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SYMPOSIUM REVIEW

Zeitgebers of skeletal muscle and implications for metabolic health

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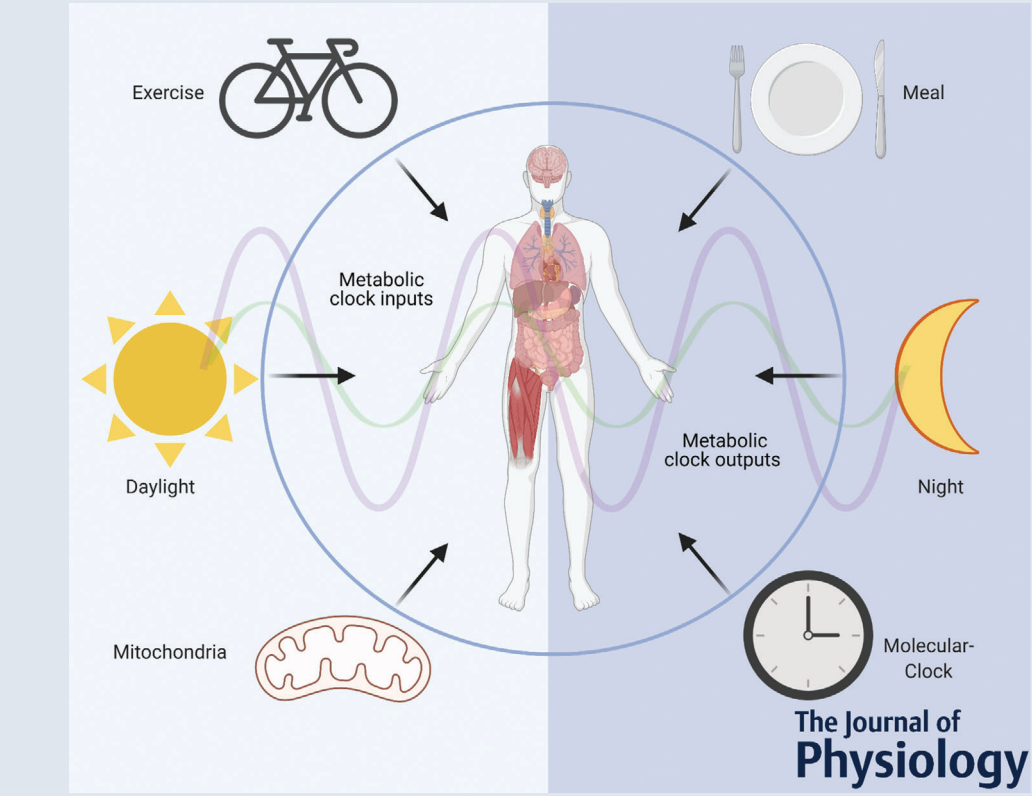

Abstract Metabolic health is a crucial area of current research, and is an outcome of innate physiology, and interactions with the environment. Environmental cues, such as the Earth's day-night rhythm, partly regulate diurnal hormones and metabolites. Circadian physiology consists of highly conserved biological processes over ∼24-h cycles, which are influenced by external cues (Zeitgebers – '*time-keepers*'). Skeletal muscle has diurnal variations of a large magnitude, owing in part to the strong nature of physical activity throughout the day and other external Zeitgebers. The orchestration of whole-body and skeletal muscle metabolism is a complex, finely tuned process, and molecular diurnal variations are regulated by a transcription-translation feedback loop controlled by the molecular clock, as well as non-transcriptional metabolic processes. The mitochondrion may play an important role in

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regulating diurnal metabolites within skeletal muscle, given its central role in the regulation of NAD^+/NADH, O_2, reactive oxygen species and redox metabolism. These molecular pathways display diurnal variation and illustrate the complex orchestration of circadian metabolism in skeletal muscle. Probably the most robust Zeitgeber of skeletal muscle is exercise, which alters glucose metabolism and flux, in addition to a range of other diurnal metabolic pathways. Indeed, performing exercise at different times of the day may alter metabolism and health outcomes in some cohorts. The objective of this Symposium Review is to briefly cover the current literature, and to speculate regarding future areas of research. Thus, we postulate that metabolic health may be optimized by altering the timing of external cues such as diet and exercise.

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Abstract figure legend A schematic of the themes covered in this Symposium Review. Illustrated are some of the major Zeitgebers of skeletal muscle and their links to metabolism.

The orchestration of diurnal metabolism

Early organisms may have evolved circadian cellular rhythms in response to selection pressure from the ionising effects of ultra-violet light (Dvornyk *et al.* 2003; Hanawalt, 2015; Negelspach *et al.* 2018). Maintenance of these rhythms in the face of the Earth's day-night cycle is an important evolutionary driver, as circadian rhythms are present in nearly all complex organisms (Andreani *et al.* 2015). At a cellular level, a set of genes with time-keeping abilities is highly conserved across evolutionary biology and has been observed in organisms ranging from prokaryotes to humans (Dvornyk *et al.* 2003). These sets of genes are expressed in nearly all cells of the body and form a cell-autonomous autoregulatory transcription translation feedback loop comprising the transcriptional activators CLOCK and BMAL1 (*ARNTL*), and target genes Period (*PER*), Cryptochrome (*CRY*), and REV-ERBα (*NR1D1*) (Fig. 1) (Panda, 2016).

The suprachiasmatic nucleus (SCN) region of the hypothalamus acts as a central co-ordinator of circadian rhythms, and manipulation of this centre drastically alters metabolic processes within the body (Ding *et al.* 2018). However, peripheral tissues are able to maintain metabolic and circadian rhythms even in the absence of the SCN (Mohawk *et al.* 2012; Koronowski *et al.* 2019). The relationship between the 'core-clock' and metabolism is demonstrated in mouse models with loss-of-function of *Clock* or *Bmal1*, which are prone to obesity and insulin resistance (Dyar *et al.* 2014; Harfmann *et al.* 2016; Schiaffino *et al.* 2016). Additionally, metabolic gene-sets are influenced by these knock-out models, and core-clock genes directly bind to the promoter regions of genes controlling pathways involved in lipid, amino acid and mitochondrial metabolism (Dyar *et al.* 2014, 2018*a*). Tissue and plasma metabolite rhythms are disrupted by loss-of-function of *Clock* or *Bmal1* (Nakahata *et al.* 2009). Extrinsic stimuli that effect circadian rhythms

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(Zeitgebers – 'time-keepers') such as food, light, heat and exercise are also important regulators of metabolic rhythms and can partly replace the time-keeping role of the SCN in some tissues (Manoogian & Panda, 2017). The regulation of metabolism, and metabolites within central and peripheral tissues, may confer an evolutionary advantage to organisms that are able to beneficially harness these rhythms to match diurnal feeding and activity cycles. Conversely, in incidences where these rhythms are dysregulated, there can be serious deleterious consequences for the health of the effected organism (Schiaffino *et al.* 2016; Gabriel & Zierath, 2019). Growing evidence suggests the deleterious consequences of dysregulated circadian rhythms (often induced by shift-work in human studies) may impinge upon metabolic health (Bescos *et al.* 2018; Vetter *et al.* 2018; Ghaben & Scherer, 2019). Poor metabolic health can be broadly defined as the dysregulated ability to process and store metabolites (e.g. impaired glucose tolerance) associated with an increased risk of developing co-morbidities such as type 2 diabetes mellitus (T2D) (McNeely *et al.* 2003; Proper *et al.* 2016; Vetter *et al.* 2018).

For clarity, in this review we define our usage of the terms 'diurnal': i.e. a diurnal cycle is any pattern that recurs every 24 h, not necessarily biological or intrinsic; and 'circadian': i.e. intrinsic, biological processes that recur naturally on a ∼24-h cycle, even in the absence of light fluctuations.

Diurnal metabolite and metabolomics analysis

Circadian control of metabolic pathways allows cells and tissues to orchestrate the processing and sequestration of pre-, and postprandial metabolites in an exact spatial and temporal manner. Oscillating metabolites can synchronise molecular clocks, alter transcriptional activity, and modify chromatin (O'Neill *et al.* 2008, 2011; Nakahata *et al.* 2009;

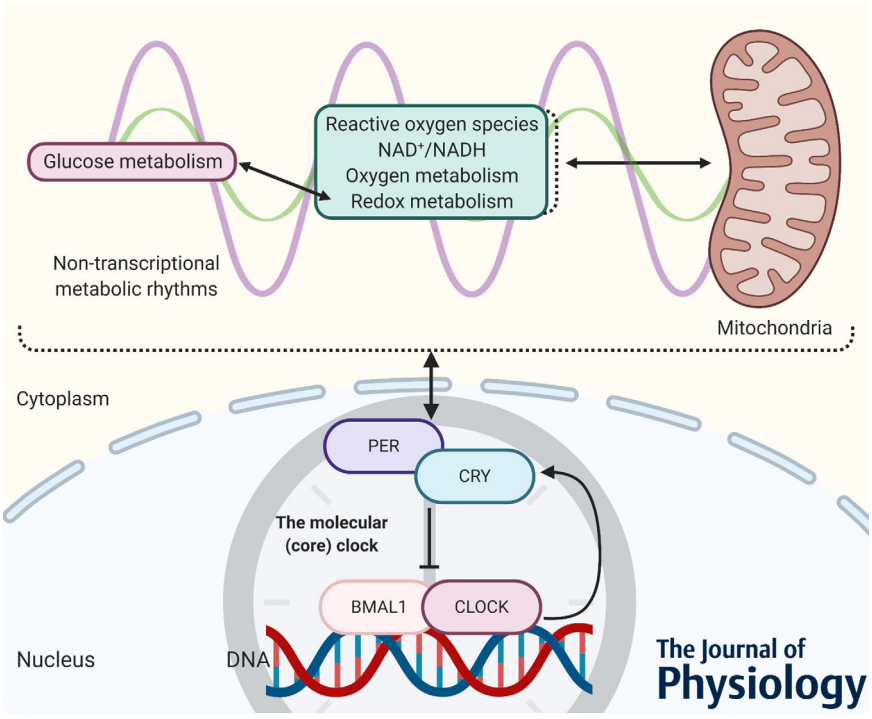

Figure 1. A schematic diagram of circadian cellular metabolism
In the nucleus, a simplified illustration of the cell-autonomous autoregulatory feedback loop comprising transcriptional activators CLOCK and BMAL1 (ARNTL) and target genes Period (PER) and Cryptochrome (CRY) (Panda, 2016; Gabriel & Zierath, 2019). In the cytoplasm, metabolites highly active within mitochondria play a key role in regulating circadian metabolism within skeletal muscle. There is bi-directional signalling between the mitochondria and the metabolism of NAD^+/NADH flux within skeletal muscle (White & Schenk, 2012). NAD^+/NADH flux oscillates in a circadian manner (Peek *et al.* 2013). Circadian hypoxia and reactive oxygen species (ROS) may be another feedback loop by which metabolism is orchestrated and sensed by circadian processes; this orchestration includes signalling to and from the mitochondria. Circadian redox rhythms appear to be driven by rhythmic glucose metabolism, including glycolysis and the pentose phosphate pathway (Ch *et al.* 2021). Whether bi-directional signalling occurs between circadian glycolytic pathways and diurnal redox metabolism is unclear, but evidence suggests this is likely.

Ramsey *et al.* 2009; Peek *et al.* 2013; Aviram *et al.* 2016). In divergent nutritional states throughout the course of a diurnal cycle, metabolic homeostasis is a crucial element in health, and the ability to sense, anticipate, and 'communicate' nutritional and metabolic requirements between tissues is a key element of the orchestration of diurnal metabolism.

Diurnal metabolites have been assessed in a variety of tissues (Dallmann *et al.* 2012; Eckel-Mahan *et al.* 2012; Aviram *et al.* 2016), although only recently has a comprehensive diurnal, metabolic atlas been produced that combines metabolite profiles from eight different tissues under standard diet and high-fat diet conditions (Dyar *et al.* 2018*b*). Applying an integrated systems biology approach, this atlas demonstrates temporal correlations between and within tissues taken from epididymal white adipose tissue, brown adipose tissue, liver, serum, cauda epididymal sperm, the SCN, gastrocnemius skeletal muscle, and the medial prefrontal cortex. However, the nutritional challenge of a 10-week high-fat diet led to a reduction of these temporal correlations, and an alteration of the metabolic signature. This atlas not only provides a comprehensive resource for circadian researchers, but also illustrates the complex orchestration of metabolism between and within a diverse range of tissues. Additionally, these data show that extrinsic factors, such as diet and obesity, can drastically alter these inter-connected systems.

Skeletal muscle, a key section of the tissue orchestra

Skeletal muscle is an important organ in metabolic health (Gabriel & Zierath, 2017), and also plays a vital role in regulating diurnal metabolism and substrate storage and utilisation (Dyar *et al.* 2014; Gabriel & Zierath, 2019). In diurnal transcriptomic studies of primates, skeletal muscle had the highest amount of oscillating transcripts of all tissues measured (Mure *et al.* 2018), indicating a high degree of diurnal metabolic oscillation in this tissue. The importance of skeletal muscle in diurnal and circadian biology is supported by findings in mice, where skeletal muscle-specific loss-of-function models of the core-clock have severely deleterious metabolic outcomes, such as insulin resistance and obesity (Dyar *et al.* 2014; Gabriel & Zierath, 2019). In a clinical-translational study of men with obesity (Lundell *et al.* 2020), skeletal muscle and serum metabolomic and transcriptomic profiling was utilised to assess the circadian effects of time-restricted feeding (TRF) in a randomised cross-over trial. TRF is a robust intervention, with data showing some promising beneficial effects on insulin sensitivity, blood pressure and oxidative stress in men with prediabetes (Sutton *et al.* 2018). TRF increased the amplitude of oscillating muscle transcripts compared to the control arm of the trial, but not serum or skeletal muscle metabolites (Lundell *et al.* 2020). Additionally, TRF induced the rhythmicity of several amino acid transporter genes and metabolites. However, gene expression of the core clock was similar between both arms of the trial. The robust effect of TRF on rhythmic amino acid metabolism in skeletal muscle is important to consider since there is a lack of a corresponding change in clock gene expression. This may lead one to ponder the intracellular conduction of communication between metabolic processes and the core clock in skeletal muscle.

The cellular orchestra: who is the conductor?

Outside of the transcriptional activity of the nucleus, skeletal muscle, and other tissues, have rhythmic metabolism within, and associated with, a variety of organelles. Probably the most studied, non-nucleic organelle in skeletal muscle is the mitochondrion. Metabolites highly active within mitochondria play a key role in regulating circadian metabolism within skeletal muscle. A large proportion of NAD^+/NADH flux within skeletal muscle occurs within mitochondria (White & Schenk, 2012), and oscillates in a circadian manner (Peek, 2020). Indeed, this appears to form part of a feedback signal by which the core-clock can sense and regulate internal cellular metabolism, as alterations in the core-clock alter NAD^+ concentrations, mitochondrial acetylation and mitochondrial rhythmic metabolism (Nakahata *et al.* 2009; Ramsey *et al.* 2009; Peek *et al.* 2013). The feedback signal of this loop is illustrated in an experimental study whereby core-clock loss-of-function mice were supplemented with extrinsic NAD^+, which allowed restoration of protein deacetylation and enhanced oxygen consumption (Peek *et al.* 2013). Additionally, NAD^+ metabolism is closely linked to respiration and oxidative metabolism, and these processes are also circadian in nature (de Goede *et al.* 2018). Mitochondrial respiration is also modulated via hypoxia-inducible factor 1-alpha (HIF1α) by the core-clock, and itself forms a feedback loop to modulate the core-clock (Peek *et al.* 2017). Hypoxia and reactive oxygen species (ROS) may be another feedback loop by which metabolism is orchestrated and sensed by circadian processes. In addition to being modulated by intracellular O_2 levels, HIF1α is regulated by ROS such as hydrogen peroxide and superoxide (Hoppeler *et al.* 2003). Therefore, O_2/ROS levels appear to be another finely balanced sensing mechanism of metabolic status for circadian processes.

Synthesising mitochondria *de novo* is a relatively resource and energy intensive procedure within a mammalian cell, and therefore it is unsurprising that biomarkers of mitochondrial biogenesis do not display circadian rhythms in skeletal muscle (de Goede *et al.* 2018). In

addition to post-translational modifications such as acetylation, morphological changes in mitochondrial membranes and associated protein structures may play a role in engendering mitochondria with diurnal metabolism (de Goede *et al.* 2018; Schmitt *et al.* 2018). There is evidence that these important metabolic changes might form part of a feedback loop, possibly mediated by metabolites such as NAD^+, O_2, and ROS. In support of this, our group (Lassiter *et al.* 2018), and others (Schmitt *et al.* 2018) have shown that disruption of proteins known to regulate the dynamics of organelle and mitochondrial membranes can alter the expression of core-clock genes. These data demonstrate that the core-clock plays a major role in regulating, and sensing, diurnal mitochondrial metabolism.

Indeed, there are many metabolic signalling pathways that fluctuate over a diurnal cycle and are affected by manipulation of the core-clock. As we have presented here (Fig. 1), several pathways have the features of both clock inputs and outputs. Since we discussed evolutionary pressures earlier in this review, the fundamentals of metabolic time-keeping in some of our ancient ancestors are interesting to consider. Some prokaryote time-keeping can be reconstituted *in vitro* with only the expression products, rather than with transcriptional activity that is normally considered essential in diurnal rhythms (Nakajima *et al.* 2005). Furthermore, a complete inhibition of transcription or even cytosolic translation is not sufficient to inhibit cellular circadian rhythms in eukaryotic cells (O'Neill *et al.* 2011). Additionally, non-transcriptional rhythms in non-nucleated human red blood cells have been described (O'Neill & Reddy, 2011). Peroxiredoxins in these cells undergo ∼24-h redox cycles, persisting in the absence of external cues. These non-transcriptional rhythmic processes appear to be highly conserved across species from unicellular green alga to humans. Peroxiredoxins and cyanobacterial KaiB are part of the thioredoxin-like superfamily which could be remnants of a proto-clock in the last common ancestor of eukaryotes and prokaryotes (O'Neill *et al.* 2011). However, although non-nucleated red blood cells display circadian redox rhythms, this appears to be driven by rhythmic glucose metabolism, including glycolysis and the pentose phosphate pathway (Ch *et al.* 2021). Inhibition of critical enzymes within these metabolic pathways abolished diurnal rhythms of metabolic flux and redox oscillations. Additionally, these metabolic flux rhythms also occur in nucleated cells, and persist when core transcriptional molecular clock activity is absent (Ch *et al.* 2021). Thus, there is some degree of 'metabolic orchestration' in regulating circadian rhythms.

Given the evidence that metabolic pathways are independently capable of maintaining circadian rhythms, clock input and output signals should be considered. Manipulating core-clock genes in a variety of tissues in mouse models has drastic consequences for the orchestration of diurnal metabolism (Bass & Lazar, 2016). Furthermore, there is sufficient evidence that 'input' signals derived from external Zeitgebers play an equally important role in the regulation of diurnal metabolism. One noteworthy example of this is the putative existence of functional light-sensitive signalling pathways in adipose cells (Ondrusova *et al.* 2017). These data suggest that exposure of adipocytes to blue light at physiological levels results in decreased lipid droplet size, increased basal lipolytic rate and alterations in adiponectin and leptin secretion. The notion that UV light at physiological levels may play a role in regulating peripheral tissue is intriguing and adds another potential input signal to circadian regulation of metabolism. However, in terms of skeletal muscle, the most robust Zeitgeber is almost certainly skeletal muscle contraction, i.e. exercise.

Influence of exercise on diurnal metabolism

Exercise is a crucial preventative and therapeutic method of improving whole-body and metabolic health (Gabriel & Zierath, 2017). We have previously reviewed how exercise interacts with circadian rhythm and the skeletal muscle core-clock (Gabriel & Zierath, 2019). The molecular clock can regulate skeletal muscle metabolism, and exercise capacity in mouse models (Jordan *et al.* 2017). Additionally, the core-clock also responds to feedback signals from exercise (Gabriel & Zierath, 2019). Physical activity has long been explored as an extrinsic entraining factor. In 1973, opportunities for spontaneous wheel running were reported to maintain more robust diurnal rhythms of locomotor activity in golden hamsters maintained in constant light compared to hamsters without *ad libitum* access to a running wheel (Aschoff *et al.* 1973). In another study (Edgar & Dement, 1991), 11 of 15 mice studied had free-running sleep-wake and drinking circadian rhythms entrained to scheduled, voluntary exercise. These data provided further evidence that the mammalian circadian time-keeping system derives physiological feedback from spontaneous activity. In humans, scheduled exercise at different times of the day can also alter circadian time-keeping, as evidenced by altered phase of physiological melatonin circadian rhythms (Van Reeth *et al.* 1994; Buxton *et al.* 1997, 2003; Barger *et al.* 2004). A recent study (Youngstedt *et al.* 2019) extended these findings by including a relatively large number of participants, with a diverse range of sex and age (48 adults aged 18−32 years (26 women and 22 men; 65% White) and 53 adults aged 59−75 years (29 women and 22 men; 91% White)). This study elegantly attempted to control for confounding external Zeitgebers, and assessed phase shifts of urinary 6-sulphatoxymelatonin (aMT6s) in response to exercise at

different times of day. Interestingly, phase response curves between young and older adults or between women and men did not differ. The most noteworthy finding from this study was that exercise performed in the morning induced a phase advance, whilst evening exercise induced a phase delay. The phase-shifting effect of exercise appears to be consistent with a previous study using a similar protocol with light as the intervention (Kripke *et al*. 2007). Intuitively, these findings appear logical, as the body's physiological rhythms respond to light and/or exercise by inducing a state of alertness/awake-ness and the timing of this state subsequently affects the peak-timing of quiescence/sleepiness.

Well-controlled studies are critical to our understanding of how the external environment interacts with whole-body physiology. One example of extrinsic Zeitgeber cues interacting with metabolic health is the increased risk of developing metabolic disease associated with shift-work (Bescos *et al*. 2018; Vetter *et al*. 2018). Indeed, just 4 days of laboratory simulated shi ft-work reduces insulin sensitivity (Bescos *et al*. 2018), consistent with a real-world setting (Lund *et al*. 2001). Thus, gaining further understanding of the interaction between physical activity and diurnal metabolism is crucial in translating circadian biological research into clinical care and treatment paradigms. Insulin sensitivity is a crucial clinical outcome in T2D, and is highly associated with glycaemic control. Perhaps unsurprisingly, insulin sensitivity and control of glycaemia display diurnal behaviour, with healthy individuals displaying reduced insulin sensitivity and glucose tolerance in the evening compared to the morning (Jarrett & Keen, 1969; Boden *et al*. 1996; Van Cauter *et al*. 1997; Mason *et al*. 2020). Despite the diurnal variation of glycaemic control and insulin sensitivity being demonstrated many decades ago (Jarrett & Keen, 1969), there has been little research assessing diurnal glycaemic regulation, and the interaction with exercise at different times of the day. In order to investigate this, our laboratory performed a study in which 11 men with T2D were recruited to a randomised cross-over trial, and performed high intensity exercise training every other day over a 2-week intervention in either the morning (08.00 h) or afternoon (16.00 h), with a 2-week 'wash-out' period between intervention arms (Savikj *et al*. 2019). The results of this trial (Savikj *et al*. 2019) provided evidence that exercise at different times of the day may regulate diurnal plasma glucose concentrations in opposing directions, compared to non-exercise conditions, i.e. afternoon exercise was more efficacious than morning exercise at improving 24-h glycaemia. This finding appears to be supported by a recent study (Mancilla *et al*. 2021) that retrospectively compared the metabolic health outcomes of morning (08.00–10.00 h) *vs*. afternoon (15.00–18.00 h) exercise in metabolically compromised subjects in a 12-week exercise program. Indeed, the fold-change of insulin sensitivity, insulin-mediated suppression of adipose tissue lipolysis, fasting plasma glucose levels, exercise performance, and fat mass was beneficially superior in individuals who completed the training study with afternoon exercise, compared to morning exercise (Mancilla *et al*. 2021). It should be noted that the study participants were not randomised to morning or afternoon exercise; instead these times were dependent on the scheduling possibilities and personal preferences. Whether these findings are recapitulated in a randomised trial with healthy subjects under differing experimental conditions or a free-living setting has yet to be determined.

The time at which one performs exercise does not exist in a vacuum, and there are several factors to consider when studying Zeitgebers such as exercise *in vivo*. For example, one must consider the type, intensity and duration of exercise or training intervention that is being assessed. In terms of chronic training, preliminary evidence suggests that habitual exercise training may be associated with a higher rhythmic amplitude of clock-associated gene expression in cultured primary muscle cells (Hansen *et al*. 2016). Furthermore, higher exercise intensity is associated with greater exercise capacity in the afternoon/evening, while this effect appears to be less apparent in lower intensity exercise (Gabriel & Zierath, 2019). If one considers the dual output/input biology of metabolic pathways that are involved in circadian biology, coupled with the fact that higher intensity exercise has larger diurnal fluctuations than lower intensity exercise, it would seem plausible that higher intensity exercise also acts as a more robust Zeitgeber. However, high intensity exercise in this context is often (although not always) performed indoors, while the converse is true of lower intensity exercise bouts. Considering the apparently additive effects of light exposure and physical activity upon circadian biology (Youngstedt *et al*. 2016), the question of which exercise modality is the most robust Zeitgeber is not straightforward.

An example of non-exercise external stimuli that are confounding factors for studies investigating time-of-day exercise effects are meal timings and content (Ruddick-Collins *et al*. 2018). Indeed, the timing of meals in relation to an exercise bout is relevant for some performance and metabolic outcomes. For example, when breakfast is 'skipped' entirely, post-exercise mental fatigue may increase (Veasey *et al*. 2013), while exercise in the morning after breakfast increases glucose flux compared to post-exercise breakfast after an overnight fast (Edinburgh *et al*. 2018). Exercise (i.e. skeletal muscle contraction) may 're-set' skeletal muscle circadian rhythms both *in vitro* (Kemler *et al*. 2020; Small *et al*. 2020) and *in vivo* (Wolff & Esser, 2012; Gabriel & Zierath, 2019; Kemler *et al*. 2020). Although there are a variety of different mechanisms likely to contribute

to this effect (Gabriel & Zierath, 2019), the 're-setting' of the skeletal muscle clock by contraction *in vitro* (Small *et al.* 2020) was shown to be partly dependent on increased contraction-induced calcium influx, resulting in binding of the phosphorylated form of cAMP response element-binding protein (CREB) to the *Per2* promoter, and a 're-setting' of *Per2* oscillating mRNA expression (Fig. 2). It should be noted that calcium homeostasis after skeletal muscle contraction may be dysregulated in T2D (Eshima *et al.* 2014). Additionally, metformin is widely prescribed for the treatment of T2D and also appears to disrupt mitochondrial membrane integrity via increased mitochondrial uptake of calcium (Loubiere *et al.* 2017). In the aforementioned study (Savikj *et al.* 2019), 9 of 11 subjects were undergoing metformin treatment. Thus, metformin treatment, alongside disease status, may interact with the time of day exercise induced effects on glycaemia (Fig. 2). Furthermore the alteration of glucose metabolism in response to exercise at different times of day (Savikj *et al.* 2019) may also play a role in molecular circadian rhythm regulation, given the role of cellular glucose metabolism in regulating non-transcriptional rhythmic processes (Ch *et al.* 2021). We speculate that within certain metabolic diseases, whereby metabolic pathways that indirectly signal and interact with the skeletal muscle clock are disrupted, the bi-directional communication from clock inputs and outputs is disturbed. Thus, optimising the timing of exercise interventions may be of higher priority in metabolically diseased populations, such as individuals with T2D.

T2D shares some characteristics with the process of ageing (Cartee *et al.* 2016). Research conducted using mouse models provides evidence to suggest that ageing is associated with dysregulated polyamine metabolism alongside an increase in the circadian period, with polyamine metabolism acting as a clock input (Zwighaft *et al.* 2015). Ageing is also associated with dysregulation of calcium metabolism (Weisleder & Ma, 2008), and mitochondrial function (Short *et al.* 2005) in skeletal muscle. Putatively, these ageing-related metabolic disruptions could also result in an elongated circadian period. Therefore, researchers studying the aetiology of ageing should be mindful of known and possible metabolic clock-inputs. Further, therapeutic exercise interventions that target ageing-related phenomena may also benefit from an optimisation of timing.

Summary

All terrestrial biology interacts in some form with the day-night rhythms of the Earth, and metabolism and metabolic health in humans is no exception. Skeletal muscle is a tissue with a large degree of plasticity and diurnal variations, owing in part to the strong diurnal nature of physical activity and other external Zeitgebers such as meal-timing. The orchestration of whole-body, and skeletal muscle metabolism, is a complex, finely tuned process, which responds strongly to environmental cues. These molecular diurnal variations are regulated by the

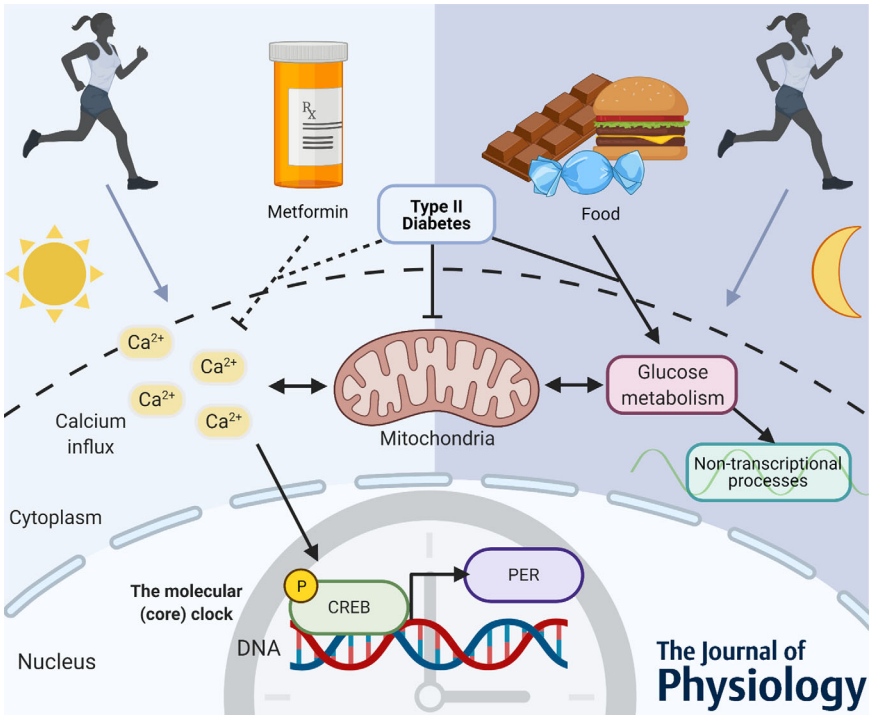

Figure 2. A schematic diagram of the speculated interactions between type 2 diabetes and skeletal muscle Zeitgebers
Contraction increases calcium influx, resulting in binding of the phosphorylated form of cAMP response element-binding protein (CREB) to the *Per2* promoter and a 're-setting' of *Per2* mRNA rhythmic expression (Small *et al.* 2020). Metformin, type 2 diabetes, exercise and ageing can all act to modulate calcium metabolism and mitochondrial function (Short *et al.* 2005; Weisleder & Ma, 2008; Eshima *et al.* 2014; Loubiere *et al.* 2017). Glucose metabolism is altered in response to exercise at different times of day (Savikj *et al.* 2019), and this may also play a role in molecular circadian rhythm regulation, given the role of cellular glucose metabolism in regulating non-transcriptional rhythmic processes (Ch *et al.* 2021). Dashed lines indicate speculated effects, continuous lines represent interactions with more evidence.

molecular clock, but also by non-transcriptional processes such as glucose metabolism. Elucidating the full spectra of metabolic interactions with the physical environment is an emerging area of research in the field of circadian biology. As this question is experimentally resolved, diet and exercise paradigms may also be developed that fine-tune metabolism by improving the timing of these modifiable lifestyle behaviours to optimise health outcomes.

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Additional information

Competing interests

The authors have no competing interests.

Author contributions

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