.** Comparing and contrasting the roles of AMPK and SIRT1 in metabolic tissues. *Cell Cycle* 7: 3669–3679, 2008.
26. **Gauthier A, Davenne D, Martin A, Cometti G, Van Hoecke J.** Diurnal rhythm of the muscular performance of elbow flexors during isometric contractions. *Chronobiol Int* 13: 135–146, 1996.

27. **Gauthier A, Davenne D, Martin A, Van Hoecke J.** Time of day effects on isometric and isokinetic torque developed during elbow flexion in humans. *Eur J Appl Physiol* 84: 249–252, 2001.
28. **Gekakis N, Staknis D, Nguyen HB, Davis FC, Wilsbacher LD, King DP, Takahashi JS, Weitz CJ.** Role of the CLOCK protein in the mammalian circadian mechanism. *Science* 280: 1564–1569, 1998.
29. **Giacomoni M, Edwards B, Bambaeichi E.** Gender differences in the circadian variations in muscle strength assessed with and without superimposed electrical twitches. *Ergonomics* 48: 1473–1487, 2005.
30. **Grimaldi B, Nakahata Y, Sahar S, Kaluzova M, Gauthier D, Pham K, Patel N, Hirayama J, Sassone-Corsi P.** Chromatin remodeling and circadian control: master regulator CLOCK is an enzyme. *Cold Spring Harb Symp Quant Biol* 72: 105–112, 2007.
31. **Guette M, Gondin J, Martin A.** Morning to evening changes in the electrical and mechanical properties of human soleus motor units activated by H reflex and M wave. *Eur J Appl Physiol* 95: 377–381, 2005.
32. **Guette M, Gondin J, Martin A.** Time-of-day effect on the torque and neuromuscular properties of dominant and non-dominant quadriceps femoris. *Chronobiol Int* 22: 541–558, 2005.
33. **Guette M, Gondin J, Martin A, Perot C, Van Hoecke J.** Plantar flexion torque as a function of time of day. *Int J Sports Med* 27: 171–177, 2006.
34. **Hastings M, O'Neill JS, Maywood ES.** Circadian clocks: regulators of endocrine and metabolic rhythms. *J Endocrinol* 195: 187–198, 2007.
35. **Hastings MH, Maywood ES, Reddy AB.** Two decades of circadian time. *J Neuroendocrinol* 20: 812–819, 2008.
36. **Hill DW, Leiferman JA, Lynch NA, Dangelmaier BS, Burt SE.** Temporal specificity in adaptations to high-intensity exercise training. *Med Sci Sports Exerc* 30: 450–455, 1998.
37. **Hirayama J, Sahar S, Grimaldi B, Tamaru T, Takamatsu K, Nakahata Y, Sassone-Corsi P.** CLOCK-mediated acetylation of BMAL1 controls circadian function. *Nature* 450: 1086–1090, 2007.
38. **Hodgson JA, Wichayanuparp S, Recktenwald MR, Roy RR, McCall G, Day MK, Washburn D, Fanton JW, Kozlovskaya I, Edgerton VR.** Circadian force and EMG activity in hindlimb muscles of rhesus monkeys. *J Neurophysiol* 86: 1430–1444, 2001.
39. **Hogenesch JB, Gu YZ, Jain S, Bradfield CA.** The basic-helix-loop-helix-PAS orphan MOP3 forms transcriptionally active complexes with circadian and hypoxia factors. *Proc Natl Acad Sci USA* 95: 5474–5479, 1998.
40. **Hogenesch JB, Panda S, Kay S, Takahashi JS.** Circadian transcriptional output in the SCN and liver of the mouse. *Novartis Found Symp* 253: 171–180; discussion 152–175, 102–179, 180–173 passim, 2003.
41. **Holzberg D, Albrecht U.** The circadian clock: a manager of biochemical processes within the organism. *J Neuroendocrinol* 15: 339–343, 2003.
42. **Hughes M, Deharo L, Pulivarthy SR, Gu J, Hayes K, Panda S, Hogenesch JB.** High-resolution time course analysis of gene expression from pituitary. *Cold Spring Harb Symp Quant Biol* 72: 381–386, 2007.
43. **Imai S, Johnson FB, Marciniak RA, McVey M, Park PU, Guarente L.** Sir2: an NAD-dependent histone deacetylase that connects chromatin silencing, metabolism, and aging. *Cold Spring Harb Symp Quant Biol* 65: 297–302, 2000.
44. **Jasper I, Hermsdorfer J.** Time-of-day effects on force control during object manipulation. *Eur J Appl Physiol* 101: 437–444, 2007.
45. **Kaeberlein M, McVey M, Guarente L.** The SIR2/3/4 complex and SIR2 alone promote longevity in Saccharomyces cerevisiae by two different mechanisms. *Genes Dev* 13: 2570–2580, 1999.
46. **King DP, Zhao Y, Sangoram AM, Wilsbacher LD, Tanaka M, Antoch MP, Steeves TD, Vitaterna MH, Kornhauser JM, Lowrey PL, Turek FW, Takahashi JS.** Positional cloning of the mouse circadian clock gene. *Cell* 89: 641–653, 1997.
47. **Ko CH, Takahashi JS.** Molecular components of the mammalian circadian clock. *Hum Mol Genet* 15, Spec No 2: R271–R277, 2006.
48. **Leighton B, Kowalchuk JM, Challiss RA, Newsholme EA.** Circadian rhythm in sensitivity of glucose metabolism to insulin in rat soleus muscle. *Am J Physiol Endocrinol Metab* 255: E41–E45, 1988.
49. **Liu C, Li S, Liu T, Borjigin J, Lin JD.** Transcriptional coactivator PGC-1alpha integrates the mammalian clock and energy metabolism. *Nature* 447: 477–481, 2007.
50. **Liu S, Cai YN, Sothern RB, Guan YQ, Chan P.** Chronobiological analysis of circadian patterns in transcription of seven key clock genes in six peripheral tissues in mice. *Chronobiol Int* 24: 793–820, 2007.
51. **Longo VD, Kennedy BK.** Sirtuins in aging and age-related disease. *Cell* 126: 257–268, 2006.
52. **Lowrey PL, Takahashi JS.** Mammalian circadian biology: elucidating genome-wide levels of temporal organization. *Annu Rev Genomics Hum Genet* 5: 407–441, 2004.
53. **Martin A, Carpentier A, Guissard N, van Hoecke J, Duchateau J.** Effect of time of day on force variation in a human muscle. *Muscle Nerve* 22: 1380–1387, 1999.
54. **Martino T, Arab S, Straume M, Belsham DD, Tata N, Cai F, Liu P, Trivieri M, Ralph M, Sole MJ.** Day/night rhythms in gene expression of the normal murine heart. *J Mol Med* 82: 256–264, 2004.
55. **Maywood ES, O'Neill JS, Chesham JE, Hastings MH.** Minireview: the circadian clockwork of the suprachiasmatic nuclei–analysis of a cellular oscillator that drives endocrine rhythms. *Endocrinology* 148: 5624–5634, 2007.
56. **Maywood ES, O'Neill JS, Reddy AB, Chesham JE, Prosser HM, Kyriacou CP, Godinho SI, Nolan PM, Hastings MH.** Genetic and molecular analysis of the central and peripheral circadian clockwork of mice. *Cold Spring Harb Symp Quant Biol* 72: 85–94, 2007.
57. **McCarthy JJ, Andrews JL, McDearmon EL, Campbell KS, Barber BK, Miller BH, Walker JR, Hogenesch JB, Takahashi JS, Esser KA.** Identification of the circadian transcriptome in adult mouse skeletal muscle. *Physiol Genomics* 31: 86–95, 2007.
58. **McWatters H, Dunlap JC, Millar AJ.** Circadian biology: clocks for the real world. *Curr Biol* 9: R633–R635, 1999.
59. **Merrow M, Spoelstra K, Roenneberg T.** The circadian cycle: daily rhythms from behaviour to genes. *EMBO Rep* 6: 930–935, 2005.
60. **Miller BH, McDearmon EL, Panda S, Hayes KR, Zhang J, Andrews JL, Antoch MP, Walker JR, Esser KA, Hogenesch JB, Takahashi JS.** Circadian and CLOCK-controlled regulation of the mouse transcriptome and cell proliferation. *Proc Natl Acad Sci USA* 104: 3342–3347, 2007.
61. **Nakahata Y, Grimaldi B, Sahar S, Hirayama J, Sassone-Corsi P.** Signaling to the circadian clock: plasticity by chromatin remodeling. *Curr Opin Cell Biol* 19: 230–237, 2007.
62. **Nakahata Y, Kaluzova M, Grimaldi B, Sahar S, Hirayama J, Chen D, Guarente LP, Sassone-Corsi P.** The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. *Cell* 134: 329–340, 2008.
63. **Nakahata Y, Sahar S, Astarita G, Kaluzova M, Sassone-Corsi P.** Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. *Science* 324: 654–657, 2009.
64. **Nicolas A, Gauthier A, Bessot N, Moussay S, Davenne D.** Time-of-day effects on myoelectric and mechanical properties of muscle during maximal and prolonged isokinetic exercise. *Chronobiol Int* 22: 997–1011, 2005.
65. **Nicolas A, Gauthier A, Trouillet J, Davenne D.** The influence of circadian rhythm during a sustained submaximal exercise and on recovery process. *J Electromyogr Kinesiol* 18: 284–290, 2008.
66. **Noshiro M, Furukawa M, Honma S, Kawamoto T, Hamada T, Honma K, Kato Y.** Tissue-specific disruption of rhythmic expression of Dec1 and Dec2 in clock mutant mice. *J Biol Rhythms* 20: 404–418, 2005.
67. **Onambele-Pearson NL, Pearson SJ.** Time-of-day effect on patella tendon stiffness alters vastus lateralis fascicle length but not the quadriceps force-angle relationship. *J Biomech* 40: 1031–1037, 2007.
68. **Oster H, Maronde E, Albrecht U.** The circadian clock as a molecular calendar. *Chronobiol Int* 19: 507–516, 2002.
69. **Panda S, Antoch MP, Miller BH, Su AI, Schook AB, Straume M, Schultz PG, Kay SA, Takahashi JS, Hogenesch JB.** Coordinated transcription of key pathways in the mouse by the circadian clock. *Cell* 109: 307–320, 2002.
70. **Pearson SJ, Onambele GN.** Influence of time of day on tendon compliance and estimations of voluntary activation levels. *Muscle Nerve* 33: 792–800, 2006.
71. **Peirson SN, Butler JN, Duffield GE, Takher S, Sharma P, Foster RG.** Comparison of clock gene expression in SCN, retina, heart, and liver of mice. *Biochem Biophys Res Commun* 351: 800–807, 2006.
72. **Preitner N, Damiola F, Lopez-Molina L, Zakany J, Duboule D, Albrecht U, Schibler U.** The orphan nuclear receptor REV-ERBalpha controls circadian transcription within the positive limb of the mammalian circadian oscillator. *Cell* 110: 251–260, 2002.
73. **Racinais S, Blonc S, Jonville S, Hue O.** Time of day influences the environmental effects on muscle force and contractility. *Med Sci Sports Exerc* 37: 256–261, 2005.

74. **Racinais S, Connes P, Bishop D, Blonc S, Hue O.** Morning versus evening power output and repeated-sprint ability. *Chronobiol Int* 22: 1029–1039, 2005.
75. **Ramsey KM, Yoshino J, Brace CS, Abrassart D, Kobayashi Y, Marcheva B, Hong HK, Chong JL, Buhr ED, Lee C, Takahashi JS, Imai S, Bass J.** Circadian clock feedback cycle through NAMPT-mediated NAD+ biosynthesis. *Science* 324: 651–654, 2009.
75a. **Reid WD, Cairns CL, Chung F, Wiggs BR, Belcastro AN.** Circadian rhythm of glycogen in the hamster diaphragm. *Can J Physiol Pharmaco* 71: 868–873, 1993.
76. **Roenneberg T, Merrow M.** Circadian clocks–from genes to complex behaviour. *Reprod Nutr Dev* 39: 277–294, 1999.
77. **Rudic RD, McNamara P, Reilly D, Grosser T, Curtis AM, Price TS, Panda S, Hogenesch JB, FitzGerald GA.** Bioinformatic analysis of circadian gene oscillation in mouse aorta. *Circulation* 112: 2716–2724, 2005.
78. **Sato TK, Panda S, Miraglia LJ, Reyes TM, Rudic RD, McNamara P, Naik KA, FitzGerald GA, Kay SA, Hogenesch JB.** A functional genomics strategy reveals Rora as a component of the mammalian circadian clock. *Neuron* 43: 527–537, 2004.
79. **Schibler U.** The 2008 Pittendrigh/Aschoff lecture: peripheral phase coordination in the mammalian circadian timing system. *J Biol Rhythms* 24: 3–15, 2009.
80. **Schibler U, Ripperger J, Brown SA.** Peripheral circadian oscillators in mammals: time and food. *J Biol Rhythms* 18: 250–260, 2003.
81. **Sedliak M, Finni T, Cheng S, Haikarainen T, Hakkinen K.** Diurnal variation in maximal and submaximal strength, power and neural activation of leg extensors in men: multiple sampling across two consecutive days. *Int J Sports Med* 29: 217–224, 2008.
82. **Sedliak M, Finni T, Peltonen J, Hakkinen K.** Effect of time-of-day-specific strength training on maximum strength and EMG activity of the leg extensors in men. *J Sports Sci* 26: 1005–1014, 2008.
83. **Souissi N, Gauthier A, Sesboue B, Larue J, Davenne D.** Effects of regular training at the same time of day on diurnal fluctuations in muscular performance. *J Sports Sci* 20: 929–937, 2002.
84. **Stavinoha MA, Rayspellicy JW, Hart-Sailors ML, Mersmann HJ, Bray MS, Young ME.** Diurnal variations in the responsiveness of cardiac and skeletal muscle to fatty acids. *Am J Physiol Endocrinol Metab* 287: E878–E887, 2004.
85. **Stokkan KA, Yamazaki S, Tei H, Sakaki Y, Menaker M.** Entrainment of the circadian clock in the liver by feeding. *Science* 291: 490–493, 2001.
86. **Storch KF, Lipan O, Leykin I, Viswanathan N, Davis FC, Wong WH, Weitz CJ.** Extensive and divergent circadian gene expression in liver and heart. *Nature* 417: 78–83, 2002.
87. **Straume M.** DNA microarray time series analysis: automated statistical assessment of circadian rhythms in gene expression patterning. *Methods Enzymol* 383: 149–166, 2004.
88. **Su MC, Chin CH, Chen YC, Hsieh YT, Wang CC, Huang YC, Lin MC.** Diurnal change of respiratory muscle strength in patients with sleep-disordered breathing. *Chang Gung Med J* 31: 297–303, 2008.
89. **Takahashi JS, Hong HK, Ko CH, McDearmon EL.** The genetics of mammalian circadian order and disorder: implications for physiology and disease. *Nat Rev Genet* 9: 764–775, 2008.
90. **Tsutsumi K, Inoue Y, Kondo Y.** The relationship between lipoprotein lipase activity and respiratory quotient of rats in circadian rhythms. *Biol Pharm Bull* 25: 1360–1363, 2002.
91. **Turek FW, Joshu C, Kohsaka A, Lin E, Ivanova G, McDearmon E, Laposky A, Losee-Olson S, Easton A, Jensen DR, Eckel RH, Takahashi JS, Bass J.** Obesity and metabolic syndrome in circadian Clock mutant mice. *Science* 308: 1043–1045, 2005.
92. **Vitaterna MH, King DP, Chang AM, Kornhauser JM, Lowrey PL, McDonald JD, Dove WF, Pinto LH, Turek FW, Takahashi JS.** Mutagenesis and mapping of a mouse gene, Clock, essential for circadian behavior. *Science* 264: 719–725, 1994.
93. **Welsh DK, Yoo SH, Liu AC, Takahashi JS, Kay SA.** Bioluminescence imaging of individual fibroblasts reveals persistent, independently phased circadian rhythms of clock gene expression. *Curr Biol* 14: 2289–2295, 2004.
94. **Yamazaki S, Numano R, Abe M, Hida A, Takahashi R, Ueda M, Block GD, Sakaki Y, Menaker M, Tei H.** Resetting central and peripheral circadian oscillators in transgenic rats. *Science* 288: 682–685, 2000.
95. **Yin L, Lazar MA.** The orphan nuclear receptor Rev-erb alpha recruits the N-CoR/histone deacetylase 3 corepressor to regulate the circadian Bmal1 gene. *Mol Endocrinol* 19: 1452–1459, 2005.
96. **Yoo SH, Ko CH, Lowrey PL, Buhr ED, Song EJ, Chang S, Yoo OJ, Yamazaki S, Lee C, Takahashi JS.** A noncanonical E-box enhancer drives mouse Period2 circadian oscillations in vivo. *Proc Natl Acad Sci USA* 102: 2608–2613, 2005.
97. **Yoo SH, Yamazaki S, Lowrey PL, Shimomura K, Ko CH, Buhr ED, Siepka SM, Hong HK, Oh WJ, Yoo OJ, Menaker M, Takahashi JS.** PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. *Proc Natl Acad Sci USA* 101: 5339–5346, 2004.
98. **Zambon AC, McDearmon EL, Salomonis N, Vranizan KM, Johansen KL, Adey D, Takahashi JS, Schambelan M, Conklin BR.** Time- and exercise-dependent gene regulation in human skeletal muscle. *Genome Biol* 4: R61, 2003.
99. **Zvonic S, Ptitsyn AA, Conrad SA, Scott LK, Floyd ZE, Kilroy G, Wu X, Goh BC, Mynatt RL, Gimble JM.** Characterization of peripheral circadian clocks in adipose tissues. *Diabetes* 55: 962–970, 2006.
100. **Zvonic S, Ptitsyn AA, Kilroy G, Wu X, Conrad SA, Scott LK, Guilak F, Pelled G, Gazit D, Gimble JM.** Circadian oscillation of gene expression in murine calvarial bone. *J Bone Miner Res* 22: 357–365, 2007.