TOPICAL REVIEW

The role of the circadian clock in regulating mitochondrial dynamics and their impact on skeletal muscle function and metabolism

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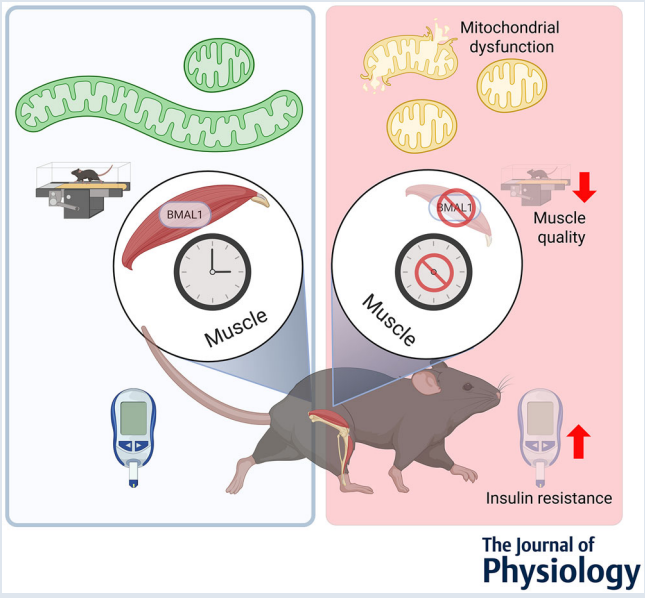

Abstract figure legend Genetic disruption of core circadian clock proteins, particularly brain and muscle Arnt-like protein-1 (BMAL1), leads to altered mitochondrial dynamics and dysfunction, accompanied by reduced skeletal muscle quality. Key hallmarks include diminished muscle fibre size, impaired contractile strength, reduced exercise capacity and insulin resistance. The molecular mechanisms linking circadian clock proteins to mitochondrial dynamics regulation, and their role in muscle deterioration, remain unresolved. Created with BioRender.com (2025).

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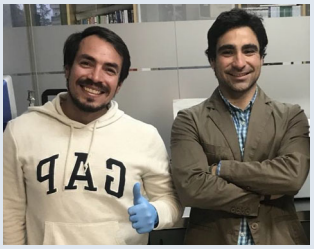

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Abstract Disruptions in both circadian clock and mitochondrial dynamics in the skeletal muscle (SkM) have been associated with insulin resistance and sarcopenia. Emerging evidence, in resting conditions and in response to metabolic challenges like exercise, suggests the intricate interplay between the circadian clock, mitochondrial dynamics and SkM function. However the molecular mechanisms that connect the circadian clock to mitochondrial dynamics and SkM function remain poorly understood. This review focuses on the role of circadian clock proteins, particularly brain and muscle Arnt-like protein-1 (BMAL1), in regulating mitochondrial dynamics and examines how their dysregulation contributes to metabolic and SkM deterioration. By exploring their interaction we aim to identify potential therapeutic targets that could improve metabolic health and muscle function.

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Introduction

Circadian clock proteins regulate essential physiological processes, including metabolism, behaviour and cellular functions. Disruptions in the function of those proteins or in circadian rhythm patterns are linked to metabolic diseases like type 2 diabetes mellitus (T2DM) (Gan et al., 2015; Stenvers et al., 2019; Turek et al., 2005). Recent evidence suggests that circadian disruption in skeletal muscle (SkM) contributes to insulin resistance and sarcopenia (Choi et al., 2019; Dyar et al., 2013; Liu et al., 2016).

SkM plays a central role in glucose and fatty acid metabolism, and its function is closely co-ordinated with circadian rhythmicity (CR). Emerging evidence in humans highlights a potential role for the circadian clock in the regulation of mitochondrial dynamics, which is critical for energy production, oxidative capacity, and SkM health (Figueroa-Toledo et al., 2024; Gemmink et al., 2023). Understanding how the circadian clock regulates mitochondrial dynamics is essential for developing targeted treatments for metabolic diseases and muscle degeneration like sarcopenia.

This review examines the emerging evidence on the interplay between circadian clock and mitochondrial dynamics in SkM, highlighting their critical roles in muscle metabolism and contractile function.

Circadian clock

Metabolism, behaviour and several other aspects of physiology exhibit circadian rhythms, that is, endogenous rhythms that follow a near-24-h cycle, which underscores the widespread influence of circadian rhythms on biological processes. CR is driven by internal clock, highly conserved timing mechanisms that enable cells, organs and organisms to anticipate and adapt to daily environmental changes, such as the day–night cycle and schedules in food intake (Challet, 2019; Partch et al., 2014). In mammals the molecular clock operates through a transcription–translation feedback loop involving the transcriptional activators brain and muscle Arnt-like protein-1 (BMAL1) and circadian locomotor output cycles kaput (CLOCK), which promote the transcription of the repressors period circadian regulator (PER) and cryptochrome circadian regulator (CRY) through the day. These repressors, in turn, inhibit the forward limb of the master clock, generating a 24-h cycle (Challet, 2019; Partch et al., 2014). This process is essential for maintaining temporal patterns in cellular physiology.

Data from 12 mouse tissues reveal that between 1000 and 3000 genes exhibit circadian expression patterns, known as clock output genes (Zhang et al., 2014). Disruption of the circadian clock machinery has severe consequences for metabolism (Stenvers et al., 2019; Turek et al., 2005). In murine models whole-body *Clock* and *Bmal1* knockout lack insulin secretion rhythm, are insulin resistant and have impaired glucose-stimulated insulin secretion (Marcheva et al., 2010). In humans epidemiological studies have shown that the relationship between intrinsic circadian rhythms and behavioural cycles, such as in night-shift workers, a model for circadian misalignment, can lead to dyslipidaemia, insulin resistance and T2DM (Dutheil et al., 2020; Gan et al., 2015). Furthermore in individuals with T2DM afternoon exercise has been shown to confer greater metabolic benefits compared to morning exercise, highlighting the importance of circadian timing in therapeutic strategies (Mancilla et al., 2021; van der Velde et al., 2023).

Circadian misalignment models in humans

Misalignment studies are conducted in various 'real-world' settings. Some misalignments are transient (e.g. daylight-saving time or time zone travel), whereas others are chronic, such as differences in sleep–wake preferences (chronotype), social jet lag, circadian rhythm sleep disorders and shift work. Shift work, now affecting at least 20% of the workforce, typically involves hours outside the 7:00 AM–6:00 PM range (IARC, 2019).

Shift work is linked to higher rates of cardiovascular disease, obesity and diabetes, likely due to a combination of circadian misalignment, sleep deprivation, increased night-time light exposure, poor eating habits and unhealthy behaviours. The exact role of shift work as a risk factor for cardiovascular disease and type 2 diabetes remains debated, with some studies showing an association whereas others do not. However the consensus is that ongoing circadian disruption and sleep loss negatively impact health (Baron & Reid, 2014).

Circadian clock in the SkM function and metabolism

SkM is the largest metabolically active tissue in the human body, accounting for approximately 40% of body mass in lean individuals. It plays a vital role in fatty acid uptake and oxidation and contributes to 80% of insulin-mediated glucose disposal (Baron et al., 1988). In addition to its metabolic functions, SkM is essential for voluntary movement, making its health fundamental to overall wellbeing. The SkM exhibits a circadian rhythm that regulates metabolic processes, allowing the body to anticipate daily cycles of activity and food intake (Martin et al., 2023).

In SkM biopsies the density and size of myocellular lipid droplets, as well as the lipid composition of whole muscle, vary in a circadian manner under normal living conditions (Held et al., 2020). Specifically day–night oscillations were observed in more than half of the diacylglycerol lipid species (Held et al., 2020). Furthermore the molecular clock is causally linked to SkM oxidative capacity, as evidenced by the day–night rhythm of ADP-stimulated oxygen consumption in permeabilized human SkM fibres, as well as the day–night rhythm of whole-body fatty acid oxidation (van Moorsel et al., 2016). Also in humans we recently showed that lower protein levels of BMAL1 and CLOCK are associated with a diminished mammalian Target of Rapamycin (mTOR)-mediated protein synthesis pathway and reduced SkM strength in humans (Figueroa-Toledo et al., 2024). Additionally short-term circadian misalignment has been shown to reduce insulin sensitivity in SkM, and siRNA-mediated reduction in CLOCK protein expression in primary myotubes decreases glucose uptake (Perrin et al., 2018).

In murine genetic models disruptions in circadian clock have been shown to impair both SkM quality and metabolic function. For example *Bmal1* knockout mice demonstrate reduced maximal muscle contraction at both the whole-muscle and single-fibre levels (Andrews et al., 2010). Additionally SkM-specific ablation of *Bmal1* and *Clock* results in both whole-body and SkM insulin resistance (Dyar et al., 2013; Liu et al., 2016). Finally in Bmal1 knockout C2C12 myotubes (a murine muscle cell line), anaerobic glycolysis, mitochondrial respiration with glycolytic substrates and transcription of hypoxia-inducible factor-1α (HIF1α) target genes are significantly reduced. Notably genetic stabilization of HIF1α also disrupts circadian gene expression, highlighting a bidirectional interaction between the circadian clock and HIF pathways in regulating metabolic adaptation (Peek et al., 2017).

Together these findings highlight the essential role of the circadian clock in SkM function and suggest that disruptions in these rhythms may contribute to metabolic dysfunction, insulin resistance, metabolic adaptation and muscle degeneration. However the precise mechanisms by which the circadian clock regulates SkM function and metabolism remain unclear.

Mitochondrial dynamics

Mitochondrial function, including oxidative phosphorylation (ATP production), regulation of programmed cell death, calcium signalling and cellular dynamics, is dependent on mitochondrial dynamics (Wai & Langer, 2016). Mitochondria exhibit a variety of morphologies, ranging from small spheres to short or long tubules, or interconnected tubules (Chen et al., 2005). These morphologies are regulated by the fine-tuned balance between the processes of fusion and fission (Chen et al., 2005; Eisner et al., 2018), allowing mitochondria to rapidly adapt to various cellular challenges (Eisner et al., 2018).

Mitochondrial fusion is regulated by mitofusins 1 and 2 (MFN1 and MFN2) and optic atrophy protein 1 (OPA1), which are located in the outer (OMM) and inner mitochondrial membranes (IMM), respectively (Liesa et al., 2009; Malka et al., 2005). MFN2 also has non-fusogenic functions, such as regulating mitochondria–endoplasmic reticulum (ER) tethering, which modulates mitochondrial activity via calcium and lipid flux (Cárdenas et al., 2010; Hernández-Alvarez et al., 2019). Fusion is crucial for mitochondrial DNA (mtDNA) integrity, respiration and apoptosis as it enables sharing of recovery proteins and cooperativeness between healthy

and damaged mitochondria (Eisner et al., 2018). The deletion of IMM pro-fusion proteins in mice is lethal to embryos (Chen et al., 2003), and organ-specific knockouts of fusion regulators cause severe dysfunction (Eisner et al., 2018).

Mitochondrial fission is driven by the recruitment of dynamin-related protein 1 (DRP1), a cytosolic GTPase to the OMM, facilitated by the mitochondrial fission protein 1 (FIS1) and other adapter proteins (Eisner et al., 2018). Mitochondrial fission, in turn, is essential for mitochondrial biogenesis and the segregation of damaged mitochondria for mitophagy, a quality control mechanism activated by low membrane potential or irreversible mtDNA damage (Kleele et al., 2021). Mitophagy is regulated by two primary mechanisms: the ubiquitin-dependent pathway, which involves PTEN-induced kinase 1 (PINK1) and PARKIN, and the ubiquitin-independent pathway, which includes proteins such as Bcl-2 interacting protein 3 (BNIP3), NIX/BNIP3L (NIP-like protein X) and FUN14 domain containing 1 (FUNDC1) (Onishi et al., 2021). Both pathways generate an 'eat-me' signal that facilitates the engulfment of damaged mitochondria into a mitophagosome, which then concludes with lysosomal degradation.

Another important aspect of mitochondrial dynamics is the morphology and folding of the IMM (mitochondrial crista formation). The shape of the cristae is not fixed; when mitochondrial oxidative phosphorylation is triggered, mitochondria transition into a 'condensed' state, expanding the crista space. This process is regulated by OPA1 and the mitocondrial contact site and cristae organizing system (MICOS) complex (Castro-Sepúlveda et al., 2021; He et al., 2022). The shape of the mitochondrial cristae also influences the assembly of mitochondrial supercomplexes, which are essential for increasing respiratory efficiency (Cogliati et al., 2013).

Physiological relevance of mitochondrial dynamics in the SkM

In SkM mitochondrial fusion is a key quality control response, maintaining bioenergetics and excitation–contraction coupling (Eisner et al., 2014). Conditional reduction in OMM fusion proteins MFN1/2 in SkM of mice leads to increased mtDNA mutations, atrophy and exercise intolerance (Chen et al., 2010). Moreover MFN2- and OPA1-deficient mice exhibit muscle atrophy, reduced strength, impaired SkM mitochondrial respiration and muscle metabolic deterioration (Sebastián et al., 2016; Tezze et al., 2017). In human SkM reduced MFN2 abundance is linked to less mitochondria–sarcoplasmic reticulum (SR) interaction, decreased crista density and lower maximal oxygen uptake (Castro-Sepulveda et al., 2021). In the context of ageing reduced protein levels of OPA1 and MFN2 correlate with decreased myofibre diameter, a hallmark of sarcopenia (Sebastián et al., 2016; Tezze et al., 2017). In terms of metabolic health SkM mitochondria in insulin-resistant individuals exhibit a reduced fusion phenotype, characterized by smaller mitochondria (Kelley et al., 2002). Finally an elongated mitochondrial phenotype is inversely associated with lipid droplet density and directly linked to increased whole-body lipid oxidation (Castro-Sepulveda et al., 2020). It is important to note that changes in mitochondrial morphology, such as the formation of smaller or elongated mitochondria, are regulated not only by mitochondrial fusion but also by mitochondrial fission.

Regarding mitochondrial fission loss of DRP1 in SkM leads to larger, dysfunctional mitochondria, muscle wasting and weakness (Dulac et al., 2021; Favaro et al., 2019). Conversely *Drp1* overexpression results in mild muscle atrophy and reduced mitochondrial quality, suggesting that maintaining DRP1 levels within a narrow range is necessary for muscle health (Dulac et al., 2021). DRP1 deficiency also reduces maximal running speed and impairs exercise adaptations (Moore et al., 2019). In obese mice DRP1 was reduced, and its inhibition improved insulin sensitivity (Jheng et al., 2012). Confirming this in an *in vitro* model, palmitate-induced mitochondrial fragmentation in muscle cells was associated with oxidative stress, reduced ATP and impaired glucose uptake, which was alleviated by DRP1 inhibition (Jheng et al., 2012). Finally *Fis1* deletion in adult SkM resulted in mitochondrial hyperfusion, respiratory chain deficiency and abnormal mitophagy, worsened by endurance exercise, highlighting the role of FIS1 in mitophagy and muscle response to exercise (Zhang et al., 2019).

The role of mitophagy in SkM health was explored by examining the effects of PARKIN deletion. *In vitro* PARKIN genetic ablation resulted in reduced mitochondrial respiration and primary myotube atrophy. Consistent with these findings tibialis anterior muscles from 4-week-old PARKIN knockout mice exhibited atrophy (Peker et al., 2018). However in 12-week-old PARKIN deletion mice, tibialis anterior muscles exhibited a trend towards type IIB fibre hypertrophy, without changes in muscle fibre-type proportions, alongside a slight but significant decrease in strength. Mitochondrial function was significantly impaired, as evidenced by reduced ADP-stimulated respiratory rates and increased mitochondrial fragmentation (Gouspillou et al., 2018). These discrepancies in the role of PARKIN in muscle atrophy may be attributed to differences in mouse age. Similarly PARKIN overexpression in SkM via adeno-associated virus injection enhances mitochondrial function, increasing maximal respiration and muscle mass (Reynaud et al., 2023). PINK1 knockout mice exhibit impaired SkM regeneration after chemical insult,

though basal muscle size remains unaffected (Cairns et al., 2024). The downregulation of BNIP3 in old mice exacerbates inflammation and muscle atrophy, whereas elevated BNIP3 expression in aged humans is associated with reduced inflammation, suggesting a protective role against age-related muscle inflammation (Irazoki et al., 2022). Finally both gain- and loss-of-function experiments in rodents and myotubes demonstrate that NIX accumulation induces mitochondrial depolarization, DRP1 activation and mitophagy, while inhibiting insulin signalling by the mTOR-RPS6KB/p70S6 kinase and insulin receptor substrate 1(IRS1) pathways (da Silva Rosa et al., 2021).

In summary the regulation of mitochondrial dynamics, including fusion, fission and mitophagy, plays a critical role in maintaining SkM function, exercise performance and metabolic homeostasis (Fig. 1). Despite its importance the molecular mechanisms that co-ordinate these dynamic processes remain incompletely understood. However recent studies suggest that circadian clock proteins may be key regulators of mitochondrial dynamics (de Goede et al., 2018; Jin et al., 2023). The following section explores the growing body of evidence highlighting the potential role of circadian clock proteins in regulating mitochondrial dynamics, and how these mechanisms, in turn, may impact SkM function and metabolism.

Role of circadian clock in the regulation of mitochondrial dynamics and SkM function

Disruptions in both circadian clock and mitochondrial dynamics contribute to similar pathophysiological changes in SkM, including impaired force production, muscle atrophy and insulin resistance. Emerging evidence underscores a critical interplay between circadian clock proteins and mitochondrial dynamics in SkM, emphasizing their combined role in modulating muscle metabolism and contractile function. We hypothesized that circadian clock proteins could regulate mitochondrial biology/dynamics in SkM (summary of evidence in Table 1).

Human evidence. A key study examining human vastus lateralis (VL) biopsies at different time points (8:00 AM, 1:00 PM, 6:00 PM, 11:00 PM and 4:00 AM) demonstrated CR in the expression of the circadian clock genes *BMAL1, PER2* and *CRY1* (van Moorsel et al., 2016). However the expression of proteins associated with mitochondrial mass, such as peroxisome proliferator-activated receptor-gamma coactivator (PGC-1α), oxidative phosphorylation complex (OXPHOS), voltage-dependent anion channel (VDAC) and translocase of outer mitochondrial membrane 20 (TOMM20), did not exhibit CR. The expression of the pro-mitochondrial dynamic proteins, FIS1, OPA1 and PINK1, did not exhibit CR, though their levels fluctuated throughout the day. These fluctuations were accompanied by changes in resting energy expenditure (REE), respiratory exchange ratio (RER), ADP-stimulated SkM respiration (van Moorsel et al., 2016) and mitochondrial network connectivity modification (Gemmink et al., 2023). In older people with metabolic compromise, the circadian clock gene expression in VL presented a similar CR than in healthy young individuals (Wefers et al., 2020). However compared to young healthy controls, older

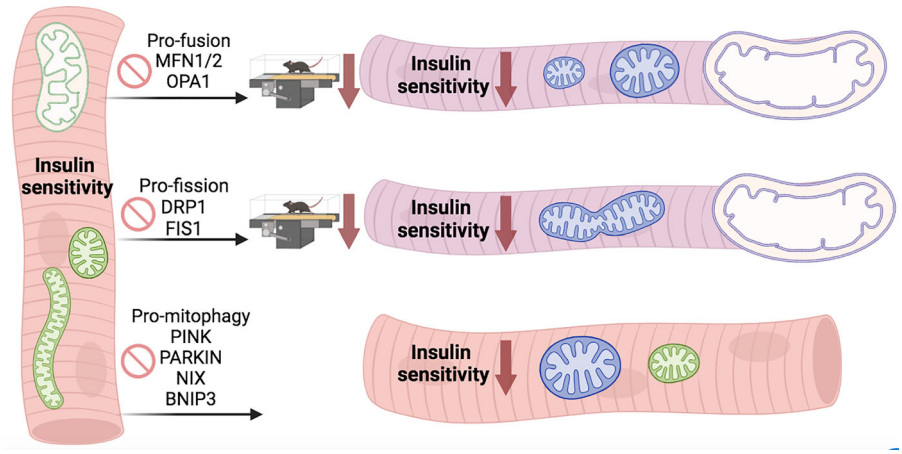

Figure 1. Summary of the effects of pro-dynamic protein deletion on mitochondrial morphology, muscle function and insulin sensitivity in SkM
Genetic deletion of proteins regulating mitochondrial fusion/fission disrupts mitochondrial morphology and ultrastructure, leading to reduced skeletal muscle (SkM) quality, including smaller fibre size, diminished exercise capacity, impaired contractile strength and insulin resistance. Similarly deletion of mitophagy regulators alters mitochondrial structure and impairs insulin sensitivity in SkM.

Table 1. Potential effects of core circadian clock proteins on mitochondrial dynamics and SkM contractile and metabolic functions.

Species/cell type	Experimental model/condition	Circadian clock/rhythm modification	Effects on mitochondrial dynamics in the SkM	Effects on genes or proteins related to mitochondrial biology in the SkM	Whole-body metabolic and physiological effects	SkM function/quality/ metabolic effects
Humans						
[19]	Healthy men biopsies at 8:00 AM, 1:00 PM, 6:00 PM, 11:00 PM and 4:00 AM in VL muscle	(CR) *Clock, Bmal1, Per2* and *Cry1*	NE	(NR) PGC-1α, OXPHOS, VDAC, TOMM20 (NR) FIS1, OPA1, PINK1, time differences	(NR) REE, time differences (NR) RER, time differences	(CR) Muscle ADP-stimulated (state 3) respiration (NR) Muscle maximally uncoupled respiration
[55]	Older people with metabolic compromise (biopsies 8:00 AM, 1:00 PM, 6:00 PM, 11:00 PM and 4:00AM in VL muscle) *versus* reference values from healthy young individuals	(=) *Clock, Bmal1, Per2 and Cry1*	NE	Loss of time effect of FIS1 and PINK1 (=) Time effect of OPA1	(↓) Day-night variation in RER	Loss of CR in muscle ADP-stimulated (state 3) respiration
[7]	Healthy men in resting condition (SkM biopsy between 8:00 and 9:00 AM in VL muscle)	Variance of BMAL1 and CLOCK	**(↓) BMAL1 associated to** (↓) Mitochondrial size and crista density (↑) Mitochondria-SR interaction **(↓) CLOCK associated to** (↑) Mitochondria-SR interaction	**(↓) CLOCK associated to** (↓) OPA1 and DRP1	**(–) BMAL1 associated to** (↑) Blood LDL-c **(–) CLOCK associated to** (↑) Blood LDL-c	**(–) CLOCK association to** (↓) Maximal muscle strength **(–) CLOCK association to** (↓) mTOR, AKT and P70S6K

(*Continued*)

Table 1. (Continued)

Species/cell type	Experimental model/condition	Circadian clock/rhythm modification	Effects on mitochondrial dynamics in the SkM	Effects on genes or proteins related to mitochondrial biology in the SkM	Whole-body metabolic and physiological effects	SkM function/quality/ metabolic effects
[56]	Healthy men and women with 4 consecutive simulated day-shift schedules (biopsies in VL muscle)	Night-shift work	NE	(=) PGC-1α, UCP3, TFAM and mitochondrial complexes II–V	(↑) Fasting plasma glucose	(↓) Insulin sensitivity assessed using euglycaemic–hyperinsulinaemic clamp (=) Muscle mitochondria respiration
Mice						
[21]	Whole-body *Clock*$^{\Delta 19}$ and *Bmal1* knockout	NE	***Clock*$^{\Delta 19}$** (↓) Mitochondrial density in GTN muscle ***Bmal1*$^{-/-}$** (↓) Mitochondrial density in GTN	***Clock*$^{\Delta 19}$** (↓) *Pgc-1*α and loss of CR in *Pgc-1*β ***Bmal1*$^{-/-}$** (↓) *Pgc-1*α	NE	***Clock*$^{\Delta 19}$** Loss of CR in *MyoD* (↓) Muscle-specific tension in EDL ***Bmal1*$^{-/-}$** Loss of CR in *MyoD* in GTN (↓) Muscle-specific tension in EDL (↓) RCR in GTN and DIA (↓) State III respiration in GTN
[57]	Whole-body *Bmal1* knockout and *Bmal1 knockout*+SKM-AAV9-*Bmal1*	***Bmal1*$^{-/-}$ *versus* WT** (↑) *Cry1*, *Clock*, *Npas2* (↓) *Per3, Nr1d1, Ror*α*, Dbp* **SKM-AAV9-Bmal1 *versus* WT** (↑) *Per2*, *Cry1*, *Cry2*, *Npas2* (↓) *Nr1d1, Nr1d2, Tcap, Mylk4*	NE	**Multiomics *Bmal1*$^{-/-}$ *versus* SKM-AAV9-Bmal1** (↓) Mitochondrial protein levels	***Bmal1*$^{-/-}$ *versus* WT** (↓) Body weight, lean mass and fat mass (↑) Glucose under glucose tolerance test (GTT) **SKM-AAV9-Bmal1 *versus* WT** (↓) Body weight, lean mass and fat mass (=) Glucose under GTT	***Bmal1*$^{-/-}$ *versus* WT** (↑) *Myod1* (↓) *Tcap, Mylk4* (↓) Wheel activity (↓) Muscle weight and CSA in QUA (↓) Specific force in EDL **SKM-AAV9-Bmal1 *versus* WT** (↓) *Tcap, Mylk4* (↓) Wheel activity (↓) Muscle weight and CSA in QUA (=) Specific force in EDL

(*Continued*)

Table 1. (Continued)

Species/cell type	Experimental model/condition	Circadian clock/rhythm modification	Effects on mitochondrial dynamics in the SkM	Effects on genes or proteins related to mitochondrial biology in the SkM	Whole-body metabolic and physiological effects	SkM function/quality/ metabolic effects
[58]	Muscle-specific *Bmal1* knockout	Loss of CR in REV-ERBα and REV-ERBβ in VL muscle	NE	Abnormal *Ucp3* CR in TA Induction of *Ucp2* CR in SOL	(↑) Lean mass (↓) Fat mass (=) Body weight (↑) Energy expenditure (↓) RER	(↑) Protein synthesis in TA (↑) Palmitate oxidation in GTN Loss of triglyceride CR in GTN (↓) Maximum catalytic activity of mitochondrial complex II+III and complex IV in VL (=) CS activity in VL
[68]	Inducible muscle-specific *Bmal1* knockout (iMS*Bmal1*) Whole-body *Bmal1* knockout (***Bmal1−/−***)	**iMS*Bmal1*** (↓) *Bmal1*, *Per2*, *Rora*, *Rev-Erb*α (↑) *Clock* (=) *Cry1*	NE	**iMS*Bmal1*** (↑) *Pgc-1*α (=) Succinate dehydrogenase (SDH)-positive fibres ***Bmal1−/−*** (↑) SDH-positive fibres	NE	**iMS*Bmal1*** (↓) Specific force and *Myod1* (=) Fibre CSA and fibres with central nuclei (=) Paired box 7 (PAX7) ***Bmal1−/−*** (↓) Specific force and fibre CSA (↑) Fibre with central nuclei (–) PAX7
[69]	iMS *Bmal1* knockout at 15 weeks of age (2 weeks post tamoxifen treatment)	NE	(↑) Mitochondrial size (↓) Mitochondrial number (↑) Mitochondrial damage	(↓) SDH-positive fibres in GTN	(=) Body weight	(↓) GTN fibre CSA (↓) Spontaneous locomotion (↑) GTN weight

(*Continued*)

Table 1. (Continued)

Species/cell type	Experimental model/condition	Circadian clock/rhythm modification	Effects on mitochondrial dynamics in the SkM	Effects on genes or proteins related to mitochondrial biology in the SkM	Whole-body metabolic and physiological effects	SkM function/quality/ metabolic effects
[60]	Muscle-specific *PER2* knockout	(↓) *Dbp, Clock, Cry2, Rorα*	NE	**Proteomics** (=) Mitochondrial protein levels	(=) Body weight, lean and fat mass, energy expenditure and total food intake (↓) RER and body temperature in the night-time (↑) Glucose under GTT in the daytime	(=) Fibre type distribution and maximal treadmill running capacity (↓) Total locomotion in the night **Proteomics** (↓) Muscle contraction protein levels

Note: Italic font has been used for mRNA levels and capital letters for protein levels.

Abbreviations: BMAL1, brain and muscle Arnt-like protein-1; CLOCK, circadian locomotor output cycles kaput; CR, circadian rhythmicity; CSA, cross-section area; DIA, diaphragm; DRP1, dynamin-related protein; EDL, extensor digitorum longus; FIS1, fission protein 1; GTN, gastrocnemius; iMS, inducible muscle specific; NE, not evaluated; NR, non-rhythmicity; OPA1, optic atrophy protein 1; OXPHOS, oxidative phosphorylation complex; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator; PINK1, PTEN-induced kinase 1; QUA, quadriceps; RCR, respiratory control ratio; REE, resting energy expenditure; RER, respiratory exchange ratio; SkM, skeletal muscle; SOL, soleus; TA, tibialis anterior; TOMM20, translocase of outer mitochondrial membrane 20; VDAC, voltage-dependent anion channel; VL, vastus lateralis; =, without changes.

individuals exhibited altered fluctuations throughout the day in FIS1 and PINK1 protein levels, as well as in RER and ADP-stimulated respiration in SkM, indicating a disruption in mitochondrial dynamics and metabolic function adaptation throughout the day (Wefers et al., 2020). These alterations in mitochondrial dynamics and metabolic function, despite unchanged CR in clock gene expression, may be explained by posttranslational modifications of circadian clock proteins.

Therefore we showed that in VL biopsies taken between 8:00 and 9:00 AM, BMAL1 protein levels are directly associated with mitochondrial size (fusion phenotype) and crista density, and indirectly with the interaction between mitochondria and SR (Figueroa-Toledo et al., 2024). Additionally CLOCK protein levels were found to correlate directly with the protein mass of OPA1 and DRP1, as well as with maximal muscle strength and key components of the protein synthesis pathway, including mTOR, AKT and P70S6K (Figueroa-Toledo et al., 2024). Another study on four consecutive simulated day-shift schedules found no changes in circadian clock gene expression in follicular cells, nor in PGC-1α, mitochondrial uncoupling protein 3 (UCP3), transcription factor A, mitochondrial (TFAM) and mitochondrial complexes II–V proteins levels, or mitochondrial respiration in SkM (Bescos et al., 2018). However shift schedules decreased SkM insulin sensitivity, as assessed by the euglycaemic–hyperinsulinaemic clamp (Bescos et al., 2018). Unfortunately mitochondrial dynamics were not evaluated in this study.

To summarize human studies have demonstrated that circadian clock gene expression exhibits CR in SkM, whereas mitochondrial density does not exhibit similar rhythmic fluctuations throughout the day. Although mitochondrial dynamics proteins fluctuate, they do not follow a clear CR. These findings also suggest that circadian clock proteins may influence mitochondrial dynamics and muscle function. However further confirmation through genetic animal models is needed.

Genetic murine models. Whole-body *Clock*Δ*19* and *Bmal1* knockout mice had a lower mitochondrial density due to lower *Pgc-1*α levels, decreased mitochondrial state III respiration and lower SkM strength associated to a loss in CR in *MyoD* (Andrews et al., 2010). Moreover a key study showed that restoring BMAL1 expression in SkM of Bmal1 knockout mice using adeno-associated viruses reactivated circadian rhythms; improved muscle strength, mobility and insulin sensitivity; and extended lifespan, without altering muscle fibre size. Multiomics analysis linked BMAL1 to enhanced mitochondrial protein expression (Gutierrez-Monreal et al., 2024). Conversely SkM-specific Bmal1 knockout impaired mitochondrial function, leading to bioactive lipid accumulation and metabolic inefficiency (Dyar et al., 2018).

However in contrast to previous studies on the role of BMAL1 in SkM size, SkM-specific *Bmal1* knockout resulted in a higher lean-to-fat mass ratio, linked to increased protein synthesis in SkM (Dyar et al., 2018). Similarly inducible SkM-specific *Bmal1* knockout mice also exhibited a higher lean-to-fat mass ratio but exhibited decreased grip strength and reduced maximal speed during an exercise test (Dyar et al., 2018). Additionally inducible SkM-specific *Bmal1* knockout demonstrated a blunted transcriptional response to acute exercise, with no upregulation of key exercise-responsive transcription factors such as *Nr4a3* and *Ppargc1a* (Viggars et al., 2024). Discrepancies in BMAL1 control of SkM size and function likely reflect model-specific effects: whole-body knockout reduces SkM mass and strength, whereas SkM-specific deletion paradoxically increases lean mass but impairs contractile performance. This suggests that BMAL1 differentially regulates muscle growth and functional capacity, potentially through distinct mechanisms, or BMAL1 SkM-specific deletion induces pseudohypertrophy, as seen in conditions such as myotonia congenita or neuromyotonia (Walters, 2017).

In contrast to BMAL1 SkM-specific *Per2* knockout mice exhibited no changes in mitochondrial protein levels, body composition or REE. However they exhibited metabolic disruptions, including reduced nocturnal RER and daytime insulin resistance. Although muscle fibre type and maximal running capacity were unaffected, *Per2* deletion decreased night-time locomotion and downregulated proteins involved in muscle contraction (Mansingh et al., 2024).

Finally the regulation of SkM function by circadian clock proteins and their interaction with mitochondrial dynamics is a complex and evolving field (Fig. 2). Both human and murine studies highlight the potential role of circadian clock proteins, particularly BMAL1, in modulating mitochondrial function and SkM performance (Fig. 2). However although circadian clock proteins are clearly involved in regulating both mitochondrial dynamics and muscle function, the precise mechanisms and interactions remain poorly understood. Investigating the effects of metabolic challenges such as ageing, obesity or exercise on circadian clock, mitochondrial dynamics and SkM function could provide valuable insights into their interconnected roles and help further elucidate their impact on muscle health and metabolism.

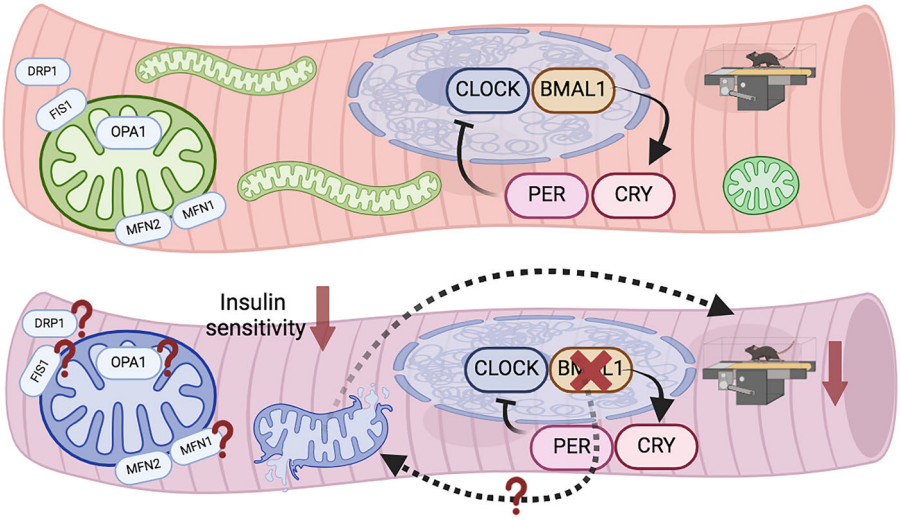

Figure 2. Effects of BMAL1 (brain and muscle Arnt-like protein-1) deletion on mitochondrial function/morphology, muscle function and insulin sensitivity in SkM (skeletal muscle)
Disruption of the core circadian clock protein BMAL1 alters mitochondrial dynamics, leading to decreased SkM quality, including diminished fibre size, exercise capacity, contractile strength and insulin sensitivity. The molecular mechanisms linking circadian clock proteins to mitochondrial dynamics regulation remain incompletely understood in SkM.

Concluding remarks and future perspectives

In this review we analysed emerging evidence that suggests that circadian clock proteins regulate mitochondrial dynamics in SkM, influencing mitochondrial function. Although these findings provide valuable insights into the complex interaction between circadian clock, especially BMAL1 protein, mitochondrial dynamics and SkM function, much remains to be understood. The molecular mechanisms underlying the regulation of mitochondrial dynamics by circadian proteins, and how these pathways contribute to metabolic and SkM health, remain incompletely understood. Previous findings show that molecular clock regulates rhythmic NAD^+ biosynthesis and mitochondrial respiration via modulation of mitochondrial protein acetylation, aligning oxidative metabolism with the 24-h fasting–feeding cycle. Specifically circadian control of the NAD^+-dependent deacetylase SIRT3 drives daily oscillations in mitochondrial respiration (Peek et al., 2013). Investigating whether similar mechanisms exist in SkM and influence performance, adaptation and disease is a promising direction for future research.

Additionally more human studies are needed to validate current findings and assess the translational potential of targeting circadian clock pathways to manage mitochondrial dynamics in metabolic disorders such as obesity, insulin resistance and sarcopenia. As an alternative to SkM biopsies, primary human myotubes offer a valuable model. A seminal study demonstrated that in T2DM myotubes, core clock gene cycling is disrupted. Genes associated with the IMM exhibit rhythmic expression in myotubes from individuals with normal glucose tolerance, but not in those with T2DM, and are positively correlated with insulin sensitivity (Gabriel et al., 2021). This highlights primary myotubes as a relevant system for investigating circadian–metabolic interactions in diabetes pathogenesis and SkM diseases. Finally to the best of our knowledge, no studies have yet examined the role of circadian clock proteins in regulating mitophagy, mitochondria–SR or lipid droplet interactions or mitochondrial supercomplex assembly in SkM.

Although significant progress has been made in understanding the interplay between the circadian clock, mitochondrial dynamics and SkM function, key questions remain unresolved. Notably it is still unclear whether BMAL1 deletion-induced mitochondrial dysregulation drives or results from SkM deterioration. Elucidating the molecular mechanisms governing these interactions, combined with clinical studies on circadian interventions, could unlock novel therapeutic strategies for metabolic and muscle-related disorders.

References

Andrews, J. L., Zhang, X., McCarthy, J. J., McDearmon, E. L., Hornberger, T. A., Russell, B., Campbell, K. S., Arbogast, S., Reid, M. B., Walker, J. R., Hogenesch, J. B., Takahashi, J. S., & Esser, K. A. (2010). CLOCK and BMAL1 regulate MyoD and are necessary for maintenance of skeletal muscle phenotype and function. *Proceedings of the National Academy of Sciences of the United States of America*, **107**(44), 19090–19095.

Baron, K. G., & Reid, K. J. (2014). Circadian misalignment and health. *International Review of Psychiatry (Abingdon, England)*, **26**(2), 139–154.

Baron, A. D., Brechtel, G., Wallace, P., & Edelman, S. V. (1988). Rates and tissue sites of non-insulin- and insulin-mediated glucose uptake in humans. *The American Journal of Physiology*, **255**(6 Pt 1), E769–E774.

Bescos, R., Boden, M. J., Jackson, M. L., Trewin, A. J., Marin, E. C., Levinger, I., Garnham, A., Hiam, D. S., Falcao-Tebas, F., Conte, F., Owens, J. A., Kennaway, D. J., & McConell, G. K. (2018). Four days of simulated shift work reduces insulin sensitivity in humans. *Acta Physiologica (Oxford, England)*, **223**(2), e13039.

Cairns, G., Thumiah-Mootoo, M., Abbasi, M. R., Gourlay, M., Racine, J., Larionov, N., Prola, A., Khacho, M., & Burelle, Y. (2024). PINK1 deficiency alters muscle stem cell fate decision and muscle regenerative capacity. *Stem Cell Reports*, **19**(5), 673–688.

Castro-Sepulveda, M., Jannas-Vela, S., Fernández-Verdejo, R., Ávalos-Allele, D., Tapia, G., Villagrán, C., Quezada, N., & Zbinden-Foncea, H. (2020). Relative lipid oxidation associates directly with mitochondrial fusion phenotype and mitochondria-sarcoplasmic reticulum interactions in human skeletal muscle. *American journal of physiology-Endocrinology and metabolism*, **318**(6), E848–E855.

Castro-Sepúlveda, M., Morio, B., Tuñón-Suárez, M., Jannas-Vela, S., Díaz-Castro, F., Rieusset, J., & Zbinden-Foncea, H. (2021). The fasting-feeding metabolic transition regulates mitochondrial dynamics. *Federation of American Societies of Experimental Biology journal: official publication of the Federation of American Societies for Experimental Biology*, **35**(10), e21891.

Castro-Sepulveda, M., Fernández-Verdejo, R., Tuñón-Suárez, M., Morales-Zúñiga, J., Troncoso, M., Jannas-Vela, S., & Zbinden-Foncea, H. (2021). Low abundance of Mfn2 protein correlates with reduced mitochondria-SR juxtaposition and mitochondrial cristae density in human men skeletal muscle: Examining organelle measurements from TEM images. *Federation of American Societies of Experimental Biology journal: official publication of the Federation of American Societies for Experimental Biology*, **35**(4), e21553.

Cárdenas, C., Miller, R. A., Smith, I., Bui, T., Molgó, J., Müller, M., Vais, H., Cheung, K. H., Yang, J., Parker, I., Thompson, C. B., Birnbaum, M. J., Hallows, K. R., & Foskett, J. K. (2010). Essential regulation of cell bioenergetics by constitutive InsP3 receptor Ca^{2+} transfer to mitochondria. *Cell*, **142**(2), 270–283.

Challet, E. (2019). The circadian regulation of food intake. *Nature reviews Endocrinology*, **15**(7), 393–405.

Chen, H., Chomyn, A., & Chan, D. C. (2005). Disruption of fusion results in mitochondrial heterogeneity and dysfunction. *The Journal of Biological Chemistry*, **280**(28), 26185–26192.

Chen, H., Detmer, S. A., Ewald, A. J., Griffin, E. E., Fraser, S. E., & Chan, D. C. (2003). Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *The Journal of Cell Biology*, **160**(2), 189–200.

Chen, H., Vermulst, M., Wang, Y. E., Chomyn, A., Prolla, T. A., McCaffery, J. M., & Chan, D. C. (2010). Mitochondrial fusion is required for mtDNA stability in skeletal muscle and tolerance of mtDNA mutations. *Cell*, **141**(2), 280–289.

Choi, Y. I., Park, D. K., Chung, J. W., Kim, K. O., Kwon, K. A., & Kim, Y. J. (2019). Circadian rhythm disruption is associated with an increased risk of sarcopenia: A nationwide population-based study in Korea. *Scientific Reports*, **9**(1), 12015.

Cogliati, S., Frezza, C., Soriano, M. E., Varanita, T., Quintana-Cabrera, R., Corrado, M., Cipolat, S., Costa, V., Casarin, A., Gomes, L. C., Perales-Clemente, E., Salviati, L., Fernandez-Silva, P., Enriquez, J. A., & Scorrano, L. (2013). Mitochondrial cristae shape determines respiratory chain supercomplexes assembly and respiratory efficiency. *Cell*, **155**(1), 160–171.

daSilva Rosa, S. C., Martens, M. D., Field, J. T., Nguyen, L., Kereliuk, S. M., Hai, Y., Chapman, D., Diehl-Jones, W., Aliani, M., West, A. R., Thliveris, J., Ghavami, S., Rampitsch, C., Dolinsky, V. W., & Gordon, J. W. (2021). BNIP3L/Nix-induced mitochondrial fission, mitophagy, and impaired myocyte glucose uptake are abrogated by PRKA/PKA phosphorylation. *Autophagy*, **17**(9), 2257–2272.

de Goede, P., Wefers, J., Brombacher, E. C., Schrauwen, P., & Kalsbeek, A. (2018). Circadian rhythms in mitochondrial respiration. *Journal of Molecular Endocrinology*, **60**(3), R115–R130.

Dulac, M., Leduc-Gaudet, J. P., Cefis, M., Ayoub, M. B., Reynaud, O., Shams, A., Moamer, A., Nery Ferreira, M. F., Hussain, S. N., & Gouspillou, G. (2021). Regulation of muscle and mitochondrial health by the mitochondrial fission protein Drp1 in aged mice. *The Journal of Physiology*, **599**(17), 4045–4063.

Dutheil, F., Baker, J. S., Mermillod, M., De Cesare, M., Vidal, A., Moustafa, F., Pereira, B., & Navel, V. (2020). Shift work, and particularly permanent night shifts, promote dyslipidaemia: A systematic review and meta-analysis. *Atherosclerosis*, **313**, 156–169.

Dyar, K. A., Ciciliot, S., Wright, L. E., Biensø, R. S., Tagliazucchi, G. M., Patel, V. R., Forcato, M., Paz, M. I., Gudiksen, A., Solagna, F., Albiero, M., Moretti, I., Eckel-Mahan, K. L., Baldi, P., Sassone-Corsi, P., Rizzuto, R., Bicciato, S., Pilegaard, H., Blaauw, B., & Schiaffino, S. (2013). Muscle insulin sensitivity and glucose metabolism are controlled by the intrinsic muscle clock. *Molecular Metabolism*, **3**(1), 29–41.

Dyar, K. A., Hubert, M. J., Mir, A. A., Ciciliot, S., Lutter, D., Greulich, F., Quagliarini, F., Kleinert, M., Fischer, K., Eichmann, T. O., Wright, L. E., Peña Paz, M. I., Casarin, A., Pertegato, V., Romanello, V., Albiero, M., Mazzucco, S., Rizzuto, R., Salviati, L., … Uhlenhaut, N. H. (2018). Transcriptional programming of lipid and amino acid metabolism by the skeletal muscle circadian clock. *PLoS Biology*, **16**(8), e2005886.

Eisner, V., Lenaers, G., & Hajnóczky, G. (2014). Mitochondrial fusion is frequent in skeletal muscle and supports excitation-contraction coupling. *The Journal of Cell Biology*, **205**(2), 179–195.

Eisner, V., Picard, M., & Hajnóczky, G. (2018). Mitochondrial dynamics in adaptive and maladaptive cellular stress responses. *Nature Cell Biology*, **20**(7), 755–765.

Favaro, G., Romanello, V., Varanita, T., Andrea Desbats, M., Morbidoni, V., Tezze, C., Albiero, M., Canato, M., Gherardi, G., De Stefani, D., Mammucari, C., Blaauw, B., Boncompagni, S., Protasi, F., Reggiani, C., Scorrano, L., Salviati, L., & Sandri, M. (2019). DRP1-mediated mitochondrial shape controls calcium homeostasis and muscle mass. *Nature Communications*, **10**(1), 2576.

Figueroa-Toledo, A. M., Gutiérrez-Pino, J., Carriel-Nesvara, A., Marchese-Bittencourt, M., Zbinden-Foncea, H., & Castro-Sepúlveda, M. (2024). BMAL1 and CLOCK proteins exhibit differential association with mitochondrial dynamics, protein synthesis pathways and muscle strength in human muscle. *The Journal of Physiology*, **602**(23), 6403–6415.

Gabriel, B. M., Altıntaş, A., Smith, J. A. B., Sardon-Puig, L., Zhang, X., Basse, A. L., Laker, R. C., Gao, H., Liu, Z., Dollet, L., Treebak, J. T., Zorzano, A., Huo, Z., Rydén, M., Lanner, J. T., Esser, K. A., Barrès, R., Pillon, N. J., Krook, A., & Zierath, J. R. (2021). Disrupted circadian oscillations in type 2 diabetes are linked to altered rhythmic mitochondrial metabolism in skeletal muscle. *Science Advances*, **7**(43), eabi9654.

Gan, Y., Yang, C., Tong, X., Sun, H., Cong, Y., Yin, X., Li, L., Cao, S., Dong, X., Gong, Y., Shi, O., Deng, J., Bi, H., & Lu, Z. (2015). Shift work and diabetes mellitus: A meta-analysis of observational studies. *Occupational and Environmental Medicine*, **72**(1), 72–78.

Gemmink, A., Daemen, S., Wefers, J., Hansen, J., van Moorsel, D., Astuti, P., Jorgensen, J. A., Kornips, E., Schaart, G., Hoeks, J., Schrauwen, P., & Hesselink, M. K. C. (2023). Twenty-four hour rhythmicity in mitochondrial network connectivity and mitochondrial respiration; a study in human skeletal muscle biopsies of young lean and older individuals with obesity. *Molecular Metabolism*, **72**, 101727.

Gouspillou, G., Godin, R., Piquereau, J., Picard, M., Mofarrahi, M., Mathew, J., Purves-Smith, F. M., Sgarioto, N., Hepple, R. T., Burelle, Y., & Hussain, S. N. A. (2018). Protective role of Parkin in skeletal muscle contractile and mitochondrial function. *The Journal of Physiology*, **596**(13), 2565–2579.

Gutierrez-Monreal, M. A., Wolff, C. A., Rijos, E. E., Viggars, M. R., Douglas, C. M., Pagala, V., Peng, J., Hunt, L. C., Ding, H., Huo, Z., Demontis, F., & Esser, K. A. (2024). Targeted Bmal1 restoration in muscle prolongs lifespan with systemic health effects in aging model. *Journal of Clinical Investigation Insight*, **9**(22), e174007.

He, B., Yu, H., Liu, S., Wan, H., Fu, S., Liu, S., Yang, J., Zhang, Z., Huang, H., Li, Q., Wang, F., Jiang, Z., Liu, Q., & Jiang, H. (2022). Mitochondrial cristae architecture protects against mtDNA release and inflammation. *Cell Reports*, **41**(10), 111774.

Held, N. M., Wefers, J., van Weeghel, M., Daemen, S., Hansen, J., Vaz, F. M., van Moorsel, D., Hesselink, M. K. C., Houtkooper, R. H., & Schrauwen, P. (2020). Skeletal muscle in healthy humans exhibits a day-night rhythm in lipid metabolism. *Molecular Metabolism*, **37**, 100989.

Hernández-Alvarez, M. I., Sebastián, D., Vives, S., Ivanova, S., Bartoccioni, P., Kakimoto, P., Plana, N., Veiga, S. R., Hernández, V., Vasconcelos, N., Peddinti, G., Adrover, A., Jové, M., Pamplona, R., Gordaliza-Alaguero, I., Calvo, E., Cabré, N., Castro, R., Kuzmanic, A., … Zorzano, A. (2019). Deficient endoplasmic Reticulum-mitochondrial phosphatidylserine transfer causes liver disease. *Cell*, **177**(4), 881–895.e17.

IARC Monographs Vol 124 group. (2019). Carcinogenicity of night shift work. *The Lancet Oncology*, **20**(8), 1058–1059.

Irazoki, A., Martinez-Vicente, M., Aparicio, P., Aris, C., Alibakhshi, E., Rubio-Valera, M., Castellanos, J., Lores, L., Palacín, M., Gumà, A., Zorzano, A., & Sebastián, D. (2022). Coordination of mitochondrial and lysosomal homeostasis mitigates inflammation and muscle atrophy during aging. *Aging Cell*, **21**(4), e13583.

Jheng, H. F., Tsai, P. J., Guo, S. M., Kuo, L. H., Chang, C. S., Su, I. J., Chang, C. R., & Tsai, Y. S. (2012). Mitochondrial fission contributes to mitochondrial dysfunction and insulin resistance in skeletal muscle. *Molecular and Cellular Biology*, **32**(2), 309–319.

Jin, Z., Ji, Y., Su, W., Zhou, L., Wu, X., Gao, L., Guo, J., Liu, Y., Zhang, Y., Wen, X., Xia, Z. Y., Xia, Z., & Lei, S. (2023). The role of circadian clock-controlled mitochondrial dynamics in diabetic cardiomyopathy. *Frontiers in Immunology*, **14**, 1142512.

Kelley, D. E., He, J., Menshikova, E. V., & Ritov, V. B. (2002). Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes*, **51**(10), 2944–2950.

Kleele, T., Rey, T., Winter, J., Zaganelli, S., Mahecic, D., Perreten Lambert, H., Ruberto, F. P., Nemir, M., Wai, T., Pedrazzini, T., & Manley, S. (2021). Distinct fission signatures predict mitochondrial degradation or biogenesis. *Nature*, **593**(7859), 435–439.

Liesa, M., Palacín, M., & Zorzano, A. (2009). Mitochondrial dynamics in mammalian health and disease. *Physiological Reviews*, **89**(3), 799–845.

Liu, J., Zhou, B., Yan, M., Huang, R., Wang, Y., He, Z., Yang, Y., Dai, C., Wang, Y., Zhang, F., & Zhai, Q. (2016). CLOCK and BMAL1 regulate muscle insulin sensitivity via SIRT1 in male mice. *Endocrinology*, **157**(6), 2259–2269.

Malka, F., Guillery, O., Cifuentes-Diaz, C., Guillou, E., Belenguer, P., Lombès, A., & Rojo, M. (2005). Separate fusion of outer and inner mitochondrial membranes. *European Molecular Biology Organization Reports*, **6**(9), 853–859.

Mancilla, R., Brouwers, B., Schrauwen-Hinderling, V. B., Hesselink, M. K. C., Hoeks, J., & Schrauwen, P. (2021). Exercise training elicits superior metabolic effects when performed in the afternoon compared to morning in metabolically compromised humans. *Physiological Reports*, **8**(24), e14669.

Marcheva, B., Ramsey, K. M., Buhr, E. D., Kobayashi, Y., Su, H., Ko, C. H., Ivanova, G., Omura, C., Mo, S., Vitaterna, M. H., Lopez, J. P., Philipson, L. H., Bradfield, C. A., Crosby, S. D., JeBailey, L., Wang, X., Takahashi, J. S., & Bass, J. (2010). Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. *Nature*, **466**(7306), 627–631.

Martin, R. A., Viggars, M. R., & Esser, K. A. (2023). Metabolism and exercise: The skeletal muscle clock takes centre stage. *Nature Reviews Endocrinology*, **19**(5), 272–284.

Mansingh, S., Maier, G., Delezie, J., Westermark, P. O., Ritz, D., Duchemin, W., Santos, G., Karrer-Cardel, B., Steurer, S. A., Albrecht, U., & Handschin, C. (2024). More than the clock: Distinct regulation of muscle function and metabolism by PER2 and rorα. *The Journal of Physiology*, **602**(23), 6373–6402.

Moore, T. M., Zhou, Z., Cohn, W., Norheim, F., Lin, A. J., Kalajian, N., Strumwasser, A. R., Cory, K., Whitney, K., Ho, T., Ho, T., Lee, J. L., Rucker, D. H., Shirihai, O., van der Bliek, A. M., Whitelegge, J. P., Seldin, M. M., Lusis, A. J., Lee, S., … Hevener, A. L. (2019). The impact of exercise on mitochondrial dynamics and the role of Drp1 in exercise performance and training adaptations in skeletal muscle. *Molecular Metabolism*, **21**, 51–67.

Onishi, M., Yamano, K., Sato, M., Matsuda, N., & Okamoto, K. (2021). Molecular mechanisms and physiological functions of mitophagy. *The European Molecular Biology Organization Journal*, **40**(3), e104705.

Peek, C. B., Affinati, A. H., Ramsey, K. M., Kuo, H. Y., Yu, W., Sena, L. A., Ilkayeva, O., Marcheva, B., Kobayashi, Y., Omura, C., Levine, D. C., Bacsik, D. J., Gius, D., Newgard, C. B., Goetzman, E., Chandel, N. S., Denu, J. M., Mrksich, M., & Bass, J. (2013). Circadian clock NAD+ cycle drives mitochondrial oxidative metabolism in mice. *Science (New York, NY)*, **342**(6158), 1243417.

Peek, C. B., Levine, D. C., Cedernaes, J., Taguchi, A., Kobayashi, Y., Tsai, S. J., Bonar, N. A., McNulty, M. R., Ramsey, K. M., & Bass, J. (2017). Circadian clock interaction with HIF1α mediates oxygenic metabolism and Anaerobic glycolysis in skeletal muscle. *Cell Metabolism*, **25**(1), 86–92.

Peker, N., Donipadi, V., Sharma, M., McFarlane, C., & Kambadur, R. (2018). Loss of Parkin impairs mitochondrial function and leads to muscle atrophy. *American journal of Physiology-Cell Physiology*, **315**(2), C164–C185.

Partch, C. L., Green, C. B., & Takahashi, J. S. (2014). Molecular architecture of the mammalian circadian clock. *Trends in Cell Biology*, **24**(2), 90–99.

Perrin, L., Loizides-Mangold, U., Chanon, S., Gobet, C., Hulo, N., Isenegger, L., Weger, B. D., Migliavacca, E., Charpagne, A., Betts, J. A., Walhin, J. P., Templeman, I., Stokes, K., Thompson, D., Tsintzas, K., Robert, M., Howald, C., Riezman, H., Feige, J. N., … Dibner, C. (2018). Transcriptomic analyses reveal rhythmic and CLOCK-driven pathways in human skeletal muscle. *eLife*, **7**, e34114.

Reynaud, O., Wang, J., Ayoub, M. B., Leduc-Gaudet, J. P., Mayaki, D., Dulac, M., Hussain, S. N. A., Bergeron, R., & Gouspillou, G. (2023). The impact of high-fat feeding and parkin overexpression on skeletal muscle mass, mitochondrial respiration, and H2O2 emission. *American journal of Physiology-Cell Physiology*, **324**(2), C366–C376.

Sebastián, D., Sorianello, E., Segalés, J., Irazoki, A., Ruiz-Bonilla, V., Sala, D., Planet, E., Berenguer-Llergo, A., Muñoz, J. P., Sánchez-Feutrie, M., Plana, N., Hernández-Álvarez, M. I., Serrano, A. L., Palacín, M., & Zorzano, A. (2016). Mfn2 deficiency links age-related sarcopenia and impaired autophagy to activation of an adaptive mitophagy pathway. *The European Molecular Biology Organization Journal*, **35**(15), 1677–1693.

Stenvers, D. J., Scheer, F. A. J. L., Schrauwen, P., la Fleur, S. E., & Kalsbeek, A. (2019). Circadian clocks and insulin resistance. *Nature Reviews Endocrinology*, **15**(2), 75–89.

Tezze, C., Romanello, V., Desbats, M. A., Fadini, G. P., Albiero, M., Favaro, G., Ciciliot, S., Soriano, M. E., Morbidoni, V., Cerqua, C., Loefler, S., Kern, H., Franceschi, C., Salvioli, S., Conte, M., Blaauw, B., Zampieri, S., Salviati, L., Scorrano, L., & Sandri, M. (2017). Age-associated loss of OPA1 in muscle impacts muscle mass, metabolic homeostasis, systemic inflammation, and epithelial senescence. *Cell Metabolism*, **25**(6), 1374–1389.e6.

Turek, F. W., Joshu, C., Kohsaka, A., Lin, E., Ivanova, G., McDearmon, E., Laposky, A., Losee-Olson, S., Easton, A., Jensen, D. R., Eckel, R. H., Takahashi, J. S., & Bass, J. (2005). Obesity and metabolic syndrome in circadian clock mutant mice. *Science (New York, NY)*, **308**(5724), 1043–1045.

van der Velde, J. H. P. M., Boone, S. C., Winters-van Eekelen, E., Hesselink, M. K. C., Schrauwen-Hinderling, V. B., Schrauwen, P., Lamb, H. J., Rosendaal, F. R., & de Mutsert, R. (2023). Timing of physical activity in relation to liver fat content and insulin resistance. *Diabetologia*, **66**(3), 461–471.

van Moorsel, D., Hansen, J., Havekes, B., Scheer, F. A. J. L., Jörgensen, J. A., Hoeks, J., Schrauwen-Hinderling, V. B., Duez, H., Lefebvre, P., Schaper, N. C., Hesselink, M. K. C., Staels, B., & Schrauwen, P. (2016). Demonstration of a day-night rhythm in human skeletal muscle oxidative capacity. *Molecular Metabolism*, **5**(8), 635–645.

Viggars, M. R., Berko, H. E., Hesketh, S. J., Wolff, C. A., Gutierrez-Monreal, M. A., Martin, R. A., Jennings, I. G., Huo, Z., & Esser, K. A. (2024). Skeletal muscle BMAL1 is necessary for transcriptional adaptation of local and peripheral tissues in response to endurance exercise training. *Molecular Metabolism*, **86**, 101980.

Walters, J. (2017). Muscle hypertrophy and pseudohypertrophy. *Practical Neurology*, **17**(5), 369–379.

Wai, T., & Langer, T. (2016). Mitochondrial dynamics and metabolic regulation. *Trends in Endocrinology and Metabolism: TEM*, **27**(2), 105–117.

Wefers, J., Connell, N. J., Fealy, C. E., Andriessen, C., de Wit, V., van Moorsel, D., Moonen-Kornips, E., Jörgensen, J. A., Hesselink, M. K. C., Havekes, B., Hoeks, J., & Schrauwen, P. (2020). Day-night rhythm of skeletal muscle metabolism is disturbed in older, metabolically compromised individuals. *Molecular Metabolism*, **41**, 101050.

Zhang, Z., Sliter, D. A., Bleck, C. K. E., & Ding, S. (2019). Fis1 deficiencies differentially affect mitochondrial quality in skeletal muscle. *Mitochondrion*, **49**, 217–226.

Zhang, R., Lahens, N. F., Ballance, H. I., Hughes, M. E., & Hogenesch, J. B. (2014). A circadian gene expression atlas in mammals: Implications for biology and medicine. *Proceedings of the National Academy of Sciences of the United States of America*, **111**(45), 16219–16224.

Additional information

Competing interests

The authors declare no competing interests.

Author contributions

M.C.-S. conducted the literature search, prepared the figures and wrote the first draft of the paper. A.C.-N., C.M.-M. and L.D. contributed to the drafting and revision of the paper. All authors approved the final version of the manuscript, agreed to be accountable for all aspects of the work, acknowledged that they qualify for authorship and confirmed that all who qualify for authorship are listed.

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Keywords

circadian clock, insulin resistance, metabolism, mitochondrial dynamics, sarcopenia, skeletal muscle

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

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