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Forum

Uncovering the muscle clock–mitochondria axis through exercise

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Emerging evidence suggests an interplay between the molecular clock and mitochondrial dynamics in skeletal muscle. In this Forum article, we hypothesize that exercise, as a metabolic challenge, provides a powerful physiological model to investigate the clock–mitochondria axis and its regulatory role in muscle function and metabolic health in humans.

Circadian clock and mitochondrial dynamics in skeletal muscle

Circadian rhythms, with a period of ~24 h, align cellular processes with environmental cycles such as light–dark and feeding–fasting. These rhythms are driven by the transcriptional activators brain and muscle Arnt-like protein-1 (BMAL1) and circadian locomotor output cycles kaput (CLOCK), which initiate a feedback loop involving the repressors period circadian regulator (PER) and cryptochrome circadian regulator (CRY). In mice, deletion of *Clock* alters the expression of 1000–3000 genes across tissues, resulting in aberrant metabolic phenotypes [1]. In humans, circadian misalignment (e.g., shift work) is associated with increased risk of sarcopenia and metabolic disorders.

Skeletal muscle, a central organ for glucose disposal and systemic metabolic regulation, displays circadian rhythms in clock genes expression alongside oscillations in mitochondrial respiration and lipid metabolism [2]. Disruption of the molecular clock, through skeletal muscle-specific deletion of *Bmal1* in mice or reduced BMAL1 and CLOCK protein levels in humans, is linked to dysregulated mitochondrial dynamics and reduced skeletal muscle strength [3,4].

Mitochondrial dynamics, mediated by fusion proteins mitofusins 1 and 2 (MFN1/2) and optic atrophy protein 1 (OPA1), and fission proteins dynamin-related protein 1 (DRP1) and mitochondrial fission protein 1 (FIS1), regulate mitochondrial morphology and are essential for maintaining mitochondrial function and turnover. An imbalance between fusion and fission processes impairs oxidative capacity and contributes to insulin resistance and skeletal muscle atrophy [5]. Emerging evidence suggests that circadian proteins may influence mitochondrial dynamics, highlighting a potential regulatory axis between the molecular clock and mitochondrial function [2].

Evidence of a molecular clock–mitochondria axis in skeletal muscle

Recent evidence has proposed a molecular axis linking the circadian clock to mitochondrial dynamics in skeletal muscle. Supporting this hypothesis, a recent human study reported that reduced levels of BMAL1 and CLOCK proteins are associated with a mitochondrial fission phenotype in skeletal muscle, as evidenced by altered fusion/fission protein level and increased mitochondrial fragmentation [3]. Interestingly, skeletal muscle-specific *Bmal1* knockout mice exhibit increased CLOCK, as a compensatory response, elevated OPA1, and reduced DRP1 protein levels, suggesting a mitochondrial fusion phenotype, although mitochondrial morphology was not assessed [6]. This resulted in a mice genetic model that contrasted with human findings, where the opposite pattern was observed [3].

These studies indicate a potential link between the molecular clock and mitochondrial dynamics in skeletal muscle. However, interspecies discrepancies may reflect compensatory mechanisms in genetic murine models or fundamental differences in biological responses, as observed in other molecular and physiological processes in skeletal muscle.

Exercise as a metabolic challenge for studying the molecular clock–mitochondria axis in skeletal muscle

Exercise, as a metabolic challenge, offers a unique model to study the interaction between the molecular clock and mitochondrial dynamics in skeletal muscle. The distinct metabolic and mechanical demands of endurance and resistance exercise, ranging from continuous low-intensity efforts to high-intensity intervals, or varying loads and repetitions, drive specific molecular adaptations [7]. These modality-specific features make exercise particularly suited for investigating the clock–mitochondria axis and its relevance to skeletal muscle health. This is especially pertinent in human studies, where translational insights are most impactful. It is expected that both acute and chronic exercise-induced modulation of the molecular clock is accompanied by synchronized changes in mitochondrial dynamics (Figure 1B).

In mice, aerobic exercise training (8 weeks, 5 days per week at ~55% V_{max} for ~70 min) led to increased protein levels of BMAL1 and PER2, enhanced glucose transporter type 4 (GLUT4) translocation, and potentially promoted mitochondrial fusion through upregulation of MFN2 in skeletal muscle [8]. However, mitochondrial morphology was not assessed, leaving potential structural changes unresolved. In rats, a single bout of heavy-load exercise (treadmill running at a −16° incline, 16 m/min for 90 min) increased protein levels of BMAL1, altered mitochondrial morphology, visually suggesting larger mitochondria, though not quantitatively assessed,

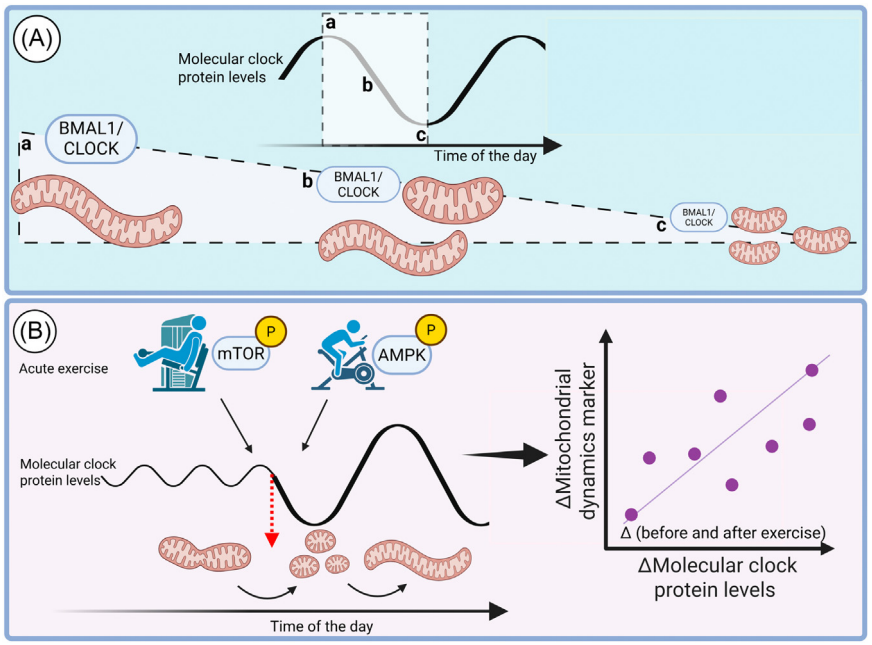

Figure 1. Experimental model proposals for studying the molecular clock–mitochondrial dynamics axis in human skeletal muscle. (A) Assessment of temporal concordance between circadian clock proteins and mitochondrial dynamics markers in human skeletal muscle. (B) Assessment of the association between exercise-induced modifications in circadian clock proteins and mitochondrial dynamics markers in human skeletal muscle. Figure created using BioRender.

and enhanced complex II activity in skeletal muscle [9].

In humans, there is currently no evidence linking the effects of acute or chronic exercise on circadian clock proteins or gene expression with markers of mitochondrial dynamics. However, a notable study demonstrated that 12 weeks of endurance high-intensity interval training (5 sessions per week at ~85% HeartRate max for 60 min) altered the rhythmic expression of molecular clock genes in skeletal muscle of insulin-resistant men. These molecular changes were accompanied by enhanced skeletal muscle insulin sensitivity and increased ADP-stimulated mitochondrial respiration [10]. Although mitochondrial dynamics were not assessed, prior studies link the high-intensity interval training to enhanced mitochondrial fusion [11]. Further research is needed to clarify exercise impact on molecular clock circadian regulation and mitochondrial dynamics.

Despite growing interest in the relationship between exercise and circadian rhythmicity, evidence of how different exercise modalities influence molecular clock components remains limited. Animal studies involving both acute and chronic exercise support the notion that increased BMAL1 protein levels promote a mitochondrial fusion phenotype, accompanied by metabolic improvements. Notably, in skeletal muscle-specific *Bmal1* knockout mice, neither acute nor chronic exercise was able to induce the expected gene expression programs or mitochondrial adaptations [12], highlighting BMAL1 as a potentially pivotal regulator of exercise-induced mitochondrial remodeling and skeletal muscle metabolic health in murine models.

Hypothesis of differential regulation of the clock–mitochondria axis by exercise modalities in human skeletal muscle

As highlighted previously, discrepancies between human and rodent studies regarding the axis between the molecular clock and mitochondrial dynamics underscore the need for direct investigation in humans. In this context, acute exercise represents a potent metabolic challenge to probe this axis. Given the null evidence available, we hypothesize that different exercise modalities may differentially regulate the clock–mitochondria axis in human skeletal muscle through distinct signaling pathways.

It is important to note that both acute and chronic adaptations to endurance and resistance training are influenced by metabolic status, training history, and age. From a simplified mechanistic perspective, acute endurance exercise activates the AMP-activated protein kinase (AMPK) signaling pathway [7] and promotes mitochondrial fission. Currently, there is a lack of evidence showing that AMPK activation influences the molecular clock in skeletal muscle. However, in the liver of mice, AMPK activation, and nuclear localization destabilized cryptochromes and altered circadian rhythms, resulting in reduced BMAL1 oscillation amplitude [13]. These findings suggest that acute endurance exercise may decrease BMAL1 protein levels while promoting a mitochondrial fission phenotype.

Resistance exercise activates the Mammalian target of rapamycin (mTOR) pathway [7] and induced mitochondrial fission [14]. While no human studies have directly examined the effects of acute resistance exercise on molecular clock components, *in vitro* data from fibroblasts indicate that mTOR inhibition reduces BMAL1 and CLOCK protein levels. However, discrepancies exist between fibroblast and liver tissue responses to mTOR

inhibition, particularly regarding gene expression versus protein levels of clock components [15]. Unpublished data from our laboratory further suggest that acute resistance exercise may dramatically reduce molecular clock protein levels in skeletal muscle, resembling the effects observed *in vitro* with serum shock synchronization protocols. These observations raise the possibility that endurance and resistance exercise can acutely reset the molecular clock at the protein level, while concurrently inducing mitochondrial fission, a key mechanism for mitochondrial turnover and improved metabolic function in skeletal muscle.

This leads to a fundamental question: how should the molecular clock be studied in human skeletal muscle? Should we focus on gene expression, protein levels, or rhythmicity?

While circadian clock genes are known to oscillate over 24-h cycles, most existing studies rely on mRNA measurements. However, this approach may be limited by the often non-linear and tissue-specific relationship between transcription and translation. Therefore, direct assessment of molecular clock proteins is essential to accurately capture circadian regulation at the functional level, particularly in the context of the molecular clock–mitochondrial dynamics axis.

We propose to investigate the molecular clock–mitochondrial dynamics axis in human skeletal muscle using two experimental models. First, by collecting muscle biopsies at multiple time points across the day, researchers can assess whether diurnal fluctuations in clock proteins are accompanied by changes in mitochondrial morphology and associated molecular mediators. Second, we suggest evaluating whether acute exercise induces modifications in molecular clock components that correlate with changes in mitochondrial dynamics markers. The integration of both approaches will provide a more comprehensive and reliable characterization of this molecular axis (Figure 1A,B). Importantly, relying solely on time-of-day sampling may be confounded by nutritional status or metabolic variability, underscoring the value of a dual-strategy design to mitigate these limitations and strengthen mechanistic insights.

Concluding remarks and future perspectives

The molecular clock–mitochondrial dynamics axis in skeletal muscle represents a promising yet underexplored area of research. Although animal models have yielded valuable mechanistic insights, species-specific differences limit the direct translation of findings to humans. In this context, endurance and resistance exercise modalities offer a unique opportunity to study this axis, as they activate distinct signaling pathways, such as AMPK and mTOR, that may regulate the molecular clock–mitochondrial dynamics axis. Thus, exercise serves as a physiologically relevant tool for investigating the interplay between circadian regulation and mitochondrial dynamics in human skeletal muscle.

Future research should prioritize temporal profiling to capture diurnal variations in clock proteins and mitochondrial dynamics, and to assess the acute effects of exercise on this molecular axis (Figure 1A, B). Importantly, we emphasize the need to evaluate mitochondrial morphology using electron microscopy or fluorescence microscopy, rather than relying solely on protein levels of fusion and fission markers, as morphology provides a more accurate and informative measure of mitochondrial dynamics. In addition to assessing mitochondrial morphology and total levels of pro-fusion and fission proteins, it will be critical to examine post-translational modifications, such as DRP1 phosphorylation, MFN1/2 GTPase activity, and OPA1 acetylation, which may play pivotal roles in the clock-dependent regulation of mitochondrial dynamics.

Translational perspective

Understanding how exercise modulates the clock–mitochondrial dynamics axis may inform novel strategies for improving skeletal muscle function, metabolic health, and resilience to circadian disruption in clinical populations, including those with insulin resistance, sarcopenia, or shift work-related disorders.

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Declaration of interests

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Declaration of Generative AI and AI-assisted technology use

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