THE IMPACT OF PHYSICAL EXERCISE ON THE SKELETAL MUSCLE CLOCK GENES

Gerardo Gabriel Mirizio[1], Rodolfo Soares Mendes Nunes[2], Ana Lucia Castillo Figueroa[3], Irineu de Sousa Junior[1], Aparecido Pimentel Ferreira[1], and Elaine Vieira[1]

[1]Postgraduate Program on Physical Education, Universidade Católica de Brasília, DF, Brazil
[2]Graduate Program on Physical Education, Unopar, Maringá, PR, Brazil
[3]Diabetes and Obesity Laboratory, IDIBAPS, Barcelona, Spain

Review
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Abstract:

The most important circadian synchronizer is the central clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus. The rhythmicity of all organs is achieved by molecular clock gene expression in the central clock and the pace is reached through neuronal and humoral signals to peripheral tissues. Skeletal muscle is one of the peripheral organs that express clock and clock controlled genes (CCGs) which display circadian rhythmicity. Recent studies have pointed out the role of clock genes in skeletal muscle function and metabolism. The expression of clock genes in skeletal muscle might be altered by several external stimuli and also by different diseases. Physical exercise is a nonphotic stimulus that can realign the skeletal muscle circadian system to the central clock, imposing a new rhythm at the organism level. This effect may be crucial to prevent or ameliorate diseases and disorders caused by disruptions of circadian rhythms. In this review, we discuss the role of clock genes in skeletal muscle function and the importance of physical exercise as a potent synchronizing stimulus for the skeletal muscle molecular clock.

Key words: *clock genes, physical exercise, skeletal muscle*

Introduction

A wide variety of physiological functions present in most living organisms exhibit an oscillatory pattern of behavior. These processes are regulated over a 24-hour period according to solar light and include sleep-wake cycles, feeding, hormone production, body temperature and DNA repair, among others. At a molecular level, such oscillations are achieved by regulating gene expression through an autonomous mechanism so to create a peak of protein expression once every 24 h and therefore controlling the timing of a physiological process (Partch, Green, & Takahashi, 2014). Thereby, circadian rhythms enable organisms to adjust many of their physiological responses to environmental changes, providing them with an anticipatory advantage when performing daily activities (Dibner, Schibler, & Albrecht, 2010; Huang, Ramsey, Marcheva, & Bass, 2011; Marcheva, et al., 2013). In mammals, these biological rhythms are orchestrated by a central pacemaker located in the suprachiasmatic nuclei (SCN) of the hypothalamus and composed of a group of approximately 20 000 neurons that exhibit independent rhythms of firing rate and gene expression.

Several studies from more than a decade ago have shown that besides the central clock in the brain, peripheral molecular clocks exist in several organs, such as liver, kidney, heart, skeletal muscle, adipose tissue and pancreas (Balsalobre, Brown, et al., 2000; Kennaway, Owens, Voultsios, Boden, & Varcoe, 2007; Kohsaka, et al., 2007; Marcheva, et al., 2010; Richards & Gumz, 2012; Vieira, Burris, & Quesada, 2014; Vieira, et al., 2008, 2012, 2013; Yoo, et al., 2004; Zambon, et al., 2003). Peripheral and central molecular clocks share the same molecular architecture and capacity to generate sustained circadian rhythms, although one key difference between them lies in the degree of their intracellular coupling (Partch, et al., 2014). These differences permit a higher robustness in central circadian rhythmicity, empowering neurons of the SCN with resistance to phase perturbations from internal factors (Liu, A.C., et al., 2007). Central and peripheral molecular clocks are linked by neuro-humoral and temperature signals and both should work in a

synchronized and coordinated manner (Balsalobre, Marcacci, & Schibler, 2000; Saini, et al., 2013). In fact, it has been shown that lesions of the SCN in transgenic mice did not abolish circadian rhythms in peripheral tissues, but instead caused phase desynchrony from tissue to tissue within each animal and among animals (Yoo, et al., 2004). Thus, tissue-specific gene expression patterns are likely to be regulated by both local and central mechanisms (Mohawk, Green, & Takahashi, 2012). In this sense, the interplay between central and peripheral circadian rhythms could represent a key physiological aspect by which certain molecular mechanisms regulate metabolic and locomotor functions in mammals.

It has been well established that biological rhythms are the result of the interaction between endogenous clocks and environmental factors. The process by which this interaction occurs is called synchronization or entrainment, so the signals which promote that biological adjustment in a particular specie are identified as circadian time synchronizers or 'zeitgebers' and play a fundamental role in entraining central and peripheral clocks (Moore, 1997). The principal zeitgebers of human biological clocks are the photic ones, such as solar light, that act adjusting the internal clock timing to the external solar day. Circadian entrainment of the central clock by photic cues occurs every day when solar light enters through the retina of the eye, causing electrical signals to pass through the retinal hypothalamic tract, which are then converted to chemical signals in the SCN (Richards & Gumz, 2012). However, besides the importance of light as the main clock synchronizer, there are also evidence for non-photic zeitgebers such as time of feeding, physical activity/exercise or social contact, that have been shown to influence molecular rhythms and behavior (Grandin, Alloy, & Abramson, 2006; Monk, 2010; Schroeder, et al., 2012; Wolff & Esser, 2012). These external signals affect, directly or indirectly, both central and peripheral clocks, altering their period length, phase or amplitude and leading to changes in circadian rhythmicity. As a nonphotic synchronizer, physical exercise has been shown to influence several metabolic- and health-related disorders in a timing dependent manner (Francois, et al., 2014; Heden, et al., 2015; Sasaki, Ohtsu, Ikeda, Tsubosaka, & Shibata, 2014), but the role of locomotor patterns in skeletal muscle circadian biology has not been studied in depth. In this sense, it is important to address the question of what are the mechanisms by which physical exercise may influence circadian biology and whether activity affects the entrainment of the muscle clock. In this review, we will discuss the current knowledge regarding the role of physical exercise on molecular clocks in the skeletal muscle.

Mammalian circadian clocks

From a molecular point of view, cell-autonomous clocks are generated by self-sustained transcriptional/translational feedback loops, involving a set of clock genes. At the basic level, *Clock*, *Bmal1*, *Cry1/2* and *Per1/2* are all core molecular components of the clock (Bass & Takahashi, 2010). These genes codify a set of four integral proteins that act as activators or repressors in the system. The primary loop is composed by CLOCK and BMAL1 transcription factors, which have the ability to heterodimerize in the nucleus, driving the transcription of other clock genes such as the Period (*Per1*, *Per2*) and Cryptochrome (*Cry1*, *Cry2*) (Reppert & Weaver, 2002). The activation of the Per and Cry promoters leads to the accumulation of Per and Cry mRNA, which in the suprachiasmatic nucleus takes place during the latter part of the day and the early part of the night. Exported to the cytoplasm, these mRNA transcripts are translated into the proteins PER1, PER2 and PER3 and CRY1 and CRY2, the concentrations of which peak during the middle of the night. Importantly, turnover of PER and CRY proteins are also tightly controlled by phosphorylation and protein degradation systems, allowing this cycle to continue every single day (Figure 1). The casein kinases CKIδ and CKIε play a critical role in controlling the rate at which PER:CRY complexes either are degraded or do enter the nucleus, and their activity is simultaneously controlled by PP1 and PP5 phosphatases that regulate the localization and net balance of these clock proteins (Partch, et al., 2014). The stability and precision of circadian rhythms is further enhanced by the interplay between different modulators of the core clock genes. *Bmal1* expression is controlled by some other transcription factors such as REV-ERBs and RORs that respond to hormones, metabolites and nutrients. In mammals, these two orphan nuclear receptors families play important roles in the transcription of the clock gene *Bmal1*. For instance, the Reverse Erythroblastosis Virus Alpha (REV-ERB ALPHA) transcription factor, so named because it is encoded by the reverse strand of the *c-erb-A* oncogene, can negatively regulate *Bmal1* expression, whereas Retinoic-acid receptor-related Orphan Receptor Alpha (ROR ALPHA) has a positive regulatory effect on *Bmal1* expression (Panda, et al., 2002; Vieira, et al., 2014). Moreover, it has been demonstrated that *Reverb* genes are similarly expressed in the thymus, skeletal muscle, and kidney, whereas *Ror* genes present distinct expression patterns, indicating that all members of the REV-ERB and ROR families are important components of the molecular circadian clock (Guillaumond, Dardente, Giguere, & Cermakian, 2005). It is worth to mention that, although this second layer of regulation is not required to drive the core feedback loop, it

provides robustness to the system in order to help maintain accurate circadian timing and also helps to generate phase delays in circadian transcriptional output. Finally, biological oscillations are also controlled by non-specific circadian genes that are regulated by core clock genes. These clock-controlled output genes (CCGs) are present in several tissues, such as skeletal muscle, and might also control the clock machinery, relaying the clock information to downstream proteins (Lefta, Wolff, & Esser, 2011).

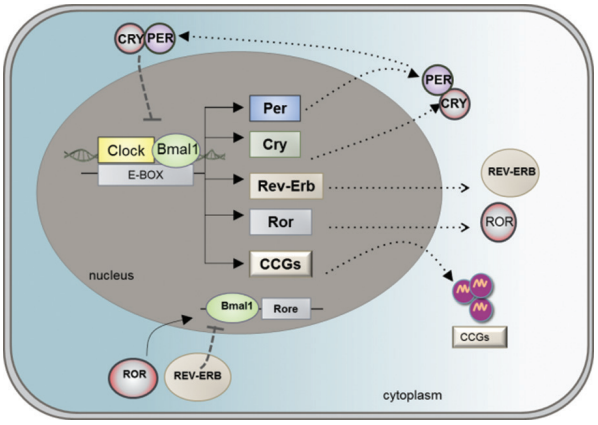

Figure 1. Molecular regulation of clock genes. CLOCK dimerizes with BMAL1 to activate transcription of Per and Cry genes that acts as negative regulators of CLOCK:BMAL1.

Clock genes and muscle function

In mammals, skeletal muscle represents a key tissue with an enormous plasticity, and there is evidence that muscle function is at least partially controlled by clock genes. In fact, several studies performed in animal models harboring clock gene deletions or mutations have highlighted the importance of circadian oscillations in skeletal muscle. Accordingly, the first study performed in order to define circadian gene expression in skeletal muscle of mice have identified a total of 267 rhythmic genes expressed in this tissue over two circadian cycles (Miller, et al., 2007), indicating that the molecular clock plays a key role in the temporal regulation of clock-controlled genes (CCGs) in skeletal muscles (Chatterjee & Ma, 2016). Subsequent results of skeletal muscle transcripts found that more than the majority of circadian genes in this tissue showed peak expression at the middle of the active phase, when mice are physically active and feeding (McCarthy, et al., 2007). Apparently, this is the result of an increased contractile activity during the active phase that may induce a higher transcription of CCGs in skeletal muscle. Furthermore, hundreds of mRNAs which are involved in a range of biological processes like transcription, lipid metabolism, protein degradation, ion transport, and vesicular trafficking revealed circadian patterns in skeletal muscle (McCarthy, et al., 2007). As is the case in other tissues, the main environmental factors which can entrain the molecular clock in skeletal muscle are light exposure, feeding timing, and activity patterns (Harfmann, Schroder, & Esser, 2015). Thus, disruption in core clock genes or CCGs rhythmicity caused either by specific clock gene mutations or new imposed circadian rhythms will lead to detrimental effects on skeletal muscle function and morphology, as well as on other skeletal muscle functionally-related tissues, such as liver and adipose tissue. These conditions can broadly affect the health status due to functional shifts at the whole organism level as a consequence of skeletal muscle defects.

Muscle cell growth and proliferation

In order to study the relationship between clock genes and muscle function, genetic strategies were developed to target circadian genes in mouse models. Results of these studies showed that many circadian mutant mouse models exhibit muscle disorders. For instance, skeletal muscle of *Clock*$^{\Delta 19}$ (bearing a dominant negative mutation in the *Clock* gene that leads to a marked metabolic phenotype of obesity, dyslipidemia and hyperglycemia [Shostak, Meyer-Kovac, & Oster, 2013]) and *Bmal1*$^{-/-}$ knockout mice (a model with a phenotype of insulin resistance, impaired glucose tolerance, reduced life span and other pathologies [Rudic, et al., 2004]) displayed ~30% reductions in normalized maximal force at the whole and single-fiber levels (Andrews, et al., 2010). Moreover, this alteration in muscle fibers was associated with decrements in exercise tolerance due to a drop in mitochondrial content and activity as well as with disruptions of myofilament sarcomeric organization, followed by changes in gene expression of important genes related to muscle function, including actin, myosin, titin, and other myogenic differentiation 1 (*MyoD1*) target genes (Andrews, et al., 2010; Zhang, et al., 2012). MyoD1 is a member of the family of transcription factors called myogenic regulatory factors (MRF), responsible for driving myogenic differentiation programs. This protein has shown to oscillate in skeletal muscle of mice due to a circadian regulation of its expression by CLOCK and BMAL1 transcriptional activators (Andrews, et al., 2010), suggesting that cell cycle control in skeletal muscle is exerted in a circadian manner.

Alterations in circadian genes expression could also be related to premature aging phenotypes and the appearance of early age-related pathologies. In fact, it has been found that *Bmal1*$^{-/-}$ mice showed a growth retardation at 16-18 weeks of age followed by sarcopenia, osteoporosis, and a shortened life span at 40-weeks of age, an effect primarily mediated by decreased adipose, bone, and muscle tissue mass (Kondratov, Kondratova, Gorbacheva, Vykhovanets, & Antoch, 2006). Furthermore, it has been noted that sciatic denervation dis-

rupts the circadian expression of many clock and CCGs via muscle atrophy in leg muscles of mice, affecting slow- and fast-twitch muscles in different ways (Nakao, et al., 2015). In this sense, it has been shown that numerous genes related to protein metabolism pathways are expressed in a circadian manner in skeletal muscle, representing around 12% of total transcripts, ~ 50% of which are associated with the ubiquitin protein degradation cycle, such as *Atrogin1* and *MuRF1* (Andrews, et al., 2010). Adding to the mentioned, most of the impairments in muscle mass growth and maintenance might be related to reductions in the proliferative capacity of satellite cells and defects in the muscle mass growth signaling. In this way, a cell-autonomous effect of *Bmal1* mediated by the embryonic and postnatal muscle growth-related Wnt signaling pathway has recently been discovered in myoblasts (Chatterjee, et al., 2013). Wnt is a highly conserved signaling pathway related to muscle development and function. Hence, the discovery of *Bmal1* as a key positive regulator of myogenesis through its interaction with several target molecules of Wnt signaling pathway opens the question about a possible strong link between *Bmal1* activity and skeletal muscle mass development. In this sense, an important role of *Bmal1* in the promotion of satellite cell expansion during muscle regeneration has also been observed since mice lacking *Bmal1* displayed a lower satellite cell number followed by an impaired muscle regeneration and an attenuated myogenic induction (Chatterjee, Yin, Nam, Li, & Ma, 2015). Interestingly, cytosolic BMAL1 has been identified as a substrate of the mTOR-effector kinase S6K1 revealing its role as a circadian regulator of protein synthesis (Lipton, et al., 2015). A possible hypothesis based on the available data is that core clock genes function as partial modulators of hypertrophy and atrophy signaling pathways, regulating daily-related processes of muscle growth and maintenance.

Apart from the hierarchical role of *Bmal1* as a component of the pro-myogenic response in skeletal muscle, other clock genes such as *Clock*, *Rev-erb alpha*, *Rev-erb beta*, *ROR alpha* and *Sharp1/Dec2* are also implicated in skeletal muscle growth related mechanisms. Accordingly, the main observed consequences have also been impairments in muscle maintenance as well as in myogenic progression and differentiation (Azmi, Ozog, & Taneja, 2004; Burke, Downes, Carozzi, Giguere, & Muscat, 1996; Ling, et al., 2012; Pircher, Chomez, Yu, Vennstrom, & Larsson, 2005; Raichur, et al., 2010).

Muscle locomotor activity

It has been documented that locomotor activity, and particularly scheduled exercise, exerts an important role in synchronization of circadian clocks throughout the body (Wolff & Esser, 2012; Zambon, et al., 2003). In fact, core clock genes expression in skeletal muscle of mice becomes altered in the absence of motor nerve activity (Nakao, et al., 2015). Moreover, it has been reported that locomotor activity, understood as motor neuron-dependent contractile activity, controls the oscillation of around 15% of skeletal muscle circadian genes independently of the core muscle clock; and that this response is at least partially mediated by changes in the calcineurin-NFAT signaling pathway, involved in the nerve activity-dependent regulation of muscle fiber-type-specific gene programs (Dyar, et al., 2015). Importantly, this study showed not only that CCGs are differentially expressed in slow and fast skeletal muscles but also that a subset of muscle circadian genes is strictly activity-dependent. In this sense, skeletal muscle fibers respond to rhythmic changes in motor neuron activity, affecting its circadian expression through a mechanism controlled by the SCN.

In accordance with the mentioned above, changes in the opposite way may also occur, as locomotor capacity of skeletal muscle may be affected by disruptions in circadian gene oscillations (Bunger, et al., 2000; Martin, et al., 2010). Regarding this, it has been observed that the REV-ERB ALPHA protein influences myosin heavy chain (MHC) isoform expression in heterozygous and homozygous mice for a REV-ERB ALPHA protein null allele (Pircher, et al., 2005) suggesting a role of circadian clock genes as regulators of contractile properties and ATPase activity in skeletal muscle. In addition, knocking of *mPer2* genes might affect the functional properties of muscles since it has been shown that loss of *mPer2* in mice was accompanied by a 20% reduction in the locomotor endurance performance without altering muscle contractility (Bae, et al., 2006). Besides, a loss of circadian rhythmicity affects the locomotor function in *Bmal1*$^{-/-}$ mice, causing tendon calcification and a decrease in body weight, longevity, and activity levels (McDearmon, et al., 2006). Interestingly, the consequences of these marked reductions in the skeletal muscle locomotor capacity could be ameliorated through muscle-specific rescue of circadian gene expression. Accordingly, a muscle-rescue of *Bmal1* expression prolonged survival of *Bmal1*-null mice in a higher rate than it did in brain-rescued models (McDearmon, et al., 2006), suggesting an important function of *Bmal1* in skeletal muscle as a regulator of activity levels, body weight maintenance, and longevity. Nevertheless, it was observed that muscle-specific *Bmal1* inactivation did not cause dramatic changes in locomotor activity in skeletal muscle phenotype of mice except for slight changes in fiber type composition and a decrease in muscle force (Dyar, et al., 2014). Results of these studies suggest that locomotor activity and skeletal muscle phenotype might be altered by changes in skeletal muscle core clock gene expression and vice versa,

although an essential role of the tissue-specific core clock machinery in driving these changes remains to be determined.

Muscle metabolic activity

Given that skeletal muscle is an essential tissue for energy metabolism homeostasis, it is not surprising that skeletal muscle circadian activity and metabolic processes are closely integrated (Bass & Takahashi, 2010; McCarthy, et al., 2007; Shavlakadze, et al., 2013). All this represents a very important aspect in circadian physiology, not only due to the fact that skeletal muscle comprises ~40% of the body mass of most mammals, but also because this organ has a critical role in regulation of glucose and lipid metabolism (Harfmann, et al., 2015). In this sense, analysis of circadian metabolic genes revealed a temporal separation of genes involved in carbohydrates and fatty acids utilization and storage over a 24-hour period, meaning that disruptions in circadian rhythmicity of these clock genes could lead to a lower ability for skeletal muscle to maintain metabolic homeostasis over a daily period (Hodge, et al., 2015; van Moorsel, et al., 2016). Moreover, a relationship has been discovered between the molecular clock and hypoxia-inducible factor 1α (HIF1α) in mouse skeletal muscle, revealing a novel mechanism by which peripheral clocks function together with oxygen-sensing molecules to promote rhythmic tissue-specific metabolic fuel selection (Peek, et al., 2017).

In order to address the interaction between metabolism and circadian rhythmicity, temporal changes in the expression of several proteins involved in muscle metabolism under different nutritional and activity conditions have been assessed. As a result, it was observed that metabolic regulation of circadian activity is controlled in part by non-transcriptional mechanisms, such as changes in cyclic AMP levels (O'Neill, Maywood, Chesham, Takahashi, & Hastings, 2008) or PI3-K signaling (Zhang, et al., 2009) and sensed at the time by incoming (AMPK and PARP-1) and outgoing (NAD^+/Sirtuin) molecules which couple nutrient availability, metabolism and the clock system (Bass & Takahashi, 2010). Essentially, most of the molecules that act as metabolic sensors in response to changes in cellular metabolic status, cell signaling and transcription might be intermediates in circadian synchrony. For instance, Vieira et al. (2008) found a specific role of AMP-activated protein kinase (AMPK) in linking circadian oscillators and energy metabolism in skeletal muscle from mice through pharmacological activation of exercise-related signaling pathways. AMPK plays a fundamental role as a sensor of AMP/ATP ratio in skeletal muscle; and together with SIRT1, PARP-1 and other molecules allow synchronization of the core molecular clock with the environment in response to changes in metabolic signals such as NAD^+ or AMP levels (Bass & Takahashi, 2010; Liu, et al., 2016; Schuldt, 2010). Importantly, this has several clinical consequences in humans since chronic circadian misalignments or phase shifts caused by feeding or activity patterns are associated with adverse metabolic consequences as an altered lipid profile or insulin resistance, leading to a higher risk of chronic diseases, such as obesity, diabetes, cardiovascular disease and metabolic syndrome (Buxton, et al., 2012; Esquirol, Bongard, Ferrieres, Verdier, & Perret, 2012; Karlsson, Knutsson, & Lindahl, 2001; Wang, Armstrong, Cairns, Key, & Travis, 2011). In this sense, experimental studies conducted in animal models with muscle-specific ablation of the *Bmal1* core clock gene identified impairments in skeletal muscle glucose uptake. Such impairments in glucose metabolism were characterized by reduced protein levels of GLUT4, the insulin dependent glucose transport, and TBC1D1, a Rab-GTPase involved in GLUT4 translocation, and accompanied by a reduction in glucose oxidation due to altered expression of PDH activity and other glycolytic intermediates (Dyar, et al., 2014). Similar effects were observed in mice lacking *Bmal1* in skeletal muscle, yet with decrements in expression of circadian genes involved in glucose utilization and adrenergic signaling; and accompanied by increases in expression of lipogenic genes (Hodge, et al., 2015). Moreover, both studies revealed that these changes were coupled with fiber-type shifts in skeletal muscle, although these findings were in opposite directions (Dyar, et al., 2014; Hodge, et al., 2015). Other studies have found that loss of *Bmal1* and *Clock* genes was accompanied by hyperphagia, hyperlipidemia, mild hyperleptinemia, and metabolic syndrome, as well as changes in body composition, glucose intolerance, and hyperglycemia in the non-fasting condition (Harfmann, et al., 2016; Kennaway, et al., 2007; Turek, et al., 2005) although some others have not found results in the same direction (Kennaway, et al., 2007; Oishi, et al., 2006).

Beyond the role of core clock genes in the metabolic programming of skeletal muscle other key factors such as the nuclear receptor REV-ERB ALPHA have been studied (Gerhart-Hines & Lazar, 2015b). This circadian clock repressor interacts mainly with the lipid oxidation process through a dependent regulation of lipoprotein lipase activity (Delezie, et al., 2012) and consequently its deficiency in muscle leads to a reduction in mitochondrial biogenesis and oxidative capacity as well as to an upregulation of autophagy (Woldt, et al., 2013). Furthermore, its reciprocal transcription activator ROR ALPHA also plays a role in lipid and carbohydrate metabolism through modulation of many genes involved in lipid metabolism such as *Carnitine palmoyltransferase-1* and *Caveolin-3* (Raichur, et al., 2010). Since most of the processes described above has been related

to changes in several signaling pathways and molecules involved in metabolic processes, it suggests that the clock machinery display a significant role in orchestrating substrate metabolism in skeletal muscle (Chatterjee & Ma, 2016; McCarthy, et al., 2007; Pastore & Hood, 2013; Shavlakadze, et al., 2013; Vieira, et al., 2008).

Short summary

In this section, we have described some of the mechanisms by which skeletal muscle physiology is regulated in a circadian manner. Basically, muscle metabolism, muscle fiber growth and proliferative capacity as well as muscle locomotor function are dependent on an autonomous mechanism present in each muscle fiber that function around a 24-hour period and might be altered by some conditions, leading to changes in muscle physiology. In this sense, animal experiments have provided evidence of potential mechanisms by which external factors such as eating habits, exercise, light exposure and sleep patterns might influence peripheral clocks in human skeletal muscle, driving changes not only at the muscle but at the whole organism level (Figure 2).

Although no direct measurements have been done at the muscle level in humans, several studies have found interactions between such external cues and muscle-related disorders. For instance, social jetlag (i.e., discrepancy between social and biological clocks), sleep restriction, desynchrony between the internal clock and the external light-dark (LD) cycle or altered timing of food intake are related to several pathologies in which skeletal muscle has a major role, such as metabolic syndrome, type 2 diabetes as well as other non-communicable diseases (Broussard & Brady, 2010; Gonzalez-Ortiz, Martinez-Abundis, Balcazar-Munoz, & Pascoe-Gonzalez, 2000; Hutchison, Wittert, & Heilbronn, 2017; Koopman, et al., 2017; Mota, Silva, Balieiro, Fahmy, & Crispim, 2017; West, et al., 2017; Wittmann, Dinich, Merrow, & Roenneberg, 2006). Consequently, in order to prevent circadian disruptions that could affect skeletal muscle development in humans, it is recommended to keep high physical activity levels administered at different times of the day (Miyazaki, Hashimoto, Masubuchi, Honma, & Honma, 2001; Richardson, Gradisar, Short, & Lang, 2017; S. D. Youngstedt, et al., 2016), be exposed to natural bright light in the morning at intensities around or up to 10 000 lux for at least 30-45 min per day (Bjorvatn & Pallesen, 2009) and avoid bad sleep patterns, such as short (<7 h) and long (>9 h) sleep duration (St-Onge, et al., 2016) given, for example, when later bed times are coupled with the requirement to wake early. Moreover, it is also recommended to avoid shifts in meal timing, such as skipping breakfast or eating erratically without consistent daily meal times (Hutchison, et al., 2017; Mukherji, et al., 2015) and exposure to blue light emitted by electronic devices, such as smartphones or laptops, before bed time (Chellappa, et al., 2013).

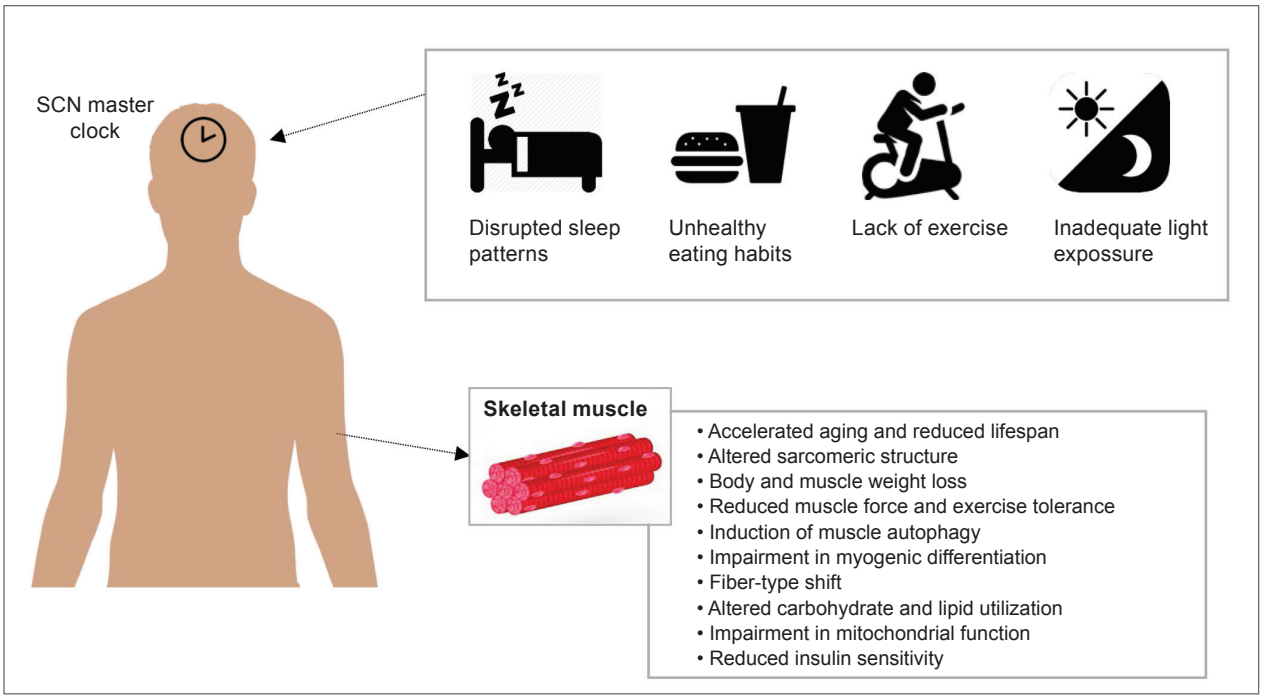

Figure 2. Potential alterations in human skeletal muscle function as a consequence of circadian misalignment. Such disturbances were observed in both clock genes disrupted animal models and animal models exposed to external factors that mediate disruption of the molecular clock.

Physical exercise and the muscle clock

The relationship between spontaneous running-wheel activity and circadian rhythms was suggested firstly by Edgar, Martin, and Dement (1991) in a mice population, since they noticed that the time of day in which spontaneous wheel running occurred was a determinant of observed circadian rhythm period. Essentially, physical exercise acts readjusting circadian rhythms when external cues, such as social behavior, force internal clocks-dependent processes leading to phase-shifts in circadian rhythmicity. For instance, a forced sleep-wake schedule of a 23 h 40 min period that was maintained for 12 cycles was accelerated after six days of physical exercise in humans (Miyazaki, et al., 2001). Moreover, three days of exercise showed to be sufficient to entrain a 9-hour shifted sleep-wake schedule (Eastman, Hoese, Youngstedt, & Liu, 1995), whereas a 8-hour shifted sleep-wake cycle was re-entrained after four days of physical exercise (Yamanaka, et al., 2010). Likewise, other experimental studies have shown that physical exercise is capable of changing the phase and synchronizing circadian rhythms of animals and humans even under constant darkness (Edgar, et al., 1991; Marchant & Mistlberger, 1996; Maywood, Mrosovsky, Field, & Hastings, 1999). In this sense, experiments performed under dim and bright light conditions showed that this circadian synchronizer effect of exercise is independent of phase-resetting effects of light (Barger, Wright, Hughes, & Czeisler, 2004; Yamanaka, et al., 2010, 2014), which means that exercise may provide an alternative or additive phase-shifting stimulus with similar potency as bright light (Youngstedt, et al., 2016; Youngstedt, Kripke, & Elliott, 2002). More importantly, entrainment effects of physical exercise are consistent even under constant conditions (i.e., without forced phase-shifts) and might be reflected by temporal changes in hormonal (i.e., melatonin, thyrotropin), temperature and sleep-wake cycles, as well as by autonomic nervous system responses and behavioral processes (i.e., alertness, cognitive performance). In this sense, studies performed with some of these circadian markers have demonstrated that exposure to nocturnal bouts of moderate and high intensity exercise normally results in a phase-delay of circadian clock (Buxton, et al., 1997; Van Reeth, et al., 1994), whereas physical exercise in the morning or in the evening cause phase-advance shifts (Buxton, Lee, L'Hermite-Baleriaux, Turek, & Van Cauter, 2003). For instance, physical exercise performed in the evening alters the onset of nocturnal melatonin secretion on the following day, providing evidence for phase-advancing effects of acute exposure to evening exercise on the human circadian system. Moreover, exercise performed in the evening attenuates nocturnal decline in body temperature and stimulates sympathetic activity in the following nocturnal sleep, whereas morning exercise has opposite effects (Yamanaka, et al., 2015). Importantly, phase-shifting effects of exercise on the circadian system are not only present in youngsters but also preserved in older adults (Baehr, et al., 2003).

Although the mechanisms responsible for this circadian synchronizing effect of physical exercise remain unknown, it has been proposed that it might be driven by exercise-induced activation and/or repression of diurnal and nocturnal genes in skeletal muscle (Zambon, et al., 2003). Accordingly, it has been identified that many putative genes, regulated in a circadian fashion in response to a resistance exercise in human quadriceps muscle, supporting the notion that physical exercise promotes circadian gene expression in skeletal muscle and that peripheral clocks can regulate themselves independently of the SCN (Zambon, et al., 2003). Furthermore, a study performed in transgenic Period2::Luciferase (*Per2::Luc*) mice have shown that aerobic exercise change the circadian expression of *Per2* gene in skeletal muscle and lung without an effect in the SCN, demonstrating that exercise acts directly in peripheral clocks (Wolff & Esser, 2012). Moreover, scheduled exercise can also regulate circadian *Per1* gene expression in skeletal muscle and lung, affecting how quickly an animal can re-entrain a new imposed light-dark cycle, an effect that might also depend on exercise timing (Yamanaka, Honma, & Honma, 2008, 2016). Looking for mechanisms behind this relation between physical exercise and skeletal muscle clocks, *in vivo* experiments have reported that forced physical exercise advances the phase of circadian rhythms in peripheral clocks through activation of the adrenal gland and the sympathetic nervous system, by increasing levels of serum corticosterone and norepinephrine in peripheral tissues (Sasaki, et al., 2016). Taken together, these findings demonstrate that physical exercise has an impact on the human circadian pacemaker, although the role of skeletal muscle clocks in mediating this response remains to be elucidated.

Short summary

In this section, we have seen that physical exercise controls circadian expression of several putative genes in skeletal muscle, demonstrating that exercise acts directly in peripheral clocks. Additionally, physical exercise has the capacity to exert an entrainment effect in circadian physiology at the whole organism level, even when phase-resetting effects of light are minimized. Circadian synchronizing effects of physical exercise occur under a variety of conditions, from moderate to high intensity exercise and when exercise is performed in the morning or in the evening. These phase-shifting effects of exercise are commonly, but not always, ac-

companied by changes in various circadian markers (e.g., melatonin, temperature, or sleep cycles). Thus, exercise may act as a strong circadian synchronizer, capable of adjust internal clocks when circadian phase-shifts have been induced. However, whether these effects are mediated by changes in skeletal muscle circadian clocks or not, remains to be determined in future studies.

Physical exercise, muscle clock and diseases

Due to the critical role that physical exercise plays in treatment of disease conditions, it is valuable to know if exercise might also mitigate the negative consequences of circadian misalignment. Physical exercise is now considered a valuable strategy to improve sleep in youngsters and adults, but also as an effective treatment for delayed sleep-wake phase disorder in young people (Richardson, et al., 2017). However, the key role of exercise as a method to treat circadian-related diseases is not restricted to sleep disorders, but also to treat metabolic and cardiovascular diseases, since they are linked with disruption of circadian rhythms (Gerhart-Hines & Lazar, 2015a; Martino & Young, 2015). In this sense, it is well known that circadian mutant mice display metabolic phenotypes of obesity, dyslipidemia, hepatic steatosis, hyperglycemia, insulin resistance and glucose intolerance as well as cardiomyopathy, sarcopenia and reduced life span, among other conditions (Mellani Lefta, Campbell, Feng, Jin, & Esser, 2012; Marcheva, et al., 2010; Rudic, et al., 2004; Turek, et al., 2005); and physical exercise has proven to be a better predictor of circadian parameters than fat mass in young men (Tranel, et al., 2015). Thus, it is important to address whether there is a role of peripheral clocks in mediating this exercise-induced response. Regarding this, some studies performed in mice and human populations have highlighted the importance of peripheral clocks as exercise targets in disease conditions. For instance, Pastore and Hood (2013) have shown a partial rescue to metabolic phenotype in skeletal muscle of *Clock* mutant mice when they were permitted access to a freely-rotating running wheel for eight weeks. More recently, a novel study performed in coronary artery disease and type 2 diabetes mellitus patients has discovered an interaction between long-term physical exercise interventions and gene expression of the *Rev-erb alpha* target protein aminolevulinate-delta-synthase-1 (ALAS1), an enzyme that influence mitochondrial biogenesis in the liver (Steidle-Kloc, et al., 2016). These findings are important since this was the first study conducted in humans to look for an interaction between physical exercise and peripheral clock genes expression in patients with metabolic diseases.

Beyond the role of skeletal muscle clocks in targeting circadian disruptions, there is a fundamental role of exercise timing in treatment of diseases. In recent years, it was demonstrated that 'exercise snacks' before (Francois, et al., 2014) or after meals (Heden, et al., 2015) result in better glycaemic and lipid control than single bouts of continuous exercise at any time of the day. In fact, a randomized crossover study reflected that advice to walk for 10 min after meals was more effective for lowering postprandial glycaemia in type 2 diabetes patients than advice to walk for 30 min at nonspecific time of the day (Reynolds, Mann, Williams, & Venn, 2016). Results from this study showed that improvements in postprandial glycemic response were particularly higher after the evening meal, when the most carbohydrate was consumed, and sedentary behaviors were highest (Reynolds, et al., 2016). Additionally, a study performed in type 2 diabetes patients observed that post-dinner resistance exercise was more effective to improve postprandial glucose and triacylglyceride (TAG) concentrations than pre-dinner resistance exercise (Heden, et al., 2015). Impressively, the TAG incremental area under the curve (iAUC) was ~92% lower when exercise was placed after meals compared with the opposite treatment (Heden, et al., 2015). Furthermore, Sasaki et al. (2014) have demonstrated that a combination of daily timing of eating and exercise was able to control high-fat diet-induced obesity in mice. Results from this experimental study suggested that eating in the morning or at noon followed by exercise in the evening could prevent weight gain more effectively than exercise in the morning followed by eating at noon or in the evening. Moreover, they found that the groups that were fed before exercise gained less body and fat weight and more skeletal muscle weight compared to the groups that exercised before eating.

Finally, therapeutic control of cardiovascular diseases might also be influenced by exercise timing and circadian rhythms. Regarding this, it is well known that risk of adverse cardiovascular events is higher in the morning (6 a.m. to noon) with a secondary peak in the evening (6 to 10 p.m.) and lowest susceptibility during the night (Scheer, et al., 2010). In this sense, it was observed that circadian system controls autonomic, hemodynamic and hemostatic risk markers at rest and during exercise in humans, suggesting a link between exercise performed at different times of the day and cardiovascular responses (Scheer, et al., 2010). Accordingly, Brito et al. (2017) observed a better ambulatory blood pressure (BP) and heart rate (HR) response after aerobic exercise performed in the evening rather than in the morning in a pre-hypertensive men population. Ultimately, circadian regulation of cardiovascular activity might be also linked with core and CCGs in skeletal muscle, as it has been shown that cardiovascular and metabolic disease in spontaneously hypertensive rats (SHR; a rodent model that

develops chronic heart failure) are associated with prolonged dysregulation of the molecular clock in skeletal muscle (Miyazaki, et al., 2011). Taken together, these findings suggest a promising role of exercise to treat human disorders caused by disruptions of circadian rhythms.

Conclusions

To summarize, skeletal muscle metabolism, muscle fiber growth and proliferative capacity as well as muscle locomotor function are dependent on an autonomous mechanism present in each muscle fiber that function around a 24-hour period and might be altered by some conditions, leading to changes in muscle physiology. In this sense, physical exercise has demonstrated to be a strong non-photic signal, capable of adjusting circadian phase shifts generated by inappropriate light exposure patterns, sleep disturbances, bad eating habits as well as other circadian misalignment responsible factors. Nevertheless, the role of skeletal muscle clock in mediating that response has not been fully understood yet. Moreover, whether there is an influence of exercise mode, volume or intensity on this molecular clock entrainment capacity is still unknown. In addition to that, exercise timing also represents a key factor in the treatment of circadian-related diseases, but the role of muscle clocks in this context is even less clear. Thus, future studies should identify the role of physical exercise and exercise timing in induction of clock gene expression in skeletal muscle to mitigate circadian disruptions and avoid muscle-related pathologies.

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Correspondence to:
Dr. Elaine Vieira
Postgraduate Program on Physical Education
Universidade Católica de Brasília-UCB, DF, Brazil
Campus I - QS 07 – Lote 01 – EPCT – Águas Claras – Brasília – DF CEP: 71966-700
Phone: + 55 (61) 33569234
E-mail: elaine.vieira@ucb.br

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