Narrative Review

Effects of Chrono-Exercise and Chrono-Nutrition on Muscle Health: Understanding the Molecular Mechanisms Activated by Timed Exercise and Consumption of Proteins and Carbohydrates

Mariarosaria Negri [1,2,*], Claudia Pivonello[3], Feliciana Amatrudo[1,2], Fabiano Cimmino[4], Giovanna Trinchese[4], Claudia Vetrani [1,2], Guido Iaccarino[2], Rosario Pivonello [2,5], Maria Pina Mollica[4], Annamaria Colao [2,5]

[1]Dipartimento di Benessere, Nutrizione e Sport, Università Telematica Pegaso, Naples 80143, Italy; [2]Dipartimento di Medicina Clinica e Chirurgia, Sezione di Endocrinologia, Diabetologia, Andrologia e Nutrizione Università Federico II di Napoli, Naples 80131, Italy; [3]Dipartimento di Sanità Pubblica, Università Federico II di Napoli, Naples 80131, Italy; [4]Department of Biology, Federico II University of Naples, Naples 80126, Italy; [5]UNESCO Chair for Health Education and Sustainable Development, Federico II University of Naples, Naples 80131, Italy

*Corresponding Author: Mariarosaria Negri, Dipartimento di Benessere, Nutrizione e Sport, Università Telematica Pegaso, Centro Direzionale, Isola F2, 80143 Naples, Italy (mariarosaria.negri@unipegaso.it).

The circadian clock is an endogenous timekeeping system that regulates various physiological and behavioral processes. Recently, it has been shown that the timing of physical activity and food intake can significantly influence metabolic muscle health. Some recent clinical evidence has shown that physical activity practiced in the late afternoon can be more effective in terms of performance and muscle strength. Preclinical studies have highlighted that the explanation for this effect lies in the different daily expression in the muscle of clock genes and clock-controlled genes involved in muscle development and hypertrophy. In conjunction with scientific advances in understanding the molecular mechanisms that regulate circadian rhythms and muscle trophy, chrono-nutrition has gained scientific resonance and has become a promising field, aimed at understanding the regulation of body metabolism. Clinical and preclinical studies have shown that protein consumption at specific circadian time points during the day, or precisely after exercise, can activate signaling pathways involved in muscle protein synthesis and, thus, favor skeletal muscle mass development as well as mitochondrial biogenesis, thereby improving skeletal muscle cell energy production and function. On the other hand, some studies have shown that the consumption of carbohydrates immediately after exercise increases insulin secretion, which facilitates glucose uptake by muscle cells to replenish glycogen. This review summarizes the current scientific literature concerning chrono-exercise and chrono-nutrition and muscle health, focusing on molecular mechanisms involving the circadian regulation of muscle mass, strength, and health. Understanding the intricate molecular relationship between circadian rhythms, exercise, nutrition, and muscle metabolism is essential for optimizing

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nutritional strategies to prevent or treat muscle wasting. In addition, tailoring protein and carbohydrate intake to timing and individual needs can improve muscle maintenance, growth, and performance.

Key words: circadian rhythm, skeletal muscle, chrono-exercise, chrono-nutrition, proteins, carbohydrates, mealtime.

INTRODUCTION

Skeletal muscle is a highly structured tissue consisting of bundles of muscle fibers, called myofibers, each containing several myofibrils. Each myofiber represents a muscle cell and is characterized by its basic unit, the sarcomere.[1] Myofibers are organized in fascicles, which, in turn, form muscle tissue surrounded by an extracellular matrix and supported by cytoskeletal networks.[1] This hierarchical organization, from myofibers to fascicles and lastly to muscle tissue, enables coordinated contraction, movement, and environmental adaptations of skeletal muscle tissue. In addition, skeletal muscle is a highly vascularized and innervated tissue that contains metabolic and regulatory components fundamental to cellular and whole-body energy production and metabolic balance.[2] The coordination between these components is crucial for muscle health, plasticity, and motor function. Disorders caused by both genetic and environmental factors can lead to muscle deterioration represented by fiber loss and decreased motor function.[2] Muscle wasting, characterized by the progressive loss of muscle mass and function, is a significant clinical problem associated with aging, chronic diseases, and various metabolic disorders.[3] In contrast, muscle injuries are associated with structural damage of muscle fibers, inflammation, pain, and impaired muscle function and usually depend on mechanical stresses that can acutely alter normal muscle structure.[4,5] It is important to note that severe muscle injuries can also contribute to subsequent muscle atrophy due to prolonged disuse or impaired recovery. Nevertheless, both muscle atrophy and injury may be reversible through various repair mechanisms, primarily involving muscle growth and regeneration.[3,6] Depending on the severity of damage, muscle growth and regeneration may be influenced by diet, exercise, and surgical interventions. With particular reference to nutritional strategies, supplementation of vitamins,[7–9] high-quality fats like omega-3 fatty acids,[10,11] polyphenols,[12] amino acids,[13] and antioxidants[14,15] significantly contributes to maintenance of muscle mass and strength. Similarly, it has been demonstrated that physical exercise promotes skeletal muscle regeneration by stimulating different molecular pathways, including oxidative stress,[16,17] metabolic reprogramming,[18] and microRNA involvement.[19,20] It should be emphasized that the type of physical exercise practiced can also influence the regenerative capacity of the damaged muscle. It has been widely established that endurance exercise induces muscle fiber–type regeneration[21] and skeletal muscle mitochondrial biogenesis,[22] contributing to muscle recovery after injury.

In the muscle regeneration process, a crucial role is driven by satellite cells, specialized stem cells localized between the sarcolemma and basal lamina of the myofiber.[6] When the muscle is injured or damaged, satellite cells are rapidly activated in response to signals released during the inflammatory phase of skeletal muscle tissue repair, with the aim to proliferate and differentiate into myocytes.[6] In this context, both nutrition and exercise may influence the synthesis, maintenance, and repair mechanisms of skeletal muscle tissue by directly stimulating quiescent satellite cells to regenerate muscle fibers.[23–26] Moreover, during the last decades, innovative strategies of surgical interventions and tissue-engineering approaches have been developed to promote muscle repair and regeneration, especially in cases of large volume of muscle mass loss.[27,28]

While the role of nutrition and of exercise in maintaining muscle health is well known,[24–26] emerging evidence suggests that the timing of food consumption as well as of physical activity may also influence muscle metabolism through direct interactions with the circadian clock expression and function.[29–32] Most mammalian cells express the circadian clock, which operates through a transcription–translation feedback loop.[33,34] The core components of the circadian clock include the Circadian Locomotor Output Cycles Kaput (Clock) and Brain and Muscle ARNT-like 1 (Bmal1) that are considered transcriptional activators[33,34] since they prime the transcription of their own repressors, such as *Period* (*Per1, Per2, Per3*) and *Cryptochrome* (*Cry1, Cry2*), through binding to E-box elements in their own promoters.[33,34] The Per and Cry proteins accumulate in the cytoplasm, in which they form protein complexes that, subsequently, translocate into the nucleus, where they inhibit *Clock-Bmal1* activity, thereby acting as transcriptional repressors and closing the loop.[35,36] The negative-feedback loop Per/Cry-dependent alternating with the positive-feedback loop Bmal1/Clock-dependent creates a daily rhythmic oscillation of gene expression.[36] Characterizing and synchronizing muscle functions with environmental and endogenous stimuli, as detectable for most body tissue organization and functions, is predominantly determined by the expression of clock-controlled genes (CCGs).[37] CCGs are genes whose expression levels rhythmically fluctuate in response to circadian rhythms governed by the internal biological clock.[37] The regulation of CCGs is essential for coordinating biological processes across different tissues and organs and simultaneously anticipating and responding to daily environmental changes, also within skeletal muscle tissue.[38] CCGs classically regulated in the skeletal muscle are implied in metabolism,[39–41] in muscle contraction,[42,43] in muscle growth and repair,[44,45] and overall in muscle physiology.[38,46]

This review aims to provide insights into the role of timed physical activity and nutrient intake in regulating muscle health and their impact on preventing or improving muscle wasting by focusing on the molecular mechanisms triggered.

CHRONO-NUTRITION AND CHRONO-EXERCISE

The term "chrono-nutrition" refers to a specialized branch of nutritional sciences that, in combination with chronobiology, investigates the relationship between food intake and the body's circadian rhythms.[47] Specifically, chrono-nutrition is the field of research that examines if the meal's timing, frequency, and composition may influence metabolic processes and overall health outcomes,[47] and gave rise to the concept of food entrainment.[48]

The circadian clock, an endogenous timing system that is ubiquitously expressed, orchestrates rhythmic physiological processes to optimize energy homeostasis and metabolic efficiency.[49] Disruptions of the circadian rhythm, such as irregular sleep–wake patterns or shift work, can lead to dysregulation of protein turnover and contribute to muscle wasting, impaired muscle repair, and decreased muscle strength.[50–52] Conversely, aligning nutrient intake, exercise, and sleep with the body's natural circadian rhythms may promote optimal muscle protein synthesis and overall muscle health.[53] At the molecular level, the so-called protein turnover is essential for maintaining muscle mass, health, and function.[54] In skeletal muscle, circadian rhythms influence the expression and activity of molecular key regulators of protein turnover, including the mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway, the ubiquitin-proteasome system (UPS), and autophagy.[55] Disruption of circadian rhythms, which generally occurs in shift workers, in jet lag conditions, or in sleep disturbances, can dysregulate these pathways, leading to impaired muscle protein synthesis and increased proteolysis, thereby predisposing individuals to muscle wasting.[51,52] Recent studies have revealed that protein synthesis exhibits circadian rhythmicity, with fluctuations across the day.[56–59] This circadian pattern of muscle protein synthesis may be influenced by factors such as nutrient availability, hormonal signaling, and cellular energy status. The levels of nocturnal protein synthesis are very low and the targeted intake of proteins during pre-established times of the day can represent a valid strategy to overcome the circadian synthesis of muscle mass.[60] In this regard, also chrono-exercise, the practice of aligning physical activity with circadian rhythms,[61] has gained considerable attention in recent years due to its potential benefits for optimizing exercise performance, skeletal muscle protein synthesis, metabolic health, and overall well-being.[61] Chrono-exercise may also have profound effects on metabolic health. Evidence indicates that the timing of physical activity relative to food intake and sleep can modulate insulin sensitivity, fat metabolism, and circadian gene expression.[62,63] For instance, performing exercise in the morning, prior to meals, has been shown to improve insulin sensitivity and glucose regulation.[62] Conversely, late-night exercise, particularly close to bedtime, can interfere with sleep patterns and disrupt the body's ability to recover and regenerate.[64] Furthermore, the integration of physical activity with circadian rhythms may help mitigate the effects of modern lifestyle disruptions, such as shift work and irregular sleep patterns, which are known to increase the risk of metabolic diseases like obesity and type 2 diabetes.[65,66]

Considering all these concerns, this review aims to explore the current scientific literature on chrono-nutrition and chrono-exercise, highlighting how the timing of food intake and physical activity, in relation to circadian rhythms, can optimize metabolic health, enhance muscle function, and potentially mitigate the adverse effects of modern lifestyle circadian rhythm disruptions.

Medline Search Strategy

The current review critically revises the scientific literature concerning the topic of chrono-exercise and chrono-nutrition with a particular attention to molecular mechanisms underpinning skeletal muscle health and metabolism control.

The relevant literature from 2010 to 2024 was screened from PubMed (Medline) using the following search terms: "skeletal muscle", "skeletal muscle physiology", "chrono-nutrition", "mealtime", "skeletal muscle and clock", "skeletal muscle and circadian rhythm", "skeletal muscle and molecular clock", "skeletal muscle metabolism and clock", "skeletal muscle strength and clock", "skeletal muscle regeneration and clock", "skeletal muscle health and clock", "meal-time and skeletal muscle", "chronobiology of skeletal muscle", "chronobiology of physical exercise/activity", "chrono-exercise", "chrono-training", "training and circadian rhythm", "training and clock genes", "protein intake and skeletal muscle", and "carbohydrate intake and skeletal muscle". Titles and abstracts were screened to identify those that investigated the relationship between chrono-nutrition and chrono-exercise and muscle structure and/or function. After screening the full text of the manuscripts, available original manuscripts, both clinical and preclinical, were included. In particular, for preclinical studies, original in vitro and in vivo molecular studies were preferred, whereas for clinical studies, clinical trials, cross-sectional, longitudinal, and population-based studies were included. The reference lists of the included studies were additionally screened to identify further relevant literature. Only English-language articles were included. Duplicates, protocol studies, second-analysis reports, articles not strictly related to chrono-nutrition and chrono-exercise and muscle structure and/or function, and articles not published in English were excluded.

From the numerous studies identified, a total of 33 articles were selected for inclusion, and were divided into preclinical and clinical sections as described in the following text. These studies were selected on the basis of their relevance, rigor, and contribution to the understanding of the interaction between circadian rhythms, exercise, diet, and muscle health and function. The preclinical studies focus primarily on in vitro and animal models and provide insights into the underlying cellular and molecular mechanisms, while the clinical studies examine human populations and provide practical implications for optimizing health outcomes through chrono-nutrition and chrono-exercise interventions. In each subsection of the article, the basic in vitro studies are described first, followed by the preclinical studies in animal models, and finally the clinical studies in humans, in this specific order.

CIRCADIAN CONTROL OF SKELETAL MUSCLE PHYSIOLOGY: EVIDENCE FROM PRECLINICAL STUDIES

The physiology of skeletal muscle is intricately linked with circadian rhythm, which, in turn, regulates various aspects of muscle function, including metabolism,[39,67–71] muscle contraction,[42,43] and muscle growth and repair,[44,45] as demonstrated by preclinical evidence. **Table 1**[45,67–71] summarizes the main molecular factors showing a circadian oscillation in the skeletal muscle. A brief and schematic description of the main circadian clock core components' role in muscle functions is summarized in **Figure 1**.

Preclinical In Vitro and Ex Vivo Studies

The following subsection provides a summary of circadian clock involvement in skeletal muscle physiology to better introduce the main topic of the current review. To examine the evidence, 3 in vitro and 4 ex vivo preclinical studies, describing the role of circadian clock components in muscle metabolism, were critically reviewed. Although most of the current scientific evidence derived from gene manipulations of the Bmal1 and Clock regulators, it should be pointed out that the skeletal muscle physiologically shows the circadian oscillation of all molecular components of the biological clock.

As demonstrated for several cell models, in vitro cultured mouse myotubes C2C12 also showed a circadian expression of clock genes after serum shock-induced synchronization, with a peak of Bmal1 expression after 12 hours of serum-free exposure and a simultaneous peak of Per and Cry expression after 22–24 hours of serum-free exposure.[39] Moreover, the same cells exposed to nonphysiological doses of hydrocortisone in the bathyphase of Bmal1 (resembling the evening time points after cell synchronization) showed significantly decreased expression of genes involved in insulin response, such as insulin receptor substrate 1 (*IRS1*) and phosphatidylinositol 3-kinase (*PI3KC*),[39] confirming the key role of both hydrocortisone timed exposure and Bmal1 expression levels in mediating muscle insulin response. In addition, synchronized C2C12 cells stimulated with electrical pulse stimulation (EPS) consisting of 30 minutes with 1 Hz frequency, 2 milliseconds in duration and 30 V, which induces expression of exercise-responsive genes, showed a significant increase in Per2 expression associated with increased intracellular calcium levels.[43] Similarly, the same in vitro model, previously synchronized with a serum shock protocol, showed a concomitant oscillation of clock genes and of the main genes involved in muscle metabolism,[71] suggesting the involvement of the biological clock in the control of transcriptomic myocyte functionality. Simultaneously, the CCGs analyzed are associated with cyclical DNA methylation, with particular attention to Cry1, a member of the core clock machinery demonstrating circadian control of transcriptomic and epigenomic muscle pathways.[71] Similarly, the lipidomic analysis from skeletal muscle biopsies of 10 healthy volunteers with a consistent daily feeding and sleeping routine demonstrated a lipid metabolite diurnal oscillation that persisted even in cultured in vitro myocytes.[69] In particular, lipids rhythmically expressed could be clustered into different subgroups based on their temporal profile,[69] emphasizing the crucial function of the molecular timekeeper in coordinating the circadian regulation of lipid metabolism in muscle. In addition, Perrin et al[70] demonstrated that, in primary cultures of skeletal

Table 1. Main Proteins and Genes Associated With Circadian Rhythmicity in Skeletal Muscle

Proteins and genes	Function	Model	Reference
CpG methylation, *ABCA5, BCD1, MBLAC1, PAIP2B, PIF1, TSPAN4* genes	Cell metabolism, regeneration and muscle adaptation	Mouse C2C12 myotubes	Altintas et al, 2020[71]
MYOD1 gene and protein	Myogenic factor	Mouse skeletal muscle	Vitale et al, 2019[45]
IL-6 secretion and gene expression	Anti-inflammatory myokine	Human skeletal myotubes	Perrin et al, 2015[67]
Oxidative phosphorylation complexes, PGC-1α, FIS1, PINK1, OPA1 protein expression	Mitochondrial activity	Human skeletal muscle biopsies	van Moorsel et al, 2016[68]
Lipids enriched in the outer leaflet of the plasma membrane and lipids metabolites secretion	Lipid metabolism	Human skeletal muscle biopsies and human skeletal myotubes	Loizides-Mangold et al, 2017[69]
Glycerophospholipid and triglyceride metabolism, storage, and transport; inositol phosphate metabolism and sphingolipid metabolism and storage	Lipid metabolism	Human skeletal myotubes	Perrin et al, 2018[70]

Abbreviations: *ABCA5*, ATP binding cassette subfamily A, member 5; *BCD1*, Beige-BEACH domain-containing protein; *FIS1*, mitochondrial fission 1 protein; *IL-6*, interleukin-6; *MBLAC1*, metallo-beta-lactamase domain-containing protein 1; *MYOD1*, myoblast determination protein 1 homolog; OPA1, mitochondrial dynamin-like GTPase; *PAIP2B*, polyadenylate-binding protein-interacting protein 2B; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; *PIF1*, petite integration frequency 1; PINK1, PTEN-induced putative kinase protein 1; *TSPAN4*, tetraspin 4.

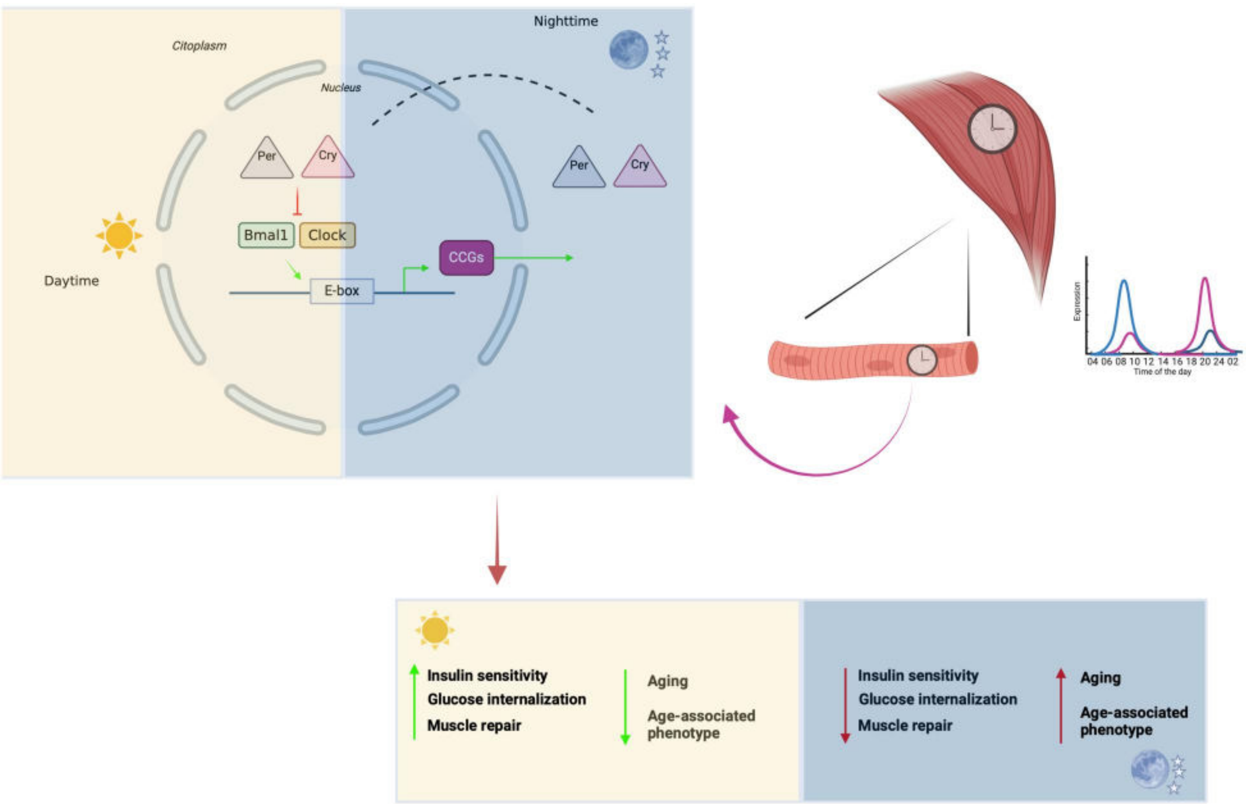

Figure 1. Molecular Components of the Circadian Clock and Their Relationship With Muscle Physiology. The core loop of the mammalian circadian clock is composed of transcriptional activators Bmal1 and Clock, which heterodimerize in the nucleus, and drive the transcription of CCGs, including *Per* and *Cry*. Upon translation, Per and Cry proteins form complexes that translocate into the nucleus and inhibit the activity of the *Bmal1–Clock* complex, thus forming a negative-feedback loop. Physiologically, in the morning, there is an increase in the expression and in the transcriptional activity of the Bmal1–Clock complex. During the day, Per and Cry undergo post-translational modifications that regulate their expression and stability, thus triggering a self-sustaining control of the circadian clock. Muscle clock positively influences physiological processes, such as myogenesis, muscle repair, insulin response, and glucose internalization in the morning. Abbreviations: Bmal1, Brain and Muscle ARNT-like 1; CCG, clock-controlled gene; Cry, Cryptochrome; Per, Period. Created with BioRender.com

myotubes obtained from 10 healthy volunteers who did not sleep during daylight hours of the testing day, the disruption of the circadian clock by targeting the *Clock* gene with small interfering RNA, led to significant changes in glucose uptake, lipid homeostasis, and myokine secretion, thus suggesting the crucial role of *Clock* in coordinating glucose uptake and lipid metabolism in skeletal muscle cells. Focusing on skeletal muscle metabolism, this transcriptomic analysis identified the rhythmic transcription patterns in a total of 4792 genes at the intronic pre-mRNA level and 956 genes at the mature mRNA level.[70] Functional analysis of these genes reveals their roles in circadian rhythm regulation, ribosome formation, lipid metabolism, protein transportation, tricarboxylic acid cycle (TCA), encoding RNA-binding proteins, and mitochondrial function. Moreover, human primary cell cultures derived from subjects with and without obesity confirmed in vitro clock genes' circadian oscillation after exposure to forskolin and dexamethasone that can act as in vitro cell synchronizers for both cell models.[67] The same in vitro cultured myocytes exposed to silencing of *Clock* showed an attenuated, but persistent, oscillation of the other molecular components of the biological clock.[67] Furthermore, silencing of the *Clock* gene caused in vitro cultured myocytes to significantly inhibit circadian interleukin (IL)-6 basal secretion, thus demonstrating the pivotal role of the molecular clock components in IL-6 plasma level regulation.[67] This type of control has also been demonstrated for several myokines, such as IL-8, monocyte chemoattractant protein-1 (MCP-1), macrophage colony-stimulating factor (M-CSF), growth regulator oncogene-α (GRO-α), vascular endothelial growth factor (VEGF), cluster of differentiation (CD44), Galectin-3, fatty acid binding protein 3 (FABP3), and tissue inhibitor of metalloproteinases-1 (TIMP-1), whose secretion was significantly impaired after silencing of *Clock*, suggesting even more clearly the crucial role of the biological clock in the secretory and regulatory capacity of skeletal muscle.[67] In addition, mitochondrial respiratory capacity measurement of cultured myocytes derived from skeletal muscle biopsies of 20 healthy subjects who had regular sleep and food habits demonstrated that skeletal muscle oxidative capacity displays a robust day–night rhythm and that mitochondrial respiration was lowest at 1 PM and highest at 11 PM.[68] Moreover, the same study reported that the peak of energy expenditure was detectable at 11 PM and the lowest energy expenditure at 4 AM,[68] thus confirming the influence of the circadian rhythm in skeletal muscle metabolism that may regulate whole-body energy homeostasis.

Preclinical In Vivo Studies

The current literature analysis included 7 preclinical studies that assessed animal models. These studies highlight molecular aspects involving circadian clock components and skeletal muscle metabolism and regeneration, providing greater insight into chrono-regulation of muscle physiology processes.

In mice lacking the *Bmal1* gene specifically in skeletal muscle, an impaired insulin-stimulated glucose uptake was observed.[72] This metabolic dysfunction was associated with reduced levels of key proteins involved in intracellular glucose transport, including glucose transporter-4 (GLUT4) and TBC1 domain family member 1 (TBC1D1), a Rab-GTPase with a crucial role in GLUT4 translocation 72. Additionally, a decreased activity of pyruvate dehydrogenase (PDH) due to altered expression of circadian genes pyruvate dehydrogenase kinase 4 (*PDK4*) and pyruvate dehydrogenase phosphatase catalytic subunit 1 (*PDP1*), which encode PDH kinase and phosphatase, respectively, was observed.[72] In mice lacking *Bmal1*, the inhibition of PDH activity caused reduced glucose oxidation and a shift of glycolytic intermediates towards alternative metabolic pathways, as revealed by metabolomics analysis. These findings suggest that the muscle-specific clock gene *Bmal1* may play a critical role in preparing skeletal muscle during the transition from resting or fasting phase to the active feeding phase, where glucose becomes the primary fuel source for muscle function. Likewise, mice lacking the *Bmal1* gene specifically in skeletal muscle exhibited progressive changes in body composition, together with glucose intolerance and non-fasting hyperglycemia.[73] Moreover, metabolomics analysis provided further evidence of reduced glycolytic flux, with decreases in TCA intermediates and elevated amino acid levels, indicating a shift towards reliance on fat as a fuel source with increased protein breakdown to support the TCA.[73]

In addition, to evaluate muscle clock autonomy and to explore potential muscle–liver interactions, RNA sequencing on liver and gastrocnemius muscles, which express predominantly fast-twitch fibers, was collected every 4 hours over the diurnal cycle from mice globally and muscle and liver specifically in *Bmal1* knock-out (KO) models.[74] The specific loss of *Bmal1* in the muscle tissue disrupted clock gene oscillations and altered glucose metabolism, while muscle *Bmal1* expression restoration only partially rescued systemic glucose regulation compared with wild-type (WT) mice.[74] Similarly, specific loss of *Bmal1* in the liver tissue led to an advanced phase shift associated with reduced amplitude expression of almost all clock genes.[74] Moreover, while both global and tissue-specific *Bmal1* loss is able to induce increased body fat mass and compromised locomotor activity, only tissue-specific Bmal1 absence is sufficient to significantly influence the tissue diurnal transcriptome. Conversely, specific loss of Bmal1 in the skeletal muscle and liver tissue is not enough to control systemic glycemic homeostasis.[74]

Accordingly, Bmal1 has been further reported as a crucial component of the clock machinery, showing a fundamental importance in skeletal muscle regeneration after injury.[75] Given that, during the initial phases of muscle differentiation and growth, the activation of myogenic differentiation 1 (MyoD1) follows a distinct circadian expression pattern in adult muscle, suggesting its role as a CCG,[44] ablation of the clock genes or key molecular clock components such as Bmal1 diminishes the circadian expression of MyoD1 and of its downstream target genes, resulting in disrupted muscle contractile function and altered organization of sarcomeric myofibers.[76] Mice globally lacking the *Bmal1* gene showed a significantly lower number of satellite cells with a lower expression of the satellite marker paired box 7 (*Pax7)*,[75] thus confirming the crucial role of Bmal1 in the muscle processes of growth, repair, and regeneration. In addition, it has been demonstrated that mice expressing the clock gene *Bmal1* during embryogenesis, but not after birth, showed significantly altered peripheral oscillatory gene expression and displayed various age-associated disorders, including impaired locomotor activity.[77] While the global loss of Bmal1 is mainly linked to accelerated premature aging, specific loss in muscle tissue does not lead to the early aging phenotype but is more associated with specific muscle tissue dysfunction.[72] This distinction underscores the tissue-specific functions of Bmal1 and its importance in regulating the aging processes or muscle functions. This is perfectly in line with data obtained from animal models lacking *Bmal1* and mutants of the *Clock* gene (*Clock*$^{\Delta 19}$) showing a 30% reduction in muscle force associated with a 40% reduction in mitochondrial size,[76] thus suggesting the important role of these clock genes in regulating muscle function by mediating mitochondrial volume and activity. The *Clock*$^{\Delta 19}$ mouse model indeed showed significantly lower levels of peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) associated with a strong decrease in mitochondrial content and of cytochrome c oxidase enzyme activity, further confirming the importance of this clock molecular component in skeletal muscle mitochondrial activity.[78]

MOLECULAR PATHWAYS INVOLVING TIMED PHYSICAL EXERCISE AND MUSCLE CLOCK

It is well known that physical performance is strongly influenced by the physiological circadian rhythm.[79] Recently, it has been demonstrated that the peak of athletic performance effectiveness occurs in the hours corresponding to late afternoon/evening.[79] This evidence has suggested the existence of a close relationship between physical activity and the biological clock, especially at the muscular level.[79] There are several factors that regulate physical performance depending on the circadian rhythm, which can be of both endogenous and environmental origin. Among the endogenous factors, in addition to diurnal behavioral pattern[80] and body temperature,[81] there are physiological circadian hormonal fluctuations that significantly influence muscle glucose and lipid metabolism.[79] In particular, the diurnal variations in testosterone and cortisol significantly influence physical performance and, consequently, regulate the circadian efficiency of skeletal muscle activity.[82] Indeed, testosterone, known for its physiological anabolic effect in the skeletal muscle and higher secretion levels in the first hours of the morning, is counterbalanced by the catabolic effect of cortisol, which reaches a peak in the morning.[83] However, it is important to point out that, unlike circulating levels, which reach the highest levels in the morning, the maximum biological activity of testosterone takes place in the afternoon hours, the period of the day when a greater effectiveness of the response to endurance sports has been demonstrated.[84] Beyond the hormonal aspects, there is certainly a direct interaction between muscle functionality and regulation of clock genes, as demonstrated by preclinical and clinical evidence also considering that human muscle strength displays maximum peaks in the late afternoon, specifically ranging between 4:00 and 8:00 PM.[85–88] At the molecular level, there is a reciprocal relationship between circadian rhythms and both acute and chronic physical activity. Acute physical activity refers to a single, brief bout of exercise or physical exertion that produces an immediate physiological response. These acute responses are transient and typically subside when the activity ceases, facilitating immediate energy needs.[89] In contrast, chronic physical activity involves repeated and sustained bouts of exercise over a longer period of time (eg, weeks or months), leading to long-term adaptations in the body. Chronic activity induces structural and functional changes that improve the body's ability to perform and recover from physical exertion.[89] This mutual interaction is essentially expressed in the ability of physical activity to regulate and harmonize the expression of the molecular components of the biological clock in the skeletal muscle[64] and the concomitant circadian oscillation of clock genes within physiological limits promoting both regeneration and muscle repair.[44] Studies have shown that acute bouts of exercise, independently from time of the day, can rapidly upregulate genes like *Per1* and *Per2*, which may cause phase shifts in the circadian rhythm of muscle cells in mouse[90] and human[90,91] models. These temporary changes in clock gene expression are mainly thought to enhance energy metabolism, optimizing the muscle's ability to meet the immediate metabolic demands of exercise. Notably, the alterations induced by acute exercise typically return to baseline after the exercise session, highlighting the transient nature of the acute response. Chronic exercise has been shown to stabilize the rhythmic expression of clock genes, thereby reinforcing the circadian rhythm in muscle tissue.[92,93] This stability promotes improved energy balance, enhances mitochondrial function, and supports greater resilience to metabolic stress.[93] Additionally, these adaptations facilitate better synchronization of peripheral clocks, like those in skeletal muscle, with the central circadian clock in the brain, thus contributing to systemic metabolic homeostasis and muscle health over time.

Preclinical In Vitro and In Vivo Studies

This subsection critically describes the scientific evidence of 1 preclinical study performed on in vitro myocytes and 5 preclinical studies conducted on animal models. It should be specified that only the studies reporting results regarding timed exercise and its effect on skeletal muscle, highlighting the involvement of the molecular components of the biological clock, are discussed and are described in **Table 2**.[42,94–96]

The importance of timed muscle contraction was primarily investigated in C2C12 myocytes previously synchronized by dexamethasone exposure.[42] C2C12 cells were stimulated to contract by electrical pulse stimulation at 3 different time points of the circadian rhythm—22, 28, and 40 hours post-synchronization—to evaluate circadian phase and molecular components of the circadian clock.[42] This in vitro study showed that C2C12 cells stimulated at 22 hours post-synchronization showed a significant decrease in phase shift, with a significant reduction in *Per2* expression.[42] Electrical pulse stimulation caused a significant decrease in *Per1*, *Per2*, and *Bmal1* expression also at 28 hours after synchronization, suggesting that muscle contraction in specific circadian time points may influence skeletal muscle circadian response. More interestingly, a preclinical study conducted on mouse models of different ages demonstrated that physical activity could serve as an effective intervention against age-related peripheral circadian misalignment.[95] Specifically, this study compared male and female adult (∼4 months) and old (∼6 months) mice subjected to voluntary wheel running and cross-checked with mice of the same age but with a sedentary lifestyle. Both male and female mice showed a delayed onset of voluntary physical activity associated with increasing age.[95] In particular, young male and female mice began running at the beginning of the active phase, with peak physical activity 2 hours after the lights were turned off, whereas old male and female mice started running later, with peak physical activity 6 and 7 hours after the lights were turned off.[95] Moreover, as suspected, younger male and female mice covered greater distances than their older counterparts, whereas female mice, irrespective of age, exhibited greater running distances compared with male mice.[95] Surprisingly, old female mice ran 2 hours into the light phase and their gastrocnemius tissues showed gene expression levels of *Per2* that were significantly higher and of *Bmal1* lower than in young female mice.[95] Wheel activity in the gastrocnemius of old female mice significantly reduced *Per2* expression and restored *Bmal1* expression to levels similar to those in young mice, suggesting that physical activity during the light phase may de-synchronize the skeletal muscle circadian clock by influencing peripheral clock gene expression. Comparing these evaluations with a sedentary group, only old female mice showed rescued peripheral clock gene expression,[95] thus suggesting a pivotal hormonal involvement in the circadian clock controlled by timed physical exercise. The well-documented and demonstrated ability of female animals to run longer distances was utilized in an additional study conducted exclusively on female Per2∷Luc C57Bl/6J mice.[42]. This study evaluated the effects of an acute bout of treadmill consisting of 1 hour of treadmill training in 3 different circadian time points (middle of light, end of light, and middle of dark phase) on the skeletal muscle molecular clock. The study demonstrated that exercise in the resting phase was able to significantly anticipate the bioluminescence phase shift, confirming that timed physical exercise may represent an important cue for peripheral circadian clock modulation.[42] Interestingly, another study in a Per2∷Luc C57Bl/6J mouse model revealed that even low-intensity, voluntary, or involuntary physical exercise, for a longer period, can impact the skeletal muscle biological clock.[94] In this study, male and female mice were first divided into 2 groups: 1 group with unrestricted food access and the other with food restriction for 2 weeks. Both groups then performed voluntary and involuntary physical activities—wheel and treadmill running, respectively—during daylight hours for 2 hours per day, for a total period of 4 weeks. Accordingly, food restriction alone caused a phase shift of approximately 3 hours in the soleus muscle, and daytime physical activity triggered a similar phase shift, advancing the peripheral clock by 2 hours in the wheel group and by 3 hours in the treadmill group.[94] The results of this study underscored that 2 distinct non-photic stimuli, such as food restriction and daylight physical activity, can effectively modulate the peripheral circadian rhythm, demonstrating the responsiveness of muscle tissue to behavioral cues independently of light exposure. To better define the interplay between timed exercise and molecular

Table 2. Physical Exercise and Muscle Clock: Preclinical In Vitro and In Vivo Studies

Model	Exercise protocol	Nutritional protocol	Intervention groups	Findings	Reference
C2C12 synchronized with dexamethasone	Electrical pulse stimulation (EPS) for 60 min at 22 h, 28 h, or 40 h post-synchronization	None	None	- EPS at 22 h post-synchronization induced a significant advance in circadian clock with a significant reduction in *Per2* expression compared with unpulsed cells - EPS at 28 h post-synchronization significantly decreased *Bmal1*, *Per1*, and *Per2*	Kemler et al, 2020[42]
Male and female C57Bl/6J mice adult (~4 mo) and old (~18 mo)	Voluntary wheel running activity for 2 wk (recorded and analyzed for 4 d)	Food and water ad libitum	Trained adult male and female mice Untrained adult male and female mice Trained old male and female mice Untrained old male and female mice	- Adult male and female mice ran a greater distance than older mice - Male and female older mice showed delayed wheel activity - Old female mice ran 2 h into the light phase - Old female mice gastrocnemius tissue showed *Per2* significantly higher than in young female mice - Trained old female mice gastrocnemius tissue showed lower *Per2* than trained and untrained young female mice - Trained old female mice gastrocnemius tissue showed higher *Bmal1* gene expression that was lower than in trained and untrained young female mice	Bruns et al, 2020[95]
Male and female adult mice (6 mo) Per2::Luc C57Bl/6J	2 h voluntary or involuntary exercise (wheel and treadmill, respectively) during the light phase for 4 wk	Food and water ad libitum group (FF) vs food-restricted (FR) group for 2 wk	Trained adult male and female Per2::Luc C57Bl/6J mice with free feeding and food restriction conditions	- FR group showed a short phase shift (~2-3 h) of Per2 in soleus tissue - The phase of the Per2::Luc bioluminescence rhythm in the soleus muscle was shifted earlier by approximately 3 h in the treadmill exercise group and by approximately 2 h in the wheel-running group; Activity increased in the RF group during the lights-on phase	Wolff and Esser, 2020[94]

(continued)

Table 2. Continued

Model	Exercise protocol	Nutritional protocol	Intervention groups	Findings	Reference
Female Per2::Luc mice	Acute bout of treadmill: 1 h of treadmill running at 15 m/min at middle of light, end of light, and middle of dark	Food and water removed at the beginning of training	Trained females at middle of light Trained females at end of light Trained females at middle of dark	- Exercise at middle and at the end of light phase anticipated bioluminescence rhythm in the skeletal muscle	Kemler et al, 2020[42]
Male and female mutant Per1, Per2, Per1/Per2, Bmal1 and WT young (∼3 mo) mice	Early vs late dark-phase 5.5-h moderate-intensity treadmill test (mice exercised with light off)	Food and water ad libitu	Female and male WT early and late Female and male *Per1,2*$^{-/-}$ early and late Female and male *Per1*$^{-/-}$ early and late Female and male *Per2*$^{-/-}$ early and late Female and male *Bmal1*$^{-/+}$ early and late Female and male *Bmal1*$^{-/-}$ early and late	- WT late group outperformed WT early group. In general, WT females performed worse than males. Muscle glycogen levels, however, were similar in the early and late groups, in both sexes. - Both sedentary and trained WT of the late phase showed food intake significantly higher than the early-phase group-*Per1,2*$^{-/-}$ and *Per2*$^{-/-}$ mice did not show time difference in exercise capacity, food consumption, glucose profile, and muscle and liver glycogen content - The *Per1*$^{-/-}$ late group outperformed the *Per1*$^{-/-}$ early group. Food consumption, glucose profile and liver glycogen content were significantly higher in *Per1*$^{-/-}$ late group - At the early time point, *Bmal1*$^{-/-}$ performed better that *Bmal1*$^{-/+}$ and WT. Food intake and liver glycogen content were higher in *Bmal1*$^{-/-}$ late group.	Adamovich et al, 2021[96]

Abbreviations: Bmal1, Brain and Muscle ARNT-like 1; Per, Period; WT, wild-type.

components of the circadian clock, an interesting study on WT and mutant mice models was performed.[96] This study on male and female mice demonstrated that, in response to exercise in the dark phase, a substantial difference between early and late periods exists. Contrary to what has been demonstrated in other evidence,[42,95] in this study, females performed worse than male mice, while, in both sexes, the late-period group outperformed the early-period group in terms of running duration in association with higher food consumption.[96] Moreover, the same study underlined the pivotal role of *Per* and *Bmal1* genes in physical activity. Furthermore, the above-mentioned mouse models were genetically crossed with mutant models in order to obtain *Per1*$^{-/-}$, *Per2*$^{-/-}$, *Per1,2*$^{-/-}$, and *Bmal1*$^{-/-}$ mice and exercise performance as well as muscle glycogen content and food consumption were evaluated.[96] This study demonstrated that clock mutant *Per1,2*$^{-/-}$ mice did not show a time difference in exercise capacity, in muscle glycogen content, or in food intake, whereas the *Per1*$^{-/-}$ late group outperformed the early group, showing that loss of *Per1* reduces exercise capacity in the early phase of the dark phase time point.[96] Conversely, the early group outperformed better in absence of *Bmal1*, strongly suggesting a role of this clock gene in ameliorating exercise response in the early phase of the dark time point.[96] The results of this interesting work have therefore defined not only the different metabolic responses based on the circadian rhythm but have also demonstrated the implications of the components of the biological clock.

Clinical In Vivo Studies

This subsection critically describes the scientific evidence from 3 clinical in vivo studies conducted in healthy subjects and that evaluated the role of chrono-exercise specifically on muscle health and functionality. In order to better define the critical role of timed exercise *di* per se on skeletal muscle, human studies without specific dietary interventions were selected, as reviewed in this subsection and detailed in **Table 3**.[93,97,98]

A multi-arm prospective study conducted on 72 healthy men (30–36 years old), undergoing combined strength and endurance physical schedules at different circadian time points for 24 weeks, underlined the different effect of exercise related to circadian rhythm. In detail, enrolled participants were divided in 4 groups: morning endurance + strength exercise, morning strength + endurance exercise, evening endurance + strength exercise, and evening strength + endurance exercise, taking into account that morning training ranged from 6:30 to 9:30 AM, while evening training ranged from 4:30 to 7:30 PM, with the aim to validate testosterone, cortisol, and muscle strength and mass measurements.[97] Although circadian variations in testosterone and cortisol remained unchanged in all the groups examined, the study demonstrated that evening physical activity significantly improved muscle mass in terms of larger gains.[97] Similarly, as demonstrated in a single-arm longitudinal study, exercise performance peaks in the afternoon when muscle tone showed maximum levels.[98] In this study, 21 healthy participants (13 men and 8 women, aged 30–35 years old) were enrolled to participate in a specific timed schedule exercise consisting of 15 minutes of warm-up and 3 subsequent exercises of an approximate duration of 15 minutes/exercise at 4 different circadian time points: 9:00 AM, 12:00 AM, 3:00 PM, 6:00 PM.[98] Independently of gender, myotonometry analysis showed that muscle tone increased after exercise at 6 PM and this result correlated with increased expression levels of the salivary *Per2* gene, not only indicating a strong correlation between physical performance and circadian rhythm but also demonstrating the pivotal role of salivary clock gene expression as predictors of individual fluctuations in daily performance.[98] Another study demonstrated that *Per* genes expression in the skeletal muscle is strongly influenced by the timing of physical activity, even in conditions of overweight and obesity.[93] This study, which was conducted on 10 overweight and obese men (40–75 years old) who underwent 12 weeks of high-intensity interval training in the afternoon (2:00–6:00 PM), showed significant variations in muscle clock gene expression induced by exercise, particularly for *Per1*, *Per2*, and *Per3*, which was significantly lower at 1:00 PM, confirming the role of physical activity as a synchronizer of muscle clock.[93] This gene modulation was associated with improved muscle mitochondrial oxidative capacity, demonstrating a strong association between clock expression and muscle metabolic activity. In addition, timed exercise in the afternoon ameliorated the clinical condition by improving body composition and exercise capacity and significantly reducing 24-hour serum levels of glucose.[93]

MOLECULAR PATHWAYS INVOLVING MEAL TIMING IN SKELETAL MUSCLE PHYSIOLOGY: FOCUS ON PROTEIN INTAKE

Different studies have shown that meal timing influences postprandial glucose tolerance, lipid metabolism, and energy expenditure.[47,99–102] The knowledge on the circadian rhythm's impact on metabolism has important implications for human health,[103,104] and the potential targeting of circadian clocks, pharmacologically or through lifestyle interventions, may offer novel therapeutic strategies for metabolic diseases.[105] Moreover, chrono-nutrition, a branch of nutrition that aims to align meal intake with circadian rhythms, has

Table 3. Physical Exercise and Muscle Clock: Clinical In Vivo Studies

Participants	Study design	Exercise protocol	Nutritional protocol	Findings	Reference
72 Healthy men (30–36 y old)	Multi-arm prospective study	Combined strength (S) and endurance (E) training for 12-24 weeks. Training in the morning (between 6:30 AM ± 30 min and 9:30 AM ± 30 min) and in the evening (between 04:30 PM ± 30 min and 07:30 PM ± 30 min). The duration of the combined endurance and strength training sessions progressively increased from 60 to 120 min.	Consume nutrients according to the national guidelines	Larger gains in muscle mass in group trained in the evening	Kuusma et al, 2018[97]
10 Overweight and obese, insulin-resistant men (40–75 y old)	Single-arm longitudinal study	12 weeks of high-intensity interval training in the afternoon (between 2:00 and 6:00 PM). One hour after each meal, participants engaged in a 15-minute low-intensity walk to incorporate standardized physical activity. Subsequently, they were instructed to remain standing for 15 minutes before being allowed to sit. At 11 PM, the lights in the respiration chamber were turned off, and participants were instructed to attempt to sleep. On the first night, whole-room indirect calorimetry was conducted.	Regular lunch and dinner at 1 and 6 PM	Improved body composition and exercise capacity, reduced 24-h plasma glucose levels, increased muscle mitochondrial oxidative capacity *Per1*, *Per2*, and *Per3* gene expression in skeletal muscle were significantly decreased after training at 1 PM	Harmsen et al, 2023[93]
21 Participants (13 men and 8 women; 30–35 y old)	Single-arm longitudinal study	A session of strength and endurance exercises consisted of: 15 min warmup and 3 exercises (approximate duration of 15 min/exercise) 3 × HST, 3 × CMJ, 1 × SRT at different times of the day (9:00 AM, 12:00 AM, 3:00 PM, and 6:00 PM).	None	Exercise performance peaks in the late afternoonfor both males and females. Muscle tone varies across the day. Molecular daily profiles correlate with daily variation in exercise performance: HST peak correlates with *Per2*	Basti et al, 2021[98]

Abbreviations: CMJ, counter-movement-jump; HST, hand-strength-test; *Per*, Period; SRT, shuttle-run-test.

shown interesting advantages for human health in recent years, especially in terms of metabolic processes, including protein synthesis, degradation, and energy metabolism.[47,101]

Preclinical In Vivo Studies

Restricting the literature search to preclinical studies investigating timed protein and amino acid ingestion and its effect on muscle resulted in a single study conducted in animal models. **Figure 2** briefly summarizes the molecular effect on muscle mass in mouse models fed branched-chain amino acids (BCCAs) at different circadian time points. **Table 4**[106–111] provides schematic details about preclinical and clinical evidence on circadian time points and consumption of protein.

A study performed on male mouse models revealed that consuming protein during the early active phase promotes muscle hypertrophy induced by overloading, and this effect is dependent on the peripheral muscle clock.[109] Wild-type, muscle-specific *Bmal1* KO (MKO), and clock mutant (Clock$^{\Delta19}$) mice aged between 5 and 10 weeks were fed a 2-meals-per-day regimen that provided different protein intakes at different times of the day. In this diet, mice were divided into 2 groups: 1 group was fed a high-protein diet (with a casein content of 20%) for breakfast and a low-protein diet (with a casein content of 3%) for dinner, and the other group received the opposite diet, with a high-protein diet for dinner.[109] It should be noted that, in mice, the 2 circadian time points corresponding to breakfast and dinner correspond to the early and the late active phases, respectively. Interestingly, the mouse group receiving the high-protein diet for breakfast showed higher plantaris muscle weight compared with the mouse group receiving the high-protein diet for dinner, suggesting that proteins consumed during the early active phase promote muscle overloading hypertrophy.[109] A similar effect was seen for mice fed BCAAs, L-isoleucine, L-leucine, and L-valine in a total dose of approximately 3%.[109] To better investigate whether this effect was dependent on the skeletal muscle clock, the same dietary regimen was applied to Clock$^{\Delta19}$ and MKO mice, which conversely did not show any skeletal muscle changes.[109] Surprisingly, muscle free glycine and histidine levels as well as insulin-like growth factor-1 (IGF-1) expression were higher in the overloaded muscles of mice fed a high-protein meal at dinner. In contrast, muscle free serine levels were higher in the overloaded muscle of mice fed a high-protein meal at breakfast, demonstrating the relevant

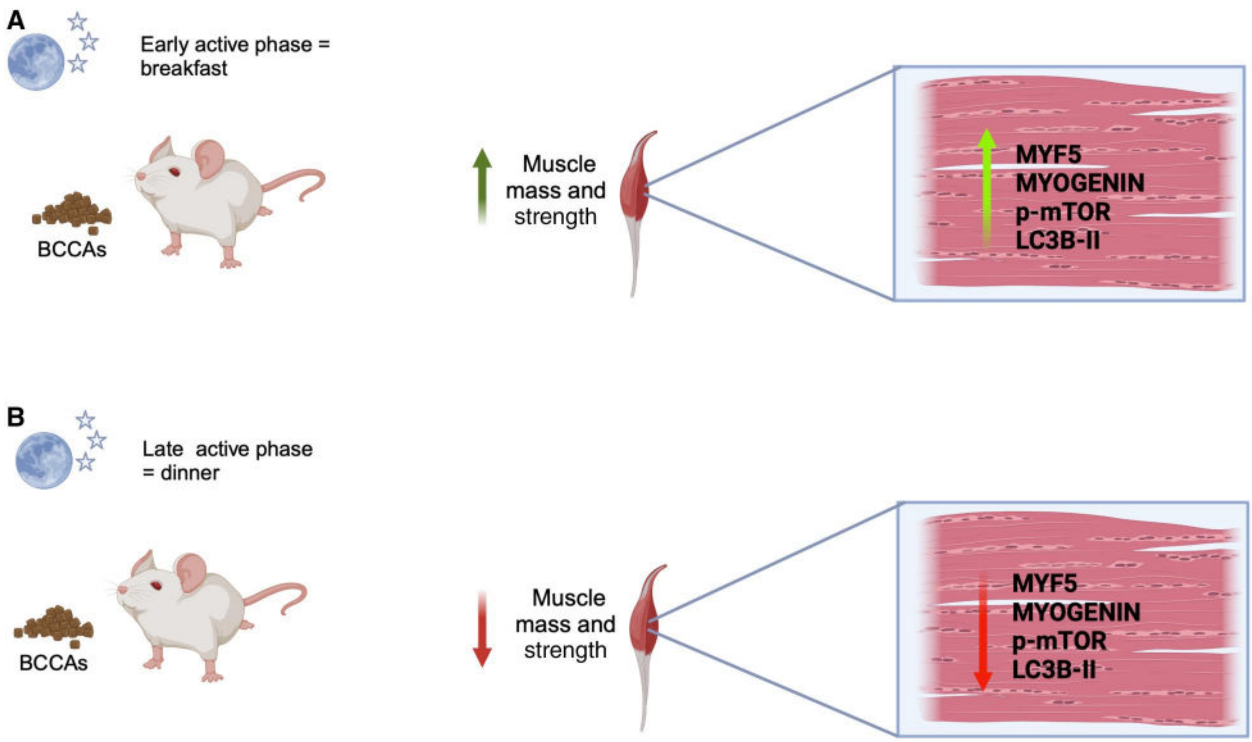

Figure 2. Timed Protein Consumption Effects on Mouse Muscle Mass. (A) Mouse models fed BCCAs in the early-active phase, corresponding to the breakfast, increase muscle mass by stimulating MYF5, myogenin, p-mTOR, and LC3B-II protein expression. (B) Mouse models fed BCCAs in the late-active phase, corresponding to dinner, decrease muscle mass by inhibiting MYF5, myogenin, p-mTOR, and LC3B-II protein expression. Abbreviations: BCCA, branched-chain amino acid; LC3B-II, microtubule-associated protein 1A/1B-light chain 3; MYF5, myogenic factor 5; p-mTOR, phosphorylated mammalian target of rapamycin. Created with BioRender.com

Table 4. Timed Protein Intake: Preclinical and Clinical In Vivo Studies

Model	Study design	Exercise protocol	Nutritional protocol	Intervention groups	Findings	Reference
Five- to 6-wk-old male WT and Clock mutant (*ClockΔ19*) mice; 5- to 10-wk-old male littermates Bmal1flox/flox and MKO C57BL/6J mice	In vivo preclinical interventional study	None	Two-meals-per-day regime: 11.5% or 8.5% of protein in a day distributed in 3 patterns: high at breakfast (early active phase), high at dinner (late active phase)	-*ClockΔ19* and WT groups consumed high levels of protein at breakfast. -*ClockΔ19* and WT consumed high levels of protein at dinner. Bmal1flox/flox and MKO groups *ClockΔ19* and WT consumed high levels of protein at breakfast. Bmal1flox/flox and MKO groups *ClockΔ19* and WT consumed high levels of protein at dinner.	High levels of protein consumed at breakfast enhanced muscle weight in the WT group but not in *ClockΔ19* . Day–night variations in locomotor activity were observed in WT mice, but not in *ClockΔ19* mice. Muscle hypertrophy by a high-protein breakfast was observed in Bmal1flox/flox mice, but not in MKO mice. The muscle free glycine and histidine levels at specific time points were higher in the overloaded muscles of mice fed a high-protein meal at dinner. Muscle free serine level was higher in the overloaded muscle of mice fed a high-protein meal at breakfast. Muscle IGF-1 expression was higher in mice fed protein at dinner.	Aoyama et al, 2021[109]
60 Healthy Japanese elderly women (65–70 y old)	Cross-sectional study (data collected from October 2017 to February 2018)	No regular exercise habits; daily physical activity levels registered via a triaxial accelerometer	Breakfast protein group (B-PRO; $n = 18$) and dinner protein group (D-PRO; $n = 42$); total amount of protein daily intake: 80 g. B-PRO group ingested a higher protein amount at breakfast (range: 25-30 g/meal)	B-PRO group and D-PRO group	Muscle mass was higher in the B-PRO group than in the D-PRO group. The skeletal muscle index and hand grip strength were significantly higher in the B-PRO group than in the D-PRO group. Skeletal muscle index and the percentage of breakfast protein intake relative to total protein intake were positively correlated.	Aoyama et al, 2021[109]

(continued)

Table 4. Continued

Model	Study design	Exercise protocol	Nutritional protocol	Intervention groups	Findings	Reference
44 Healthy young men (20–24 y old)	NCT02222415 (clinical-trials.gov) randomized double-blind trial	Supervised resistance-type exercise training was conducted three times per week over a 12-week period. Each session began with a 5-minute warm-up on a cycle ergometer, followed by four sets on both the leg press and leg extension machines. Additionally, two sets on the chest press and horizontal row were alternated with vertical pull-down and shoulder press exercises in each session. Training concluded with a 5-minute cool-down on the cycle ergometer. All training sessions were performed in the evening between 8:00 and 9:00 PM or 9:00 and 10:00 PM. During the first week of the training period, the workload was gradually increased from 70% (10–15 repetitions) of 1 RM to 80% of 1 RM (8–10 repetitions).	Subjects consumed a 300-mL bottle containing either a placebo drink (PLA group) or protein drink (PRO group) daily immediately before sleep. The protein drink contained 13.75 g of casein hydrolysate, 13.75 g of casein, 15 g of carbohydrate, and 0.1 g of fat (total energy: 746 kJ). The placebo drink is a noncaloric beverage. All participants ate a standardized meal the evening before each test day and a snack immediately after every training session, including a cheese sandwich, an apple, and a noncaloric beverage (total energy intake: 1151 kJ; 37 g of carbohydrates, 10 g of protein, and 9 g of fat).	PLA group and PRO group	The percentage of type II muscle fiber area increased significantly in PRO group. Results of the lateral pull-down machine showed significantly greater muscle strength gains in the PRO group than in the PLA group. The PRO group showed greater gains in muscle quadriceps cross-sectional area.	Snijders et al, 2015[107]

(continued)

Table 4. Continued

Model	Study design	Exercise protocol	Nutritional protocol	Intervention groups	Findings	Reference
16 Healthy elderly men (73–75 y old)	Randomized open-label study	Any exhaustive physical activity	Fasted over the previous night. A standardized dinner was ingested at 7:00 PM. Subjects went to sleep at midnight. From 2:00 to 2:05 AM, each subject received 400 mL water with (PRO) or without (PLA) 40 g intrinsically L-[1-13C]phenylalanine–labeled casein protein via a nasogastric tube.	PLA group and PRO group	Overnight muscle protein fractional synthesis rates were much greater in the PRO group than in PLA group.	Groen et al, 2012[106]
1741 Elderly subjects: 827 men and 914 women- (67–84 y old)	Long-term, longitudinal, population-based study (from NuAge study)	No forced exercise protocol; evaluation of physical performance as muscle strength (handgrip, arm, and leg) and mobility functions (timed up-and-go, chair stand, and walking speed)	Examination at time (T) 0 (baseline) and every year for 3 y (T3) via recall registrations (description of all the foods and beverages consumed during the previous day). Protein was evenly consumed: ∼20-30 g/meal. Protein consumed was skewed: ∼10 g for breakfast and 30 g for dinner	The same group at baseline vs T3	More-evenly distributed protein intake, independent of the total quantity, was associated with a higher muscle strength in both sexes throughout follow-up. The association with mobility function was demonstrated only in man	Farsijani et al, 2017[108]
828 Elderly subjects (70–80 y old)	SOMMA longitudinal study	No forced exercise protocol; evaluation of physical performance as muscle strength: grip strength and leg power	Dietary intake based on the proportion of each macronutrient; dietary intake was self-reported by each participant via the ASA24 website; grams of protein intake/kilogram of body weight	The same group at baseline vs 4–6-wk window	Earlier food intake and longer eating window were associated with greater higher grip strength.	Mao et al, 2023[111]
34 Young adults (18–39 y old); 12 males and 22 females	Pre-post single-arm intervention study; 4-wk intervention with 8-h time-restricted eating (TRE)	No forced exercise protocol	Participants were invited to choose the eating window that best suited their lifestyle. 20 g of protein per day were provided weekly as snacks.	The same group at baseline vs 4 wk	No change in muscle mass (bioelectrical impedance method) after the intervention study	Kim and Song, 2022[110]

Abbreviations: ASA24, Automated Self-Administered Dietary Assessment Tool; Bmal1, Brain and Muscle ARNT-like 1; IGF-1, insulin-like growth factor 1; MKO, muscle-specific knock-out; RM, repetition maximum; SOMMA, Study of Muscle, Mobility and Ageing; WT, wild-type.

effect of protein intake during the late-active phase of mice.[109] In addition, the same study analyzed the main molecular pathway involved in skeletal muscle metabolism, demonstrating that the expression levels of IGF-1, myogenin, and myogenic factor 5 (Myf5) and the autophagy marker microtubule-associated protein 1A/1B-light chain 3 (LC3B-II) were higher in the overloaded muscles of mice fed a high-protein diet in the early-active phase,[109] underlining the importance of protein-timing intake on the regeneration and resistance pathway.

Clinical Studies

This subsection describes clinical studies conducted on human models that evaluated the effects of timed protein ingestion on muscle. The analysis was restricted to 6 studies in which the enrolled participants did not engage in any physical exercise, aiming to isolate the impact of protein-intake timing on muscle outcomes.

Recent studies indicate that protein intake synchronized with the body's natural circadian fluctuations may enhance muscle anabolism, recovery, and overall function, especially when paired with exercise. A randomized double-blind trial conducted on 41 healthy young participants (20–24 years old), randomly assigned to either receive a protein supplement beverage or a noncaloric placebo before sleep in association with resistance-type training for a period of 12 weeks, evaluated the effects of a timed protein intake schedule on muscle mass and strength.[107] Participants were asked to consume 300-mL bottle containing either a placebo drink (PLA group) or protein drink (PRO group) daily immediately before sleep. While the placebo was a noncaloric drink, whereas the protein beverage contained a total amount of casein of approximately 27.5 g. In addition, during the period of 12 weeks, participants performed a supervised resistance-type exercise training 3 times per week.[107] As demonstrated by skeletal muscle biopsies, the percentage of type II muscle fiber area increased significantly as well as muscle strength, measured on the lateral pull-down machine, was significantly higher in the PRO group compared with the PLA group.[107] These results suggest that the timing of protein intake, combined with constant resistance-type training, might improve muscle strength independently of muscle mass modification.

A randomized open-label study demonstrated that nighttime protein intake can significantly improve muscle protein synthesis in older adults (73–75 years old).[106] After a standardized dinner, 16 healthy elderly participants were enrolled to randomly received 40 g of intrinsically L-[1-^{13}C]phenylalanine–labeled casein protein (PRO) or placebo (PLA) via a nasogastric tube during overnight sleep, specifically from 2:00 to 2:05 AM.[106] Interestingly, the PRO group showed a higher incorporation of the amino acids ingested via the intrinsically labeled protein and, concomitantly, muscle protein synthesis rates significantly improved compared with the PLA group,[106] demonstrating that protein supplementation during sleep may represent a promising strategy to mitigate age-related muscle mass loss. Conversely, a cross-sectional study assessed on elderly women demonstrated that higher levels of protein consumed in the morning positively influenced muscle mass and strength.[109] Sixty healthy elderly women were enrolled and divided into 2 groups: a breakfast protein group (B-PRO; $n = 18$) and a dinner protein group (D-PRO; $n = 42$). The former was invited to consume a large amount of protein for breakfast, the latter was asked to consume a large amount of protein for dinner.[109] The daily total amount of protein was not significantly different between the 2 groups, although the B-PRO group ingested a large quantity of protein for breakfast compared with the D-PRO group (30 g vs 20 g, respectively).[109] This study showed that, compared with the D-PRO group, muscle mass in the B-PRO group was greater, concomitantly with skeletal muscle index and grip strength.[109] The significant positive correlation between skeletal muscle index percentage of breakfast protein intake relative to total protein intake strongly suggests the pivotal importance of timed protein intake in the regulation of muscle health. Despite the gender differences in the two studies described above and the potential hormonal interference, they nonetheless raise the possibility of consuming proteins at night to stimulate protein synthesis and in the morning to assess the increase in muscle mass and strength. A different approach was demonstrated in a study comparing muscle protein synthesis in participants receiving daily protein intakes according to 2 distributions: evenly and skewed.[112] The randomized controlled trial was conducted on 8 healthy subjects (25–55 years old; 5 males and 3 females) and aimed to evaluate the skeletal muscle protein content after different portioning of daily protein amounts for 7 days.[112] According to this distribution, protein consumed during the day was either equally divided between the 3 different meals (defined as "evenly") or with a higher intake at dinner (defined as "skewed").[112] This study demonstrated that, on days 1 and 7, muscle protein synthesis, expressed as mixed muscle protein fractional synthesis rates and measured by direct incorporation of L-[ring-$^{13}C_6$]phenylalanine into protein, is higher in response to the breakfast meal containing 30-g protein meal (evenly) than with the 10-g protein meal (skewed) at the same time point.[112] This study found that the distribution of protein intake over 24 hours of the day contributed significantly to muscle protein synthesis and potentially to muscle mass formation.

Similar results have been obtained in studies of elderly people, where it is important to note that there a physiological decline in protein synthesis and a loss of muscle mass associated with the aging of cellular processes. **Figure 3**[108] shows the skeletal muscle effects of timed protein consumption in elderly subjects. A long-term evaluation assessed in a large sample (1741; of which 827 were men and 914 were women) of elderly subjects (67–84 years old) showed that the more-even daily distribution of protein may be associated with improved muscle strength, evaluated as handgrip, arm, and leg strengths, throughout the entire follow-up without gender differences.[108] In addition, mobility function, such as normal and fast running, after 3 years increased in both sexes, again demonstrating how the equally distributed intake of protein throughout the day has a significant role in muscle function. The same group was evaluated in a 2-year follow-up observational study. Consistently, another study conducted in 828 elderly subjects (70–80 years old) derived from the Study of Muscle Mobility and Ageing (SOMMA) , a longitudinal study evaluated the influence of eating habits related to the timing of food intake on muscle health.[111] Food consumption was self-recorded by study participants and expressed as a percentage of kilocalories per day. Statistical analysis showed that elderly participants who consumed protein equally distributed during the day and ranging from 16% to 17% protein showed significantly increased muscle strength, calculated by grip strength and leg power. The study considered not only the nutrients ingested during the day but also the time between the first food intake and the last food intake of the day, the so-called eating window, as well as the time of the first and last fluid intake of the day.[111] The analysis showed that earlier food intake and the longer eating window were associated with greater hand-grip strength, strongly suggesting that the time of food ingestion is critically related to better muscle mass and

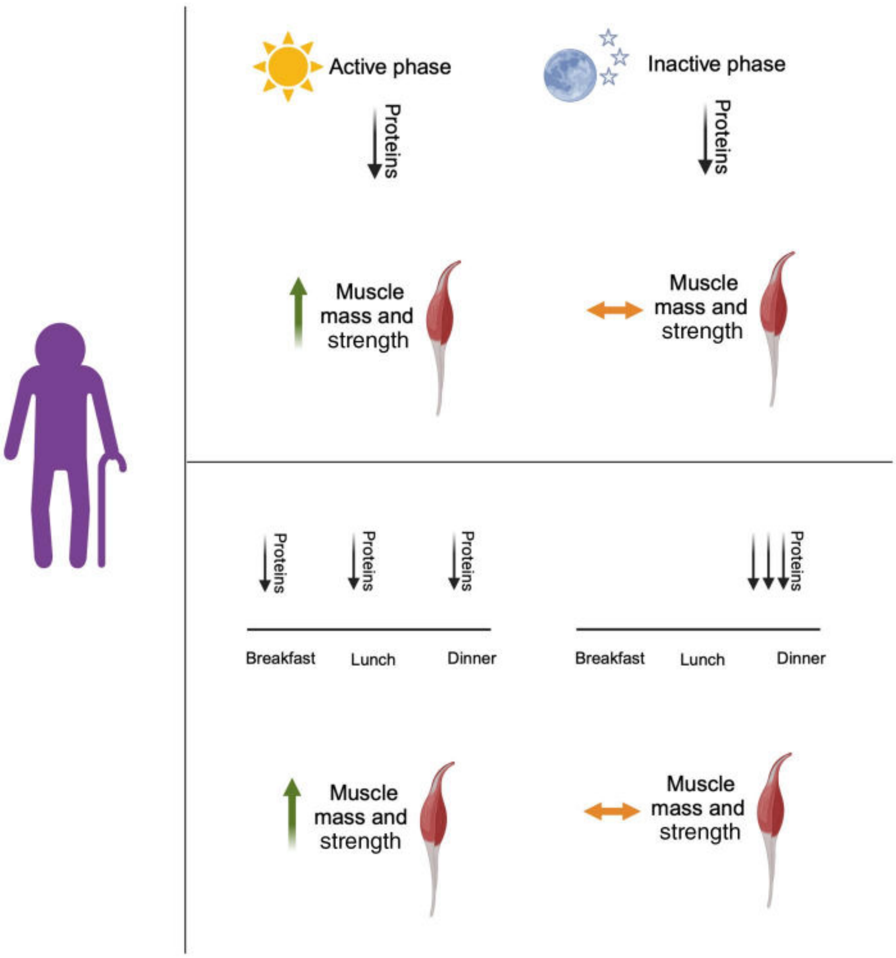

Figure 3. Timed Protein Consumption Effects on Muscle Mass and Strength in Elderly Subjects. Elderly subjects consuming proteins in the morning showed greater muscle mass and strength than those consuming proteins in the evening. Similarly, elderly subjects distributing proteins during the different meals of the day displayed increased muscle mass and strength compared with elderly subjects consuming the same amount of protein in a single meal of the day. Created with BioRender.com

function in older adults.[111] Moreover, a pre-post single-arm intervention study involving 34 healthy and young participants (18–39 years old) evaluated body composition according to self-reported time-restriction feeding of 8 hours and the supplementation of supplied snack packages containing 20 g of protein per day. In particular, enrolled participants were asked to select the eating window that best fit their lifestyle and were invited to follow an early time-restricted eating diet of 8 hours. The study showed that, although the group who started eating before noon experienced significant reductions in body weight, fat mass, fasting glucose, fasting insulin, and serum triglyceride levels compared with baseline, no changes were shown in muscle mass, suggesting that an early time-restricted eating diet may be a feasible and effective strategy for managing body composition and cardiometabolic risk factors without significant changes in muscle characteristics.[110]

MOLECULAR PATHWAYS INVOLVING MEAL TIMING IN SKELETAL MUSCLE PHYSIOLOGY: FOCUS ON CARBOHYDRATE INTAKE

Although in the last decades the scientific literature on chrono-nutrition has strengthened, the molecular knowledge concerning the timing of the intake of carbohydrates seems to be poorer when compared with studies performed on the timed intake of protein. However, the timing of carbohydrate consumption relative to skeletal muscle is an important concern, particularly in the field of sports nutrition and exercise physiology, as it can influence muscle glycogen storage, protein synthesis, and overall muscle adaptation to exercise.[113] The few existing studies on circadian carbohydrate intake are combined with physical activity. For this reason, 2 preclinical in vivo studies and 1 clinical in vivo study were reviewed and critically examined for the following subsection, as detailed in **Table 5**.[114–116]

Preclinical and Clinical In Vivo Studies

In the preclinical field, an interesting study conducted in a male mouse model investigated the role of timed glucose administration on muscle glycogen recovery associated with treadmill running of 20 m/minute for 60 minutes.[116] In particular, after the treadmill running exercise to deplete muscle and liver glycogen, glucose was administered either as a bolus or as a pulse.[116] The results showed that, in the mouse group receiving the bolus, the blood glucose concentration was significantly lower and plasma insulin concentration was significantly higher compared with the mouse group receiving pulse feeding. Muscle glycogen concentration in the mouse group receiving the bolus was 25.3% higher than in the mouse group receiving pulse feeding at 60 minutes after glucose ingestion, indicating enhanced muscle glycogen recovery,[116] and demonstrating that ingesting a large amount of glucose immediately after exercise promotes insulin secretion and enhances muscle glycogen recovery, whereas frequent ingestion of small amounts of glucose enhances liver glycogen recovery. More specifically related to circadian rhythm, a preclinical study performed in male mice investigated timed food consumption associated with voluntary wheel-running effects on obesity and muscle circadian clock components.[114] In summary, this preclinical study performed on 6-week-old male mice aimed at comparing metabolic changes induced by time-imposed feeding under conditions of voluntary wheel-running versus sedentary activity in mice undergoing different time-point feeding schedules, with the main aim of understanding the impact of exercise on obesity.[114] In particular, mice were housed either with running wheels (RW) or without running wheels (SED). After 2 weeks, 8-week-old mice were fed a high-fat, high-sucrose diet (containing 20.0% wt/wt sucrose) ad libitum for 2 weeks. For the following 2 weeks, mice were feeding-restricted. The SED and RW groups were then separated into groups that were fed with the diet only during the sleep phase (daytime feeding [DF]) or only during the active phase (nighttime feeding [NF]) for 10 days.[114] The study demonstrated that the RW group consumed more food than the SED group independently of feeding time, but muscle weight was significantly lower in the DF groups independently of physical activity.[114] It was shown that chronic voluntary exercise on running wheels had a minimal effect on obesity and adiposity caused by daily feeding at unusual times, suggesting that the timing of food intake is more important than physical exercise in obesity pathogenesis.[114] In addition, during the second half of the dark period, nocturnal wheel-running activity was maintained to a lesser extent, confirming a less efficacious physical performance in this circadian time point. Surprisingly, in this animal model, daytime feeding tissue affect the rhythmic expression of clock genes only in liver and adipose tissue, suggesting that change in muscle weight was not related to clock-controlled gene activity in the skeletal muscle.[114]

To confirm these findings, a clinical study investigated the effects of a high-carbohydrate nocturnal meal on body composition and muscle cross-sectional area in 10 male Japanese wrestlers (20 years old). Male wrestlers usually experience body mass loss 1 week before their fights by rapidly ingesting fluids and food. Specifically, in this study, the impact of an overnight high-carbohydrate recovery meal following severe rapid weight loss (baseline condition) on muscle glycogen

Table 5. Timed Carbohydrate Intake: Preclinical and Clinical In Vivo Studies.

Model	Study design	Exercise protocol	Nutritional protocol	Intervention groups	Findings	Reference
Six-week-old male ICR mice (sample size not indicated)	In vivo interventional preclinical study	20 m/min for 60 min of treadmill followed by a recovery of 60 min	A bolus containing 1.2 mg/g ofbody weight glucose treatment immediately after exercise and 1.2 mg/g of body weight glucose treatment via pulse ingestion (every 15 min × 4 times) during active phase	Bolus ingestion group Pulse ingestion group	Plantaris muscle glycogen concentration in the group receiving the bolus was 25.3% higher than in the group receiving pulse	Matsunaga et al, 2021[116]
Six-week-old male C57BL/6J mice without running wheel (SED) and with running wheel (RW) fed normal feed pellets; After 2 weeks, mice fed high-fat, high-sucrose diet (containing 20.0% wt/wt sucrose) ad libitum for 2 wk	In vivo interventional preclinical study	Running wheel (RW) recorded throughout the study at 1-min intervals in 1-h bins for 6 wk	Six-week-old male C57BL/6J mice without running wheel (SED) and with running wheel (RW) fed normal feed pellets. After 2 wk, mice were fed with a high-fat, high-sucrose diet (containing 20.0% wt/wt sucrose) ad libitum for 2 wk. SED and RW groups were then separated into groups that were fed with the diet only during the sleep phase (daytime feeding [DF])or only during the active phase (nighttime feeding [NF]) for 10 days. For another 2 weeks, mice were feed-restricted.	Sedentary (SED) and running (RW) groups consumed the same high-fat, high-sucrose diet. Subsequently, they were divided into 2 groups: DF and NF.	The RW group consumed more food than the SED group independently of feeding time. Muscle weight was significantly lower in DF groups independently of physical activity. During the second half of the dark period, nocturnal wheel-running activity was maintained to a lesser extent. Daytime feeding influenced the rhythmic expression of clock genes in the liver and in skeletal muscle.	Okauchi et al, 2018[114]
Ten Japanese male wrestlers (∼20 y old)	Interventional single-arm study	None	Participants consumed meals between 5:30 and 11:00 PM: (R0–R2) 5:30–7:00 PM, 290 g carbohydrates; (R2–R4) 9:00–10:00 PM, 200 g carbohydrates; (R4–R13)10:30–11:00 PM, 33 g carbohydrates	The same group at baseline (BL), R0, R2, R4, and R13 time points	-Compared with BL, at the R0 time point body mass significantly decreased, whereas compared to R0 time point, at R2, R4, and R13 time points participants showed significantly increased body mass. -Muscle cross-sectional area, significantly decreased at the R0 time point compared with BL and significantly increased at R2, R4, and R13 compared with the R0 time point. -Muscle glycogen concentration was significantly lower at R0 compared with BL. It remained lower than BL at R2, R4, and R13.	Kondo et al, 2021[115]

Abbreviations: BW, body weight; ICR, Institute of Cancer Research.

concentration was investigated.[115] Participants were asked to consume 3 predefined meals within 5 hours overnight, using the following time and carbohydrate-dose schedule: (R [recovery] 0–R2) 5:30–7 PM, 290 g carbohydrates; (R2–R4) 9:00–10:00 PM, 200 g carbohydrates; (R4–R13) 10:30–11:00 PM, 33 g carbohydrates.[115] Compared with baseline, at the R0 period body mass significantly decreased, whereas compared with the R0 period, at R2, R4, and R13 participants showed significantly increased body mass. Moreover, muscle cross-sectional area significantly decreased at the R0 period compared with baseline and significantly increased at R2, R4, and R13 compared with the R0 period.[115] In addition, muscle glycogen content was significantly lower at R0 compared with baseline and did not recover by R2 or R4, and also remained lower than baseline at R13. This study suggested that a nocturnal meal with a high carbohydrate content was not sufficient to restore the glycogen content of muscles during a 13-hour recovery period.

In conclusion, as demonstrated by preclinical and clinical studies, skeletal muscle exhibits rhythmic changes in gene expression involved in muscle metabolism, reflecting the influence of the circadian clock system, and suggesting that the integration of muscle physiology with circadian control may impact response to nutrient intake, muscle performance, and adaptation to exercise. Thus, understanding the connection between skeletal muscle physiology and circadian clock control is crucial for optimizing exercise and nutritional strategies aimed at enhancing muscle health and performance.

CONCLUSION

The circadian clock regulates physiological processes in skeletal muscle influencing metabolism, protein synthesis, and repair mechanisms. Disruptions of the circadian rhythm, such as irregular sleep patterns or shift work, can impair muscle function and contribute to muscle wasting. Concomitantly, disruption of specific muscle clock contributes to muscle de-structure and dysfunction. Recent studies suggest that chrono-exercise and chrono-nutrition may strongly contribute to muscle health maintenance. In particular, protein distribution throughout the day influences muscle metabolism, considering that consuming protein evenly across meals, rather than in large amounts all at once, may optimize muscle protein synthesis and overall muscle health. Moreover, timed consumption of carbohydrates may support physical exercise by restoring glycogen balance. Understanding the interactions between chronobiology, chrono-nutrition, and skeletal muscle health has important implications for preventing and treating conditions like sarcopenia and muscle wasting. Future studies exploring the molecular mechanisms underlying these relationships will guide effective strategies to promote muscle health and function through optimized meal timing and circadian rhythm alignment.

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Conflicts of Interest

None declared.

Data availability

This review is based on previously published studies and does not involve the collection of new data. All data supporting the findings of this study are available in the cited references.

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