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Review

# Clocking In, Working Out: Circadian Regulation of Exercise Physiology

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**Research over the past century indicates that the daily timing of physical activity impacts on both immediate performance and long-term training efficacy. Recently, several molecular connections between circadian clocks and exercise physiology have been identified. Circadian clocks are protein-based oscillators that enable anticipation of daily environmental cycles. Cell-autonomous clocks are present in almost all cells of the body, and their timing is set by a variety of internal and external signals, including hormones and dietary intake. Improved understanding of the relationship between molecular clocks and exercise will benefit professional athletes and public health guidelines for the general population. We discuss here the role of circadian clocks in exercise, and explore time-of-day effects and the proposed molecular and physiological mechanisms.**

## Highlights

Exercise performance is subject to diurnal variation, with peak capacity generally occurring towards the late afternoon or early evening. However, this can be modified by training habits and by the inherent chronotype of each individual.

Time-of-day-dependent differences in exercise capacity are associated with diurnal oscillations in core body temperature, cardiorespiratory responses, muscle mechanics, and tissue metabolism.

The muscle-specific circadian clock is emerging as an integral mediator of exercise adaptations.

Nuclear receptors are based at the crossroads of metabolism and the circadian system; they may become an important consideration for exercise-based interventions and a promising target for chronotherapeutics.

## It Is All in the Timing

An important safeguard against the development of metabolic disease and age-related physical decline is the maintenance of lean body mass, which can be achieved in part through regular exercise. In addition exercise type, volume, and frequency, the daily timing of physical activity impacts on its efficacy. Exercise performance is significantly influenced by our internal biological clock, known as the circadian system. The circadian clock represents a series of biological rhythms that oscillate over a 24 h period, enabling an organism to anticipate daily changes in its environment and adapt its physiology and behavior accordingly. Although the primary circadian pacemaker is located in the **suprachiasmatic nucleus** (SCN, see Glossary), autonomous, self-sustaining oscillators (Box 1) are also present in almost all cells of the body [1]. The timing of these peripheral clocks is set by both neuroendocrine signals from the master clock and by local **zeitgebers**, such as feeding–fasting cycles or locomotor activity [2]. Improved understanding of the relationship between chronobiology and exercise physiology will benefit professional sports organizations aimed at enhancing athletic performance, as well as aiding public health authorities in designing population-wide exercise recommendations. In the following we discuss the role of circadian clocks in exercise, exploring time-of-day effects, proposed physiological mechanisms, and potential inputs from the molecular clock, with a focus on recent developments in these areas. This focus does not directly include the consequences for metabolic health and disease, which have recently been evaluated elsewhere [3]. In addition, given inevitable editorial constraints, we regrettably cannot include all key studies in this field, thus we refer to other relevant reviews where appropriate.

## Exercise Performance and Training Efficacy across the Day

Several studies in human subjects have demonstrated significantly enhanced performance in evening exercise of various types compared to the same exercise performed in the morning [4–7]. Many of these reports have come from dynamic types of exercise such as cycling, running, and swimming which incorporate both aerobic and anaerobic elements. In compound resistance exercises, this phenomenon is somewhat less robust and is only observed at submaximal loads [8–10]. In highly technical sports, the evidence suggests that there could be a trade-off between

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### Box 1. The Molecular Circadian Clock

The core cellular machinery of the mammalian circadian system consists of interconnected transcription–translation feedback loops (TTFLs). The primary loop is composed of a positive arm with two activator proteins (CLOCK and BMAL1) and a negative arm with two repressor proteins (CRY and PER). *CLOCK* (circadian locomotor output control kaput) and *BMAL1* (brain muscle ARNT-like 1) form the heterodimeric transcriptional complex CLOCK:BMAL1 and initiate transcription of the cryptochrome *(Cry1*, *Cry2)* and period *(Per1*, *Per2*, *Per3)* genes, as well as a host of other clock-controlled genes. CRYs and PERs heterodimerize to form the repressive complex, subsequently translocating to the nucleus to repress further CLOCK:BMAL1 activity [93]. Proteasomal degradation of CRYs and PERs relieves the repression of CLOCK:BMAL1, allowing the transcriptional cycle to repeat, all of which occurs over approximately 24 h. A secondary TTFL consists of CLOCK:BMAL1 driving the expression of the nuclear hormone receptors REV-ERBα/β and RORα/γ, which in turn repress or activate BMAL1 expression, respectively [94,95] (Figure 1).

timing of peak cognitive physical abilities, such that measures of accuracy (i.e., in dart-throwing or soccer goal aim) may be greater earlier in the day compared to the typical evening peak in measures of more purely physical attributes such as strength, speed, and endurance [11,12]. Although it can be difficult to distinguish between the many complex and intertwined factors that influence exercise performance, morning–evening differences in swim performance are at least partly attributable to the internal circadian clock of the body [13].

Some studies have incorporated a period of targeted training to establish whether the timing of habitual practice aligns with peak performance and can influence diurnal variation. Indeed, in children and young adults such training time-specific adaptations align with measures of aerobic and anaerobic capacity, as well as with muscle force [5]. These data suggest that those involved in competitive events would benefit from aligning daily training schedules with the timing of future competitions. If competition time is unknown or spans multiple days and times, morning training may be the best option: evening performance will improve (albeit to a lesser degree) and diurnal differences will be mitigated. In the case of professional sports involving travel across time zones, jet lag causes misalignment of internal clocks and can negatively affect athletic performance. The impairment appears to depend on the direction of air travel, the number of time zones crossed, and the specific sport in question [14,15].

## Morning Larks versus Night Owls

It is well established that people vary widely in the timing of their natural behavioral alignment to daily light–dark cycles. Colloquially we refer to someone who wakes up early as being 'a morning person' or a 'lark', and to people whose natural inclination is to stay up late and sleep in as 'night owls'. Several methods of quantifying the propensity of an individual for early or late alignment with environmental light–dark cycles have been developed, including the Horne–Ostberg Questionnaire [16] and the Munich ChronoType Questionnaire [17]. When analysis of **chronotype** is included in the study design, the data suggest that overall performance and other related measures are greatest at times that align with the chronotype of the subject [18–20], and that this effect may be further influenced by habitual training time [21,22]. Thus, exercise capacity and chronotype may exhibit reciprocal feedback (Box 2). As opposed to the clear diurnality of humans, most experimental strains of mice are nocturnal. In addition, mice display numerous short episodes of sleep and activity during the 24 h cycle, thus making it difficult to reliably determine chronotype differences and stratify in the context of exercise capacity [23].

## Proposed Physiological Mechanisms Underlying Diurnal Variation

### Body Temperature

One of the circadian mechanisms put forward to explain improved evening performance in humans is the well-established rhythmic daily oscillation (~0.4–1.0°C) in core body temperature (CBT) [24]. The pattern of anaerobic power output across the day closely mirrors that for body temperature [13,25,26]; moreover, prewarming or precooling of the limbs can alter diurnal differences in muscle strength [27,28]. Studies of isolated rodent skeletal muscles have suggested that

### Glossary

**Chronotype:** the behavioral manifestation of the internal circadian clock of an individual. It is reflected in the timing of the sleep–wake cycle within the 24 h period and is commonly determined by a morningness–eveningness questionnaire.

**Myokines:** cytokines and other proteins produced in and released by skeletal muscle. Myokines can influence physiology through autocrine, paracrine, or endocrine effects.

**Peroxisome proliferator-activated receptors (PPARs):** a family of nuclear receptors (NRs) that regulate gene transcription in response to activation by specific ligands. Variants of these receptors are present in different tissues, including liver, adipose tissue, muscle, hepatocytes, and endothelial cells, where they play an important role in glucose and lipid metabolism.

**Suprachiasmatic nucleus (SCN):** a group of neurons in the anterior hypothalamus which act as the principal circadian pacemaker in mammals. This autonomous 'master clock' is necessary for daily rhythms and coordinates peripheral cellular clocks through behavioral, neural, and endocrine signals.

**Zeitgeber:** any external cue that synchronizes the circadian clock of an organism with its environment. For example, light is the predominant zeitgeber for the SCN. The term originates from German, meaning 'time-giver'.

Box 2. From Genotype, to Chronotype, to Phenotype

In recent years the elucidation of molecular clock gene polymorphisms as a genetic basis of chronotype has become a consideration for sports performance. A length polymorphism in the human *PER3* gene (in addition to the endogenous circadian expression of the gene) has been associated with altered morning–evening preferences in the general population; the longer allele being linked to morningness and the shorter allele with eveningness [96]. These behavioral correlates are further supported by the preponderance of the shorter-form allele in patients with delayed sleep phase syndrome (DSPS) [96], a condition also now linked to *CRY1* coding variants [97]. These relevant differences in *PER3* allele length are driven by a variable number of tandem repeats (VNTR). Analysis of this polymorphism in endurance athletes has revealed a greater prevalence of the longer *PER3(5)* allele relative to control subjects, with the VNTR being predictive of self-reported chronotype distribution [98] and time-of-day differences in exercise performance [21,99]. It is unclear whether this correlation reflects a direct effect of chronotype on performance or whether there are two independent effects of the *PER3(5)* variant allele. Analysis of male and female elite athletes from a variety of sports reveals an over-representation of morning-types in sports that generally take place in the morning, such as marathon running, cycling, and triathlon [100,101]. These findings suggest that those with strong morning preference may be more drawn to sporting activities that take place during the early morning. Alternatively, those with chronotypes that radically oppose the performance schedules of that sport may simply not progress to the very elite level, resulting in a selection bias.

elevation in CBT (and subsequently muscle temperature) alleviates muscle stiffness and improves thermodynamic muscular efficiency [29], and this could contribute to improved evening performance in humans.

### Substrate Utilization

Diurnal differences in muscle function could also arise from changes in substrate utilization and cellular metabolism. Although circadian rhythms in glycogen accumulation have been well documented in rodents [30], the extent to which tissue glycogen content cycles diurnally in humans is less clear [31,32], possibly owing to differences in overall muscle mass and fiber-type composition, as well as lifestyle factors such as diet and amount of sleep [33].

Both glycogenolysis and gluconeogenesis during intense/prolonged exercise are mediated, in part, by sympathoadrenal responses. Long-term training adaptations to intense exercise can include enhanced epinephrine release [34], increased gluconeogenesis, and greater lactate clearance [35,36]. However, although there is a clear circadian rhythm in the production and circulation of hormones such as cortisol and catecholamines [37], the peaks and nadirs of these hormones do not align reliably with the diurnal variation in human exercise capacity. Mouse studies have revealed that molecular clock components can influence the response to cortisol [38] and otherwise influence substrate selection [39] in peripheral organs, and this may also contribute to daytime–dependent differences in glucose, lipid, and glycogen storage and/or utilization. Diurnal variation in muscle fatigue has been evaluated elsewhere [40] and falls outside the scope of this review.

### Cardiovascular Effects

Sustained physical activity is highly dependent on the delivery of oxygen and nutrients to, and the removal of waste products from, the working musculature. Thus, diurnal variation in exercise capacity could be linked to alterations in blood flow. Endothelium-dependent and -independent vasodilation in the forearm varies significantly across the day, and is related to the circadian oscillation of endothelin-1 (ET-1), a major endogenous vasoconstrictor [41,42]. However, the time of peak perfusion may differ between vascular beds [43,44], and thus standard experimental measures from the brachial artery may not be representative of diurnal changes in muscles that are heavily engaged in athletics, such as the legs and trunk.

### Neuromuscular Recruitment and Central Governance

Time-of-day differences in exercise performance can be influenced by both peripheral (e.g., cardiovascular, muscular) and neurological mechanisms. Studies of different muscle groups in humans have demonstrated that, although contractile force increases over the course of the

day, there is no difference in electromyographic parameters of muscle excitation, suggesting minimal diurnal changes in neural drive [45,46]. Other relevant aspects of central command include cognitive processing and reaction times in complex tasks; these may be subject to circadian regulation but are beyond the scope of this review.

## Molecular Clock–Exercise Reciprocal Regulation

The central circadian pacemaker is located in the SCN at the base of the anterior hypothalamus, and its timing is set primarily by light cues. Molecular clocks in peripheral tissues oscillate in a cell-autonomous fashion. Moreover, elegant studies in mice have demonstrated that these decentralized clocks can be dissociated from SCN-generated rhythms and entrained by other environmental cues, including the timing of food availability [47]. Because acute exercise and long-term training adaptations are whole-body challenges, the application of chronobiology to athletic performance and sports medicine must consider these multilevel circadian concepts. Namely, exercise may be governed centrally by generalized behavioral rhythms and entrained by photoperiod (e.g., sleep–wake cycles and travel across time-zones). It may also be influenced by tissue-specific clocks in organs such as skeletal muscle and the liver (including any crosstalk), and exercise could itself act as a physiological cue to entrain the circadian system. Rodent studies have revealed that habitual, voluntary running-wheel activity at specific times of day is sufficient to synchronize behavioral rhythms in mice and regulate the expression of core clock genes in the SCN [48]. Phase-shifting effects of exercise have also been observed in humans [49–51], suggesting that exercise could be a valuable tool to mitigate the effects of transmeridian travel (i.e., jet-lag) on sleep timing and quality.

### Muscle Rhythms

Hundreds of transcripts, representing approximately 4% of all expressed genes, undergo circadian oscillations of expression in mouse skeletal muscle [52], many of them being involved in processes relevant to exercise capacity. Genes encoding components of fatty acid (FA) transport and β-oxidation peak in the middle of the inactive phase, whereas lipogenic genes peak late into the active phase, likely in response to feeding. Transcripts involved in glucose uptake and glycogen storage are most highly expressed in the active phase [53], consistent with previously documented rhythms in skeletal muscle glycogen [30]. However, most studies have focused on large muscle groups of mixed fiber types (e.g., gastrocnemius or quadriceps). Humans and rodents have many different types of muscle, each of which influences exercise in different ways. The vast diversity of muscle subtypes was recently elegantly demonstrated by transcriptional profiling of a wide array of muscle groups in rodents [54]. When several mouse muscle groups were analyzed for rhythmic transcript expression in parallel (e.g., plantaris, soleus, tibialis anteriorus), the group of transcripts that undergo circadian oscillation was found to be specific to each muscle group [55,56].

Biopsy samples have revealed that human skeletal muscle transcription is also regulated in a time-of-day-dependent manner, resulting in rhythmic expression of transcripts involved in substrate storage and utilization, as well as in the basal production of various **myokines** [57,58]. Given that many of these pathways are known to be important for exercise capacity and training adaptations, these findings imply that the timing of exercise may be optimized to coincide with a rhythmic metabolic milieu. Supporting this idea, mitochondrial oxidative capacity of human muscle fibers and whole-body energy expenditure exhibit synchronous circadian rhythms that peak in the evening, consistent with peaks in exercise performance [59].

### CLOCK:BMAL1 Control

In addition to observed rhythmicity, the direct influence of the intrinsic circadian clock on muscle function has been highlighted in models employing genetic manipulation of core molecular

components. Several studies have now examined the impact of deleting *Bmal1* specifically in mouse skeletal muscles, enabling robust interpretation of tissue-autonomous clock disruption in the context of normal sleep–wake cycles, and feeding and activity rhythms. Deletion of *Bmal1* selectively in skeletal muscles impairs insulin sensitivity and causes a switch from carbohydrate to lipid oxidation. These changes are accompanied by decreased muscle force generation, despite only modest changes in fiber type distribution [53,60,61]. Analysis of global RNA expression in mice lacking BMAL1 specifically in muscles revealed sweeping changes in metabolic transcriptional programs. These changes likely reflect a loss of both direct CLOCK:BMAL1 target gene regulation and impaired hypoxia-responsive transcription because *Bmal1*$^{-/-}$ myotubes express much less HIF1α than control samples [62]. The interaction of the circadian clock with HIF1α has strong implications for intense exercise, when there is increased reliance on glycolytic metabolism and a potential drop in the partial pressure of oxygen in the muscle. Indeed, wild-type mice exercised to exhaustion have variable induction of transcripts regulated by HIF1α in skeletal muscle depending on the time of day; the lowest transcriptional response is in the middle of the inactive phase [62]. Further supporting a role for cell-autonomous muscle clocks in driving oscillations of gene expression and metabolic functions, disruption of *CLOCK* in cultured human myotubes leads to upregulation of genes encoding lipid transport and downregulation of genes involved in exercise- and insulin-stimulated glucose uptake, together with altered myokine production [57,58] and potential changes to hypertrophic signaling (Box 3).

### Transcription–Translation Feedback Loop Negative Arms

A role for the primary and secondary negative arms of the core clock in muscle function and exercise physiology has also been recognized. Deletion of *Per2*, but not *Per1*, appears to increase muscle mass and reduce running endurance capacity of mice [63]. Conversely, mice deficient in CRY1 and CRY2 have improved capacity for sprint exercise, but do not exhibit increased running endurance. Skeletal muscle and liver from these mice exhibited greater glycogen storage, whereas *Cry1*$^{-/-}$;*Cry2*$^{-/-}$ myotubes displayed upregulation of genes involved in FA utilization. These findings suggest that CRY1/2 help to regulate substrate switching [39]. Loss of input from REV-ERB also impacts on skeletal muscle morphology and metabolism, as well as on exercise ability. *Rev-erbα*$^{-/-}$ mice have disrupted fuel partitioning [64], reduced skeletal muscle mitochondrial content, and impaired running endurance [65]. Because all these studies have been performed in animals harboring germline disruption of repressive clock components, further analysis will be necessary to determine the muscle-intrinsic roles of PERs, CRYs, and REV-ERBs in exercise physiology.

### Input from Other Peripheral Clocks

Exercise requires an integrated response across many organ systems, and peak performance therefore likely depends on the synchronized function of multiple peripheral clocks. The liver supplies substrates to working muscles at all levels of exercise intensity, and circadian rhythms in liver have been extensively studied [66]. The importance of the clock in this process is apparent even at rest because liver-specific deletion of *Bmal1* in mice leads to impaired glucose homeostasis

#### Box 3. Clock Control of Anabolic Responses

A crucial aspect of training adaptation (particularly for strength-based athletes) and the efficacy of exercise with age is the hypertrophic response within the muscle. Although extensive research suggests that athletic performance peaks in the evening, it is unclear whether the muscle-intrinsic hypertrophic response is affected by the time of day at which resistance training occurs [102]. Hypertrophy is correlated with mTOR (mechanistic target of rapamycin) signaling [103], which exhibits circadian regulation in mouse liver [104]. Myogenic genes such as myogenin (*Myog*) and *Myod* are also under circadian control in mouse skeletal muscle [105], and circadian expression of the corresponding genes in human (*MYOG* and *MYOD*) may contribute to resistance exercise-induced muscle hypertrophy [106].

throughout the day [67]. In addition, $NAD^+$ biosynthesis and oxidative metabolism in the liver is under clock control [68,69], which may influence exercise physiology [70]. Energy stressors such as exercise activate AMPK, which has been shown to phosphorylate and degrade CRY1, thereby altering hepatocyte rhythms [71]. Mitochondria are the powerhouse for energy production in cells and are subject to a surprisingly high-amplitude oscillation in proteome composition in mouse liver [72,73]. Moreover, hepatic BMAL1 seems to be required for directing mitochondrial dynamics in the liver and for defending redox balance during different nutrient conditions [74]. Together, these data describe circadian regulation of key metabolic pathways in the liver, many of which converge to appropriately match the timing of energy supply to physiological demand. Circadian regulation of several other tissues important for exercise physiology, including bone [75,76] and adipose tissue [77–80], may also contribute to daily changes in physical performance.

### Role of Nuclear Receptors

One of the major targets of the molecular clock, and a well-established pathway for regulating metabolic health and exercise capacity, is the superfamily of nuclear hormone receptors (NRs) [81]. Multiple core clock proteins interact with NRs, thus providing a molecular bridge connecting the circadian clock to endocrine and metabolic outputs [38,39,82–84]. REV-ERBα/β and RORα/γ are NRs that participate directly in core clock function. Genetic manipulation of REV-ERBα in mice alters skeletal muscle expression of the NR transcriptional coactivator PGC-1α [**peroxisome proliferator-activated receptor** (PPAR) γ coactivator 1α, Box 4], leading to changes in mitochondrial biogenesis and running endurance [65].

Exercise and circadian clock synchronization both rely on responses to neurohumoral inputs, many of which signal through NRs. Circulating glucocorticoids are one of the major hormonal inputs, and these oscillate diurnally to mediate fuel catabolism and their secretion is upregulated in response to stress. CRYs repress glucocorticoid receptor (GR)-mediated transcription to modulate hepatic glucose production and overall metabolic homeostasis in mice [38]. Because glucocorticoids are released in response to intense exercise and may mediate some of the phase-shifting effects of physical activity in rodents [85], optimal clock control of GR signaling

#### Box 4. NR Coregulators

The ability of NRs to respond to and coordinate circadian and metabolic signals is regulated by transcriptional coactivators and corepressors. For example, PPARγ coactivators 1α (PGC-1α) and 1β (PGC-1β) cooperate to promote oxidative metabolism in skeletal muscle and liver via transcription of mitochondrial genes [107,108]. Furthermore, there are at least four isoforms of PGC-1α that are differentially expressed in response to exercise and confer variable levels of transcriptional activation on associated NRs [109]. Transgenic expression of the truncated PGC-1α4 isoform in mouse skeletal muscles enhances muscle size and strength [110]. In addition, PGC-1α undergoes circadian oscillation of expression in mouse liver nuclei [111], and is subject to post-translational modification by AMP-activated protein kinase (AMPK) [112], one of the best-established effectors of acute exercise stimulation [113,114]. It is unclear whether circadian clocks modulate specific isoforms of PGC-1α and/or PGC-1β or whether the time of day impacts on their phosphorylation by AMPK, which is also subject to circadian regulation [115], both transcriptionally [71] and post-translationally [116]. The steroid receptor coactivators (SRC-1, 2, and 3) stimulate the transcriptional activity of many NRs and exert broad effects on energy homeostasis. SRC-2 protein exhibits daily oscillation in mouse liver, and its interactions with chromatin overlap to a surprisingly large extent with those of BMAL1 [117]. Furthermore, SRC-2 may promote rhythms of chromatin accessibility, thus broadly enabling rhythmic transcription [118].

Among NR corepressors, a direct role has been demonstrated for nuclear receptor corepressor 1 (NCoR1) in exercise performance, where muscle-specific ablation in mice leads to improved endurance running, associated with increased muscle mass and oxidative capacity, that could largely be attributed to enhanced activities of PPARδ and ERRs [119]. Germline deletion of the clock components and NR corepressors CRY1 and CRY2 [82] also leads to hyperactivation of PPARδ in muscle and enhanced exercise capacity [39]. PER2 also seems to repress NRs, and its deletion causes abnormal cycling of gluconeogenic genes, hepatic glycogen storage, and adipose tissue metabolism [83,84], which could also impact on exercise physiology. Together, these data reveal widespread crosstalk between NRs, coregulators, and molecular clock components; these relationships likely coordinate the response to physiological stressors such as exercise.

has implications for overtraining in athletes and circadian alignment during transmeridian travel. This is supported by the identification of many rhythmic genes in rat skeletal muscle that also happen to be regulated by glucocorticoids. Moreover, several of these genes encode exercise-responsive pathways such as glycolysis, oxidative phosphorylation, and glutamine and lipid metabolism [86]. Additional hormones can regulate metabolism and exercise through their respective NRs (e.g., androgen receptor, AR; and thyroid hormone receptor, THR), which are also repressed by CRYs [82], but it is less clear how these are modulated by tissue-specific clocks.

A prominent link between the circadian clock and energy metabolism is the PPAR subfamily of NRs. The PPARδ isoform has received much attention given that pharmacological activation or genetic overexpression in mice has been shown to protect against obesity and dramatically improve running endurance. This response seems to involve PGC-1α coactivation, leading to increased FA oxidation in skeletal muscle and concomitant sparing of glucose [87]. A role for the circadian clock in this axis was revealed by the observation of PPARδ derepression following loss of CRY1/2, subsequently allowing glycogen storage and improved high-intensity exercise performance [39]. In addition, tissue-specific loss of BMAL1 in skeletal muscle alters the transcriptional programming of PPARδ targets, leading to upregulation of genes involved in lipid transport and oxidation [88]. Oscillations of PPARδ in other peripheral tissues could similarly impact on systemic metabolism and exercise capacity; for example, liver PPARδ coordinates

## Key Figure

### Circadian Regulation of Exercise Capacity

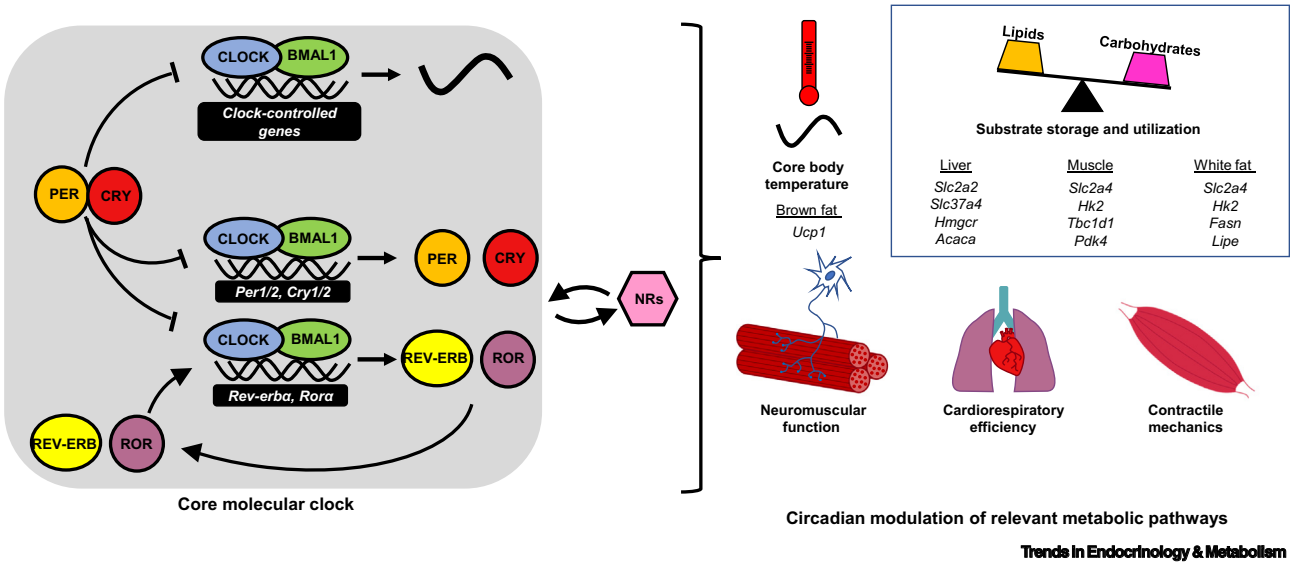


Figure 1. Differences in exercise performance across the day are influenced by several aspects of physiology. The core molecular clock consists of a transcription and translation feedback loop that coordinates systemic and tissue-specific metabolic processes. Clocks are intimately linked to the activity of nuclear hormone receptors (NRs), which are crucial mediators of both circadian rhythms and energy metabolism. Circadian modulation of gene expression and physiology has been studied in detail in several organs. We highlight some of the oscillating transcripts that are controlled by circadian clocks in peripheral organs and that contribute to daily rhythms in physiological programs that likely influence exercise performance. The circadian molecular mechanisms underlying other rhythmic phenomena remain to be uncovered in future studies.

hepatic lipogenesis and muscle FA uptake [89]. PPARα and PPARγ are major regulators of lipid storage and utilization [90,91], as well as of gluconeogenesis [91], and are also repressed by CRYs [39,82] and PERs [83,84,92].

## Concluding Remarks and Future Perspectives

The increasingly recognized role of the circadian system in exercise physiology has far-reaching consequences for basic research and applied sports medicine. Although time-of-day differences in human exercise performance have been a topic of considerable interest, very few studies have directly investigated the underlying regulation by central and tissue-specific clocks. Elucidating the molecular clock-controlled mechanisms of skeletal muscle function will be crucial for the future of this field, as well as for understanding the necessary integration with other peripheral clocks, the circadian pacemaker, and neurohormonal output (see Outstanding Questions). The physiological demands of behavioral practices (particularly intense training schedules, dietary regimens, and sleeping habits) will also require careful attention. These personally and socially enforced entrainment cues could shape molecular clock function over time through reciprocal feedback. The ability of the circadian clock to coordinate and respond to targeted exercise likely requires complex metabolic signaling, particularly at the outer edges of performance. Centrally placed in this metabolic network are the NRs, some of which directly control substrate flux and molecular timekeeping (Figure 1, Key Figure). These cellular components are therefore appealing targets for pharma- and nutraceuticals, which could conceivably modulate exercise capacity in humans if delivery is optimized for circadian phase and tissue-specificity. This represents an exciting frontier for circadian biology because chronotherapeutic strategies considered in the context of lifestyle and genotype hold the promise of optimizing health, performance, and longevity.

### Outstanding Questions

What are the crucial central or peripheral mechanisms that dictate the diurnal variation in exercise performance and response to training? Do these differ between exercise types?

To what extent can the circadian system be modified by long-term exercise habits?

How can existing and prospective ergogenic aids (supplements, light therapy, etc.) be optimized to mitigate the decline in performance due to circadian disruption (e.g., sleep loss, jet-lag).

How do circadian clock function and/or the interaction between clocks and metabolism differ between muscle groups and fiber types?

What is the crosstalk between skeletal muscle circadian clocks and other peripheral clocks (e.g., liver, white and brown adipose tissue, cardiovascular, etc.) in coordinating the physiological response to exercise?

Will it someday be possible for military leadership, sports organizations, and public health authorities to harness chronobiological data to push the boundaries of human performance and formulate evidence-based exercise recommendations?

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### References

1. Yoo, S.H. *et al.* (2004) PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues. *Proc. Natl. Acad. Sci. U. S. A.* 101, 5339–5346
2. Stratmann, M. and Schibler, U. (2006) Properties, entrainment, and physiological functions of mammalian peripheral oscillators. *J. Biol. Rhythm.* 21, 494–506
3. Gabriel, B.M. and Zierath, J.R. (2019) Circadian rhythms and exercise – re-setting the clock in metabolic disease. *Nat. Rev. Endocrinol.* 15, 197–206
4. Bessot, N. *et al.* (2006) The effect of pedal rate and time of day on the time to exhaustion from high-intensity exercise. *Chronobiol. Int.* 23, 1009–1024
5. Chtourou, H. and Souissi, N. (2012) The effect of training at a specific time of day: a review. *J. Strength Cond. Res.* 26, 1984–2005
6. Fernandes, A.L. *et al.* (2014) Effect of time of day on performance, hormonal and metabolic response during a 1000-M cycling time trial. *PLoS One* 9, e109954
7. Dalton, B. *et al.* (1997) Circadian rhythms have no effect on cycling performance. *Int. J. Sports Med.* 18, 538–542
8. Mora-Rodriguez, R. *et al.* (2012) Caffeine ingestion reverses the circadian rhythm effects on neuromuscular performance in highly resistance-trained men. *PLoS One* 7, e33807
9. Teo, W. *et al.* (2011) The effects of circadian rhythmicity of salivary cortisol and testosterone on maximal isometric force, maximal dynamic force, and power output. *J. Strength Cond. Res.* 25, 1538–1545
10. Pallares, J.G. *et al.* (2015) Pseudoephedrine and circadian rhythm interaction on neuromuscular performance. *Scand. J. Med. Sci. Sports* 25, e603–e612
11. Masmoudi, L. *et al.* (2016) Effect of time of day on soccer specific skills in children: psychological and physiological responses. *Biol. Rhythm. Res.* 47, 59–68
12. Blatter, K. and Cajochen, C. (2007) Circadian rhythms in cognitive performance: methodological constraints, protocols, theoretical underpinnings. *Physiol. Behav.* 90, 196–208
13. Kline, C.E. *et al.* (2007) Circadian variation in swim performance. *J. Appl. Physiol. (1985)* 102, 641–649
14. Leatherwood, W.E. and Dragoo, J.L. (2013) Effect of airline travel on performance: a review of the literature. *Br. J. Sports Med.* 47, 561–567
15. Song, A. *et al.* (2017) How jet lag impairs major league baseball performance. *Proc. Natl. Acad. Sci. U. S. A.* 114, 1407–1412
16. Horne, J.A. and Ostberg, O. (1976) A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *Int. J. Chronobiol.* 4, 97–110
17. Roenneberg, T. *et al.* (2003) Life between clocks: daily temporal patterns of human chronotypes. *J. Biol. Rhythm.* 18, 80–90
18. Kunorozva, L. *et al.* (2014) Perception of effort in morning-type cyclists is lower when exercising in the morning. *J. Sports Sci.* 32, 917–925
19. Tamm, A.S. *et al.* (2009) Chronotype influences diurnal variations in the excitability of the human motor cortex and the ability to generate torque during a maximum voluntary contraction. *J. Biol. Rhythm.* 24, 211–224
20. Facer-Childs, E. and Brandstaetter, R. (2015) The impact of circadian phenotype and time since awakening on diurnal performance in athletes. *Curr. Biol.* 25, 518–522
21. Rae, D.E. *et al.* (2015) Factors to consider when assessing diurnal variation in sports performance: the influence of chronotype and habitual training time-of-day. *Eur. J. Appl. Physiol.* 115, 1339–1349
22. Brown, F.M. *et al.* (2008) Collegiate rowing crew performance varies by morningness-eveningness. *J. Strength Cond. Res.* 22, 1894–1900

23. Pfeffer, M. *et al.* (2015) Owls and larks in mice. *Front. Neurol.* 6, 101
24. Aschoff, J. (1983) Circadian control of body temperature. *J. Therm. Biol.* 8, 143–147
25. Souissi, N. *et al.* (2004) Circadian rhythms in two types of anaerobic cycle leg exercise: force-velocity and 30-s Wingate tests. *Int. J. Sports Med.* 25, 14–19
26. Deschodt, V.J. and Arsac, L.M. (2004) Morning vs. evening maximal cycle power and technical swimming ability. *J. Strength Cond. Res.* 18, 149–154
27. Taylor, K. *et al.* (2011) Warm-up affects diurnal variation in power output. *Int. J. Sports Med.* 32, 185–189
28. Robinson, W.R. *et al.* (2013) Does lowering evening rectal temperature to morning levels offset the diurnal variation in muscle force production? *Chronobiol. Int.* 30, 998–1010
29. Ranatunga, K.W. (1982) Temperature-dependence of shortening velocity and rate of isometric tension development in rat skeletal muscle. *J. Physiol.* 329, 465–483
30. Benavides, A. *et al.* (1998) Circadian rhythms of lipoprotein lipase and hepatic lipase activities in intermediate metabolism of adult rat. *Am. J. Phys.* 275, R811–R817
31. Takahashi, H. *et al.* (2015) $^{13}$C MRS reveals a small diurnal variation in the glycogen content of human thigh muscle. *NMR Biomed.* 28, 650–655
32. Macauley, M. *et al.* (2015) Diurnal variation in skeletal muscle and liver glycogen in humans with normal health and type 2 diabetes. *Clin. Sci. (Lond.)* 128, 707–713
33. Skein, M. *et al.* (2011) Intermittent-sprint performance and muscle glycogen after 30 h of sleep deprivation. *Med. Sci. Sports Exerc.* 43, 1301–1311
34. Kjaer, M. *et al.* (1985) Effect of exercise on epinephrine turnover in trained and untrained male subjects. *J. Appl. Physiol. (1985)* 59, 1061–1067
35. Bergman, B.C. *et al.* (2000) Endurance training increases gluconeogenesis during rest and exercise in men. *Am. J. Physiol. Endocrinol. Metab.* 278, E244–E251
36. MacRae, H.H. *et al.* (1995) Effects of endurance training on lactate removal by oxidation and gluconeogenesis during exercise. *Pflugers Arch.* 430, 964–970
37. Scheer, F.A. *et al.* (2010) Impact of the human circadian system, exercise, and their interaction on cardiovascular function. *Proc. Natl. Acad. Sci. U. S. A.* 107, 20541–20546
38. Lamia, K.A. *et al.* (2011) Cryptochromes mediate rhythmic repression of the glucocorticoid receptor. *Nature* 480, 552–556
39. Jordan, S.D. *et al.* (2017) CRY1/2 Selectively repress PPARdelta and limit exercise capacity. *Cell Metab.* 26, 243–255
40. Chtourou, H. *et al.* (2013) Effect of time-of-day on muscle fatigue: a review. *J. Nov. Physiother.* 3, 1–10
41. Elherik, K. *et al.* (2002) Circadian variation in vascular tone and endothelial cell function in normal males. *Clin. Sci. (Lond.)* 102, 547–552
42. Etsuda, H. *et al.* (1999) Morning attenuation of endothelium-dependent, flow-mediated dilation in healthy young men: possible connection to morning peak of cardiac events? *Clin. Cardiol.* 22, 417–421
43. Conroy, D.A. *et al.* (2005) Daily rhythm of cerebral blood flow velocity. *J. Circadian Rhythms* 3, 3
44. Johnston, J.G. and Pollock, D.M. (2018) Circadian regulation of renal function. *Free Radic. Biol. Med.* 119, 93–107
45. Guette, M. *et al.* (2005) Time-of-day effect on the torque and neuromuscular properties of dominant and non-dominant quadriceps femoris. *Chronobiol. Int.* 22, 541–558
46. Chtourou, H. *et al.* (2011) Diurnal variation in Wingate-test performance and associated electromyographic parameters. *Chronobiol. Int.* 28, 706–713
47. Damiola, F. *et al.* (2000) Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes Dev.* 14, 2950–2961
48. Schroeder, A.M. *et al.* (2012) Voluntary scheduled exercise alters diurnal rhythms of behaviour, physiology and gene expression in wild-type and vasoactive intestinal peptide-deficient mice. *J. Physiol.* 590, 6213–6226
49. Youngstedt, S.D. *et al.* (2016) Circadian phase-shifting effects of bright light, exercise, and bright light plus exercise. *J. Circadian Rhythms* 14, 2
50. Buxton, O.M. *et al.* (2003) Exercise elicits phase shifts and acute alterations of melatonin that vary with circadian phase. *Am. J. Phys. Regul. Integr. Comp. Phys.* 284, R714–R724
51. Youngstedt, S.D. *et al.* (2019) Human circadian phase–response curves for exercise. *J. Physiol.* Published online February 19, 2019. https://doi.org/10.1113/JP276943
52. Zhang, R. *et al.* (2014) A circadian gene expression atlas in mammals: implications for biology and medicine. *Proc. Natl. Acad. Sci. U. S. A.* 111, 16219–16224
53. Hodge, B.A. *et al.* (2015) The endogenous molecular clock orchestrates the temporal separation of substrate metabolism in skeletal muscle. *Skelet. Muscle* 5, 17
54. Terry, E.E. *et al.* (2018) Transcriptional profiling reveals extraordinary diversity among skeletal muscle tissues. *Elife* 7, e34613
55. Dyar, K.A. *et al.* (2015) The calcineurin–NFAT pathway controls activity-dependent circadian gene expression in slow skeletal muscle. *Mol. Metab.* 4, 823–833
56. Dyar, K.A. *et al.* (2018) Atlas of circadian metabolism reveals system-wide coordination and communication between clocks. *Cell* 174, 1571–1585
57. Perrin, L. *et al.* (2018) Transcriptomic analyses reveal rhythmic and CLOCK-driven pathways in human skeletal muscle. *Elife* 7, e34114
58. Perrin, L. *et al.* (2015) Human skeletal myotubes display a cell-autonomous circadian clock implicated in basal myokine secretion. *Mol. Metab.* 4, 834–845
59. van Moorsel, D. *et al.* (2016) Demonstration of a day–night rhythm in human skeletal muscle oxidative capacity. *Mol. Metab.* 5, 635–645
60. Dyar, K.A. *et al.* (2014) Muscle insulin sensitivity and glucose metabolism are controlled by the intrinsic muscle clock. *Mol. Metab.* 3, 29–41
61. Harfmann, B.D. *et al.* (2016) Muscle-specific loss of Bmal1 leads to disrupted tissue glucose metabolism and systemic glucose homeostasis. *Skelet. Muscle* 6, 12
62. Peek, C.B. *et al.* (2017) Circadian clock interaction with HIF1alpha mediates oxygenic metabolism and anaerobic glycolysis in skeletal muscle. *Cell Metab.* 25, 86–92
63. Bae, K. *et al.* (2006) Differential effects of two period genes on the physiology and proteomic profiles of mouse anterior tibialis muscles. *Mol. Cells* 22, 275–284
64. Delezie, J. *et al.* (2012) The nuclear receptor REV-ERBalpha is required for the daily balance of carbohydrate and lipid metabolism. *FASEB J.* 26, 3321–3335
65. Woldt, E. *et al.* (2013) Rev-erb-alpha modulates skeletal muscle oxidative capacity by regulating mitochondrial biogenesis and autophagy. *Nat. Med.* 19, 1039–1046
66. Zwighaft, Z. *et al.* (2016) The liver in the eyes of a chronobiologist. *J. Biol. Rhythm.* 31, 115–124
67. Lamia, K.A. *et al.* (2008) Physiological significance of a peripheral tissue circadian clock. *Proc. Natl. Acad. Sci. U. S. A.* 105, 15172–15177
68. Ramsey, K.M. *et al.* (2009) Circadian clock feedback cycle through NAMPT-mediated $NAD^+$ biosynthesis. *Science* 324, 651–654
69. Peek, C.B. *et al.* (2013) Circadian clock $NAD^+$ cycle drives mitochondrial oxidative metabolism in mice. *Science* 342, 1243417
70. White, A.T. and Schenk, S. (2012) $NAD^+$/NADH and skeletal muscle mitochondrial adaptations to exercise. *Am. J. Physiol. Endocrinol. Metab.* 303, E308–E321
71. Lamia, K.A. *et al.* (2009) AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. *Science* 326, 437–440
72. Neufeld-Cohen, A. *et al.* (2016) Circadian control of oscillations in mitochondrial rate-limiting enzymes and nutrient utilization by PERIOD proteins. *Proc. Natl. Acad. Sci. U. S. A.* 113, E1673–E1682
73. Bugge, A. *et al.* (2012) Rev-erbalpha and Rev-erbbeta coordinately protect the circadian clock and normal metabolic function. *Genes Dev.* 26, 657–667
74. Jacobi, D. *et al.* (2015) Hepatic Bmal1 regulates rhythmic mitochondrial dynamics and promotes metabolic fitness. *Cell Metab.* 22, 709–720

75. Xu, C. *et al.* (2016) Circadian clock regulates bone resorption in mice. *J. Bone Miner. Res.* 31, 1344–1355
76. Yang, N. and Meng, Q.J. (2016) Circadian clocks in articular cartilage and bone: a compass in the sea of matrices. *J. Biol. Rhythm.* 31, 415–427
77. Paschos, G.K. *et al.* (2012) Obesity in mice with adipocyte-specific deletion of clock component Arntl. *Nat. Med.* 18, 1768–1777
78. Henriksson, E. and Lamia, K.A. (2015) Adipose clocks: burning the midnight oil. *J. Biol. Rhythm.* 30, 364–373
79. Gerhart-Hines, Z. *et al.* (2013) The nuclear receptor Rev-erbalpha controls circadian thermogenic plasticity. *Nature* 503, 410–413
80. Christou, S. *et al.* (2019) Circadian regulation in human white adipose tissue revealed by transcriptome and metabolic network analysis. *Sci. Rep.* 9, 2641
81. Yang, X. *et al.* (2007) Nuclear receptors, metabolism, and the circadian clock. *Cold Spring Harb. Symp. Quant. Biol.* 72, 387–394
82. Kriebs, A. *et al.* (2017) Circadian repressors CRY1 and CRY2 broadly interact with nuclear receptors and modulate transcriptional activity. *Proc. Natl. Acad. Sci. U. S. A.* 114, 8776–8781
83. Grimaldi, B. *et al.* (2010) PER2 controls lipid metabolism by direct regulation of PPARgamma. *Cell Metab.* 12, 509–520
84. Schmutz, I. *et al.* (2010) The mammalian clock component PERIOD2 coordinates circadian output by interaction with nuclear receptors. *Genes Dev.* 24, 345–357
85. Sasaki, H. *et al.* (2016) Forced rather than voluntary exercise entrains peripheral clocks via a corticosterone/noradrenaline increase in PER2::LUC mice. *Sci. Rep.* 6, 27607
86. Almon, R.R. *et al.* (2008) Relationships between circadian rhythms and modulation of gene expression by glucocorticoids in skeletal muscle. *Am. J. Phys. Regul. Integr. Comp. Phys.* 295, R1031–R1047
87. Fan, W. *et al.* (2017) PPARdelta promotes running endurance by preserving glucose. *Cell Metab.* 25, 1186–1193
88. Dyar, K.A. *et al.* (2018) Transcriptional programming of lipid and amino acid metabolism by the skeletal muscle circadian clock. *PLoS Biol.* 16, e2005886
89. Liu, S. *et al.* (2013) A diurnal serum lipid integrates hepatic lipogenesis and peripheral fatty acid use. *Nature* 502, 550–554
90. Muoio, D.M. *et al.* (2002) Peroxisome proliferator-activated receptor-alpha regulates fatty acid utilization in primary human skeletal muscle cells. *Diabetes* 51, 901–909
91. Kersten, S. *et al.* (1999) Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. *J. Clin. Invest.* 103, 1489–1498
92. Costa, M.J. *et al.* (2011) Circadian rhythm gene period 3 is an inhibitor of the adipocyte cell fate. *J. Biol. Chem.* 286, 9063–9070
93. Partch, C.L. *et al.* (2014) Molecular architecture of the mammalian circadian clock. *Trends Cell Biol.* 24, 90–99
94. Preitner, N. *et al.* (2002) The orphan nuclear receptor REV-ERBalpha controls circadian transcription within the positive limb of the mammalian circadian oscillator. *Cell* 110, 251–260
95. Sato, T.K. *et al.* (2004) A functional genomics strategy reveals Rora as a component of the mammalian circadian clock. *Neuron* 43, 527–537
96. Archer, S.N. *et al.* (2003) A length polymorphism in the circadian clock gene Per3 is linked to delayed sleep phase syndrome and extreme diurnal preference. *Sleep* 26, 413–415
97. Patke, A. *et al.* (2017) Mutation of the human circadian clock gene CRY1 in familial delayed sleep phase disorder. *Cell* 169, 203–215
98. Kunorozva, L. *et al.* (2012) Chronotype and PERIOD3 variable number tandem repeat polymorphism in individual sports athletes. *Chronobiol. Int.* 29, 1004–1010
99. Anderson, A. *et al.* (2018) Circadian effects on performance and effort in collegiate swimmers. *J. Circadian Rhythms* 16, 8
100. Lastella, M. *et al.* (2016) The chronotype of elite athletes. *J. Hum. Kinet.* 54, 219–225
101. Henst, R.H. *et al.* (2015) A chronotype comparison of South African and Dutch marathon runners: the role of scheduled race start times and effects on performance. *Chronobiol. Int.* 32, 858–868
102. Sedliak, M. *et al.* (2018) Morphological, molecular and hormonal adaptations to early morning versus afternoon resistance training. *Chronobiol. Int.* 35, 450–464
103. Mitchell, C.J. *et al.* (2013) Muscular and systemic correlates of resistance training-induced muscle hypertrophy. *PLoS One* 8, e78636
104. Jouffe, C. *et al.* (2013) The circadian clock coordinates ribosome biogenesis. *PLoS Biol.* 11, e1001455
105. Shavlakadze, T. *et al.* (2013) Impact of fasting on the rhythmic expression of myogenic and metabolic factors in skeletal muscle of adult mice. *Am. J. Phys. Cell Physiol.* 305, C26–C35
106. Thalacker-Mercer, A. *et al.* (2013) Cluster analysis reveals differential transcript profiles associated with resistance training-induced human skeletal muscle hypertrophy. *Physiol. Genomics* 45, 499–507
107. Kamei, Y. *et al.* (2003) PPARgamma coactivator 1beta/ERR ligand 1 is an ERR protein ligand, whose expression induces a high-energy expenditure and antagonizes obesity. *Proc. Natl. Acad. Sci. U. S. A.* 100, 12378–12383
108. Lin, J. *et al.* (2003) PGC-1beta in the regulation of hepatic glucose and energy metabolism. *J. Biol. Chem.* 278, 30843–30848
109. Martinez-Redondo, V. *et al.* (2015) The hitchhiker's guide to PGC-1alpha isoform structure and biological functions. *Diabetologia* 58, 1969–1977
110. Ruas, J.L. *et al.* (2012) A PGC-1alpha isoform induced by resistance training regulates skeletal muscle hypertrophy. *Cell* 151, 1319–1331
111. Liu, C. *et al.* (2007) Transcriptional coactivator PGC-1alpha integrates the mammalian clock and energy metabolism. *Nature* 447, 477–481
112. Jager, S. *et al.* (2007) AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. *Proc. Natl. Acad. Sci. U. S. A.* 104, 12017–12022
113. Gabriel, B.M. and Zierath, J.R. (2017) The limits of exercise physiology: from performance to health. *Cell Metab.* 25, 1000–1011
114. Mounier, R. *et al.* (2015) Expanding roles for AMPK in skeletal muscle plasticity. *Trends Endocrinol. Metab.* 26, 275–286
115. Jordan, S.D. and Lamia, K.A. (2013) AMPK at the crossroads of circadian clocks and metabolism. *Mol. Cell. Endocrinol.* 366, 163–169
116. Henriksson, E. *et al.* (2017) The liver circadian clock modulates biochemical and physiological responses to metformin. *J. Biol. Rhythm.* 32, 345–358
117. Stashi, E. *et al.* (2014) SRC-2 is an essential coactivator for orchestrating metabolism and circadian rhythm. *Cell Rep.* 6, 633–645
118. Zhu, B. *et al.* (2015) Coactivator-dependent oscillation of chromatin accessibility dictates circadian gene amplitude via REV-ERB loading. *Mol. Cell* 60, 769–783
119. Yamamoto, H. *et al.* (2011) NCoR1 is a conserved physiological modulator of muscle mass and oxidative function. *Cell* 147, 827–839