HIGHLIGHTED TOPIC | The Role of Clock Genes in Cardiometabolic Disease

Working around the clock: circadian rhythms and skeletal muscle

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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 co-activator-1

CIRCADIAN RHYTHMS

The term circadian comes from the Latin circa, “around”, and diei, “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.

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 periphr...
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: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). 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 repressing 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 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-1α 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-1β 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).

Table 1.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Ref. No.</th>
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<tbody>
<tr>
<td>Core clock genes</td>
<td>Bmal1, Clock, Per1, Per2, Cry1, Cry2, Rora, Rev-erba, Csnk1d, Fbx13</td>
</tr>
<tr>
<td>Clock-controlled genes</td>
<td>Dhp, Tef, Nampt, p21, Id1, Dec1</td>
</tr>
<tr>
<td>Circadian genes in muscle</td>
<td>UCP3, PDK4, Atrogin, MuRF1, Pank1, Dga2, MHC IX, Thra, Tbc1d4</td>
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molecular clock and circadian function is SIRT1, the nicotinamide adenine dinucleotide (NAD+)-dependent histone deacetylase (8, 62, 63, 75). Mammalian SIRT1 (Sir2a) 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+. 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 Ddx4, 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, Atoh1 (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 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 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 mitochondrial 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 PGC1α 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, adipose 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, glycogenolysis, 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 glycolyis (used as an indicator of glucose uptake). However, the concentration of insulin required to stimulate glycology 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.

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