Optimising intermittent fasting: Evaluating the behavioural and metabolic effects of extended morning and evening fasting

D. J. Clayton iD **, W. J. A. Mode and T. Slater** iD

School of Science and Technology, Nottingham Trent University, Nottingham, UK

Abstract

This article describes the aims of a new study funded by the British Nutrition Foundation Drummond Pump Priming Award. This study will explore the independent metabolic, endocrine and behavioural effects of extended morning and evening fasting. In an obesogenic society, there is an urgent need to identify effective strategies for preventing obesity-related diseases, such as type 2 diabetes. Implementing extended periods of fasting and restricted time permitted for food intake may be an efficacious method for weight management and improving metabolic health. However, recent research suggests that the success of this intervention may be influenced by when the fasting window occurs, with evening fasting appearing to elicit superior metabolic benefits compared to morning fasting. The mechanisms driving these time-dependent outcomes are not yet clear but may be due to circadian variations in metabolic physiology and in behaviours known to influence energy balance. To date, no study has directly compared the acute metabolic and behavioural responses to morning and evening fasting with those of a control trial. Research on evening fasting is also currently restricted to individuals living with overweight or obesity, emphasising a need for research in lean individuals aiming to maintain a healthy bodyweight and improve metabolic health. This article highlights the need for alternative nutritional interventions to improve public health, before reviewing the existing literature linking extended fasting, circadian rhythms and behavioural and metabolic outcomes. The final part of this article outlines the aims, methodology and intended outcomes of the current research project.

Keywords: chrono-nutrition, energy balance, meal timing, metabolic health, time-restricted eating, weight management

Correspondence: Dr. David J. Clayton, Lecturer/Senior Lecturer in Nutrition and Exercise Physiology, School of Science and Technology, Clifton Campus, Nottingham Trent University, Nottingham, Nottinghamshire NG11 8NS, UK.
E-mail: David.Clayton@ntu.ac.uk

Introduction

Sixty-three per cent of the UK population are living with overweight or obesity and are at increased risk of numerous metabolic diseases, making this a significant public health priority (NHS Digital 2020). Maintaining a healthy bodyweight requires careful management of energy balance, with weight gain occurring when

energy intake exceeds energy expenditure over a prolonged period of time. If not arrested, this energy surplus can lead to a series of metabolic disorders related to the accumulation of adipose tissue. There is evidence that weight gain occurs incrementally throughout life, such that many individuals who are classified as 'normal weight' (BMI <25 kg/m^2) as young adults, have a strong possibility of becoming overweight or obese later in adulthood (Østbye *et al.* 2011). Therefore, it is crucial that preventative methods which successfully control energy balance are implemented prior to becoming overweight or obese.

Weight loss requires the creation of an energy deficit, typically achieved by reducing energy intake (*i.e.* energy restriction) and/or increasing energy expenditure (*i.e.* exercise). However, the long-term success of such interventions is poor, likely impeded by persistently elevated appetite stimulating an increase in energy intake (Polidori *et al.* 2016), as well as reduced physical activity and a disproportionate reduction in basal metabolism, suppressing energy expenditure (Hall *et al.* 2012). These compensatory behavioural adaptations hinder weight loss progress, often culminating in reduced adherence (Dansinger *et al.* 2005) and poor long-term success (Curioni & Lourenco 2005). Research has typically focussed on the magnitude of the energy deficit created but there is growing evidence that when food is consumed can influence the metabolic response (Johnston *et al.* 2016). This is due to complex interactions between nutrient consumption, circadian biology and metabolism, an area of research termed 'chrono-nutrition' (Ruddick-Collins *et al.* 2018).

'Chrono-nutrition' – meal timing independently influences metabolism

Mammalian physiology has evolved to respond seamlessly to the catabolic and anabolic fluctuations associated with daily life (Mohawk *et al.* 2012). Endogenous circadian timing systems regulate biological pathways and adjust essential physiological and behavioural mechanisms (Potter *et al.* 2016). The suprachiasmatic nucleus (SCN), situated within the hypothalamus, is referred to as the 'master clock' and is regulated by external light-dark cycles (Ruddick-Collins et al. 2018). The SCN is the primary factor in synchronising human diurnal physiology (Mohawk *et al.* 2012), but clock genes have also been identified in several peripheral tissues (Dibner *et al.* 2010). Interestingly, there appears to be little crossover between rhythmic gene expression in different tissues (Storch

et al. 2002), highlighting not only the importance of the SCN in coordinating these clocks, but also the broad range of biological processes that are subject to circadian influence (*e.g.* rate of digestion, metabolism and appetite regulation) (Ruddick-Collins *et al.* 2018).

Circadian clocks are malleable and synchronise with daily life in response to external environmental entrainment cues, referred to as zeitgebers. Light-dark cycles are the dominant zeitgeber for the SCN (Mohawk *et al.* 2012), but, although data are largely derived from rodent studies, nutrient intake also appears to be an important peripheral zeitgeber, with some evidence for this effect recently emerging in humans (Lewis *et al.* 2020). The uncoupling of the light-entrained SCN rhythm from feeding-entrained peripheral rhythms has been implicated in the development of obesity and metabolic disorders (Stenvers *et al.* 2019). For example, shift workers are at a greater risk of developing obesity and metabolic disorders such as type 2 diabetes (Antunes *et al.* 2010). These data suggest that temporally disordered behavioural rhythms can incur metabolic consequences, likely due, in part, to an uncoupling of centrally and peripherally located clocks.

Human intervention studies have also revealed that the metabolic responses to meals are profoundly influenced by the time of day at which they are consumed (Garaulet *et al.* 2013; Jakubowicz *et al.* 2013). Postprandial glycaemic control peaks in the morning, before gradually reducing to its nadir in the evening (Van Cauter *et al.* 1997), likely due to decreased peripheral insulin sensitivity and impaired β-cell responsiveness later in the day (Saad *et al.* 2012). Moreover, greater weight loss has been observed in participants with overweight or obesity when energy intake was biased towards early, rather than late in the day, despite no differences in self-reported energy intake (Garaulet *et al.* 2013; Jakubowicz *et al.* 2013). These weight loss findings imply an alteration in energy balance kinetics in response to energy distribution. One possibility is increased dietary induced thermogenesis (DIT), which is the energy expended digesting and assimilating nutrients. DIT has been shown to be greater in the initial 2-hour postprandial period in the morning compared to the evening (Morris *et al.* 2015), although differences may be lost when assessed over longer periods (Weststrate *et al.* 1989). It is also possible that undetected changes in energy expenditure and/or energy intake may have influenced weight loss findings. Indeed, physical activity was not closely monitored in these studies and there are well-reported issues with assessing food intake from self-

report methods (Dhurandhar *et al.* 2015). Nevertheless, these data suggest that encouraging food intake at specific times of the day and reducing it at others may be conducive to weight loss, but the behavioural responses which could mediate these outcomes are not well understood. Understanding how altered meal timing affects behaviours influencing energy balance is of paramount importance for developing practical and achievable guidelines on how meal timing can be optimised to improve metabolic health.

Intermittent fasting – a possible strategy to improve diet adherence

Whilst making small, daily reductions in energy intake (*i.e.* continuous energy restriction) or altering energy distribution can be an effective means of managing bodyweight and improving metabolic health (Most *et al.* 2017), many individuals struggle with the daily adherence required by such interventions. Interestingly, these adherence problems may be partly due to the requirement to limit energy intake across the entire day or at specific mealtimes, rather than abstain from food intake completely (Templeman *et al.* 2020). The absence of satiety after eating smaller meals (Alajmi *et al.* 2016) and calorie-counting difficulties (Das *et al.* 2007) may be considerable barriers for some individuals, and therefore, dietary interventions that require complete energy intake abstinence may offer a practical advantage over energy restriction (Johnstone 2015; Parr *et al.* 2020). Intermittent fasting requires a transition from the traditional Western meal pattern of three or more evenly spaced meals per day to a meal pattern that shortens periods of energy consumption and extends periods of fasting. There are several sub-types of intermittent fasting (Table 1), and whilst each type differs slightly in its application, all involve extended periods of severe (≤25% energy requirements) or complete (*i.e.* fasting) energy restriction, alternated with periods of adequate or *ad libitum* energy intake.

Evidence from randomised controlled trials indicates that intermittent fasting is as effective as continuous energy restriction for weight loss and improving metabolic health (Barnosky *et al.* 2014). However, the majority of long-term studies (>8 weeks) have utilised versions of intermittent fasting that permit small energy intakes (400–600 kcal) within fasting windows, such as 5:2 and alternate-day modified fasting (Carter *et al.* 2016; Trepanowski *et al.* 2017). Despite this approach having only a minor impact on energy balance, interrupting the fasting period may preclude the attainment of health benefits that extend beyond simple calorie restriction. Researchers postulate that fasting periods exceeding 12 hours will accelerate lipolysis and oxidation of endogenous lipid-derived substrates, known as 'flipping the metabolic switch' (Anton *et al.* 2018). Research in rodents has shown that imposing a daily fasting period of 16 hours can elicit several health benefits, including improved insulin sensitivity, reduced hyperlipidaemia, reduced inflammation and reduced bodyweight, without the need to restrict absolute energy intake (Hatori *et al.* 2012). Prolonged periods of fasting are uncommon within the traditional Western meal paradigm, which is characterised by short intervals between meals. This results in the majority of the day being spent in the postprandial state, concomitant with elevated glucose, insulin and triglyceride concentrations, producing a lipogenic state conducive to fat mass accretion (Saponaro *et al.*

Table 1 Characteristics of popular continuous and intermittent methods of dieting as frequently implemented in research

Diet	Characteristics
Continuous Energy Restriction	Daily reduction (typically ∼ 25%) in energy intake below baseline intake.
Intermittent Fasting	An umbrella term for diets involving extended periods of severe or complete energy restriction, alternated with periods of adequate or *ad libitum* energy intake.
Time-Restricted Eating	Daily fasting periods within defined time windows during the day with adequate or *ad libitum* energy intake during non-fasting periods.
5:2	Severe energy restriction (typically consuming 400–600 kcal per day) on two consecutive or non-consecutive days of the week with adequate or *ad libitum* energy intake on the remaining 4 days.
Alternate-Day Fasting	Complete fasting for 24-hour periods, alternated with 24-hour periods of adequate or *ad libitum* energy intake.
Modified Alternate-Day Fasting	Severe energy restriction (typically ≤25% energy requirements) for 24-hour periods, alternated with 24-hour periods of adequate or *ad libitum* energy intake.

2015). Therefore, despite the current consensus that the benefits of intermittent fasting are largely driven by energy restriction, interventions involving fasting intervals of sufficient duration to elicit a metabolic shift may be able to unlock independent health benefits (Anton et al. 2018).

Time-restricted eating – skipping meals to improve metabolic health

Extended morning fasting

Assuming a regular sleep pattern, the most practical approach to sustaining a fast of more than 12 hours is to extend the morning fast by delaying the first intake of energy and/or to extend the evening fast by advancing the final intake of energy. One approach to extending the morning fast is to skip breakfast. It has been reported that 36% of the UK population either sometimes or regularly skip breakfast (Reeves et al. 2013), implying that morning fasting is a frequently utilised form of intermittent fasting. Compared to fasting, consuming a morning meal appears to acutely increase resting energy expenditure beyond that of the natural morning elevation in resting energy expenditure, a response which is likely driven entirely by an increase in DIT following nutrient ingestion (Kobayashi et al. 2014; Clayton et al. 2016; Nas et al. 2017). However, when assessing 24-hour metabolism in a respiratory chamber under isocaloric dietary conditions, morning fasting has been shown to have no effect (Kobayashi et al. 2014), or to slightly increase (+41 kcal/day) energy expenditure, whilst also increasing fat oxidation (Nas et al. 2017). Moro et al. (2016) assessed the effects of 8 weeks of extended morning fasting (meals consumed at 13:00, 16:00 and 20:00) with a control trial (meals consumed at 08:00, 13:00 and 20:00) under isocaloric conditions in a sample of healthy men performing resistance training 3 times per week. Delaying the first meal until 13:00 resulted in greater fat mass loss compared to the control trial, which was concomitant with a relative increase in the contribution of fat oxidation to resting energy expenditure. Moreover, reductions in fasting glucose and insulin concentrations were observed only in the morning fasting trial. This evidence supports the notion that routinely fasting beyond 12 hours can elicit a metabolic shift and may lead to improvements in health outcomes.

Although cross-sectional studies have regularly associated skipping breakfast with a higher BMI (Odegaard et al. 2013), several acute studies have reported that skipping breakfast reduces energy intake within a 24-hour period (Clayton et al. 2015; Chowdhury et al. 2015). These studies also found that whilst appetite is significantly elevated in the morning after skipping breakfast, the next meal of the day (typically lunch) appears to offset appetite completely, resulting in no differences in appetite following this meal (Clayton et al. 2015; Chowdhury et al. 2015). Thus, these studies suggest that skipping breakfast may be an effective method of modulating energy intake and may assist in appetite control (Clayton & James 2016).

Despite breakfast skipping acutely reducing energy intake, some evidence suggests that 6 to 16 weeks of routine morning fasting does not significantly improve anthropometric measurements compared to morning meal consumption in either lean adults (Betts et al. 2014; Tinsley et al. 2019), or adults living with obesity (Schlundt et al. 1992; Chowdhury et al. 2016). However, these findings are not unequivocal. A recent 8-week study in adults with obesity reported a ~ 3% reduction in body mass when unrestricted eating was permitted from 13:00–19:00, compared to unrestricted eating with no timing constraints (Cienfuegos et al. 2020). It should be noted that the reduction in body mass observed in this study is smaller than expected, given the reported ~ 550 kcal/day reduction in energy intake in the morning fasting group. The minor or null body mass changes observed in these studies are likely due to passive compensatory reductions in components of energy expenditure, including resting energy expenditure, DIT and, perhaps most significantly, physical activity energy expenditure (PAEE) (Hall et al. 2012). Betts et al. (2014) investigated 6 weeks of fasting until midday or consuming breakfast (>700 kcal before 11:00) in lean individuals under free-living conditions. Results found that 24-hour energy intake was lower in the morning fasting trial compared to the breakfast trial, however, PAEE was also lower in the fasting trial. This reduction in PAEE fully compensated for the lower energy intake, leading to no change in anthropometric measurements between trials. When the same intervention was applied to individuals with obesity, there were trends for lower energy intake and PAEE in the morning fasting trial compared to the breakfast trial, although these did not reach statistical significance (Chowdhury et al. 2016). Nevertheless, these results indicate that the association previously identified between skipping breakfast and higher BMI may be causally related to compensatory changes in behaviour.

There is also tentative evidence from short-term intervention studies that repeated morning fasting

negatively affects insulin sensitivity and glycaemic control. Farshchi *et al.* (2005) found that fasting until 11:00 for 2 weeks decreased insulin sensitivity, compared to an isocaloric trial with first meal consumption at 08:00. Additionally, Betts *et al.* (2014) reported that fasting until midday under free-living conditions for 6 weeks resulted in higher glucose variability in the evening, compared to consuming at least 700 kcal before 11:00. It is possible that the detrimental glycaemic response to routine extended morning fasting is a product of adaptations to the acute response. Specifically, a larger postprandial elevation of plasma glucose, either with or without a concomitant increase in insulin concentrations, is typically observed in response to lunch, when breakfast has been omitted – often referred to as the 'second meal effect' (Jovanovic *et al.* 2009). This effect may be mediated by the upregulation of lipolysis in response to a prolonged overnight fast, causing a subsequent increase in plasma non-esterified fatty acid (NEFA) concentrations, which if sustained, can inhibit the insulin response (Grill & Qvigstad 2000). Consuming breakfast will stimulate the release of insulin and consequently suppress lipolysis during the morning, permitting greater insulin-stimulated glucose uptake into active tissues at lunch (Jovanovic *et al.* 2009).

The impact of morning fasting on the circadian system may also drive the metabolic outcomes. Rhythmic expression of the core clock and clock-controlled genes of the circadian system are strongly implicated in the successful regulation of glucose homeostasis (Gachon *et al.* 2017). Accordingly, rodent and human studies have revealed associations between desynchronised or absent expression of circadian clock genes with reduced insulin secretion, β-cell proliferation and insulin resistance (Vieira *et al.* 2014). As highlighted earlier in this article, temporally disordered feeding patterns which likely cause misalignment between feeding-driven peripheral clocks and the light-driven central clock lead to deleterious metabolic outcomes (Antunes *et al.* 2010). Similarly, clock and clock-controlled gene expression was adversely altered during the morning when breakfast was omitted compared to when breakfast was consumed, in both healthy participants and participants with type 2 diabetes (Jakubowicz *et al.* 2017). These adverse effects on gene expression were not restored by the consumption of a lunch meal at 12:00, and glycaemic control was impaired at lunch in the breakfast skipping trial, suggesting that early meal consumption after the overnight fast may have a fundamental role in regulating normal circadian oscillations and glucose control. Moreover, even when the fasting duration is equidistant, NEFA concentrations display circadian variations and are typically greater in the afternoon and evening, compared to the morning (Morgan *et al.* 1999; Hutchison *et al.* 2019). As such, circadian regulation of lipid metabolism may be a mediating factor in the second meal effect.

In summary, time-restricted eating which omits food intake in the morning is shown to suppress acute daily energy intake, without compensatory elevations in appetite. However, its long-term effects on weight loss are equivocal due to concomitantly reduced PAEE, and some evidence suggests that glycaemic control may be impaired. It is important to note that implementing time-restricted eating by morning fasting and skipping breakfast are not synonymous and skipping breakfast may be associated with other deleterious behaviours (Keski-Rahkonen *et al.* 2003). However, morning fasting may be considered the most feasible method of achieving a >12 hour fast, therefore, research should seek to explore whether the benefits of extended fasting can be harnessed when implemented in the morning.

Extended evening fasting

Restricting eating opportunities to a window of 8 hours or less by morning fasting appears to be an effective method of increasing lipid oxidation and has been shown to improve insulin sensitivity when accompanied by regular exercise (Moro *et al.* 2016). However, outside experimentally controlled trials, extended morning fasting may not necessarily equate to time-restricted eating. For example, late-night eating may be a contributing factor to the poor long-term metabolic health outcomes associated with skipping breakfast (Kutsuma *et al.* 2014), and in the absence of a structured exercise regime, reductions in PAEE may also occur (Betts *et al.* 2014). Kelly *et al.* (2020) found that moving the breakfast meal from 08:00 to 22:00, whilst fixing lunch and dinner time, reduced 24-hour lipid oxidation, indicating different metabolic effects of consuming the same meal at a different time of the day. By contrast, studies which have restricted evening energy intake have shown improvements in several markers of metabolic health (Antoni *et al.* 2018; Gabel *et al.* 2018; Sutton *et al.* 2018; Parr *et al.* 2020) and achieved weight loss (LeCheminant *et al.* 2013; Gabel *et al.* 2018). Additionally, consuming all daily energy before 14:00, compared to 20:00, improved circadian clock and clock-controlled gene expression

(Jamshed *et al.* 2019), which is in contrast to the negative effects on clock gene expression observed with morning fasting (Jakubowicz *et al.* 2017). Numerous metabolic markers display 24-hour circadian variations, including plasma lipids (Morgan *et al.* 1999) and insulin sensitivity (Saad *et al.* 2012), which appears to ameliorate in the morning and decline in the evening. This suggests that time-restricted eating interventions restricting energy intake to an earlier eating window may be particularly efficacious for health.

Rodent studies have shown that restricting feeding to the early hours of the active phase can elicit numerous health benefits, including improvements in insulin sensitivity and blood lipid profile, and resistance to weight gain (Hatori *et al.* 2012). In rodents, these effects may be partly driven by increased 24-hour energy expenditure when meals are consumed exclusively within the early active phase (Hatori *et al.* 2012), but a similar effect does not appear to occur in humans. Four days of consuming all meals between 08:00–14:00, compared to 08:00–20:00, increased 24-hour fat oxidation, but had no effect on energy expenditure (Ravussin *et al.* 2019). Similar findings were reported in an older population (Kelly *et al.* 2020), and although one study reported that evening fasting marginally increased (+91 kcal/day) 24-hour energy expenditure compared to a 12-hour eating window (Nas *et al.* 2017), the fasting interval prior to assessment was not standardised. Discrepancies between human and rodent findings may be due to differences in physiology and/or behaviour; for example, rodents exhibit food-seeking tendencies during periods of food restriction (Mitchell *et al.* 2016), whereas humans appear to reduce physical activity in these circumstances (Betts *et al.* 2014; James *et al.* 2020). Moreover, the respiratory chamber method typically employed when assessing 24-hour energy expenditure in humans severely impedes free-living energy expenditure estimates, due to physical constraints imposed by their small size. As such, the effects of evening fasting compared to a traditional meal pattern on habitual PAEE, the most malleable component of energy expenditure, is not known.

Fasting after 19:00 for 2 weeks was shown to reduce daily energy intake by ~ 250 kcal and significantly reduced bodyweight compared to a control trial with no eating time restrictions (LeCheminant *et al.* 2013). Two further studies have extended the evening fast by advancing dinner by 1.5 hours, alongside delaying breakfast by 1.5 hours, for 10 weeks (Antoni *et al.* 2018), or by terminating eating at 18:00 until 10:00 the following day for 8 weeks (Gabel *et al.*

2018). Both studies observed reductions in self-reported energy intake, however, only one reported significant weight loss (Gabel *et al.* 2018). The reasons for these different findings are unclear but may relate to differences in study design or suggest a mediating role of altered energy expenditure. Self-reported energy intake should also be interpreted carefully due to potential issues with accuracy and sensitivity (Dhurandhar *et al.* 2015). Furthermore, the concomitant restriction of morning energy intake in the studies by Antoni *et al.* (2018) and Gabel *et al.* (2018) make it difficult to elucidate the specific effects of evening fasting on free-living energy intake.

Ravussin *et al.* (2019) observed that evening fasting from 14:00 compared to eating from 08:00 to 20:00 reduced mean fasting concentrations of the hunger-stimulating hormone ghrelin and increased the satiety hormone PYY in the evening, as well as reducing subjective appetite. Similar reductions in PYY and subjective appetite were observed by Sutton *et al.* (2018) in response to 5 weeks of evening fasting. However, infrequent appetite hormone assessment was a limitation of both studies. Only fasted blood samples were taken, and the proximity of sampling to the prior meal was not standardised between trials. Therefore, the effects of evening fasting on indices of energy balance, particularly outside strict dietary and experimental control, are not well understood.

In the most comprehensive study to date, eight men with pre-diabetes underwent 5 weeks of consuming energy and macronutrient matched diets within either a 6-hour (08:00–14:00) or a 12-hour (08:00–20:00) eating window (Sutton *et al.* 2018). Extended evening fasting increased insulin sensitivity and improved blood lipid profile in the absence of weight loss, suggesting that evening fasting can induce metabolic benefits without altering energy balance. Unfortunately, the 7-week washout period employed in this study was seemingly insufficient to normalise baseline measurements following the evening fasting trial, and a trial order effect was observed for baseline insulinaemia. Whilst hindering interpretation of the results, this suggests that 5 weeks of evening fasting may have a profound and long-lasting positive effect on insulin sensitivity in this population.

Only one study has directly compared morning and evening fasting. Fifteen men with overweight or obesity and at elevated risk of type 2 diabetes completed 7 days of morning fasting (eating between 12:00–21:00) and 7 days of evening fasting (eating between 08:00–17:00), in randomised crossover order (Hutchison *et al.* 2019). To ensure equidistant

fasting periods prior to sampling, the pre- and post-intervention metabolic assessments occurred in conjunction with the first meal of the day for each trial and consequently commenced at different times (08:00 or 12:00). Results found that both time-restricted eating methods reduced postprandial plasma glucose and fasting triglyceride concentrations and tended to reduce postprandial insulin, although there were no differences between trials. Mean fasting blood glucose concentrations over the intervention period (assessed with continuous glucose monitoring) were reduced following evening fasting only. These findings provide some support to the notion that reducing the window between the first and final meal of the day can improve metabolic profile, but advancing the final meal may be preferable for 24-hour glucose control. Both trials similarly resulted in~ 1 kg body mass loss. Energy intake was not closely monitored in this study, although measurement of several appetite hormones revealed no differences between trials. Estimates of free-living PAEE from wrist- and arm-worn accelerometers were also not different between trials, although wrist- and arm-worn accelerometers have previously shown questionable accuracy (O'Driscoll et al. 2020). Therefore, whilst time-restricted eating by extending the overnight fast appears to be a potent method of improving glycaemic control, the specific effects of evening and morning fasting on energy balance remain unclear.

At present, time-restricted eating implemented either as morning or evening fasting appears to elicit a metabolic shift towards lipid oxidation, which may convey benefits to health. However, the outlook on the long-term effects of morning fasting is less positive and associated behaviour changes, such as late-night eating and reduced PAEE, may outweigh potential benefits. By contrast, evening fasting appears to elicit several benefits to metabolic health, but the effects on behaviour are poorly understood. Therefore, the proposed study will aim to elucidate and compare the acute effects of morning and evening fasting on parameters of metabolic health and behaviours influencing energy balance. Additionally, this study will explore time-restricted eating in a lean population, providing evidence on the acute effects these interventions might have if employed infrequently.

Study objectives

Early evidence suggests that time-restricted eating could be optimised if implemented as an evening fast (Sutton et al. 2018; Jamshed et al. 2019), however, very little is currently known about how evening fasting influences indices of energy balance. In addition, the majority of studies in this field have been conducted in adults with overweight or obesity, often with underlying health problems (Sutton et al. 2018; Hutchison et al. 2019; Jamshed et al. 2019), and there is a paucity of data in lean individuals. This is despite evidence that weight gain begins in early adulthood, indicating that nutritional interventions to regulate energy balance should be implemented early to prevent weight gain later in life (Østbye et al. 2011). Therefore, the focus of this study is to comprehensively evaluate the acute effects of morning and evening fasting on indices of energy balance and metabolic health, in lean adults. The key objectives of the study are represented schematically in Figure 1.

The project will compare how physiology and behaviour are influenced by an acute episode of extended morning and evening fasting, as well as a control trial. There are two overriding objectives:

- to assess how energy balance parameters, including energy intake and PAEE, respond to extended morning and evening fasting and explore underlying endocrinal mechanisms influencing eating behaviour;
- to investigate the acute effects of morning and evening fasting on important indices of metabolic health, including glycaemic control, blood lipid profile and substrate oxidation.

This study will expand current understanding by exploring how singular, infrequent exposure to morning and evening fasting will influence acute energy balance and metabolic parameters. This holds particular relevance for lean, metabolically healthy individuals, who are only likely to adopt such interventions occasionally as a preventative measure against weight gain. An overview of the literature, research gaps and aims of the study are outlined in Figure 2. Based on the outlined evidence and data in UK adults showing that ~ 40% of daily energy intake is consumed in the evening (Almoosawi et al. 2016), the principal hypothesis for this study is that evening fasting will reduce energy intake and elevate markers of appetite, but will improve glycaemic control, relative to morning fasting and a control trial.

Methodology

Lean, young and healthy individuals (age: 18–30 years; BMI: 20–27 kg/m^2; body fat: <25%) will complete a randomised crossover study, involving two consecutive
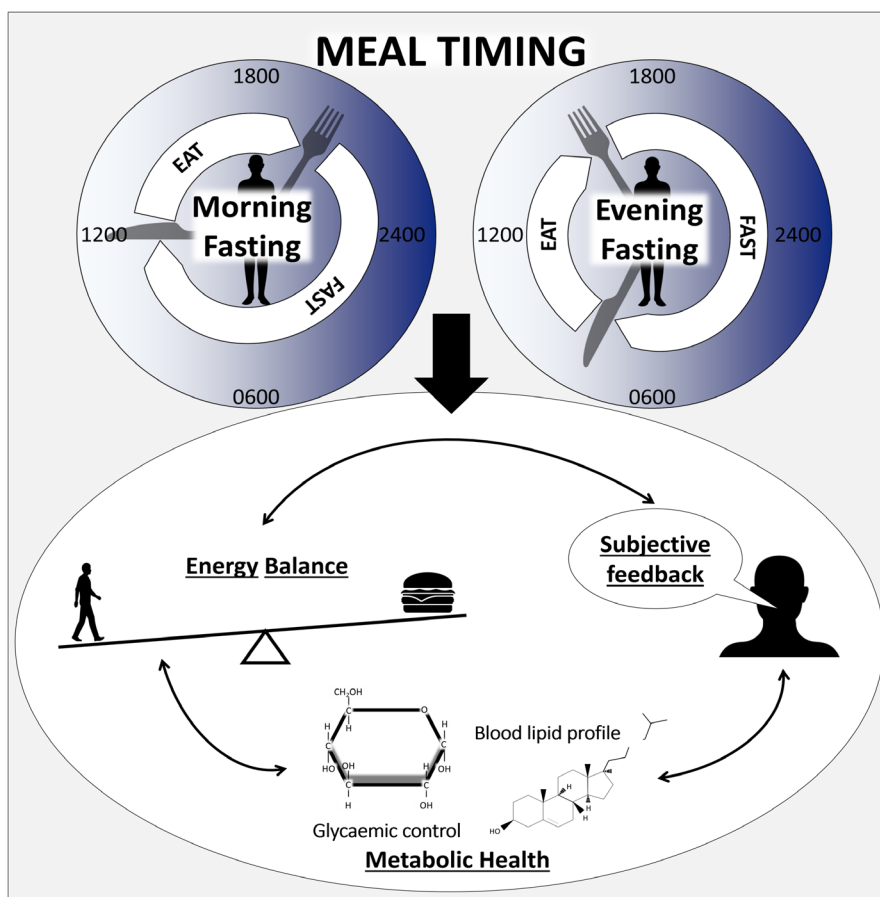
Figure 1 Key objectives of the study are to understand how acute morning and evening fasting influences energy balance and key markers of metabolic health. Specifically, this study will focus on how these interventions influence behaviour, and obtain additional subjective experience information, providing comprehensive pilot data on the acute response. This will support chronic fasting interventions exploring weight management and metabolic health.

days of either morning fasting (mFAST; all calories consumed between 12:00–20:00), evening fasting (eFAST; all calories consumed between 08:00–16:00), or a control trial (CON; all calories consumed between 08:00–20:00), separated by at least 7 days.

Day 1: After collection of a baseline fasted blood sample, expired gas sample and appetite perceptions (Flint *et al.* 2000), participants will be fitted with an Actiheart monitor to continuously measure PAEE throughout the trial period. Participants will consume a 24-hour standardised diet (50% carbohydrate, 30% fat, 20% protein), containing 100% of energy requirements determined by predictive equations, distributed over the day in accordance with the experimental trial. Participants will fast (consuming only a prescribed amount of plain water) outside of the permitted eating window, with appetite perceptions collected periodically throughout.

Day 2: Participants will return to the laboratory after a 12-hour (CON) or 16-hour overnight fast (mFAST and eFAST). To ensure equidistant fasting durations between the two fasting trials, participants will attend the laboratory at 07:30 (eFAST and CON) or 11:30 (mFAST). Baseline measurements will be repeated in the fasted state, after which participants will consume a mixed macronutrient meal (~700 kcal; 70% carbohydrate, 15% fat, 15% protein) followed by repeated blood, expired gas and appetite perception sampling for 3.5 hours. After the postprandial assessment, participants will be given access to an *ad libitum* buffet and permitted to eat until they feel satisfied. Upon leaving the laboratory, participants will adhere to the same eating window as day 1, weighing and recording all subsequent food and drink intake in a food diary. A full study schematic is shown in Figure 3.
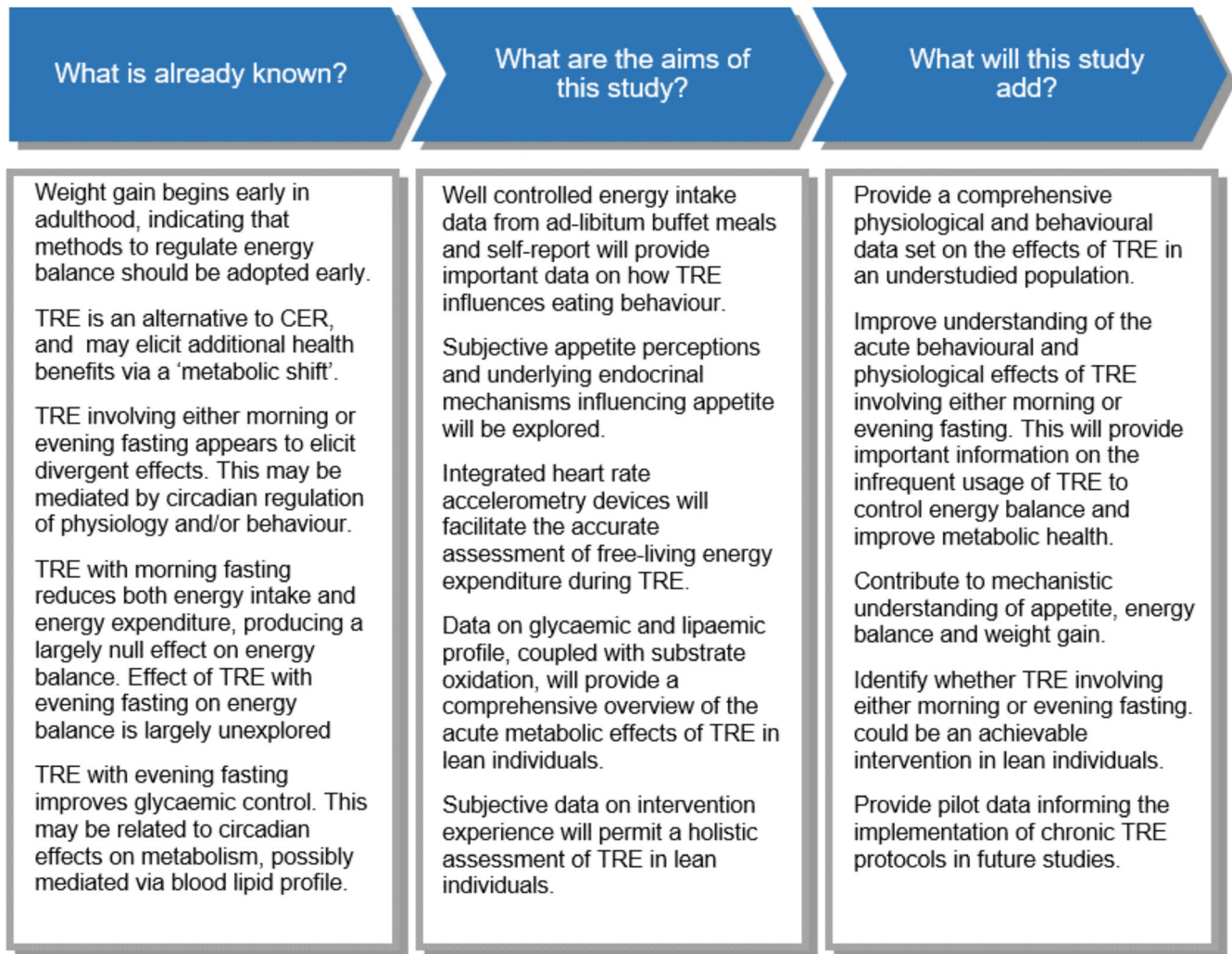
What is already known?	What are the aims of this study?	What will this study add?
Weight gain begins early in adulthood, indicating that methods to regulate energy balance should be adopted early. TRE is an alternative to CER, and may elicit additional health benefits via a 'metabolic shift'. TRE involving either morning or evening fasting appears to elicit divergent effects. This may be mediated by circadian regulation of physiology and/or behaviour. TRE with morning fasting reduces both energy intake and energy expenditure, producing a largely null effect on energy balance. Effect of TRE with evening fasting on energy balance is largely unexplored TRE with evening fasting improves glycaemic control. This may be related to circadian effects on metabolism, possibly mediated via blood lipid profile.	Well controlled energy intake data from ad-libitum buffet meals and self-report will provide important data on how TRE influences eating behaviour. Subjective appetite perceptions and underlying endocrinal mechanisms influencing appetite will be explored. Integrated heart rate accelerometry devices will facilitate the accurate assessment of free-living energy expenditure during TRE. Data on glycaemic and lipaemic profile, coupled with substrate oxidation, will provide a comprehensive overview of the acute metabolic effects of TRE in lean individuals. Subjective data on intervention experience will permit a holistic assessment of TRE in lean individuals.	Provide a comprehensive physiological and behavioural data set on the effects of TRE in an understudied population. Improve understanding of the acute behavioural and physiological effects of TRE involving either morning or evening fasting. This will provide important information on the infrequent usage of TRE to control energy balance and improve metabolic health. Contribute to mechanistic understanding of appetite, energy balance and weight gain. Identify whether TRE involving either morning or evening fasting. could be an achievable intervention in lean individuals. Provide pilot data informing the implementation of chronic TRE protocols in future studies.

Figure 2 Summary of current research, the aims of the proposed study and what this study will contribute to current understanding. TRE, time-restricted eating; CER, continuous energy restriction.

Outcomes

This study will provide a comprehensive data set to assess the acute effects of morning and evening fasting in lean individuals, whilst also generating important pilot data to inform long-term interventions assessing whether infrequent implementation of fasting can elicit adaptations in this cohort. Important information regarding eating behaviour will be obtained by combining rigorously controlled laboratory eating protocols alongside low-burden participant self-reported data, in accordance with best practice recommendations (Blundell *et al.* 2010). Supporting this, subjective appetite perceptions will be evaluated periodically throughout each intervention period and mechanistic data on the endocrinal regulation of appetite will be assessed (including acylated ghrelin, GLP-1 and PYY). Individually calibrated Actiheart monitors, which combine accelerometry and heart rate to yield the most accurate estimation of free-living energy expenditure from any device (Chowdhury *et al.* 2017), will provide novel data on PAEE in response to evening fasting. An advantage of Actiheart monitors over doubly labelled water is that they can detect subtle temporal changes in PAEE and are not confounded by alterations in substrate oxidation, both of which might be anticipated with a dietary intervention involving fasting (Betts *et al.* 2014; Anton *et al.* 2018). Plasma concentrations of glucose, insulin, NEFA and triglycerides, alongside fat and carbohydrate oxidation data from expired gas samples, will provide a comprehensive metabolic profile, permitting the
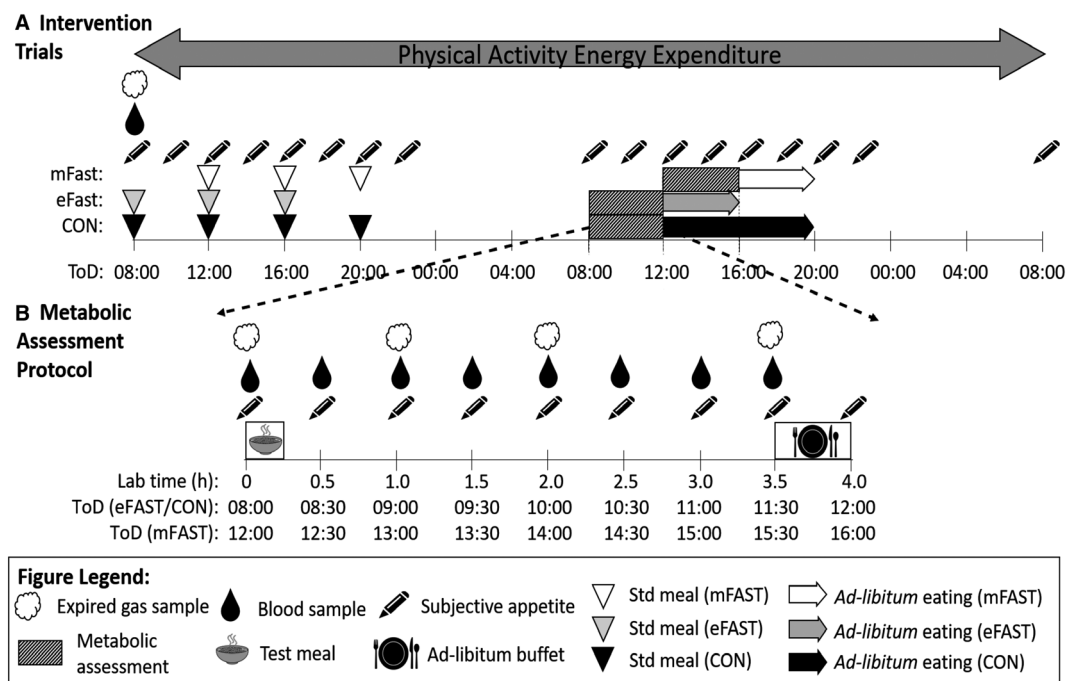
Figure 3 Schematic representation of study protocol. (a) Each intervention arm involves two days adherence to morning fasting (mFAST; all calories consumed between 12:00–20:00), evening fasting (eFAST; all calories consumed between 08:00–16:00) or a control eating schedule (CON; all calories consumed between 08:00–20:00). On day 2 of each trial, participants will attend the laboratory for a metabolic assessment protocol (b), with blood, gas and subjective appetite sampled for 3.5 hours after consuming a test meal. Following this, participants eat freely from an *ad libitum* buffet, before leaving the laboratory and recording all subsequent food intake within their eating window. ToD, time of day.

examination of glycaemic and lipaemic responses to acute morning and evening fasting. Finally, subjective data relating to participant experience of the interventions will contribute an improved holistic understanding about the utility of periodic fasting to improve metabolic health outcomes in this population.

Conclusions

Early evidence indicates that evening fasting is an efficacious way to implement intermittent fasting to improve metabolic health. This study aims to further understand how evening fasting influences energy balance, whilst also providing a comprehensive examination of the acute metabolic responses in lean individuals. This information will contribute to current understanding and may lead to innovative knowledge on the optimisation of intermittent fasting methods in the context of weight management.

Acknowledgements

Conflict of interest

None.

Funding

Author contributions

David Clayton, William Mode and Tommy Slater all contributed to the writing and preparation of the manuscript.

References

Alajmi N, Deighton K, King JA *et al.* (2016) Appetite and energy intake responses to acute energy deficits in females versus males. *Medicine and Science in Sports and Exercise* 48: 412–20.

Almoosawi S, Vingeliene S, Karagounis LG *et al.* (2016) Chrono-nutrition: a review of current evidence from observational studies on global trends in time-of-day of energy intake and its association with obesity. *Proceedings of the Nutrition Society* 75: 487–500.

Anton SD, Moehl K, Donahoo WT *et al.* (2018) Flipping the metabolic switch: understanding and applying the health benefits of fasting. *Obesity* **26**: 254–68.

Antoni R, Robertson TM, Robertson MD *et al.* (2018) A pilot feasibility study exploring the effects of a moderate time-restricted feeding intervention on energy intake, adiposity and metabolic physiology in free-living human subjects. *Journal of Nutritional Science* **7**: 1–6.

Antunes LC, Levandovski R, Dantas G *et al.* (2010) Obesity and shift work: chronobiological aspects. *Nutrition Research Reviews* **23**: 155–68.

Barnosky AR, Hoddy KK, Unterman TG *et al.* (2014) Intermittent fasting vs daily calorie restriction for type 2 diabetes prevention: a review of human findings. *Translational Research* **164**: 302–11.

Betts JA, Richardson JD, Chowdhury EA *et al.* (2014) The causal role of breakfast in energy balance and health: a randomized controlled trial in lean adults. *The American Journal of Clinical Nutrition* **100**: 539–47.

Blundell J, De Graaf C, Hulshof T *et al.* (2010) Appetite control: methodological aspects of the evaluation of foods. *Obesity Reviews* **11**: 251–70.

Carter S, Clifton PM & Keogh JB (2016) The effects of intermittent compared to continuous energy restriction on glycaemic control in type 2 diabetes; a pragmatic pilot trial. *Diabetes Research and Clinical Practice* **122**: 106–12.

Chowdhury EA, Richardson JD, Holman GD *et al.* (2016) The causal role of breakfast in energy balance and health: a randomized controlled trial in obese adults. *The American Journal of Clinical Nutrition* **103**: 747–56.

Chowdhury EA, Richardson JD, Tsintzas K *et al.* (2015) Carbohydrate-rich breakfast attenuates glycaemic, insulinaemic and ghrelin response to ad libitum lunch relative to morning fasting in lean adults. *British Journal of Nutrition* **114**: 98–107.

Chowdhury EA, Western MJ, Nightingale TE *et al.* (2017) Assessment of laboratory and daily energy expenditure estimates from consumer multi-sensor physical activity monitors. *PLoS One* **12**: e0171720.

Cienfuegos S, Gabel K, Kalam F *et al.* (2020) Effects of 4-and 6-h Time-Restricted Feeding on Weight and Cardiometabolic Health: A Randomized Controlled Trial in Adults with Obesity. *Cell Metabolism* **32**: 366–78.

Clayton DJ, Barutcu A, Machin C *et al.* (2015) Effect of breakfast omission on energy intake and evening exercise performance. *Medicine & Science in Sports & Exercise* **47**: 2645–52.

Clayton DJ & James LJ (2016) The effect of breakfast on appetite regulation, energy balance and exercise performance. *Proceedings of the Nutrition Society* **75**: 319–27.

Clayton DJ, Stensel DJ & James LJ (2016) Effect of breakfast omission on subjective appetite, metabolism, acylated ghrelin and GLP-17-36 during rest and exercise. *Nutrition* **32**: 179–85.

Curioni CC & Lourenco PM (2005) Long-term weight loss after diet and exercise: a systematic review. *International Journal of Obesity* **29**: 1168–74.

Dansinger ML, Gleason JA, Griffith JL *et al.* (2005) Comparison of the Atkins, Ornish, Weight Watchers, and Zone diets for weight loss and heart disease risk reduction: a randomized trial. *Journal of the American Medical Association* **293**: 43–53.

Das SK, Gilhooly CH, Golden JK *et al.* (2007) Long-term effects of 2 energy-restricted diets differing in glycemic load on dietary adherence, body composition, and metabolism in CALERIE: a 1-y

randomized controlled trial. *The American Journal of Clinical Nutrition* **85**: 1023–30.

Dhurandhar NV, Schoeller D, Brown AW *et al.* (2015) Energy balance measurement: when something is not better than nothing. *International Journal of Obesity* **39**: 1109–13.

Dibner C, Schibler U & Albrecht U (2010) The mammalian circadian timing system: organization and coordination of central and peripheral clocks. *Annual Review of Physiology* **72**: 517–49.

Farshchi HR, Taylor MA & Macdonald IA (2005) Deleterious effects of omitting breakfast on insulin sensitivity and fasting lipid profiles in healthy lean women. *The American Journal of Clinical Nutrition* **81**: 388–96.

Flint A, Raben A, Blundell JE *et al.* (2000) Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International Journal of Obesity* **24**: 38–48.

Gabel K, Hoddy KK, Haggerty N *et al.* (2018) Effects of 8-hour time restricted feeding on body weight and metabolic disease risk factors in obese adults: a pilot study. *Nutrition and Healthy Aging* **4**: 345–53.

Gachon F, Loizides-Mangold U, Petrenko V *et al.* (2017) Glucose homeostasis: regulation by peripheral circadian clocks in rodents and humans. *Endocrinology* **158**: 1074–84.

Garaulet M, Gómez-Abellán P, Alburquerque-Béjar JJ *et al.* (2013) Timing of food intake predicts weight loss effectiveness. *International Journal of Obesity* **37**: 604–11.

Grill V & Qvigstad E (2000) Fatty acids and insulin secretion. *British Journal of Nutrition* **83**: S79–S84.

Hall KD, Heymsfield SB, Kemnitz JW *et al.* (2012) Energy balance and its components: implications for body weight regulation. *The American Journal of Clinical Nutrition* **95**: 989–94.

Hatori M, Vollmers C, Zarrinpar A *et al.* (2012) Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metabolism* **15**: 848–60.

Hutchison AT, Regmi P, Manoogian EN *et al.* (2019) Time-restricted feeding improves glucose tolerance in men at risk for type 2 diabetes: a randomized crossover trial. *Obesity* **27**: 724–32.

Jakubowicz D, Barnea M, Wainstein J *et al.* (2013) High caloric intake at breakfast vs. dinner differentially influences weight loss of overweight and obese women. *Obesity* **21**: 2504–12.

Jakubowicz D, Wainstein J, Landau Z *et al.* (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**: 1573–79.

James R, James LJ & Clayton DJ (2020) Anticipation of 24 h severe energy restriction increases energy intake and reduces physical activity energy expenditure in the prior 24 h, in healthy males. *Appetite* **152**: 104719.

Jamshed H, Beyl RA, Della Manna DL *et al.* (2019) Early time-restricted feeding improves 24-hour glucose levels and affects markers of the circadian clock, aging, and autophagy in humans. *Nutrients* **11**: 1234.

Johnston JD, Ordovás JM, Scheer FA *et al.* (2016) Circadian rhythms, metabolism, and chrononutrition in rodents and humans. *Advances in Nutrition* **7**: 399–406.

Johnstone A (2015) Fasting for weight loss: an effective strategy or latest dieting trend? *International Journal of Obesity* **39**: 727–33.

Jovanovic A, Leverton E, Solanky B *et al.* (2009) The second-meal phenomenon is associated with enhanced muscle glycogen storage in humans. *Clinical Science* **117**: 119–27.

Kelly KP, McGuinness OP, Buchowski M *et al.* (2020) Eating breakfast and avoiding late-evening snacking sustains lipid oxidation. *PLoS Biology* **18**: e3000622.

Keski-Rahkonen A, Kaprio J, Rissanen A *et al.* (2003) Breakfast skipping and health-compromising behaviors in adolescents and adults. *European Journal of Clinical Nutrition* **57**: 842–53.

Kobayashi F, Ogata H, Omi N *et al.* (2014) Effect of breakfast skipping on diurnal variation of energy metabolism and blood glucose. *Obesity Research & Clinical Practice* **8**: E249–57.

Kutsuma A, Nakajima K & Suwa K (2014) Potential association between breakfast skipping and concomitant late-night-dinner eating with metabolic syndrome and proteinuria in the Japanese population. *Scientifica* **2014**: 253581.

LeCheminant JD, Christenson E, Bailey BW *et al.* (2013) Restricting night-time eating reduces daily energy intake in healthy young men: a short-term cross-over study. *British Journal of Nutrition* **110**: 2108–13.

Lewis P, Oster H, Korf HW *et al.* (2020) Food as a circadian time cue – evidence from human studies. *Nature Reviews Endocrinology* **16**: 213–23.

Mitchell SE, Delville C, Konstantopedos P *et al.* (2016) The effects of graded levels of calorie restriction: V. Impact of short term calorie and protein restriction on physical activity in the C57BL/6 mouse. *Oncotarget* **7**: 19147–70.

Mohawk JA, Green CB & Takahashi JS (2012) Central and peripheral circadian clocks in mammals. *Annual Review of Neuroscience* **35**: 445–62.

Morgan LM, Aspostolakou F, Wright J *et al.* (1999) Diurnal variations in peripheral insulin resistance and plasma non-esterified fatty acid concentrations: a possible link? *Annals of Clinical Biochemistry* **36**: 447–50.

Moro T, Tinsley G, Bianco A *et al.* (2016) Effects of eight weeks of time-restricted feeding (16/8) on basal metabolism, maximal strength, body composition, inflammation, and cardiovascular risk factors in resistance-trained males. *Journal of Translational Medicine* **14**: 290.

Morris CJ, Garcia JI, Myers S *et al.* (2015) The human circadian system has a dominating role in causing the morning/evening difference in diet-induced thermogenesis. *Obesity* **23**: 2053–8.

Most J, Tosti V, Redman LM *et al.* (2017) Calorie restriction in humans: an update. *Ageing Research Reviews* **39**: 36–45.

Nas A, Mirza N, Hägele F *et al.* (2017) Impact of breakfast skipping compared with dinner skipping on regulation of energy balance and metabolic risk. *The American Journal of Clinical Nutrition* **105**: 1351–61.

NHS Digital (2020) *Statistics on Obesity, Physical Activity and Diet.* Available at: https://digital.nhs.uk/data-and-information/publications/statistical/statistics-on-obesity-physical-activity-and-diet/england-2020/part-3-adult-obesity-copy (accessed 13 July 2020).

O'Driscoll R, Turicchi J, Beaulieu K *et al.* (2020) How well do activity monitors estimate energy expenditure? A systematic review and meta-analysis of the validity of current technologies. *British Journal of Sports Medicine* **54**: 332–40.

Odegaard AO, Jacobs DR, Steffen LM *et al.* (2013) Breakfast frequency and development of metabolic risk. *Diabetes Care* **36**: 3100–106.

Østbye T, Malhotra R, Landerman LR *et al.* (2011) Body mass trajectories through adulthood: results from the National Longitudinal Survey of Youth 1979 Cohort (1981–2006). *International Journal of Epidemiology* **40**: 240–50.

Parr EB, Devlin BL, Radford BE *et al.* (2020) A delayed morning and earlier evening time-restricted feeding protocol for improving glycemic control and dietary adherence in men with overweight/obesity: a randomized controlled trial. *Nutrients* **12**: 505.

Polidori D, Sanghvi A, Seeley RJ *et al.* (2016) How strongly does appetite counter weight loss? Quantification of the feedback control of human energy intake. *Obesity* **24**: 2289–95.

Potter GD, Cade JE, Grant PJ *et al.* (2016) Nutrition and the circadian system. *British Journal of Nutrition* **116**: 434–42.

Ravussin E, Beyl RA, Poggiogalle E *et al.* (2019) Early time-restricted feeding reduces appetite and increases fat oxidation but does not affect energy expenditure in humans. *Obesity* **27**: 1244–54.

Reeves S, Halsey LG & McMeel Y (2013) Breakfast habits, beliefs and measures of health and wellbeing in a nationally representative UK sample. *Appetite* **60**: 51–57.

Ruddick-Collins LC, Johnston JD, Morgan PJ *et al.* (2018) The Big Breakfast Study: Chrono-nutrition influence on energy expenditure and bodyweight. *Nutrition Bulletin* **43**: 174–83.

Saad A, Dalla Man C, Nandy DK *et al.* (2012) Diurnal pattern to insulin secretion and insulin action in healthy individuals. *Diabetes* **61**: 2691–2700.

Saponaro C, Gaggini M, Carli F *et al.* (2015) The subtle balance between lipolysis and lipogenesis: a critical point in metabolic homeostasis. *Nutrients* **7**: 9453–74.

Schlundt DG, Hill JO, Sbrocco T *et al.* (1992) The role of breakfast in the treatment of obesity: a randomized clinical trial. *The American Journal of Clinical Nutrition* **55**: 645–51.

Stenvers DJ, Scheer FA, Schrauwen P *et al.* (2019) Circadian clocks and insulin resistance. *Nature Reviews Endocrinology* **15**: 75–89.

Storch KF, Lipan O, Leykin I *et al.* (2002) Extensive and divergent circadian gene expression in liver and heart. *Nature* **417**: 78–83.

Sutton EF, Beyl R, Early KS *et al.* (2018) Early time-restricted feeding improves insulin sensitivity, blood pressure, and oxidative stress even without weight loss in men with prediabetes. *Cell Metabolism* **27**: 1212–21.

Templeman I, Gonzalez JT, Thompson D *et al.* (2020) The role of intermittent fasting and meal timing in weight management and metabolic health. *Proceedings of the Nutrition Society* **79**: 76–87.

Tinsley GM, Moore ML, Graybeal AJ *et al.* (2019) Time-restricted feeding plus resistance training in active females: a randomized trial. *The American Journal of Clinical Nutrition* **110**: 628–40.

Trepanowski JF, Kroeger CM, Barnosky A *et al.* (2017) Effect of alternate-day fasting on weight loss, weight maintenance, and cardioprotection among metabolically healthy obese adults: a randomized clinical trial. *Journal of the American Medical Association –. Internal Medicine* **177**: 930–38.

Van Cauter E, Polonsky KS & Scheen AJ (1997) Roles of circadian rhythmicity and sleep in human glucose regulation. *Endocrine Reviews* **18**: 716–38.

Vieira E, Burris TP & Quesada I (2014) Clock genes, pancreatic function, and diabetes. *Trends in Molecular Medicine* **20**: 685–93.

Weststrate JA, Weys PJ, Poortvliet EJ *et al.* (1989) Diurnal variation in postabsorptive resting metabolic rate and diet-induced thermogenesis. *The American Journal of Clinical Nutrition* **50**: 908–14.