

SYMPOSIUM REVIEW

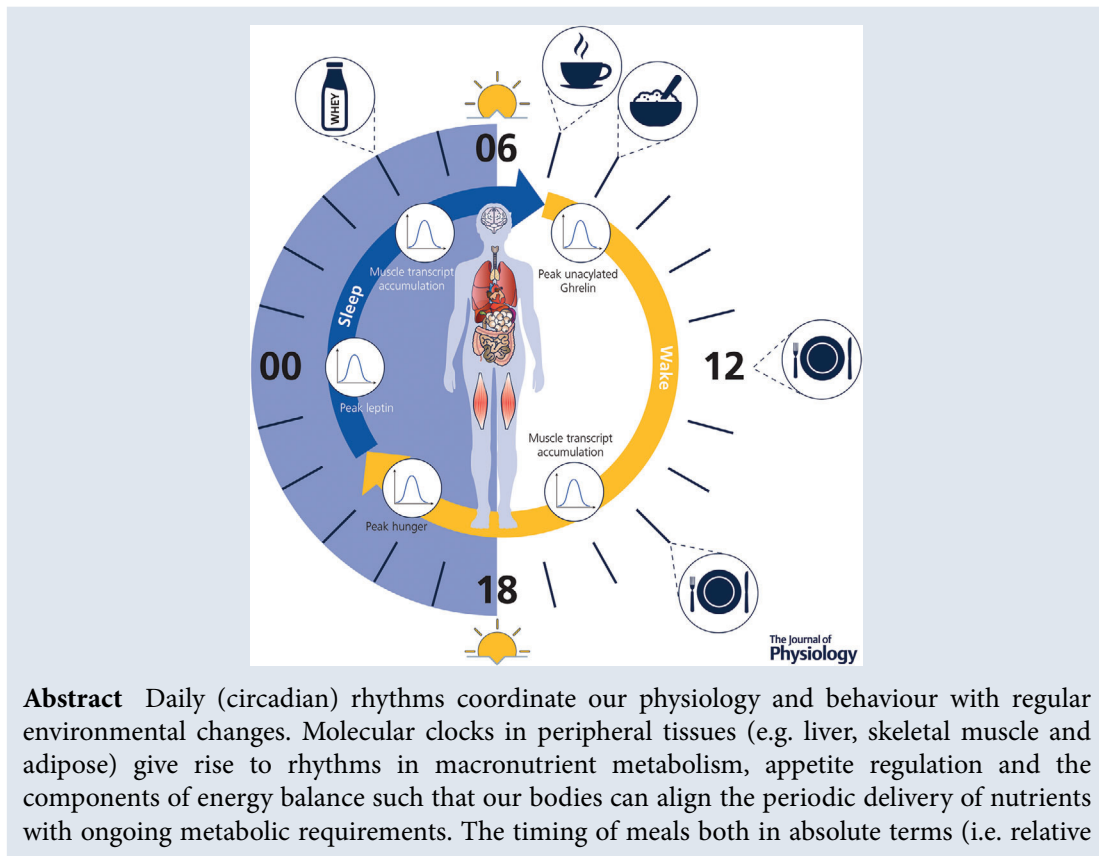
Nutrient timing and metabolic regulation

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to clock time) and in relative terms (i.e. relative to other daily events) is therefore relevant to metabolism and health. Experimental manipulation of feeding–fasting cycles can advance understanding of the effect of absolute and relative timing of meals on metabolism and health. Such studies have extended the overnight fast by regular breakfast omission and revealed that morning fasting can alter the metabolic response to subsequent meals later in the day, whilst also eliciting compensatory behavioural responses (i.e. reduced physical activity). Similarly, restricting energy intake via alternate-day fasting also has the potential to elicit a compensatory reduction in physical activity, and so can undermine weight-loss efforts (i.e. to preserve body fat stores). Interrupting the usual overnight fast (and therefore also the usual sleep cycle) by nocturnal feeding has also been examined and further research is needed to understand the importance of this period for either nutritional intervention or nutritional withdrawal. In summary, it is important for dietary guidelines for human health to consider nutrient timing (i.e. *when we eat*) alongside the conventional focus on nutrient quantity and nutrient quality (i.e. *how much we eat* and *what we eat*).

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Abstract figure legend Timing of meal-intake across the day can be considered in both absolute (i.e. clock time) and relative terms (i.e. to other events across the day). In particular meals can be considered relative to predictable cycles of sleep-wake (e.g. nocturnal feeding) and fasting-feeding (e.g. breakfast and intermittent fasting). Likewise, the timing of meal-intake throughout the day can also be considered relative to peaks in the rhythmic control of physiology (e.g. muscle transcript accumulation and/or appetite regulation). Collectively, consideration of these factors provides insight into the complexity of metabolic regulation within the context of nutrient timing.

Introduction

Life on earth has evolved within the context of a repetitive cycle of *ca* 24 h, whereby environmental variables such as light exposure predictably oscillate during each daily period. As such, natural selection has provided almost all organisms on this planet with endogenous circadian rhythms to help anticipate impending environmental challenges and thus pre-emptively adjust our physiology, metabolism and/or behaviour accordingly (Jagannath *et al.* 2017). The mammalian circadian timing system comprises both a central ‘master’ clock located in the suprachiasmatic nucleus of the hypothalamus and an integrated network of peripheral clocks located throughout various organs, tissues and cell-types (Albrecht, 2017). Collectively, these molecular clocks facilitate the coordinated disposal, degradation, synthesis and recycling of metabolic substrates in order that our periodic delivery of dietary nutrients (i.e. meal times) can appropriately meet our ongoing physiological requirements (Frayn, 2019). The objective of this review is to briefly summarise the mammalian circadian timing system and the daily rhythmicity of macronutrient metabolism, energy expenditure and appetite regulation, before considering how the alignment of daily feeding patterns with these underlying rhythms can impact human health.

The mammalian circadian timing system

The suprachiasmatic nucleus can translate repeating environmental stimuli, such as photic input, into the appropriate biological rhythms via a variety of signalling pathways, such as autonomic stimulation, endocrine action and body temperature modification (Lewy *et al.* 1999; Brown *et al.* 2002; Berson, 2003; Buhr *et al.* 2010; Slominski *et al.* 2012). Translation of murine work to humans highlights that molecular regulation of circadian rhythms at a cellular level involves the expression of clock genes, which can maintain approximate 24 h rhythmicity via interlocking transcriptional–translational feedback loops with both positive and negative limbs (Mazzocchi *et al.* 2012; McGinnis & Young, 2016). The positive limb is characterised by the proteins circadian locomotor output cycles kaput (CLOCK), its paralogue neuronal PAS domain protein 2 (NPAS2), and brain and muscle ARNT-like 1 (BMAL1), which are typically found in the nucleus (Kwon *et al.* 2006). Whilst this positive part of the loop targets clock-controlled genes, it also activates rhythmic transcription within the negative limb, including the *Period* (*PER*) and *Cryptochrome* (*CRY*) genes (Mohawk *et al.* 2012); this serves to inhibit the activity of CLOCK:BMAL1 prior to degradation, thereby ending repression of the positive aspect and initiating a new cycle of transcription (Table 1) (Sahar & Sassone-Corsi, 2012;

Table 1. Name, definition and basic function of the 'core' circadian clock machinery involved in the transcription–translation feedback loop

Name	Definition	Function	Reference
Ebox	Enhancer box	Promoter region that regulates cellular transcriptional activity	Hao <i>et al.</i> (1997)
RORE	Retinoic acid-related orphan receptor response element.	Promoter region that regulates cellular transcriptional activity	Cook <i>et al.</i> (2015)
CLOCK	Circadian locomotor output cycles kaput	Forms heterodimer with BMAL1 which binds to and activates the Ebox thereby stimulating transcription and translation of Per and Cry	Buhr & Takahashi (2013)
NPAS2	Neuronal PAS domain protein 2	Paralogue of CLOCK. Forms heterodimer with BMAL1 which binds to and activates the Ebox thereby activating transcription and translation of Per and Cry	Buhr & Takahashi (2013)
BMAL1 (Arntl)	Brain and muscle ARNT-like 1	Forms heterodimer with CLOCK which binds to and activates the Ebox thereby activating transcription and translation of Per and Cry	Buhr & Takahashi (2013)
Cry1,2,3	Cryptochrome 1, 2, 3	Form a complex with Period proteins. Inactivates Ebox thereby inhibiting transcription and translation of CLOCK and BMAL1	Ko & Takahashi (2006)
Per1, 2, 3	Period 1, 2, 3	Form a complex with cryptochrome proteins. Inactivates Ebox thereby inhibiting transcription and translation of CLOCK and BMAL1	Ko & Takahashi (2006)
NR1D1/2 (REV-ERB α/β)	Nuclear receptor subfamily 1 group D member 1/2	Repression of <i>BMAL1</i> gene expression through binding with RORE sites	Guillaumond <i>et al.</i> (2005)
ROR- $\alpha/\beta/\gamma$	Retinoic acid-related orphan receptors	Transcriptional activator for <i>BMAL1</i> through binding with RORE sites	Guillaumond <i>et al.</i> (2005)

Buhr & Takahashi, 2013; St John *et al.* 2014). The broad importance of proper circadian alignment is clearly apparent in the expression of this core clock machinery throughout mammalian biology, with 3–16% of all mRNA exhibiting rhythmic daily expression (Mohawk *et al.* 2012; Albrecht, 2017; Dierickx *et al.* 2018).

Circadian rhythmicity is particularly evident in signalling pathways within peripheral tissues that are vital for effective metabolic regulation (e.g. liver, muscle, and adipose tissue) (Fig. 1). Specifically, approximately 6–10% of genes in murine hepatocytes display robust circadian rhythms in a tissue-specific manner, with gene clusters targeting carbohydrate and lipid metabolism (Akhtar *et al.* 2002; Robles *et al.* 2014). Likewise, genome-wide transcriptome analysis of skeletal muscle samples from humans reveals high amplitude oscillations for the core clock genes *ARNTL* (*BMAL1*), *NPAS2*, *CLOCK*,

PER2, *PER3*, *CRY2*, *NR1D1* (*REV-ERB α*) and *ROR- α* (Perrin *et al.* 2018). Notably, these peaks in transcript accumulation clustered at 16.00 h (for genes implicated in muscle force production and mitochondrial activity) and at 04.00 h (for genes implicated in immune function and inflammation), with rhythmicity also present for genes linked to glucose, lipid and protein homeostasis (Perrin *et al.* 2018). Lastly, approximately 10–20% of the white adipose tissue transcriptome displays 24 h variation, with meaningful temporal oscillations present in both core clock (*PER1*, *PER2*, *PER3*, *CRY2*, *BMAL1* and *DBP*) and metabolic (*REVERB α* , *RIP140* and *PGC1 α*) genes under diurnal and constant conditions (Ptitsyn *et al.* 2006; Zvonic *et al.* 2006; Otway *et al.* 2011; Christou *et al.* 2019). Within adipocytes, these core clock genes play an important role in regulating lipolysis, adipogenesis and adipocyte hypertrophy, and so are central to proper

understanding of nutrient balances and obesity (Grimaldi *et al.* 2010; Shimba *et al.* 2011; Guo *et al.* 2012; Paschos *et al.* 2012).

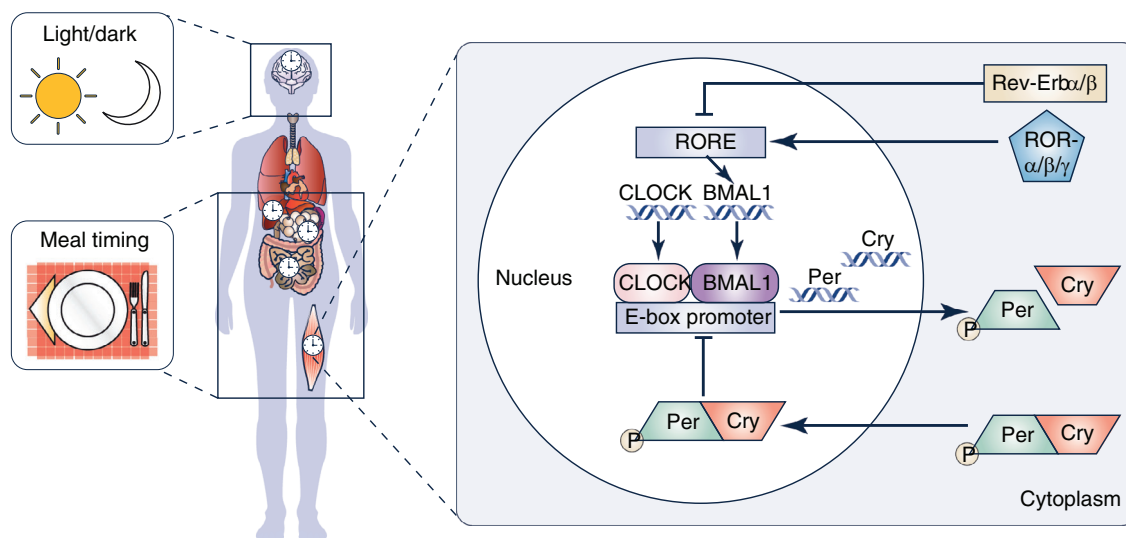
Rhythms in macronutrient metabolism

With regard to carbohydrate metabolism, whilst basal blood glucose can be relatively elevated upon waking (i.e. the dawn phenomenon), post-prandial glucose tolerance is generally lower in the evening than in the morning (Van Cauter *et al.* 1989, 1992, 1997; Simon *et al.* 1994; Qian & Scheer, 2016). The former is subject to endocrine regulation and driven by hepatic glycogenolysis and gluconeogenesis (Radziuk & Pye, 2006), whereas the latter is primarily regulated by the positive and negative limbs of the transcriptional feedback loop that drives diurnal rhythms in β -cell responsiveness, insulin secretion/clearance and insulin sensitivity (Baker & Jarrett, 1972; Aparicio *et al.* 1974; Boden *et al.* 1996; Asher *et al.* 2008; Lamia *et al.* 2009; Saad *et al.* 2012; Morris *et al.* 2015b; Perrin *et al.* 2018).

By contrast, lipid metabolism favours progressively elevated circulating non-esterified fatty acids, triglyceride and cholesterol later in the day and overnight (Zimmet *et al.* 1974; Morgan *et al.* 1999; Pan & Hussain, 2007; Ang *et al.* 2012; Dallmann *et al.* 2012; Yoshino *et al.* 2014), which is a reflection of diurnal rhythms in lipid storage and mobilisation as opposed to recent

food intake (Yoshino *et al.* 2014; Held *et al.* 2020). Specifically, a combination of animal and human studies suggests that a net shift in fatty acid metabolism from oxidation towards lipogenesis occurs throughout the day, with circadian regulation of intestinal triglyceride absorption, acylcarnitines, mitochondrial oxidative capacity, very-low-density lipoprotein secretion and insulin secretion all contributing to this daily variance (Marrino *et al.* 1987; Lee *et al.* 1992; Pan & Hussain, 2007; Ang *et al.* 2012; Pan *et al.* 2013; Yoshino *et al.* 2014; van Moorsel *et al.* 2016; Sprenger *et al.* 2021).

Finally, in relation to protein metabolism, the majority of amino acids (including all essential, some non-essential and some conditionally essential) display circadian rhythmicity, with peak values occurring between 12.00 and 20.00 h and with lowest values at 04.00–08.00 h (Feigin *et al.* 1967; Wurtman *et al.* 1967; Feigin *et al.* 1968; Grant *et al.* 2019). Variation in the generation and release of amino acids from assorted tissues may underpin this rhythm, including rhythmicity in protein digestion, and absorption (Barattini *et al.* 1993; Fiorucci *et al.* 1995; Qandeel *et al.* 2009a,b). The net effect of this variance in amino acid availability on tissue turnover is that protein synthesis is higher during the day and protein oxidation higher at night, with no clear temporal variance in the rate of protein breakdown (Garlick *et al.* 1980; Adam & Oswald, 1981; Kelu *et al.* 2020). This apparent day–night rhythm of muscle protein synthesis is not modulated by the relative absence of dietary protein at night, nor



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Figure 1. The central clock is located in the brain in the suprachiasmatic nucleus (SCN) and is robustly driven by regular cycles of light and dark

Core clock machinery is also present in numerous metabolically important peripheral tissues such as the liver, skeletal muscle, adipose tissue and gut. Given the role of these tissues in processing ingested nutrients, it is perhaps unsurprising that the effects of meal timing on metabolism are mediated by these peripheral clocks.

the overnight endocrine response (Beelen *et al.* 2008; Betts *et al.* 2011) but is consistent with the rhythmic regulation of MyoD (a myogenic transcription factor) by the CLOCK:BMAL1 complex (Andrews *et al.* 2010; Perrin *et al.* 2018).

Rhythms in energy expenditure

In stark contrast to the periodic arrival of dietary nutrients from regular daily meals, our ongoing metabolic requirements present a relentless need for continuous energy expenditure. Nonetheless, although unceasing, the rate of thermogenesis also exhibits variability over time and is integral to circadian regulation. For example, elevated body temperature is generally observed during daylight/waking hours, with lower temperature coincident with the dark/sleeping phase amongst most humans, which contributes to synchronising central and peripheral clock machinery (Edwards *et al.* 2002; Buhr *et al.* 2010). Indeed, constant routine protocols (removal of environmental/behavioural stimuli through prolonged wakefulness and even distribution of energy intake) reveal that heat production, oxygen uptake (\dot{V}_{O_2}), and carbon dioxide production (\dot{V}_{CO_2}) are all highest during the biological morning (Krauchi & Wirz-Justice, 1994; Spengler *et al.* 2000), whereas a recent forced desynchrony protocol (non-standard daily behavioural patterns under dim light conditions) demonstrated that resting metabolic rate is lowest during the late biological night and highest ~ 12 h later in the biological afternoon/evening (Zitting *et al.* 2018). Interestingly resting energy expenditure also changes overnight with differing stages of sleep (as assessed by sleep encephalography). Generally energy expenditure tends to be highest during lighter/earlier phases, and lowest during the deepest/late stages of sleep (Brebba & Altshuler, 1965; Fontvieille *et al.* 1994), but some studies have failed to replicate any differences between stages of sleep (Webb & Hiestand, 1975; Haskell *et al.* 1981; White *et al.* 1985; Palca *et al.* 1986; Jung *et al.* 2011). Beyond basal metabolic requirements (i.e. under fasted and resting conditions), an endogenously driven daily rhythm has been reported in diet-induced thermogenesis (i.e. the thermic effect of feeding), with ~ 20 – 44% higher values in the morning relative to the evening (Romon *et al.* 1993; Bo *et al.* 2015; Morris *et al.* 2015a). However, recent evidence indicates that this is apparent rhythmicity in diet-induced thermogenesis can be accounted for by the underlying circadian variation in resting metabolic rate (Ruddick-Collins *et al.* 2021). Finally, although highly individual, a range of contrasting diurnal patterns of physical activity thermogenesis have been identified, with more intense physical activity often favoured earlier in the day (Maddison *et al.* 2009; Sartini *et al.* 2015; Jansen *et al.* 2018).

Rhythms in appetite regulation

Evidence for circadian rhythms in appetite and appetite regulatory peptides has been generated using experimental protocols involving both the constant routine and forced desynchrony protocols introduced above. These studies have revealed that hunger is typically lowest in the morning (~ 08.00 h) and peaks in the evening (~ 20.00 h), when satiety also tends to be lowest (Scheer *et al.* 2013; Sargent *et al.* 2016; Rynders *et al.* 2020; Templeman *et al.* 2021b). This robust rhythmicity in appetite ratings occurs independent of time since waking, inter-meal intervals and the energy content of meals (Scheer *et al.* 2013), but is nonetheless entirely consistent with the typical feeding pattern in westernised societies, whereby energy intake tends to be lowest in the morning and highest in the evening (NHANES, 2016).

Our recent work employed a semi-constant routine (i.e. continuous feeding throughout waking hours) to examine the 24 h profile of appetite regulatory hormones (Templeman *et al.* 2021b). In that study we reported diurnal rhythms in leptin (peak 00.32 h) and unacylated ghrelin (peak 08.26 h) (Fig. 2). Notably, despite nominally being classified as a hunger hormone, the observed rhythm of ghrelin was approximately antiphasic with that of subjective hunger and ratings of prospective food consumption, which peaked as expected in the evening (i.e. *ca.* 20.00–21.00 h) – this phase separation between peaks in appetite ratings and appetite hormones was also evident in another recent study (Rynders *et al.* 2020). In addition to leptin and ghrelin, such daily rhythmicity has also been identified for other appetite regulatory peptides, such as: glucagon-like peptide-1 (peak ~ 10.00 h, nadir ~ 17.00 h), peptide YY (peak at ~ 14.00 h, nadir

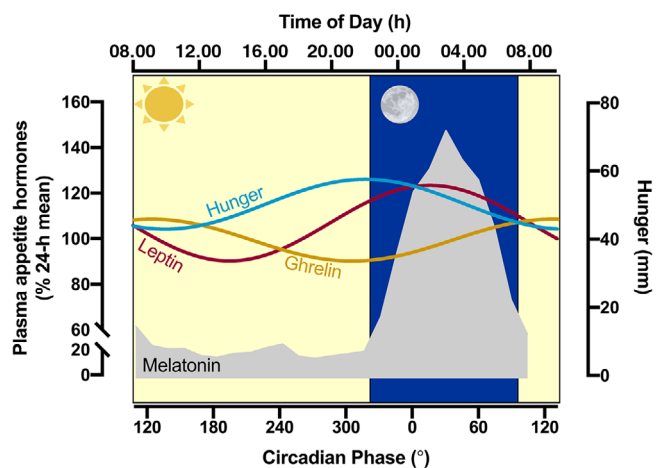


Figure 2. Diurnal profiles of hunger

Diurnal rhythms in unacylated ghrelin, leptin and subjective hunger under conditions of semi-constant routine (i.e. hourly feeding during waking hours only) relative to melatonin profile (grey) and light/dark (yellow/blue respectively)

~04.00 h) and pancreatic polypeptide (peak ~15.00 h, nadir ~09.00 h) (Johns *et al.* 2006; Hill *et al.* 2011; Galindo Munoz *et al.* 2015; Rynders *et al.* 2020).

Nutrient timing

Endogenously controlled rhythms are entrained to environmental time cues known as zeitgebers or 'time givers' (Aschoff, 1954; Aschoff & Pohl, 1978). These include naturally repeating cycles of light–dark, waking–sleeping and activity–rest but also our transitions between the fed–fasted state. As such, the scheduling/alignment of eating occasions (i.e. chrono-nutrition; Flanagan *et al.* 2021), and thus the availability of exogenous nutrients, relative to other regular daily events can serve as a powerful signal to help entrain the endogenous rhythms described in the previous sections (la Fleur *et al.* 2001; Zambon *et al.* 2003; Duffy & Czeisler, 2009; Figueiro *et al.* 2012; Leproult *et al.* 2014; Cheung *et al.* 2016; Tanaka *et al.* 2020). Therefore, in addition to the conventional focus of dietary guidelines for human health regarding nutrient quantity and nutrient quality (i.e. *how much* we eat and *what* we eat), it is also important to consider nutrient timing (i.e. *when* we eat).

Nutrient timing can be understood in terms of both absolute timing (i.e. objective time-of-day, clock time) and relative timing (i.e. with respect to when other relevant events occur and/or usually occur, e.g. wake/sleep, exercise, other meals). The physiological responses to identical meals consumed at different times of day can vary dramatically. For example, as noted earlier, carbohydrate, lipid and protein metabolism all exhibit marked morning–evening differences (Van Cauter *et al.* 1992; Yoshino *et al.* 2014; Morris *et al.* 2015a; Leung *et al.* 2019), yet the complete absence of daily food intake for 24 h (i.e. fasting) can eradicate the circadian rhythm in hepatic gene expression that would otherwise occur with a regular meal pattern (Vollmers *et al.* 2009). Even just a short delay in habitual meal timing can alter *Per2* phase in adipose tissue, with corresponding phase shifts in systemic metabolites and hormones but without altering the temporal pattern of melatonin or cortisol (robust markers of the central clock) – all consistent with the idea that peripheral rhythms are closely matched to the absolute time of feeding each day (Schoeller *et al.* 1997; Wehrens *et al.* 2017; Gu *et al.* 2020). Indeed, feeding responsive hormones such as insulin, glucagon and insulin-like growth factor 1 appear to be especially potent modulators of clock gene and/or protein expression in multiple tissues – at least in murine models, but emerging evidence is now beginning to demonstrate this in humans (Tahara *et al.* 2010; Mukherji *et al.* 2015; Sun *et al.* 2015; Ikeda *et al.* 2018; Crosby *et al.* 2019; Tuvia *et al.* 2021).

Extended overnight fasting. In terms of relative nutrient timing, the 'other relevant events' that can both influence and be influenced by the response to feeding may include light exposure, sleeping, exercise and, critically, other eating occasions. Breakfast is an eating occasion with particular potential to serve as a zeitgeber and to modify subsequent responses, since this first meal of the day generally marks the end of the overnight period of darkness, sleeping, resting and fasting, whilst also preceding all other daily events. The capacity of breakfast to exert a marked influence on metabolic control later in the day is perhaps best illustrated by the 'second-meal effect', which describes how the glycaemic and insulinaemic responses to repeated carbohydrate ingestion are attenuated relative to an initial meal hours earlier (Hamman & Hirschman, 1919). This phenomenon was first observed using sequential oral glucose tolerance tests but has since been replicated with intravenous infusions (Szabo *et al.* 1969) and mixed macronutrient breakfasts relative to extended morning fasting (Gonzalez, 2014; Chowdhury *et al.* 2015, 2016b; Jakubowicz *et al.* 2017). Interestingly the availability of systemic glucose across the morning has been suggested as a possible determinant of physical activity levels in breakfast 'consumers' relative to 'skippers' (Betts *et al.* 2014; Chowdhury *et al.* 2016a). Whilst the precise mechanisms underpinning the second-meal effect remain the subject of current investigations (Lee *et al.* 2011; Edinburgh *et al.* 2017; Edinburgh *et al.* 2018), the study by Jakubowicz *et al.* (2017) supports that maintenance of rhythmic clock gene expression plays a role, since the expected pattern of core clock gene expression in leukocytes is disrupted when habitual breakfast consumers omit their usual morning meal.

Further to the acute metabolic effects of breakfast on the responses to subsequent meals later within the same day, recent research has also explored the longer-term effects (i.e. 6 weeks) of regular daily breakfast consumption *versus* extended morning fasting on free-living behavioural responses and any accumulated adaptation in metabolic control. In brief, complete omission of breakfast (i.e. zero energy intake until midday) every day for 6 weeks resulted in significantly lower physical activity thermogenesis than when a regular morning feeding was prescribed – a finding that has been replicated amongst both lean adults and those with obesity (Betts *et al.* 2014; Chowdhury *et al.* 2016a). However, other than some evidence in these studies of more stable glycaemia and altered adipose tissue gene expression in lean individuals and improved insulin sensitivity in obese individuals (Gonzalez *et al.* 2018), there were no other effects of regular breakfast on markers of cardiometabolic health nor any metabolic adaptation (Chowdhury *et al.* 2018, 2019). (For a more detailed overview of this series of studies, see Betts *et al.* 2016.)

Intermittent fasting. Whilst skipping breakfast is often considered an unhealthy dietary approach (notwithstanding the lack of empirical support for that view), regularly omitting the same meal and/or restricting energy intake to the same set times each day (i.e. time restricted eating) may at least be conducive to the entrainment of endogenous rhythms to that consistently repeating feeding pattern. By contrast, numerous other contemporary approaches to intermittent fasting, often employed as a means to control body weight through weight loss or maintenance, can involve irregular or chaotic patterns of feeding and fasting within each 24 h period (Templeman *et al.* 2020), so are not easily anticipated by the circadian timing system and thus complicate effective metabolic regulation. Popular forms of intermittent fasting within this category include the 5:2 diet (fasting on two non-consecutive days each week) and alternate day fasting (i.e. never feeding on consecutive days). Part of the challenge in understanding the potential effects of any diet based upon intermittent fasting is that the extended periods of complete energy restriction typically culminate in a net energy deficit and therefore weight loss. It therefore becomes difficult to determine whether any observed effects on cardio-metabolic health, appetite regulation or other relevant outcomes are attributable to fasting *per se* or simply to the consequences of negative energy balance and reduced adipose tissue mass.

We recently conducted a randomised controlled trial in lean participants expressly to isolate the independent effects of intermittent fasting and net energy restriction (Templeman *et al.* 2021a). This was achieved by having some participants impart a prescribed degree of energy restriction but without fasting (i.e. consuming 75% of usual energy intake at each regular meal), whilst others fasted completely every other 24 h but, critically, were re-fed on the alternate days either to match the first group for net energy restriction (i.e. 50% more food than usual on fed days) or to replace the energy 'missed' through fasting altogether (i.e. 100% more food than usual on fed days). Prescribing additional food to minimise or even completely prevent weight loss is understandably not intended to reflect a diet that might be advocated in the real world, but this unusual approach does provide the required experimental design needed to understand the separate and combined effects of fasting and energy (im)balance.

Through the above approach it was possible to determine that standard daily dieting (i.e. without fasting) elicited almost 2 kg of weight loss over 3 weeks and, moreover, that almost all of that change in total body mass was attributable to reductions in body fat content. By contrast, imposing the same prescribed degree of energy restriction via alternate-day fasting resulted in a similar (albeit slightly lower) rate of overall weight loss but this

was accounted for in equal measure by reductions in both fat mass and fat-free mass. Part of the explanation for this apparent difference in energy balance despite ostensibly similar reductions in energy intake is that energy expenditure is not constant but rather has the capacity to compensate for extended periods of fasting to preserve endogenous energy reserves. Specifically, consistent with the adaptive behavioural responses to breakfast omission described earlier, achieving an energy deficit via intermittent fasting can spontaneously inhibit physical activity energy expenditure (i.e. skeletal muscle force production; Westerterp, 2013; Ruddick-Collins *et al.* 2020) to below habitual levels, whereas there was no such change in physical activity levels when the same degree of energy restriction was achieved without fasting (it remains to be seen whether similar behavioural responses occur in obese individuals). Nonetheless, unlike the previously described effect of breakfast omission, there was no difference between any of the interventions in relation to systemic indices of cardio-metabolic health, gut hormones, or the expression of key genes in subcutaneous adipose tissue. Overall, the data reported in Templeman *et al.* (2021a) further illustrate the complexity of metabolic regulation within the context of nutrient timing since the potential physiological consequences of intermittent fasting may depend upon the interaction between circadian rhythms and related compensatory responses to a modified feeding–fasting pattern.

Nocturnal interventions. Excepting the above rather extreme forms of prolonged fasting, most individuals remain in a permanently post-prandial (fed) state for the entirety of daylight/waking hours and so the overnight/sleep phase typically coincides with the longest period of fasting in any given 24 h cycle (Ruge *et al.* 2009). According to the circadian timing system described earlier, this may reflect an entirely natural and properly synchronised alignment between the fed–fasted cycle and all other daily light–dark, wake–sleep and activity–rest cycles. However, it might also be reasoned that this extended period of nutritional withdrawal presents a possible opportunity for dietary intervention. For example, the 'dawn phenomenon' noted earlier highlights how blood glucose may be elevated upon waking, whereas the 'second-meal effect' highlights how prior feeding can be employed to prime the system in preparation for subsequent meals; this begs questions such as whether a nocturnal pre-load can be used to improve glycaemic control in response to breakfast. An initial investigation into such possibilities examined whether waking briefly at 04.00 h to consume a bolus of whey protein might improve metabolic control at breakfast; paradoxically, that nocturnal feeding intervention actually

resulted in impaired glucose tolerance at breakfast, along with elevated lipid oxidation but no effect on appetite (Smith *et al.* 2021). This surprising finding may be partly attributable to the relatively large dose of protein, which was delivered at a time when an abundance of exogenous amino acids is neither required nor expected by the circadian timing system. Consequently, whilst nocturnal feeding presents a possible opportunity for nutritional intervention, it also is a useful paradigm through which we can further understand the relationship between misaligned eating and the increased risk of cardio-metabolic disease.

In addition to balancing the potential benefits and apparent risks of applying nutritional interventions at night, it is also important to consider the indirect impact of interfering with habitual sleep patterns. Indeed, sleep appears to be inherently linked to metabolic regulation, obesity and associated comorbidities, with chronic sleep disorders exerting a potent negative effect on glycaemic control (Briancon-Marjollet *et al.* 2015). For example, to begin with the more extreme model of total sleep deprivation (i.e. remaining awake for one or more nights), fasted glucose concentrations are progressively elevated after 24–120 h of sleeplessness (Kuhn *et al.* 1969; Vondra *et al.* 1981; Wehrens *et al.* 2010; Benedict *et al.* 2011). Post-prandial metabolic control is even more profoundly affected by such models of total sleep restriction, with elevated glycaemic and insulinaemic responses and reduced insulin sensitivity clearly evident after a single night of complete nocturnal wakefulness (Kuhn *et al.* 1969; VanHelder *et al.* 1993; Wehrens *et al.* 2010; Benedict *et al.* 2011). Disrupted sleep may perturb next day metabolism through a multitude of proposed mechanisms; these include, but are not limited to alterations in brain glucose utilization and changes in hormonal secretion profile (Scheen *et al.* 1996), sympathetic nervous stimulation (Spiegel *et al.* 2004), and/or inflammation (Meier-Ewert *et al.* 2004; Vgontzas *et al.* 2004).

Partial sleep deprivation (i.e. a shorter total sleep duration than usual) is a more common occurrence in the real world and can also perturb glycaemic control the following morning, with evidence of impaired glucose clearance and whole-body insulin sensitivity after even a single night of limited sleep (Donga *et al.* 2010; Gonnissen *et al.* 2013; Wang *et al.* 2016; Sweeney *et al.* 2017). Sleep duration can be limited by simply going to bed later and/or getting up earlier or via sleep fragmentation. The latter refers to when sleep is intermittently disrupted by brief waking periods and has the potential to interrupt progression through the various stages of the sleep cycle even if total sleep duration is not substantially curtailed (Tasali *et al.* 2008). We tested the effect of fragmented sleep in our recent work but found post-prandial glucose and insulin responses upon waking to be unaffected by having

woken hourly throughout the prior 8-h sleep opportunity (Smith *et al.* 2020). Interestingly, based on the reasoning that a strong coffee is a common remedy following a night of broken sleep, we also investigated the effects of caffeine within the context of the above experimental model. Consistent with the established effects of caffeine on insulin sensitivity independent of sleep deprivation (Robertson *et al.* 2015; Robertson *et al.* 2018), consuming a cup of coffee following a night of sleep fragmentation resulted in a ~50% higher glycaemic response and ~15% higher insulinaemic response at breakfast than either a night of uninterrupted sleep or a matched sleep fragmentation protocol without caffeine prior to breakfast (Smith *et al.* 2020). Further work is therefore needed to better understand whether the potential opportunity for nutritional intervention at night can be harnessed with minimal disruption of sleep patterns, circadian rhythms and next-day metabolic responses.

Conclusion

Molecular clocks allow for temporal coordination between environmental, metabolic and behavioural cues. Meal patterns are a key element of this system and so considerations regarding nutrient timing should be incorporated into dietary guidelines alongside the conventional focus on nutrient quantity and nutrient quality. Research over the past decade has explored various aspects of nutrient timing and identified several promising approaches to human health improvement involving chrono-nutrition. Further novel insight will be possible through examining the physiological responses of human participants over complete 24-h monitoring cycles, including sequential meal tests, nocturnal feeding and with assessments under free-living conditions.

References

- Adam K & Oswald I (1981). Diurnal pattern of protein and energy metabolism in man: some doubts. *Am J Clin Nutr* **34**, 1624–1628.
- Akhtar RA, Reddy AB, Maywood ES, Clayton JD, King VM, Smith AG, Gant TW, Hastings MH & Kyriacou CP (2002). Circadian cycling of the mouse liver transcriptome, as revealed by cDNA microarray, is driven by the suprachiasmatic nucleus. *Curr Biol* **12**, 540–550.
- Albrecht U (2017). The circadian clock, metabolism and obesity. *Obes Rev* **18** 25–33.
- Andrews JL, Zhang X, McCarthy JJ, McDearmon EL, Hornberger TA, Russell B, Campbell KS, Arbogast S, Reid MB, Walker JR, Hogenesch JB, Takahashi JS & Esser KA (2010). CLOCK and BMAL1 regulate MyoD and are necessary for maintenance of skeletal muscle phenotype and function. *Proc Natl Acad Sci U S A* **107**, 19090–19095.

- Ang JE, Revell V, Mann A, Mantele S, Otway DT, Johnston JD, Thumser AE, Skene DJ & Raynaud F (2012). Identification of human plasma metabolites exhibiting time-of-day variation using an untargeted liquid chromatography-mass spectrometry metabolomic approach. *Chronobiol Int* **29**, 868–881.
- Aparicio NJ, Puchulu FE, Gagliardino JJ, Ruiz M, Llorens JM, Ruiz J, Lamas A & De Miguel R (1974). Circadian variation of the blood glucose, plasma insulin and human growth hormone levels in response to an oral glucose load in normal subjects. *Diabetes* **23**, 132–137.
- Aschoff J (1954). Zeitgeber der tierischen Tagesperiodik. *Naturwissenschaften* **41**, 49–56.
- Aschoff J & Pohl H (1978). Phase relations between a circadian rhythm and its zeitgeber within the range of entrainment. *Naturwissenschaften* **65**, 80–84.
- Asher G, Gatfield D, Stratmann M, Reinke H, Dibner C, Kreppel F, Mostoslavsky R, Alt FW & Schibler U (2008). SIRT1 regulates circadian clock gene expression through PER2 deacetylation. *Cell* **134**, 317–328.
- Baker IA & Jarrett RJ (1972). Diurnal variation in the blood-sugar and plasma-insulin response to tolbutamide. *Lancet* **2**, 945–947.
- Barattini P, Larsen KR, Moore JG & Dayton MT (1993). Circadian rhythm of pepsin efflux in the fasting rat stomach. *Chronobiol Int* **10**, 403–409.
- Beelen M, Tieland M, Gijsen AP, Vandereydt H, Kies AK, Kuipers H, Saris WH, Koopman R & van Loon LJ (2008). Coingestion of carbohydrate and protein hydrolysate stimulates muscle protein synthesis during exercise in young men, with no further increase during subsequent overnight recovery. *J Nutr* **138**, 2198–2204.
- Benedict C, Hallschmid M, Lassen A, Mahnke C, Schultes B, Schiöth HB, Born J & Lange T (2011). Acute sleep deprivation reduces energy expenditure in healthy men. *Am J Clin Nutr* **93**, 1229–1236.
- Berson DM (2003). Strange vision: ganglion cells as circadian photoreceptors. *Trends Neurosci* **26**, 314–320.
- Betts JA, Beelen M, Stokes KA, Saris WH & van Loon LJ (2011). Endocrine responses during overnight recovery from exercise: impact of nutrition and relationships with muscle protein synthesis. *Int J Sport Nutr Exerc Metab* **21**, 398–409.
- Betts JA, Chowdhury EA, Gonzalez JT, Richardson JD, Tsintzas K & Thompson D (2016). Is breakfast the most important meal of the day? *Proc Nutr Soc* **75**, 464–474.
- Betts JA, Richardson JD, Chowdhury EA, Holman GD, Tsintzas K & Thompson D (2014). The causal role of breakfast in energy balance and health: a randomized controlled trial in lean adults. *Am J Clin Nutr* **100**, 539–547.
- Bo S, Fadda M, Castiglione A, Ciccone G, De Francesco A, Fedele D, Guggino A, Parasiliti Caprino M, Ferrara S, Vezio Boggio M, Mengozzi G, Ghigo E, Maccario M & Broglio F (2015). Is the timing of caloric intake associated with variation in diet-induced thermogenesis and in the metabolic pattern? A randomized cross-over study. *Int J Obes* **39**, 1689–1695.
- Boden G, Ruiz J, Urbain JL & Chen X (1996). Evidence for a circadian rhythm of insulin secretion. *Am J Physiol* **271**, E246–E252.
- Brebbia DR & Altshuler KZ (1965). Oxygen consumption rate and electroencephalographic stage of sleep. *Science* **150**, 1621–1623.
- Briancon-Marjollet A, Weiszenstein M, Henri M, Thomas A, Godin-Ribuot D & Polak J (2015). The impact of sleep disorders on glucose metabolism: endocrine and molecular mechanisms. *Diabetol Metab Syndr* **7**, 25.
- Brown SA, Zimbrunn G, Fleury-Olela F, Preitner N & Schibler U (2002). Rhythms of mammalian body temperature can sustain peripheral circadian clocks. *Curr Biol* **12**, 1574–1583.
- Buhr ED & Takahashi JS (2013). Molecular components of the mammalian circadian clock. *Handb Exp Pharmacol*, 3–27.
- Buhr ED, Yoo SH & Takahashi JS (2010). Temperature as a universal resetting cue for mammalian circadian oscillators. *Science* **330**, 379–385.
- Cheung IN, Zee PC, Shalman D, Malkani RG, Kang J & Reid KJ (2016). Morning and evening blue-enriched light exposure alters metabolic function in normal weight adults. *PLoS One* **11**, e0155601.
- Chowdhury EA, Richardson JD, Gonzalez JT, Tsintzas K, Thompson D & Betts JA (2019). Six weeks of morning fasting causes little adaptation of metabolic or appetite responses to feeding in adults with obesity. *Obesity* **27**, 813–821.
- Chowdhury EA, Richardson JD, Holman GD, Tsintzas K, Thompson D & Betts JA (2016a). The causal role of breakfast in energy balance and health: a randomized controlled trial in obese adults. *Am J Clin Nutr* **103**, 747–756.
- Chowdhury EA, Richardson JD, Tsintzas K, Thompson D & Betts JA (2015). Carbohydrate-rich breakfast attenuates glycaemic, insulinaemic and ghrelin response to ad libitum lunch relative to morning fasting in lean adults. *Br J Nutr* **114**, 98–107.
- Chowdhury EA, Richardson JD, Tsintzas K, Thompson D & Betts JA (2016b). Effect of extended morning fasting upon ad libitum lunch intake and associated metabolic and hormonal responses in obese adults. *Int J Obes* **40**, 305–311.
- Chowdhury EA, Richardson JD, Tsintzas K, Thompson D & Betts JA (2018). Postprandial metabolism and appetite do not differ between lean adults that eat breakfast or morning fast for 6 weeks. *J Nutr* **148**, 13–21.
- Christou S, Wehrens SMT, Isherwood C, Moller-Levet CS, Wu H, Revell VL, Bucca G, Skene DJ, Laing EE, Archer SN & Johnston JD (2019). Circadian regulation in human white adipose tissue revealed by transcriptome and metabolic network analysis. *Sci Rep* **9**, 2641.
- Cook DN, Kang HS, Jetten AM (2015). Retinoic acid-related orphan receptors (RORs): regulatory functions in immunity, development, circadian rhythm, and metabolism. *Nucl Receptor Res* **2**, <https://doi.org/10.11131/2015/101185>
- Crosby P, Hamnett R, Putker M, Hoyle NP, Reed M, Karam CJ, Maywood ES, Stangherlin A, Chesham JE, Hayter EA, Rosenbrier-Ribeiro L, Newham P, Clevers H, Bechtold DA & O'Neill JS (2019). Insulin/IGF-1 drives PERIOD synthesis to entrain circadian rhythms with feeding time. *Cell* **177**, 896–909.e20.
- Dallmann R, Viola AU, Tarokh L, Cajochen C & Brown SA (2012). The human circadian metabolome. *Proc Natl Acad Sci U S A* **109**, 2625–2629.

- Dierickx P, Van Laake LW & Geijsen N (2018). Circadian clocks: from stem cells to tissue homeostasis and regeneration. *EMBO Rep* **19**, 18–28.
- Donga E, van Dijk M, van Dijk JG, Biermasz NR, Lammers GJ, van Kralingen KW, Corssmit EP & Romijn JA (2010). A single night of partial sleep deprivation induces insulin resistance in multiple metabolic pathways in healthy subjects. *J Clin Endocrinol Metab* **95**, 2963–2968.
- Duffy JF & Czeisler CA (2009). Effect of light on human circadian physiology. *Sleep Med Clin* **4**, 165–177.
- Edinburgh RM, Betts JA, Burns SF & Gonzalez JT (2017). Concordant and divergent strategies to improve post-prandial glucose and lipid metabolism. *Nutrition Bulletin* **42**, 113–122.
- Edinburgh RM, Hengist A, Smith HA, Travers RL, Koumanov F, Betts JA, Thompson D, Walhin JP, Wallis GA, Hamilton DL, Stevenson EJ, Tipton KD & Gonzalez JT (2018). Pre-exercise breakfast ingestion versus extended overnight fasting increases postprandial glucose flux after exercise in healthy men. *Am J Physiol Endocrinol Metab* **315**, E1062–E1074.
- Edwards B, Waterhouse J, Reilly T & Atkinson G (2002). A comparison of the suitabilities of rectal, gut, and insulated axilla temperatures for measurement of the circadian rhythm of core temperature in field studies. *Chronobiol Int* **19**, 579–597.
- Feigin RD, Klainer AS & Beisel WR (1967). Circadian periodicity of blood amino-acids in adult men. *Nature* **215**, 512–514.
- Feigin RD, Klainer AS & Beisel WR (1968). Factors affecting circadian periodicity of blood amino acids in man. *Metabolism* **17**, 764–775.
- Figueiro MG, Plitnick B & Rea MS (2012). Light modulates leptin and ghrelin in sleep-restricted adults. *Int J Endocrinol* **2012**, 530726.
- Fiorucci S, Distrutti E, Di Matteo F, Brunori P, Santucci L, Mallozzi E, Bigazzi U & Morelli A (1995). Circadian variations in gastric acid and pepsin secretion and intra-gastric bile acid in patients with reflux esophagitis and in healthy controls. *Am J Gastroenterol* **90**, 270–276.
- Flanagan A, Bechtold DA, Pot GK & Johnston JD (2021). Chrono-nutrition: from molecular and neuronal mechanisms to human epidemiology and timed feeding patterns. *J Neurochem* **157**, 53–72.
- Fontvieille AM, Rising R, Spraul M, Larson DE & Ravussin E (1994). Relationship between sleep stages and metabolic rate in humans. *Am J Physiol* **267**, E732–E737.
- Frayn K (2019). *Human Metabolism: A Regulatory Perspective*. Wiley-Blackwell, Hoboken, NJ.
- Galindo Munoz JS, Jimenez Rodriguez D & Hernandez Morante JJ (2015). Diurnal rhythms of plasma GLP-1 levels in normal and overweight/obese subjects: lack of effect of weight loss. *J Physiol Biochem* **71**, 17–28.
- Garlick PJ, Clugston GA, Swick RW & Waterlow JC (1980). Diurnal pattern of protein and energy metabolism in man. *Am J Clin Nutr* **33**, 1983–1986.
- Gonnissen HK, Hursel R, Rutters F, Martens EA & Westerterp-Plantenga MS (2013). Effects of sleep fragmentation on appetite and related hormone concentrations over 24 h in healthy men. *Br J Nutr* **109**, 748–756.
- Gonzalez JT (2014). Paradoxical second-meal phenomenon in the acute postexercise period. *Nutrition* **30**, 961–967.
- Gonzalez JT, Richardson JD, Chowdhury EA, Koumanov F, Holman GD, Cooper S, Thompson D, Tsintzas K & Betts JA (2018). Molecular adaptations of adipose tissue to 6 weeks of morning fasting vs. daily breakfast consumption in lean and obese adults. *J Physiol* **596**, 609–622.
- Grant LK, Ftouni S, Nijagal B, De Souza DP, Tull D, McConville MJ, Rajaratnam SMW, Lockley SW & Anderson C (2019). Circadian and wake-dependent changes in human plasma polar metabolites during prolonged wakefulness: a preliminary analysis. *Sci Rep* **9**, 4428.
- Grimaldi B, Bellet MM, Katada S, Astarita G, Hirayama J, Amin RH, Granneman JG, Piomelli D, Leff T & Sassone-Corsi P (2010). PER2 controls lipid metabolism by direct regulation of PPAR γ . *Cell Metab* **12**, 509–520.
- Gu C, Brereton N, Schweitzer A, Cotter M, Duan D, Børshiem E, Wolfe RR, Pham LV, Polotsky VY & Jun JC (2020). Metabolic effects of late dinner in healthy volunteers—A randomized crossover clinical trial. *J Clin Endocrinol Metab* **105**, 2789–2802.
- Guillaumond F, Dardente H, Giguère V & Cermakian N (2005). Differential control of Bmal1 circadian transcription by REV-ERB and ROR nuclear receptors. *J Biol Rhythms* **20**, 391–403.
- Guo B, Chatterjee S, Li L, Kim JM, Lee J, Yechoor VK, Minze LJ, Hsueh W & Ma K (2012). The clock gene, brain and muscle Arnt-like 1, regulates adipogenesis via Wnt signaling pathway. *FASEB J* **26**, 3453–3463.
- Hamman L & Hirschman I (1919). Studies on blood sugar. Effects upon the blood sugar of the repeated ingestion of glucose. *Johns Hopkins Hospital Bull* **344**, 306–308.
- Hao H, Allen DL & Hardin PE (1997). A circadian enhancer mediates PER-dependent mRNA cycling in *Drosophila melanogaster*. *Mol Cell Biol* **17**, 3687–3693.
- Haskell EH, Palca JW, Walker JM, Berger RJ & Heller HC (1981). Metabolism and thermoregulation during stages of sleep in humans exposed to heat and cold. *J Appl Physiol* **51**, 948–954.
- Held NM, Wefers J, van Weeghel M, Daemen S, Hansen J, Vaz FM, van Moorsel D, Hesselink MKC, Houtkooper RH & Schrauwen P (2020). Skeletal muscle in healthy humans exhibits a day-night rhythm in lipid metabolism. *Mol Metab* **37**, 100989.
- Hill BR, De Souza MJ & Williams NI (2011). Characterization of the diurnal rhythm of peptide YY and its association with energy balance parameters in normal-weight premenopausal women. *Am J Physiol Endocrinol Metab* **301**, E409–E415.
- Ikeda Y, Kamagata M, Hirao M, Yasuda S, Iwami S, Sasaki H, Tsubosaka M, Hattori Y, Todoh A, Tamura K, Shiga K, Ohtsu T & Shibata S (2018). Glucagon and/or IGF-1 production regulates resetting of the liver circadian clock in response to a protein or amino acid-only diet. *EBioMedicine* **28**, 210–224.
- Jagannath A, Taylor L, Wakaf Z, Vasudevan SR & Foster RG (2017). The genetics of circadian rhythms, sleep and health. *Hum Mol Genet* **26**, R128–R138.

- Jakubowicz D, Wainstein J, Landau Z, Raz I, Ahren B, Chapnik N, Ganz T, Menaged M, Barnea M, Bar-Dayan Y & Froy O (2017). Influences of breakfast on clock gene expression and postprandial glycemia in healthy individuals and individuals with diabetes: a randomized clinical trial. *Diabetes Care* **40**, 1573–1579.
- Jansen FM, van Kollenburg GH, Kamphuis CBM, Pierik FH & Ettema DF (2018). Hour-by-hour physical activity patterns of adults aged 45–65 years: a cross-sectional study. *J Public Health* **40**, 787–796.
- Johns CE, Newton JL, Westley BR & May FE (2006). Human pancreatic polypeptide has a marked diurnal rhythm that is affected by ageing and is associated with the gastric TFF2 circadian rhythm. *Peptides* **27**, 1341–1348.
- Jung CM, Melanson EL, Frydendall EJ, Perreault L, Eckel RH & Wright KP (2011). Energy expenditure during sleep, sleep deprivation and sleep following sleep deprivation in adult humans. *J Physiol* **589**, 235–244.
- Kelu JJ, Pipalia TG & Hughes SM (2020). Circadian regulation of muscle growth independent of locomotor activity. *Proc Natl Acad Sci U S A* **117**, 31208–31218.
- Ko CH & Takahashi JS (2006). Molecular components of the mammalian circadian clock. *Hum Mol Genet* **15**(suppl_2), R271–R277.
- Krauchi K & Wirz-Justice A (1994). Circadian rhythm of heat production, heart rate, and skin and core temperature under unmasking conditions in men. *Am J Physiol* **267**, R819–R829.
- Kuhn E, Brodan V, Brodanová M & Rysánek K (1969). Metabolic reflection of sleep deprivation. *Act Nerv Super* **11**, 165–174.
- Kwon I, Lee J, Chang SH, Jung NC, Lee BJ, Son GH, Kim K & Lee KH (2006). BMAL1 shuttling controls transactivation and degradation of the CLOCK/BMAL1 heterodimer. *Mol Cell Biol* **26**, 7318–7330.
- la Fleur SE, Kalsbeek A, Wortel J, van der Vliet J & Buijs RM (2001). Role for the pineal and melatonin in glucose homeostasis: pinealectomy increases night-time glucose concentrations. *J Neuroendocrinol* **13**, 1025–1032.
- Lamia KA, Sachdeva UM, DiTacchio L, Williams EC, Alvarez JG, Egan DF, Vasquez DS, Juguilon H, Panda S, Shaw RJ, Thompson CB & Evans RM (2009). AMPK regulates the circadian clock by cryptochrome phosphorylation and degradation. *Science* **326**, 437–440.
- Lee A, Ader M, Bray GA & Bergman RN (1992). Diurnal variation in glucose tolerance. Cyclic suppression of insulin action and insulin secretion in normal-weight, but not obese, subjects. *Diabetes* **41**, 750–759.
- Lee SH, Tura A, Mari A, Ko SH, Kwon HS, Song KH, Yoon KH, Lee KW & Ahn YB (2011). Potentiation of the early-phase insulin response by a prior meal contributes to the second-meal phenomenon in type 2 diabetes. *Am J Physiol Endocrinol Metab* **301**, E984–E990.
- Leproult R, Holmbäck U & Van Cauter E (2014). Circadian misalignment augments markers of insulin resistance and inflammation, independently of sleep loss. *Diabetes* **63**, 1860–1869.
- Leung GKW, Huggins CE & Bonham MP (2019). Effect of meal timing on postprandial glucose responses to a low glycemic index meal: a crossover trial in healthy volunteers. *Clin Nutr* **38**, 465–471.
- Lewy AJ, Cutler NL & Sack RL (1999). The endogenous melatonin profile as a marker for circadian phase position. *J Biol Rhythms* **14**, 227–236.
- Maddison R, Hoorn SV, Jiang Y, Mhurchu CN, Exeter D, Dorey E, Bullen C, Utter J, Schaaf D & Turley M (2009). The environment and physical activity: the influence of psychosocial, perceived and built environmental factors. *Int J Behav Nutr Phys Act* **6**, 19.
- Marrino P, Gavish D, Shafrir E & Eisenberg S (1987). Diurnal variations of plasma lipids, tissue and plasma lipoprotein lipase, and VLDL secretion rates in the rat. A model for studies of VLDL metabolism. *Biochim Biophys Acta* **920**, 277–284.
- Mazzoccoli G, Paziienza V & Vinciguerra M (2012). Clock genes and clock-controlled genes in the regulation of metabolic rhythms. *Chronobiol Int* **29**, 227–251.
- McGinnis GR & Young ME (2016). Circadian regulation of metabolic homeostasis: causes and consequences. *Nat Sci Sleep* **8**, 163–180.
- Meier-Ewert HK, Ridker PM, Rifai N, Regan MM, Price NJ, Dinges DF & Mullington JM (2004). Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk. *J Am Coll Cardiol* **43**, 678–683.
- Mohawk JA, Green CB & Takahashi JS (2012). Central and peripheral circadian clocks in mammals. *Annu Rev Neurosci* **35**, 445–462.
- Morgan LM, Aspostolakou F, Wright J & Gama R (1999). Diurnal variations in peripheral insulin resistance and plasma non-esterified fatty acid concentrations: a possible link? *Ann Clin Biochem* **36**, 447–450.
- Morris CJ, Garcia JI, Myers S, Yang JN, Trienekens N & Scheer FA (2015a). The human circadian system has a dominating role in causing the morning/evening difference in diet-induced thermogenesis. *Obesity* **23**, 2053–2058.
- Morris CJ, Yang JN, Garcia JI, Myers S, Bozzi I, Wang W, Buxton OM, Shea SA & Scheer FA (2015b). Endogenous circadian system and circadian misalignment impact glucose tolerance via separate mechanisms in humans. *Proc Natl Acad Sci U S A* **112**, E2225–E2234.
- Mukherji A, Kobiita A & Chambon P (2015). Shifting the feeding of mice to the rest phase creates metabolic alterations, which, on their own, shift the peripheral circadian clocks by 12 hours. *Proc Natl Acad Sci U S A* **112**, E6683–E6690.
- NHANES (2016). What We Eat in America, NHANES 2015–2016. <https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/wweianhanes-overview/>
- Otway DT, Mantele S, Bretschneider S, Wright J, Trayhurn P, Skene DJ, Robertson MD & Johnston JD (2011). Rhythmic diurnal gene expression in human adipose tissue from individuals who are lean, overweight, and type 2 diabetic. *Diabetes* **60**, 1577–1581.

- Palca JW, Walker JM & Berger RJ (1986). Thermoregulation, metabolism, and stages of sleep in cold-exposed men. *J Appl Physiol* **61**, 940–947.
- Pan X & Hussain MM (2007). Diurnal regulation of microsomal triglyceride transfer protein and plasma lipid levels. *J Biol Chem* **282**, 24707–24719.
- Pan X, Jiang XC & Hussain MM (2013). Impaired cholesterol metabolism and enhanced atherosclerosis in clock mutant mice. *Circulation* **128**, 1758–1769.
- Paschos GK, Ibrahim S, Song WL, Kunieda T, Grant G, Reyes TM, Bradfield CA, Vaughan CH, Eiden M, Masoodi M, Griffin JL, Wang F, Lawson JA & Fitzgerald GA (2012). Obesity in mice with adipocyte-specific deletion of clock component Arntl. *Nat Med* **18**, 1768–1777.
- Perrin L, Loizides-Mangold U, Chanon S, Gobet C, Hulo N, Isenegger L, Weger BD, Migliavacca E, Charpagne A, Betts JA, Walhin JP, Templeman I, Stokes K, Thompson D, Tsintzas K, Robert M, Howald C, Riezman H, Feige JN, Karagounis LG, Johnston JD, Dermitzakis ET, Gachon F, Lefai E & Dibner C (2018). Transcriptomic analyses reveal rhythmic and CLOCK-driven pathways in human skeletal muscle. *Elife* **16**, 34114.
- Ptitsyn AA, Zvonic S, Conrad SA, Scott LK, Mynatt RL & Gimble JM (2006). Circadian clocks are resounding in peripheral tissues. *PLoS Comput Biol* **2**, e16.
- Qandeel HG, Alonso F, Hernandez DJ, Duenes JA, Zheng Y, Scow JS & Sarr MG (2009a). Role of vagal innervation in diurnal rhythm of intestinal peptide transporter 1 (PEPT1). *J Gastrointest Surg* **13**, 1976–1985.
- Qandeel HG, Duenes JA, Zheng Y & Sarr MG (2009b). Diurnal expression and function of peptide transporter 1 (PEPT1). *J Surg Res* **156**, 123–128.
- Qian J & Scheer F (2016). Circadian system and glucose metabolism: implications for physiology and disease. *Trends Endocrinol Metab* **27**, 282–293.
- Radziuk J & Pye S (2006). Diurnal rhythm in endogenous glucose production is a major contributor to fasting hyperglycaemia in type 2 diabetes. Suprachiasmatic deficit or limit cycle behaviour? *Diabetologia* **49**, 1619–1628.
- Robertson TM, Clifford MN, Penson S, Chope G & Robertson MD (2015). A single serving of caffeinated coffee impairs postprandial glucose metabolism in overweight men. *Br J Nutr* **114**, 1218–1225.
- Robertson TM, Clifford MN, Penson S, Williams P & Robertson MD (2018). Postprandial glycaemic and lipaemic responses to chronic coffee consumption may be modulated by CYP1A2 polymorphisms. *Br J Nutr* **119**, 792–800.
- Robles MS, Cox J & Mann M (2014). In-vivo quantitative proteomics reveals a key contribution of post-transcriptional mechanisms to the circadian regulation of liver metabolism. *PLoS Genet* **10**, e1004047.
- Romon M, Edme JL, Boulenguez C, Lescroart JL & Frimat P (1993). Circadian variation of diet-induced thermogenesis. *Am J Clin Nutr* **57**, 476–480.
- Ruddick-Collins LC, Flanagan A, Johnston JD, Morgan PJ & Johnstone AM (2021). Circadian rhythms in resting metabolic rate account for apparent daily rhythms in thermic effect of food. *J Clin Endocrinol Metab* (in press; DOI: 10.1210/clinem/dgab654.
- Ruddick-Collins LC, Morgan PJ & Johnstone AM (2020). Mealtime: a circadian disruptor and determinant of energy balance? *J Neuroendocrinol* **32**, e12886.
- Ruge T, Hodson L, Cheeseman J, Dennis AL, Fielding BA, Humphreys SM, Frayn KN & Karpe F (2009). Fasted to fed trafficking of fatty acids in human adipose tissue reveals a novel regulatory step for enhanced fat storage. *J Clin Endocrinol Metab* **94**, 1781–1788.
- Rynders CA, Morton SJ, Bessesen DH, Wright KP Jr & Broussard JL (2020). Circadian Rhythm of substrate oxidation and hormonal regulators of energy balance. *Obesity* **28** S104–S113.
- Saad A, Dalla Man C, Nandy DK, Levine JA, Bharucha AE, Rizza RA, Basu R, Carter RE, Cobelli C, Kudva YC & Basu A (2012). Diurnal pattern to insulin secretion and insulin action in healthy individuals. *Diabetes* **61**, 2691–2700.
- Sahar S & Sassone-Corsi P (2012). Regulation of metabolism: the circadian clock dictates the time. *Trends Endocrinol Metab* **23**, 1–8.
- Sargent C, Zhou X, Matthews RW, Darwent D & Roach GD (2016). Daily rhythms of hunger and satiety in healthy men during one week of sleep restriction and circadian misalignment. *Int J Environ Res Public Health* **13**, 170.
- Sartini C, Wannamethee SG, Iliffe S, Morris RW, Ash S, Lennon L, Whincup PH & Jefferis BJ (2015). Diurnal patterns of objectively measured physical activity and sedentary behaviour in older men. *BMC Public Health* **15**, 609.
- Scheen AJ, Byrne MM, Plat L, Leproult R & Van Cauter E (1996). Relationships between sleep quality and glucose regulation in normal humans. *Am J Physiol* **271**, E261–E270.
- Scheer FA, Morris CJ & Shea SA (2013). The internal circadian clock increases hunger and appetite in the evening independent of food intake and other behaviors. *Obesity* **21**, 421–423.
- Schoeller DA, Cella LK, Sinha MK & Caro JF (1997). Entrainment of the diurnal rhythm of plasma leptin to meal timing. *J Clin Invest* **100**, 1882–1887.
- Shimba S, Ogawa T, Hitosugi S, Ichihashi Y, Nakadaira Y, Kobayashi M, Tezuka M, Kosuge Y, Ishige K, Ito Y, Komiyama K, Okamatsu-Ogura Y, Kimura K & Saito M (2011). Deficient of a clock gene, brain and muscle Arnt-like protein-1 (BMAL1), induces dyslipidemia and ectopic fat formation. *PLoS One* **6**, e25231.
- Simon C, Brandenberger G, Saini J, Ehrhart J & Follenius M (1994). Slow oscillations of plasma glucose and insulin secretion rate are amplified during sleep in humans under continuous enteral nutrition. *Sleep* **17**, 333–338.
- Slominski AT, Zmijewski MA, Skobowiat C, Zbytek B, Slominski RM & Steketeer JD (2012). Sensing the environment: regulation of local and global homeostasis by the skin's neuroendocrine system. *Adv Anat Embryol Cell Biol* **212**, v, vii, 1–115.
- Smith ES, Adama E, Clayton K, Holbrey J, Palubiski G, Smith HA, Gonzalez JT & Betts JA (2021). Nocturnal whey protein ingestion impairs post-prandial glucose tolerance at breakfast. *Br J Nutr* **125**, 669–677.

- Smith HA, Hengist A, Thomas J, Walhin JP, Heath P, Perkin O, Chen YC, Gonzalez JT & Betts JA (2020). Glucose control upon waking is unaffected by hourly sleep fragmentation during the night, but is impaired by morning caffeinated coffee. *Br J Nutr* **124**, 1114–1120.
- Spengler CM, Czeisler CA & Shea SA (2000). An endogenous circadian rhythm of respiratory control in humans. *J Physiol* **3**, 683–694.
- Spiegel K, Leproult R, L'Hermite-Balériaux M, Copinschi G, Penev PD & Van Cauter E (2004). Leptin levels are dependent on sleep duration: relationships with sympathovagal balance, carbohydrate regulation, cortisol, and thyrotropin. *J Clin Endocrinol Metab* **89**, 5762–5771.
- Sprenger RR, Hermansson M, Neess D, Becciolini LS, Sørensen SB, Fagerberg R, Ecker J, Liebisch G, Jensen ON, Vance DE, Færgeman NJ, Klemm RW & Ejsing CS (2021). Lipid molecular timeline profiling reveals diurnal crosstalk between the liver and circulation. *Cell Rep* **34**, 108710.
- St John PC, Hirota T, Kay SA & Doyle FJ 3rd (2014). Spatiotemporal separation of PER and CRY post-translational regulation in the mammalian circadian clock. *Proc Natl Acad Sci U S A* **111**, 2040–2045.
- Sun X, Dang F, Zhang D, Yuan Y, Zhang C, Wu Y, Wang Y & Liu Y (2015). Glucagon-CREB/CRTC2 signaling cascade regulates hepatic BMAL1 protein. *J Biol Chem* **290**, 2189–2197.
- Sweeney EL, Jeromson S, Hamilton DL, Brooks NE & Walshe IH (2017). Skeletal muscle insulin signaling and whole-body glucose metabolism following acute sleep restriction in healthy males. *Physiol Rep* **5**, e13498.
- Szabo AJ, Maier JJ, Szabo O & Camerini-Davalos RA (1969). Improved glucose disappearance following repeated glucose administration. Serum insulin growth hormone and free fatty acid levels during the Staub-Traugott effect. *Diabetes* **18**, 232–237.
- Tahara Y, Hirao A, Moriya T, Kudo T & Shibata S (2010). Effects of medial hypothalamic lesions on feeding-induced entrainment of locomotor activity and liver Per2 expression in Per2::luc mice. *J Biol Rhythms* **25**, 9–18.
- Tanaka Y, Ogata H, Kayaba M, Ando A, Park I, Yajima K, Araki A, Suzuki C, Osumi H, Zhang S, Ishihara A, Takahashi K, Shoda J, Nabekura Y, Satoh M & Tokuyama K (2020). Effect of a single bout of exercise on clock gene expression in human leukocyte. *J Appl Physiol* **128**, 847–854.
- Tasali E, Leproult R, Ehrmann DA & Van Cauter E (2008). Slow-wave sleep and the risk of type 2 diabetes in humans. *Proc Natl Acad Sci U S A* **105**, 1044–1049.
- Templeman I, Smith HA, Chowdhury E, Chen YC, Carroll H, Johnson-Bonson D, Hengist A, Smith R, Creighton J, Clayton D, Varley I, Karagounis LG, Wilhelmsen A, Tsintzas K, Reeves S, Walhin JP, Gonzalez JT, Thompson D & Betts JA (2021a). A randomized controlled trial to isolate the effects of fasting and energy restriction on weight loss and metabolic health in lean adults. *Sci Transl Med* **13**, eabd8034.
- Templeman I, Smith HA, Walhin JP, Middleton B, Gonzalez JT, Karagounis LG, Johnston JD & Betts JA (2021b). Unacylated ghrelin, leptin, and appetite display diurnal rhythmicity in lean adults. *J Appl Physiol* **130**, 1534–1543.
- Templeman I, Gonzalez J, Thompson D & Betts J (2020). The role of intermittent fasting and meal timing in weight management and metabolic health. *Proc Nutr Soc* **79**, 76–87.
- Tuvia N, Pivovarovva-Ramich O, Murahovschi V, Lück S, Grudziecki A, Ost AC, Kruse M, Nikiforova VJ, Osterhoff M, Gottmann P, Gögebakan Ö, Sticht C, Gretz N, Schupp M, Schürmann A, Rudovich N, Pfeiffer AFH & Kramer A (2021). Insulin directly regulates the circadian clock in adipose tissue. *Diabetes* **70**, 1985–1999.
- Van Cauter E, Desir D, Decoster C, Fery F & Balasse EO (1989). Nocturnal decrease in glucose tolerance during constant glucose infusion. *J Clin Endocrinol Metab* **69**, 604–611.
- Van Cauter E, Polonsky KS & Scheen AJ (1997). Roles of circadian rhythmicity and sleep in human glucose regulation. *Endocr Rev* **18**, 716–738.
- Van Cauter E, Shapiro ET, Tillil H & Polonsky KS (1992). Circadian modulation of glucose and insulin responses to meals: relationship to cortisol rhythm. *Am J Physiol* **262**, E467–E475.
- VanHelder T, Symons JD & Radomski MW (1993). Effects of sleep deprivation and exercise on glucose tolerance. *Aviat Space Environ Med* **64**, 487–492.
- van Moorsel D, Hansen J, Havekes B, Scheer F, Jorgensen JA, Hoeks J, Schrauwen-Hinderling VB, Duez H, Lefebvre P, Schaper NC, Hesselink MKC, Staels B & Schrauwen P (2016). Demonstration of a day-night rhythm in human skeletal muscle oxidative capacity. *Mol Metab* **5**, 635–645.
- Vgontzas AN, Zoumakis E, Bixler EO, Lin HM, Follett H, Kales A & Chrousos GP (2004). Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines. *J Clin Endocrinol Metab* **89**, 2119–2126.
- Vollmers C, Gill S, DiTacchio L, Pulivarthy SR, Le HD & Panda S (2009). Time of feeding and the intrinsic circadian clock drive rhythms in hepatic gene expression. *Proc Natl Acad Sci U S A* **106**, 21453–21458.
- Vondra K, Brodan V, Bass A, Kuhn E, Teisinger J, Anděl M & Veselková A (1981). Effects of sleep deprivation on the activity of selected metabolic enzymes in skeletal muscle. *Eur J Appl Physiol Occup Physiol* **47**, 41–46.
- Wang X, Greer J, Porter RR, Kaur K & Youngstedt SD (2016). Short-term moderate sleep restriction decreases insulin sensitivity in young healthy adults. *Sleep Health* **2**, 63–68.
- Webb P & Hiestand M (1975). Sleep metabolism and age. *J Appl Physiol* **38**, 257–262.
- Wehrens SM, Hampton SM, Finn RE & Skene DJ (2010). Effect of total sleep deprivation on postprandial metabolic and insulin responses in shift workers and non-shift workers. *J Endocrinol* **206**, 205–215.
- Wehrens SMT, Christou S, Isherwood C, Middleton B, Gibbs MA, Archer SN, Skene DJ & Johnston JD (2017). Meal timing regulates the human circadian system. *Curr Biol* **27**, 1768–1775.e3.
- Westerterp KR (2013). Physical activity and physical activity induced energy expenditure in humans: measurement, determinants, and effects. *Front Physiol* **4**, 90.
- White DP, Weil JV & Zwillich CW (1985). Metabolic rate and breathing during sleep. *J Appl Physiol* **59**, 384–391.

- Wurtman RJ, Chou C & Rose CM (1967). Daily rhythm in tyrosine concentration in human plasma: persistence on low-protein diets. *Science* **158**, 660–662.
- Yoshino J, Almeda-Valdes P, Patterson BW, Okunade AL, Imai S, Mittendorfer B & Klein S (2014). Diurnal variation in insulin sensitivity of glucose metabolism is associated with diurnal variations in whole-body and cellular fatty acid metabolism in metabolically normal women. *J Clin Endocrinol Metab* **99**, E1666–E1670.
- Zambon AC, McDearmon EL, Salomonis N, Vranizan KM, Johansen KL, Adey D, Takahashi JS, Schambelan M & Conklin BR (2003). Time- and exercise-dependent gene regulation in human skeletal muscle. *Genome Biol* **4**, R61.
- Zimmet PZ, Wall JR, Rome R, Stimmler L & Jarrett RJ (1974). Diurnal variation in glucose tolerance: associated changes in plasma insulin, growth hormone, and non-esterified fatty acids. *Br Med J* **1**, 485–488.
- Zitting KM, Vujovic N, Yuan RK, Isherwood CM, Medina JE, Wang W, Buxton OM, Williams JS, Czeisler CA & Duffy JF (2018). Human resting energy expenditure varies with circadian phase. *Curr Biol* **28**, 3685–3690.
- Zvonic S, Ptitsyn AA, Conrad SA, Scott LK, Floyd ZE, Kilroy G, Wu X, Goh BC, Mynatt RL & Gimble JM (2006). Characterization of peripheral circadian clocks in adipose tissues. *Diabetes* **55**, 962–970.

Additional information

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Author contributions

Both authors contributed to the writing of this review. Both authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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