Effects of intermittent fasting on glucose and lipid metabolism

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Effects of intermittent fasting on glucose and lipid metabolism

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Two intermittent fasting variants, intermittent energy restriction (IER) and time-restricted feeding (TRF), have received considerable interest as strategies for weight-management and/or improving metabolic health. With these strategies, the pattern of energy restriction and/or timing of food intake are altered so that individuals undergo frequently repeated periods of fasting. This review provides a commentary on the rodent and human literature, specifically focusing on the effects of IER and TRF on glucose and lipid metabolism. For IER, there is a growing evidence demonstrating its benefits on glucose and lipid homeostasis in the short-to-medium term; however, more long-term safety studies are required. Whilst the metabolic benefits of TRF appear quite profound in rodents, findings from the few human studies have been mixed. There is some suggestion that the metabolic changes elicited by these approaches can occur in the absence of energy restriction, and in the context of IER, may be distinct from those observed following similar weight-loss achieved via modest continuous energy restriction. Mechanistically, the frequently repeated prolonged fasting intervals may favour preferential reduction of ectopic fat, beneficially modulate aspects of adipose tissue physiology/morphology, and may also impinge on circadian clock regulation. However, mechanistic evidence is largely limited to findings from rodent studies, thus necessitating focused human studies, which also incorporate more dynamic assessments of glucose and lipid metabolism. Ultimately, much remains to be learned about intermittent fasting (in its various forms); however, the findings to date serve to highlight promising avenues for future research.

Intermittent fasting: Glucose: Lipids: Weight-loss

We are currently in the midst of a global obesity epidemic, with its prevalence more than doubling since 1980[1]. The development of obesity is closely associated with a number of metabolic complications including insulin resistance and dyslipidaemia, which can in turn increase an individual's risk of developing type 2 diabetes mellitus (T2DM) and CVD[2]. Glucose and lipid homeostasis can be improved through modest weight-loss, which due to current accepted dietary advice, is most commonly achieved via a modest continuous (daily) energy restriction (CER)[3]. Independently of weight-loss, the metabolic regulation of glucose and lipids can also be influenced by other dietary manipulations, including alterations in meal timing[4] as well as abrupt changes

in energy status such as fasting, which when protracted, elicits profound shifts in fuel utilisation that persist during the subsequent refeeding period[5].

In recent years, several intermittent fasting variants, including intermittent energy restriction (IER) and time-restricted feeding (TRF) have received considerable interest as alternative dietary strategies for weight-management and improving metabolic health. With these dietary strategies, the pattern of energy restriction and/or timing of food intake are altered, so that individuals undergo frequently repeated periods of fasting (Table 1). Their basic premise compared to CER is that an individual may not need to energy restrict either every day, or at all, to attain metabolic benefits. With

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Abbreviations: CER, continuous energy restriction; FAO, fatty-acid oxidation; FFA, free-fatty acid; HOMA-IR, homeostasis model assessment of insulin resistance; IER, intermittent energy restriction; T2DM, type 2 diabetes mellitus; TRF, time-restricted feeding.
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Table 1. Overview of weekly fasting schedule for the most commonly studied intermittent fasting protocols. 'Fast' is used to denote periods of substantial (total or partial, ≥70%) energy restriction. Intermittent energy restriction (IER) protocols involving more modest energy restriction, or IER–refeeding cycles not included in the review

	Mon	Tues	Wed	Thurs	Fri	Sat	Sun
5:2 diet	Fast	Fast	Feed	Feed	Feed	Feed	Feed
Alternate day fasting	Feed	Fast	Feed	Fast	Feed	Fast	Feed
Time-restricted feeding	≥12 h fast	≥12 h fast	≥12 h fast	≥12 h fast	≥12 h fast	≥12 h fast	≥12 h fast

these points in mind, the current review aims to provide a commentary on studies within the intermittent fasting literature, with a specific focus on the effects of IER and TRF on glucose and lipid metabolism. Where possible, the relative contribution of weight-loss/energy restriction and the intermittent fasting eating pattern *per se* to observed metabolic changes will be discussed. In addition, the review highlights potential avenues for future research.

Intermittent energy restriction

Overview and effects on body weight

The majority of rodent studies[6–21] and a small number of human studies[22–26] have used IER protocols, which completely restrict energy intake (i.e. 100% energy restriction) every other day, with fasting intervals ranging between 20 and 36 h. However, the long-term sustainability of this alternate day total fasting approach in human subjects is questionable due to the persistent hunger reported[24]. Subsequently, the IER protocols used by most human studies[27–41], and by some rodent studies[11,14,42], have allowed a small amount of 'fast' day intake, so that energy is substantially (≥70%) but not completely restricted. This is often referred to as modified fasting, such that, the term fasting in this IER context denotes periods of severe (total or partial) energy restriction. These modified fasting forms of IER have predominantly been utilised as weight-loss strategies, whilst a small number of studies have also used protocols intended to encourage weight maintenance[37,39,41]. The most well-studied examples include alternate day modified fasting and the 5:2 diet, which entails two modified fast days a week (Table 1), although many variations exist. Intakes on non-restricted ('feed') days among these studies have ranged from *ad libitum*[27–29,31–34,36,38], hypoenergetic (approximately 15–30% of energy requirements)[34,41], isoenergetic[30,37,40] or hyperenergetic (approximately 125–175% of energy requirements)[35,39]. Compliance is reportedly high[29,37], with both acute[5] and chronic[29,30,37,40] studies demonstrating a lack of full compensatory hyperphagia following modified fasting days. In overweight/obese[26–35,37,38,40,41] or combined healthy/overweight[36] cohorts, the average reported weight-loss through IER (70–100% energy restriction) has ranged between approximately 4 and 10% over dieting periods of 4–24 weeks. There are also some data demonstrating the successful applications of IER as a weight-maintenance

strategy following weight-loss for periods of up to 1 year[37,39].

Effects of intermittent energy restriction on glucose and lipid metabolism

Rodent studies. Although findings from rodent studies cannot be readily extrapolated to human subjects, they do permit a greater level of control over dietary intakes and environmental confounders. Rodent studies of IER have mostly used alternate day total fasting protocols. Over study intervals of 4 weeks to 1 year, this form of IER can reduce levels of fasting glucose[6,8,9,21], insulin[8,9,16–18,21] and insulin resistance (measured via homeostasis model assessment of insulin resistance (HOMA-IR))[16,17], which in turn reflects improved hepatic insulin sensitivity. Additionally, lipid profiles are also favourably altered in wild-type rodents[7,10,16], with similar observations made by one alternate day modified (85% energy restriction) fasting study[42]. Findings from a number studies, which have performed more dynamic assessments of glycaemic indices, through use of gold-standard hyperinsulinaemic–euglycaemic glucose clamp techniques to assess insulin sensitivity or glucose/insulin tolerance testing, have been mixed. Several studies have reported improvements in glycaemic control in rodents fed normal or low-fat chow[13,19], whilst some[17,20] (but not all[16]) studies have shown that IER is able to protect against high-fat diet-induced insulin resistance. In contrast, one study conducted in obesity-prone Sprague–Dawley rats noted a marked impairment in glucose tolerance after 8 months of IER (normal chow)[15].

Human studies: alternate day total fasting. A small number of studies have assessed the metabolic impacts of alternate day total fasting using a range of experimental techniques. Most recently, an 8-week pilot study completed by twenty-six obese male and female participants reported an average weight-loss of 9%, accompanied by beneficial reductions in LDL-cholesterol and fasting TAG[26]. The earlier 2[22,25]–3[23,24]-week studies conducted in small groups (*n* ⩽ 16) of healthy and overweight participants were not originally designed as weight-loss studies, although participants on the 3-week study struggled to sustain prescribed energy intakes on feed days, and so incurred a modest weight-loss[24]. No post-treatment changes in fasting levels of insulin and glucose (when measured after a feed day) were observed by these

studies[22,23,25], whereas one study reported improved lipid profiles[24]. Two studies used hyperinsulinaemic–euglycaemic glucose clamp techniques to assess changes in glycaemic control, yielding conflicting results. The hyperinsulinaemic–euglycaemic glucose clamp method involves concomitant infusions of insulin (to achieve hyperinsulinaemia) and glucose (to achieve a constant basal level of plasma glucose). Under steady-state euglycaemic conditions, the glucose infusion rate equals glucose uptake by all tissues and is therefore a measure of tissue sensitivity to exogenous insulin. Halberg et al.[22] reported an increase (improvement) in insulin-mediated whole-body glucose uptake after 2 weeks in healthy men (BMI 25·7 (SD 0·4) kg/m^2). In contrast, Soeters et al.[25], the only group among these earlier studies to have used a (cross-over) controlled study design, found no change in insulin sensitivity in lean men following the same duration of time. In a 3-week study by Heilbronn et al.[23], which was conducted in a mixed sex healthy/overweight cohort, significant post-treatment reductions in postprandial insulin responses to a test-meal challenge was noted among male participants, whereas there was a contrasting decline in glucose tolerance among female participants (with no change in the insulinaemic response). On closer examination of the study design, post-treatment metabolic assessments were performed following a fast day (i.e. after 36 h of fasting), whereas baseline assessments were conducted after a shorter 12-h overnight fast. As such, the unstandardised fasting periods makes it difficult to ascribe these findings as a true chronic treatment effect, given that prolonged fasting can also acutely alter postprandial substrate responses during the subsequent refeeding period[5]. Ultimately, the uncontrolled study designs represent a significant limitation of most of these earlier studies.

Human studies: modified fasting. To date, all IER studies allowing fast day intake have solely measured fasting blood markers of cardiometabolic risk. Some[27,30–33,35–37,41] but not all of these studies[28,29,34,40] have included CER (standard treatment) or *ad libitum* (no intervention) control groups, whilst others have compared two or more different IER protocols[35,38,39]. In individuals with T2DM, IER has been shown to improve glycaemic control and lipid profiles[27]. By contrast, many of the IER studies within heterogeneous non-T2DM populations, which have spanned from 4–24 weeks, have failed to show any significant effect on fasting glucose levels[28,34,37–40] or glycated Hb[37]; unsurprising findings given that glucose levels are usually kept under tight physiological control. However, improvements in other indices of insulin sensitivity have been observed, including reductions in fasting insulin[30,37,39,40] and/or HOMA-IR[30,37,40]. IER additionally elicits favourable alterations in fasted lipid profiles[28–33,35–38,41] including shifts in LDL sub-fraction distribution and/or size towards larger less atherogenic particles[31–33,36,38], even in the absence of overall change in total LDL[38]. Glycaemic and lipaemic outcomes do not appear to be influenced by meal timing on modified fast days[38], or by dietary composition on feed days, although in this particular study vascular function worsened slightly when a high-fat (45 % total energy) diet was consumed[35]. In healthy/overweight participants, Wegman et al.[39] compared 3 weeks of IER with antioxidant supplementation or placebo using a randomised cross-over design. The protocol consisted of an energy intake of 25 and 175 % of energy requirements on alternating days, designed to maintain energy balance. The study observed a modest within-treatment decline in fasting insulin following the IER plus placebo intervention only, but did not conduct statistical comparisons between antioxidant and placebo legs.

Outstanding questions

Weight-management is an integral part of reducing cardiometabolic risk; even modest amounts of weight-loss can improve glycaemic control and lipid profiles, which reflect improved glucose and lipid homeostasis. The weight-loss efficacy of IER is not in doubt however, from a metabolic standpoint, a number of questions remain outstanding:

Can intermittent energy restriction influence metabolism in the absence of overall energy restriction or weight-loss?. If IER could be shown to improve metabolic control in the absence of overall energy restriction or weight-loss, it would highlight additional applications of IER in healthy weight populations. There is some supportive evidence in the rodent literature to suggest that IER is able to improve glycaemic control markers, to a similar extent as CER (40 % energy restriction), despite no overall energy deficit[8]. In healthy/overweight human subjects, improvements in insulin sensitivity have been observed after 2–3 weeks of alternate day total and modified fasting in the absence of weight-change; however, these studies lacked appropriate *ad libitum* control group/interventions[22,39]. There is clear paucity of data in this area and so more controlled studies in healthy weight populations are required to address this particular question.

Does the mode of energy restriction (intermittent v. continuous) influence metabolic responses during weight-loss?. Ultimately it is the degree of dietary adherence and sustainability, rather than the type of dietary strategy, which will predict weight-loss outcomes. Understanding metabolic differences between dieting approaches is nonetheless important as it may identify potential applications of IER within certain patient subgroups. Rodent studies (examining glycaemic indices) have demonstrated comparable short-term outcomes between IER and CER[8,15,17], but also worse longer-term outcomes with IER[15], which is discussed further in the following section. Considering the rodent literature in its entirety, a large proportion of studies have reported a lack of full energy compensation on feed days, irrespective of background diet composition[9,10,13,16–19]. Inclusion of a separate pair-fed group, i.e. a group fed an identical quantity of food but on a daily basis, is one way in which future

studies could ascertain the relative contribution of the IER eating pattern and overall energy restriction to observed metabolic changes.

Of the relatively few human studies to directly compare IER to CER, some studies have demonstrated comparable metabolic outcomes between the two dietary approaches[26,27]. The 5:2 diet studies, which were conducted in overweight/obese women, found reductions in fasting insulin and HOMA-IR following IER that have exceeded those of CER (25 % energy restriction/day) after 3 months[39] and 6 months[32], with both studies reporting comparable average weight-loss between the two dieting groups. In a study by Varady *et al.*[32], this time using an alternate day modified fasting regimen, IER led to a significant greater reduction in fasting TAG than CER after 12 weeks, with both dieting groups attaining a comparable 5 % weight-loss.

From a metabolic perspective, without standardisation for weight-loss between dieting groups, it becomes difficult to interpret to what extent any underlying differences can be explained simply by the extent of weight-loss. Whilst most dietary comparison studies have reported similar average weight-loss between IER and CER groups overall, the degree of weight-loss can vary considerably at an individual level, as exemplified by one such study, which found a proportionally greater number of IER participants tending to attain a ⩾5 % weight-loss[37]. Notwithstanding, these data do highlight the potential for underlying differences between the two modes of energy restriction, which requires focused metabolic studies, which conduct assessments following standardised weight-loss as opposed to diet duration.

By what mechanisms does intermittent energy restriction alter metabolism, and how does this compare with continuous (daily) energy restriction?

Acute disturbances in fuel management. Over an acute timescale, fasting (both total and modified) elicits reciprocal shifts in fuel utilisation in a seemingly dose-responsive manner; free-fatty acid (FFA) mobilisation, fatty-acid oxidation (FAO) and ketogenesis progressively rise, favouring the conservation of glucose as its demand drops[5]. During the subsequent refeeding period after a prolonged (36 h) fast, postprandial fatty-acid partitioning remains shifted towards whole-body FAO and hepatic ketogenesis, translating to a marked reduction in postprandial lipaemia[5]. From a chronic perspective, these acute disturbances in fuel management (experienced repeatedly during IER) may elicit very distinct alterations in glucose and lipid metabolism when compared with CER, and may not necessitate an overall energy restriction.

For instance, the premise of IER is that the repeated periods of FAO may promote or augment improvements in insulin sensitivity via the preferential reduction in the ectopic accumulation of lipid and associated cytosolic intermediaries, which are causally implicated in the development of insulin resistance[43]; by extension, this would also be expected to favourably impact other aspects of metabolism, such as the regulation of hepatic TAG metabolism[44]. In support of this theory are findings from the previously discussed human studies demonstrating superior reductions in HOMA-IR[30,37] and fasting TAG[32] with IER *v.* CER, whilst another study found suggestions of increased mitochondrial fatty-acid transporter expression in skeletal muscle after 3 weeks of IER, which reflects long-term adaptation to the repeated elevations in FFA/FAO[23]. In rodents, post-treatment reductions in hepatic steatosis have been observed[17,19], but the effects in human subjects remains unknown.

Whilst diminished FAO capacity has been implicated as a key instigator of insulin resistance, an alternative mitochondrial overload model by Koves *et al.*[45] proposes that increased FFA delivery is conducive to excessive and incomplete FAO and it is this that instead precipitates skeletal muscle insulin resistance. A major contributory factor is thought to be lipid-induced mitochondrial stress, which is the primary site of endogenous reactive oxygen species production. In Koves' model, the liver is considered relatively resilient to lipid-induced mitochondrial stress due to its capacity to channel FFA towards alternate metabolic pathways, with the ketogenic pathway being of particular relevance to fasting. Whilst Koves' theory is framed in the context of obesity/high-fat feeding, it draws parallels with IER in that metabolic tissues are repeatedly exposed to elevations in FFA/FAO with each fast–refeed cycle. Indeed the studies (in both rodents and human subjects), which report impaired glucose tolerance, all of which have used alternate day total fasting protocols, have also found increased markers of oxidative stress[15], oxidative insulin receptor modification[15], reduced GLUT content[16], or indications of reduced mitochondrial function markers[23] in skeletal muscle. Interestingly, insights into the time-course of glycaemic changes were provided by one of the rodent studies, which found that despite an initial improvement at 4 weeks, glucose tolerance then deteriorated over time[15]. These data highlight the potential for harm, as well as the potential for tissue-specific responses to IER given that two of these studies reported no change in fasting (hepatic) glycaemic indices[16,23].

Based on these data, we speculate that the alternate day total fasting paradigm may present too great or frequent metabolic challenge, which could potentially over time lead to aberrant rather than beneficial changes in peripheral insulin sensitivity. In human subjects, the effects of this form of IER on glycaemic control beyond 8 weeks (where no change was observed) is unknown[26]. Further research over longer timescales is now required to evaluate the potential harms and benefits of IER protocols that in fast frequency and the severity of fast day energy restriction, as well as tissue-specific changes in insulin sensitivity and ectopic lipid content. In addition, dynamic assessment of glucose and lipid metabolism, such as hyperinsulinaemic–euglycaemic glucose clamp techniques and postprandial challenges, may help to detect any underlying differences between IER and CER, which may not be immediately apparent when solely looking at singular blood measurements taken in the fasted state.

Change in adipose tissue morphology, physiology and distribution

Adipose tissue is integral to the maintenance of glucose and lipid homeostasis; not only does it act as important sink and storage organ for FFA, but also as a source of an array of adipokines capable of influencing whole-body metabolism. An important driver of obesity-associated metabolic disorders appears to be the pathological expansion of adipose tissue among predisposed individuals, the manifestations of which include: impaired adipogenesis and excessive adipocyte hypertrophy in subcutaneous depots; macrophage infiltration, resulting in low grade inflammation and altered adipokine secretion; impaired insulin action and dysregulated lipid storage; visceral adipose tissue accumulation. The result is the creation of an unfavourable metabolic and hormonal milieu, which drives the development of systemic insulin resistance and associated metabolic disorders[46].

Several studies have shown that rodents maintained on IER exhibit reduced visceral adipose tissue[14,42], as well as favourable reductions in fat cell size and augmented adipocyte differentiation within subcutaneous and visceral fat pads[11]. Levels of adiponectin, which has anti-atherogenic and insulin-sensitising properties, are usually reduced in states of obesity but increased through IER[14,21]. On the basis of one study, these beneficial changes in adipose tissue physiology and morphology appear to be comparable with CER[14], but what was unique to IER is that that it did not necessitate an overall energy deficit, which is perhaps attributable to the alternating periods of feeding and fasting. In human subjects, one uncontrolled alternate day total fasting found improved insulin-mediated lipolysis inhibition, reflecting improved insulin action, after 2 weeks[22]. In addition to reductions in total adiposity, IER weight-loss studies have reported reductions in visceral adipose tissue[38], truncal adiposity[26] as well as beneficial changes in circulating adipokine profiles. For example, pro-atherogenic adipokines, including leptin[26,30,36,37] are reduced, whilst adiponectin levels are increased[30,36]. Among studies comparing changes in adiposity measures (total, truncal and waist circumference)[26,27,30,37] and/or circulating adipokines[26,30,37] between IER and CER, no consistent differences have been observed; however, additional studies employing robust assessments of regional adiposity are needed.

Time-restricted feeding

Overview

Over the past few years, there has been an emergence of interest in the concept of chrononutrition, i.e. the interaction between meal timing and our circadian system, which comprise self-sustained approximately 24 h oscillations in physiology, metabolism and behaviour[47]. Examples include the daily rhythms in glucose homeostasis and insulin sensitivity, which declines over the course of the day[48]. These rhythms are driven by a series of molecular 'clocks'[47]. The 'master' clock is located in a small brain region within the anterior hypothalamus, the suprachiasmatic nuclei. Light is the dominant entrainer (Zeitgeber) of the suprachiasmatic nuclei, which controls many essential physiological processes, including the sleep–wake cycle and endocrine rhythms[47]. Peripheral clocks are located in many tissues integral to glucose and lipid metabolism such as the liver, pancreas, skeletal muscle and adipose tissue. Feeding time acts as the major Zeitgeber of peripheral clocks, with the suprachiasmatic nuclei acting as the 'central conductor', ensuring correct synchronisation between peripheral clocks[48]. Possession of such rhythms permits effective co-ordination of endogenous processes to changes in the environment such as the daily light–dark cycle, and consequent cyclical food availability. TRF is one example of a timed dietary approach, which also falls under the intermittent fasting umbrella (Table 1). TRF involves limiting food intake to within a shortened window of time, which thereby extends the length of the daily fasting interval. In contrast to IER, TRF is usually performed on a daily basis and does not necessarily entail a prescribed energy restriction; however, the two approaches are not mutually exclusive, indeed, alternate day total fasting can be seen as a prolonged form of TRF. An overview of findings from the rodent and human literature is discussed next.

Effects of time-restricted feeding on glucose and lipid metabolism

Rodents. Being nocturnal animals, mice consume the majority of their energy during the dark/active phase. TRF (⩽12 h) where food intake is consolidated to the dark phase, has been shown to both protect against and reverse the harmful metabolic consequences of diverse nutritional challenges, including high-fat and high-sugar obesogenic diets[49,50]. TRF mice display reduced adiposity and liver steatosis, as well as improved glucose tolerance and reduced cholesterol levels when compared with mice fed *ad libitum* with the same high-fat diet; these changes may reflect improved homeostasis in multiple tissues[49,50]. Importantly, these improvements occur in the absence of changes in energy intake.

Human studies. In human individuals, TRF has been achieved through avoidance of night-time intake[51], by limiting food intake to one evening meal daily (referred to herein as evening TRF)[52,53], or by allowing participants to self-select their own shortened (10–11 h) eating window[54]. Islamic religious fasting restricts food access to nocturnal hours, between sunrise and sunset. Recent meta-analyses of these Ramadan studies, which by their nature have been observational by design, demonstrate improvements in some biochemical risk markers despite the nocturnal eating pattern[55]. With regard to interventional TRF studies, only two publications derived from the same cross-over controlled study conducted within a healthy-weight middle-aged study cohort, have reported metabolic outcomes. Stote *et al.*[53] found both pro-atherogenic

(increasing LDL-cholesterol) and anti-atherogenic (increasing HDL-cholesterol and decreasing TAG) changes following 8 weeks of evening TRF. Findings may have been confounded by circadian variations in these parameters, as baseline and post-intervention blood measurements were taken in the morning and evening, respectively. In the second publication, the group also reported significant elevations in fasting and postprandial glycaemia[52]. In this instance, oral glucose tolerance testings were conducted in the morning, following a period of time in which participants may have become metabolically adapted to this evening eating pattern. In addition, as there was no standardisation of dietary intakes on the day preceding their two metabolic assessments, participants would have consumed a greater quantity of food the night before their post-intervention visit, which might explain the rise in fasting glucose relative to baseline.

Outstanding questions

Whilst pervasive rodent data exist highlighting the substantive health benefits of TRF, human data are sparse and their interpretation hindered by methodological issues. As such, a number of fundamental questions remain outstanding:

By what mechanisms might time-restricted feeding reduce disease risk, and to what extent can any metabolic benefits be attributable to fasting?. Misalignment between our circadian biology and behavioural patterns, for example through consumption of food outside of the appropriate phase of the endogenous circadian cycle, is thought to be a major contributor to obesity and metabolic disease risk[48]. Defined daily feeding and fasting rhythms support robust oscillation in components of the circadian clock and their output genes, but become dampened in rodent models of diet induced obesity as they start to consume a greater proportion of their food intake in the light (inactive) phase[49,50]. Rhythmicity can be restored in these rodent models through TRF, which is made possible by the extensive cross-talk and interaction between molecular clock components and the feed/fasting responsive signals of cellular energy status. For example, fasting induces the activities of the AMP-activated protein kinase and cAMP-response element-binding protein, which promote ATP production during low-energy availability. In contrast, feeding stimulates the mechanistic target of rapamycin pathway, which promotes anabolic processes during increased energy availability. These feed/fast responsive elements can influence the expression of peripheral clock components and vice versa[56]. In support of this, TRF has been shown to improve cAMP-response element-binding protein, mechanistic target of rapamycin, and AMP-activated protein kinase rhythms, and hence promote robust oscillations of circadian clock gene components[49]. The metabolic benefits of TRF may additionally be attributed to the accompanying elongation of daily fasting intervals, which as previously discussed, may favourably impact on regional and ectopic adiposity. In the comprehensive rodent study conducted by Chaix *et al.*[50], the magnitude of the metabolic benefits of TRF were proportional to the length of the fasting interval. Whilst highly suggestive, there are no comparable human data. In addition, whilst one purported benefit of TRF is that it constrains energy intake to the most appropriate phase of the endogenous circadian cycle, in human subjects, the impact of earlier TRF meal timings (when metabolic control is more optimal[48]), has yet to be assessed.

Can time-restricted feeding alter metabolism in the absence of energy restriction in human subjects?. One major difference between human and rodent studies is that human subjects tend to behaviourally reduce food intake during TRF when permitted *ad libitum/* self-selected intake[51,54]. Similarly, many Ramadan studies report a mild energy deficit, although this is not a universal finding[55,57]. Where energy restriction is absent, or minimal, current evidence suggests that TRF can influence metabolism (both positively and negatively), necessitating further study into the optimal fasting window[52,53]. Although this often unintentional energy restriction makes metabolic interpretations difficult, the significance cannot be disregarded as it highlights the potential utility of TRF as a 'small changes' strategy for weight management. However, from a metabolic standpoint, further studies enforcing tighter control and monitoring over both energy intake and expenditure would be needed to fully explore this.

Conclusion

Put together, there is a growing evidence base demonstrating the benefits of IER over the short-to-medium term on glucose and lipid homeostasis, meriting further research in population groups with (or are at high risk of) T2DM and CVD, including healthy-weight individuals. Whilst the metabolic benefits of TRF appear quite profound in rodents, studies in human subjects are sparse and subject to some methodological issues, which hinder their interpretation. There is some suggestion that the metabolic changes elicited by the two intermittent fasting approaches can occur in the absence of energy restriction, and in the context of IER, may be distinct to those observed following similar weight-loss achieved via modest CER. Specifically, some studies have found greater improvements in indices of hepatic insulin sensitivity and TAG metabolism with IER. The metabolic benefits of intermittent fasting are likely to stem in part from the frequently repeated prolonged fasting intervals, which may favour the preferential reduction of ectopic fat, beneficially modulate aspects of adipose tissue physiology/morphology and distribution (in addition to any changes in overall mass), and may also impinge on circadian clock regulation. However mechanistic evidence is largely limited to findings from rodent studies, thus necessitating focused, controlled, human studies, which also incorporate more dynamic assessments of glucose and lipid metabolism in addition to steady-state measurements. Long-term studies are

also needed, which may yet uncover the potential for adverse health consequences, as evidenced by a small number of studies. Ultimately, much remains to be learned about intermittent fasting (in its various forms); however, the positive findings to date serve to highlight promising avenues for future research.

Financial Support

Conflicts of Interest

K. L. J. is the Head of Nutrition and Research at Lighterlife.

Authorship

R. A. wrote the manuscript. K. L. J., A. L. C. and M. D. R. assisted with manuscript editing.

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