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The role of intermittent fasting and meal timing in weight management and metabolic health

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Obesity remains a major public health concern and intermittent fasting is a popular strategy for weight loss, which may present independent health benefits. However, the number of diet books advising how fasting can be incorporated into our daily lives is several orders of magnitude greater than the number of trials examining whether fasting should be encouraged at all. This review will consider the state of current understanding regarding various forms of intermittent fasting (e.g. 5:2, time-restricted feeding and alternate-day fasting). The efficacy of these temporally defined approaches appears broadly equivalent to that of standard daily energy restriction, although many of these models of intermittent fasting do not involve fed-fasted cycles every other 24 h sleep–wake cycle and/or permit some limited energy intake outside of prescribed feeding times. Accordingly, the intervention period therefore may not regularly alternate, may not span all or even most of any given day, and may not even involve absolute fasting. This is important because potentially advantageous physiological mechanisms may only be initiated if a post-absorptive state is sustained by uninterrupted fasting for a more prolonged duration than applied in many trials. Indeed, promising effects on fat mass and insulin sensitivity have been reported when fasting duration is routinely extended beyond sixteen consecutive hours. Further progress will require such models to be tested with appropriate controls to isolate whether any possible health effects of intermittent fasting are primarily attributable to regularly protracted post-absorptive periods, or simply to the net negative energy balance indirectly elicited by any form of dietary restriction.

Eating pattern: Circadian rhythms: Time-restricted feeding: Weight loss

Obesity is a prevalent health concern throughout the world[1,2], which arises due to chronic positive energy balance[3–5]. Any energy surplus is stored primarily in the form of TAG within adipocytes, thus leading to adipose tissue expansion[6,7] predominantly as a result of adipocyte hypertrophy[8]. If sustained over time, this hypertrophic expansion can lead to adipocyte dysfunction, hyperglycaemia, hyperlipidaemia, ectopic lipid deposition, chronic low-grade systemic inflammation and insulin resistance[9–15], thereby fostering comorbidities such as type 2 diabetes and CVD[16,17]. To remedy this metabolic dysfunction, interventions often seek to redress the underlying energy imbalance by reducing energy intake and/or increasing expenditure, which can improve health outcomes[18,19]. However, these improvements are hampered by compensatory changes in appetite and energy use[4,20–22], as well as poor adherence[23,24], resulting in poor long-term success rates[4,25,26].

Strategies that exploit nutrient timing as a means of achieving weight loss and/or improving metabolic health have been the subject of considerable public interest in recent years[27]. Intermittent fasting is an umbrella term that may be used to describe these approaches, which involve a complete or partial restriction of energy within defined temporal windows on a recurrent basis[27,28]. Thus far, the therapeutic potential of intermittent fasting

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has been largely overshadowed by direct manipulation of the principal components of the energy balance equation[29]. However, advances in the understanding of circadian rhythms suggest that this could be a particularly effective approach for tackling obesity and the accompanying dysfunction[30,31], in addition to arguably being more acceptable in practice than conventional alternatives[32–36]. To explore this notion, this review will consider the literature on meal timing and intermittent fasting as it relates to metabolic health.

Meal timing

In Western cultures, consuming three or more meals daily is generally accepted as a societal norm[37,38]. However, this typically results in an anabolic state predominating each day[39,40]. The postprandial metabolic response to a mixed-macronutrient meal in metabolically healthy participants is characterised by a peak in glycaemia within the first hour followed by a steady return to fasted glycaemia over the ensuing 2 h[41,42]. This is paralleled by an accompanying peak in insulin secretion within the first hour followed by a decrease over the next 4 h[42]. Conversely, plasma TAG concentrations rise steadily to a peak after 3–5 h and generally remain 50 % higher than baseline even after 6 h[41]. When a subsequent meal is ingested approximately 5 h after the first (as is common in Western diets), glucose peaks at a similar time after feeding, albeit an attenuated absolute peak[43]. However, glucose then takes slightly longer to return to baseline as the day progresses, a pattern that is largely mirrored by insulin concentrations[44]. Plasma TAG on the other hand does not peak until shortly after the second meal is ingested; it then falls rapidly due to the insulinaemic response to the second meal, before peaking again about 5 h after the second meal[44].

These responses suggest that, even with just two meals daily, plasma TAG is elevated continuously for 12 h, with this pattern then propagated when further extended to include a third meal. This is well-demonstrated by Ruge *et al.*[45], who examined the 24 h circulating profiles of glucose, TAG and insulin in response to three successive meals at 10.00, 15.00 and 20.00 hours. Within this model, TAG remained elevated until 02.00 hours, along with insulin and glucose concentrations. Similarly, McQuaid *et al.*[46] showed that TAG extraction by adipose tissue in response to three meals daily is elevated for over 16 h. The net effect of this is that the majority of each 24 h day is spent in a postprandial and lipogenic state, which is conducive to fat accretion[39,40]. By extension, this provides fewer opportunities for net lipolysis and the predominance of lipid-derived substrates in energy metabolism, thereby favouring positive fat balance.

Ultimately, this results in a scenario wherein those adhering to conventional dietary meal timing patterns are attempting to achieve energy balance using a feeding schedule that is inherently biased towards fat accretion. Conventional diet and exercise interventions aim to reduce the amplitude of postprandial excursions in order to provide more opportunities for the liberation and utilisation of endogenous lipid reservoirs. However, the imbalance between the daily fasting window and the daily feeding window remains largely unperturbed. Comparatively, the omission of meals is typically necessitated by intermittent fasting and eliminates a subset of these postprandial excursions, thereby providing greater equilibrium between fasting and feeding opportunities and a better platform for achieving energy balance.

Further to this, the routine extension of fasting periods has been associated with metabolic benefits which are independent of net energy balance[27,28,47], constituting a secondary therapeutic dimension to these strategies. Specifically, Anton *et al.*[28] argue that the depletion of hepatic glycogen reserves and the ensuing transition towards metabolism of endogenous, lipid-derived substrates (i.e. NEFA, glycerol, ketone bodies) prompt a series of adaptive processes conducive to improved health outcomes, including improvements in body composition and insulin sensitivity. Considering that this transition does not take place in most instances until the uninterrupted fasting duration proceeds beyond 12–14 h[28,48], these adaptive processes are not often invoked by the conventional meal patterns described earlier.

Based on the afore-mentioned reasoning, it is conceivable that intermittent fasting may constitute an efficacious strategy for tackling obesity and the metabolic disorders associated with excess adiposity. To date, however, studies exploring these facets of intermittent fasting are scarce and inconsistent.

Eating frequency

Perhaps the most widely researched dimension of nutrient timing within the context of obesity in human subjects is eating frequency. Early work by Fabry *et al.*[49] deployed a cross-sectional approach to explore the relationship between intake frequency and metabolic health. Interestingly, in a cohort of 440 men, higher eating frequency broadly corresponded to a healthier profile of BMI, cholesterol concentrations and fasting glucose. Contrary to this, using data from the National Health and Nutrition Examination Survey, Murakami and Livingstone[50] observed that those eating on more than four occasions daily were approximately 50 % more likely to be overweight or obese by BMI relative to those eating on less than three occasions daily. Such discrepancies are a consistent theme throughout these cross-sectional studies; a recent systematic review by Canuto *et al.*[51] analysed data from thirty-one such studies containing a collective sample of over 130 000 participants. Of these thirty-one studies, fourteen established an inverse association, ten showed no association and seven revealed a positive association, which the authors ascribe to the spectrum of approaches employed.

Upon shifting to prospective methodologies, the pattern appears to be largely the same; two recent systematic reviews conclude that the majority of studies reveal no association between eating frequency and subsequent obesity[52,53]. The review of Raynor *et al.*[52] makes a

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particularly strong case, given that these authors only included human studies in which food was provided or intake monitored in a laboratory setting. However, of the studies covered in these reviews, most evaluated the impact of increased meal frequency on metabolic health, wherein three meals daily is used as the reference for lower frequency. Therefore, upon framing these studies within the context of the 24 h metabolite profiles discussed previously, the lack of a consensus is perhaps not surprising. In fact, only one of the studies reported is likely to have resulted in the predominance of a fasting state over the course of 24 h[54].

Specifically, the study of Stote *et al.*[54] explored the impact of reducing meal frequency to one meal daily under conditions of energy balance. Briefly, fifteen normal-weight participants completed two 8-week intervention periods in a randomised crossover design with an 11-week washout interval. In one treatment, all energy was consumed in a single meal between 17.00 and 21.00 hours, whilst the other treatment separated the same foods into a conventional breakfast, lunch and dinner format. To facilitate compliance, the dinner in both conditions was consumed under supervision and all foods were provided. The diets were matched for both energy and macronutrient content and targeted weight maintenance, with daily adjustment of prescribed intake based on body weight measurements, which were then mirrored in the opposing trial. No differences in body mass, body composition or health markers were apparent at the outset of each treatment and no differences in energy intake, macronutrient balance or physical activity were noted between the two conditions. Despite these null findings, body mass and fat mass (as assessed by bioelectrical impedance) were reduced by 1·4 and 2·1 kg, respectively, following the one meal daily condition but not the three meals daily condition. However, the reduction in adiposity was not accompanied by improvements in lipid profile or glycaemia[55]. This is consistent with the prior suggestion that extending the daily fasting period may result in increased utilisation of lipid-derived substrates in energy metabolism and favourable effects on fat balance[28].

The afore-mentioned interpretation suggests that, in much the same way as a protracted daily feeding window may be conducive to an energy surplus, prolonged fasting on a routine basis could be an effective strategy to counter fat accretion. However, what is particularly interesting here is that this observation was made under carefully matched conditions. Whilst this does not exclude any possibility of some amalgamation of undetectable changes in the various components of energy balance[56], it is also plausible that the protracted fasting period is exerting impacts on energy metabolism that are independent of net energy balance[28,57]. The current literature on intermittent fasting provides a useful platform for exploring this notion further.

Intermittent fasting

The umbrella term intermittent fasting refers to a series of therapeutic interventions which target temporal feeding restrictions, nominally categorised as: the 5:2 diet, modified alternate-day fasting, time-restricted feeding and complete alternate-day fasting[27]. Irrespective of the rationale for each, such approaches have been subject to growing popularity in recent years, yet experimental data to support their application are comparatively sparse[27,36]. Bluntly, the number of diet books advising how intermittent fasting can be incorporated into our daily lives is several orders of magnitude greater than the number of scientific papers examining whether intermittent fasting should be encouraged at all[27].

The 5:2 diet

Amongst the most coveted forms of intermittent fasting is the 5:2 diet, wherein severe energy restriction is imposed on 2 d/week with *ad libitum* consumption on the remaining five. The study of Carter *et al.*[58] randomised sixty-three adults with overweight or obesity and type 2 diabetes to 12 weeks of either daily energy restriction or a 5:2 approach. The 5:2 group reduced their intake to 1674–2510 kJ (400–600 kcal) for two non-consecutive days per week and followed their habitual diet on the remaining five, whilst the daily restriction group simply reduced their intake to 5021–6485 kJ (1200–1550 kcal) every day. Although the extent to which prescriptions were achieved was not reported, main effects of time but not group were seen for reductions in body mass, fat mass and fat-free mass, as well as improvements in glycated Hb concentration and the use of diabetic medications. Similar conclusions were also drawn by two recent studies which compared this 5:2 approach (i.e. 1674–2510 kJ (400–600 kcal) on two non-consecutive days per week) against daily energy restriction over 6 months[59,60].

This pattern of results indicates a broad equivalency between the metabolic impacts of the 5:2 diet and daily energy restriction, arguing against any special properties of the fasting element *per se*. However, this is not a consistent finding throughout the literature. Upon comparing the 5:2 approach (requiring two consecutive days of 75 % energy restriction per week) against daily energy restriction (requiring 25 % energy restriction every day) over 6 months, Harvie *et al.*[61] observed differential changes in fasting insulin and fasting indices of insulin resistance. Despite similar reductions in body mass and fat mass, the modest reductions in fasting insulin and insulin resistance seen in both groups were more pronounced with the 5:2 method. Although this may reflect a more potent influence of using two consecutive days of severe energy restriction (as opposed to non-consecutive), there were also greater reductions in energy and carbohydrate intake in this group, which complicate the interpretation.

Using a similar approach, Antoni *et al.*[62] sought to compare the effects of intermittent energy restriction (implemented using the 5:2 approach) against daily energy restriction when matched for net energy balance and thus weight losses, in order to minimise the confounding influence of such factors on metabolic health. Furthermore, this study featured dynamic indices of

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metabolic control, building upon the prior studies which only featured fasted measures. Briefly, twenty-seven participants with overweight or obesity were randomised to undertake either an intermittent or a continuous energy restriction diet. The 5:2 condition restricted participants to 2636 kJ (630 kcal)/d for two consecutive days each week, with a self-selected euenergetic diet on the remaining five. Comparatively, the continuous restriction implemented a self-selected diet intended to reduce energy intake by 2510 kJ (600 kcal)/d. As opposed to returning to the laboratory after a fixed period, participants were reassessed upon achieving a 5 % weight loss. Despite larger reductions in energy intake in the intermittent condition, the design meant that changes in body mass were similar between groups. Body composition and fasting biochemical outcomes were also similarly affected by the two diets, showing good agreement with previous studies. However, the intermittent diet resulted in significant reductions in postprandial TAG concentrations relative to daily energy restriction, whilst postprandial C-peptide concentration also showed a tendency for greater reductions in the intermittent feeding group. The authors concluded that this highlights a potential superiority of intermittent relative to continuous energy restriction.

Based on the afore-mentioned studies of the 5:2 approach to intermittent fasting, it seems that the manner in which the fast is applied is a key determinant of the impacts on metabolic health. When the fast is undertaken on consecutive days, there is an apparent superiority relative to daily energy restriction[61,62], whilst applying the fast on non-consecutive days results in broadly equivalent effects[58–60]. Upon considering this in terms of the resultant uninterrupted fasting duration, this would appear to fit with the proposition of Anton *et al.*[28], as fasting on consecutive days is more likely to result in an uninterrupted fast of over 12–14 h when compared with fasting on non-consecutive days. However, as these interventions do not confine the permitted intake during fasting to a specific time window (e.g. 1674–2510 kJ (400–600 kcal) consumed between 12.00 and 14.00 hours on fasting days), this makes it difficult to establish the exact duration of absolute fasting achieved.

Modified alternate-day fasting

The majority of human studies which examine intermittent fasting have centred upon a strategy referred to as modified alternate-day fasting[27]. It differs from the 5:2 diet in two key regards: the severe restriction is applied during alternating days (nominally 24 h, although practically more varied to accommodate sleep); and any permitted energy during fasting is provided in a single meal (thereby ensuring a tangible extension of the typical overnight fast). Much of the work undertaken in this field originates from pioneering experiments by Varady *et al.*, in which participants were required to alternate between 24 h periods of fasting and *ad libitum* feeding, with a single 2510–3347 kJ (600–800 kcal) meal permitted between 12.00 and 14.00 hours on non-feeding days.

The effects of this approach on body mass were initially explored by Varady *et al.*[32] in a single-arm trial, where twelve obese participants completed 8 weeks of modified alternate-day fasting. Reported adherence to the fasting protocol remained high throughout, with energy intake averaging 26 % of habitual[32,35]. Comparatively, intake on feeding days reached 95 % of the habitual level, resulting in a 37 % net energy restriction on average. This led to body mass losses of 5·6 kg, 5·4 kg of which was accounted for by decreases in fat mass[32]. Total cholesterol, LDL-cholesterol and TAG were also reduced by at least 20 %, effects which were associated with improvements in adipokine profile[63]. Subsequent work by the same group neatly demonstrates that these outcomes are similar when applied to cohorts of adults who are overweight[64], when meal timing on the fasting day is varied[65], and that concurrent macronutrient manipulation does not exert additive effects[66].

Collectively, these data suggest that modified alternate-day fasting may be a viable means of improving cardiometabolic health in adults who are overweight or obese. However, without a comparative daily energy restriction group, it is difficult to isolate any independent effects of the fasting periods from the effects of energy restriction and/or associated weight loss. This was addressed recently by a comparison of the two methods under isoenergetic conditions relative to a no intervention control group[67,68]. Briefly, sixty-nine adults with obesity were randomised to undertake 6 months of modified alternate-day fasting or daily energy restriction. The alternate-day fasting diet restricted participants to a single meal containing 25 % of their measured energy requirements between 12.00 and 14.00 hours during fasting periods, but prescribed 125 % of energy requirements on feeding days. Conversely, the daily energy restriction diet prescribed a 25 % reduction in energy intake every day, resulting in an equivalent reduction in energy intake of 25 % in both groups. Macronutrient balance was preserved in both instances and the attained energy restriction was 21 and 24 % for alternate-day fasting and daily energy restriction, respectively. The observed body mass loss of 6·8 % was also similar between the two groups, a pattern driven by changes in both fat mass and lean mass. Fasted markers of metabolic health were also largely unaffected by either intervention, including lipid profile, inflammatory markers, adipokines, glucose concentration and insulin resistance[67,68]. Furthermore, few differences emerged during an ensuing 6-month weight maintenance period in which the feeding patterns were maintained but the prescriptions modified to fulfil energy requirements (i.e. no energy deficit).

This once again indicates that intermittent fasting and daily energy restriction exert similar effects on most health outcomes, as concluded previously for the 5:2 approach. However, during the modified alternate-day fasting intervention, participants consistently overconsumed on fasting days and under-consumed on fed days, in what the authors describe as *de facto* energy restriction[67]. Consequently, over the duration of the study, the difference in reported energy intake between feeding and fasting days was <2092 kJ (500 kcal) on average[69]. Yet when the thirty-four participants that undertook alternate-day fasting were stratified into those who lost more or less than 5 % body mass, those closest to the prescribed intake targets showed larger decreases in body mass despite consuming more energy overall[69]. Unfortunately, the mechanisms underpinning this are unclear. The observation could reflect increased use of lipid-derived substrates or lower levels of adaptive thermogenesis with intermittent methods, or perhaps it simply reflects poorer dietary reporting by those with lower adherence.

Nonetheless, data emerging from studies of modified alternate-day fasting do not allude to a superiority relative to daily energy restriction. Although, the use of single-arm trials and poor adherence to fasting prescriptions leave this question open to further study.

Time-restricted feeding

Ironically, the adherence issues that appear common to modified alternate-day approaches may lie in the imposition of a severe restriction as opposed to a complete fast, which in being an absolute (albeit more severe) could in fact facilitate compliance[32,33,36]. Drawing from this premise, time-restricted feeding is another method of intermittent fasting which has emerged recently[27] and requires no knowledge of food composition or restraint at eating occasions, only awareness of the time at which eating occasions are permitted at all. This approach aims to restrict food intake to a temporal window (typically ≤10 h) within the waking phase, thereby reducing feeding opportunities and extending the overnight fast to at least 14 h daily[70].

Work in our laboratory explored the impact of extending the overnight fast on energy balance and nutrient metabolism, thereby providing several insights regarding the effects of such strategies[71–74]. Initially, thirty-three adults who were of healthy weight were randomised to 6 weeks of either consuming breakfast, defined as at least 2929 kJ (700 kcal) before 11.00 hours daily (with half consumed within 2 h of waking), or extended morning fasting up until 12.00 hours[73]. Interestingly, improvements in anthropometric parameters and fasting health markers were not meaningfully different between interventions. In agreement, a panel of hormones implicated in the regulation of energy balance showed little change following the two interventions, although specific measures of adipose tissue insulin sensitivity suggested an improvement in the breakfast group only[71].

These largely null findings relative to prior research could be explained by the free-living approach used to study compensatory changes in components of energy balance. The fasting group consumed less energy than the breakfast group when averaged throughout each 24 h period, but this was compensated for by lower physical activity thermogenesis. Upon applying this protocol to a cohort of adults with obesity[72], extended fasting resulted in a slightly greater compensatory increase in energy intake following fasting (although still not adequate to offset the energy consumed or omitted at breakfast), whilst daily fasting was again causally related to lower physical activity energy expenditure in the morning. Interestingly, in this cohort with obesity breakfast did result in improved insulinaemic responses during an oral glucose tolerance test relative to the fasting condition. However, this test was aligned for circadian cycle rather than feeding cycle, so the observed finding could simply reflect better alignment with anticipated events in the breakfast condition.

Other studies have applied time-restricted feeding under euenergetic conditions, much like the study of Stote *et al.*[54]. Focusing on energy metabolism, Moro *et al.*[75] randomised thirty-four men to 8 weeks of time-restricted feeding or a control diet. Diets were matched for energy and macronutrient content and aimed to provide 100 % of energy requirements across three meals in both conditions. In the control condition, meals were consumed at 08.00, 13.00 and 20.00 hours, whilst in the experimental condition, meals were consumed at 13.00, 16.00 and 20.00 hours to give a 16 h fast. The time-restricted approach resulted in reductions in fat mass relative to controls, which were partnered by decreases in RER, indicating a shift towards fat oxidation. Interestingly, however, despite accompanying reductions in leptin and hypothalamic–pituitary–thyroid signalling, resting energy expenditure was maintained. This reinforces the notion that nutrient timing impacts upon nutrient metabolism, whilst also highlighting that this appears to occur to a greater degree with a 16 h fast relative to a 12 h fast. Considering this in light of the typical post-prandial nutrient profile discussed previously, the increase in fasting duration may provide more opportunities for the metabolism of substrates derived from endogenous lipids. This again points to the possibility that routine extension of the fasting period beyond 12–14 h may be key to these benefits, which was not necessarily achieved by the 5:2 or modified alternate-day methods discussed earlier. The pivotal question is whether these improvements are enhanced with even longer durations of complete fasting.

More prolonged and complete fasting was recently examined by Sutton *et al.*[70], who hypothesised that circadian rhythms in energy metabolism would potentiate the effects of time-restricted feeding when eating times are confined to earlier stages of the waking phase. Using a repeated-measures crossover design, they compared the effect of consuming all daily energy within a 6 h window and a 12 h window over 5 weeks in men with pre-diabetes. The diets were prescribed based on energy requirements to maintain energy balance and were also matched for energy and macronutrient content. Compliance to the two conditions was high and the extended fasting period was accompanied by reductions in fasting insulin, peak insulin and insulin resistance during an oral glucose tolerance test. However, it appears the magnitude and persistence of any treatment effects may have required a longer wash-out interval between repeated treatments, as the impacts on insulinaemia were seemingly affected by baseline differences arising from a trial order effect. Combined with the fact that the fasting duration preceding post-intervention measurements was not standardised across trials, further investigations are warranted to verify these intriguing possibilities.

Based on all the afore-mentioned findings, the evidence does point to an effect of extended fasting intervals on fat mass independent of energy balance, particularly when the fasting interval is extended to at least 16 h, as shown by Stote *et al.*[54] and Moro *et al.*[75]. In both cases, this produced significant reductions in fat mass relative to a routine 12 h fast, which implicates extended fasting beyond 12 h as a key factor. However, the importance of such changes for metabolic health is less clear due to a series of confounding influences.

Complete alternate-day fasting

Thus far, the intermittent fasting strategies discussed typically permit the consumption of energy within each 24 h cycle to some degree, meaning that the fasting interval is only extended by a few hours[76]. This is primarily to facilitate adherence[32,34] but it also replenishes hepatic glycogen stores and reduces the utilisation of lipid-derived substrates (i.e. ketone bodies), which may mask several proposed benefits of intermittent fasting[28]. Furthermore, this disruption is profoundly asymmetric, in that even a short feeding occasion immediately suppresses lipolysis and ketogenesis, which then do not return for a number of hours[41,42]. It is worthy of note at this juncture that the inclusion of physical activity or exercise during the fasted period may serve to accelerate the restoration of these pathways to some degree, although the concurrent application of intermittent fasting alongside exercise interventions is beyond the scope of this review. Nonetheless, the 20 h fasting interval used by Stote *et al.*[54] is likely to have led to a greater reliance on these lipid-derived substrates over the course of 24 h, which may explain the reduction in fat mass despite euenergetic intake.

Building upon this premise, Halberg *et al.*[47] applied a 20 h fast on alternate days from 22.00 to 18.00 hours, representing an integration of the strategies employed by Stote *et al.*[54] and Varady *et al.*[32]. Fasting prohibited all intake with the exception of water, whilst during the intervening feeding periods, participants were told to double their habitual intake to maintain body mass. Although dietary intake was not monitored, blood samples collected in a subset of fasting periods confirmed compliance with the fasting protocol, with corresponding changes in systemic concentrations of glucose, NEFA, glycerol, adiponectin and leptin. Although both body mass and fat mass were unchanged, the glucose infusion rate during a euglycaemic–hyperinsulinaemic clamp increased in the final 30 min of the sampling period, suggesting enhanced insulin sensitivity following complete alternate-day fasting. Accordingly, this was accompanied by more rapid suppression of adipose tissue lipolysis during the insulin infusion. While the lack of an effect on body mass and fat mass relative to prior studies may reflect the disparity in cumulative fasting time, the authors were nonetheless able to conclude that this approach to intermittent fasting can improve metabolic health even in the absence of detectable changes in body mass.

Employing a similar approach, Soeters *et al.*[77] recruited eight males of healthy weight to a repeated-measures crossover study. This compared the effects of 2 weeks of a standard weight maintenance diet against 2 weeks of an intermittent fasting diet, using the same fasting protocol as Halberg *et al.*[47]. In this instance, a more prescriptive approach was adopted to the feeding cycles, with liquid meals used to bolster intake and adjustment of prescriptions in the event of meaningful weight change. Accordingly, body mass and composition were unaltered, yet there were no significant changes in glucose, lipid or protein kinetics in the basal state, or during a two-stage euglycaemic–hyperinsulinaemic clamp. In actuality, the only difference was a slight decrease in resting energy expenditure following the intermittent fasting arm.

Contrary to the studies of Halberg *et al.*[47] and Stote *et al.*[54], the afore-mentioned findings suggest that recurrent extension of the fasting period exerts no influence on energy or nutrient metabolism, aside from a possible decline in resting energy use. Whilst there are some discrepancies in terms of the approach to feeding cycles and assessment of nutrient metabolism under dynamic conditions, attributing to such factors would suggest the effect is unlikely to be clinically meaningful. However, work by Heilbronn *et al.* provides interesting insights that could explain such stark contrasts between ostensibly similar approaches[34,78]. Their study applied an intermittent fasting intervention to a cohort of sixteen adults who were not obese which involved fasting from midnight to midnight on alternating days for 3 weeks, with fasting periods only permitting energy-free drinks and sugar-free gum (fed periods were *ad libitum*). Assessments of body composition, a mixed-meal test and muscle biopsies were carried out at baseline and follow-up, with an additional set of measurements collected after a 36 h fast to explore the physiological impact of individual fasting periods on energy metabolism.

Although energy intake was not reported, the intervention reduced body mass by 2·5 %, approximately two-thirds of which was accounted for by reduced fat mass. However, the majority of fasting parameters, including plasma glucose concentration, RMR, substrate oxidation and muscle GLUT4 content showed no notable change[34,78]. Key exceptions were sex-specific alterations in cholesterol profile, with women experiencing an increase in HDL-cholesterol concentration and men exhibiting reductions in fasting TAG. Values collected after 36 h of fasting confirmed increased fatty acid oxidation, raising the question of why the routine up-regulation of fat metabolism combined with body mass losses resulted in no consistent changes in metabolic health. However, this pattern of sexual dimorphism continued into postprandial outcomes, with increases in glucose area under curve for females and reductions in insulin area under curve for males[78].

It might then be suggested that males and females respond differently to complete alternate-day fasting. However, there were a number of baseline differences between men and women in that study which should be considered in this interpretation, with men exhibiting higher glucose, insulin and TAG concentrations in the fasted state[34]. Upon contextualising this in the physiology of insulin resistance[9–14], it seems plausible that the

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metabolic state of male participants at baseline may stand to benefit more from the routine extension of fasting (notwithstanding the possibility of statistical regression). In these individuals, the shift toward fat oxidation seen in response to prolonged fasting could help to clear lipid intermediaries from non-adipose tissues, thereby enhancing insulin sensitivity. This is supported by the reported increase in carnitine palmitoltransferase-1 protein content in muscle tissue after the intervention[78,79].

Extending this premise to the studies of Halberg *et al.*[47] and Soeters *et al.*[77], the average body fat percentage of their cohorts was 20·1 and 14·8 %, respectively. This may therefore support the notion that those with lower levels of adiposity may not benefit from such interventions. Consequently, it is imperative to consider the seemingly distinct responses seen between leaner and more overweight cohorts when interpreting the results of similar studies. This is not only because the potential for weight loss and health gain may vary, but also because the presentation as lean or obese at baseline may be symptomatic of a predisposition towards various compensatory adjustments that predict responsiveness to treatment[5,72–74,80].

Furthering this line of enquiry, Catenacci *et al.*[81] undertook a randomised controlled trial of complete alternate-day fasting in a sample of adults with obesity. Briefly, twenty-six participants were randomised to undertake 8 weeks of either daily energy restriction (requiring a reduction in energy intake of 1674 kJ (400 kcal)/d) or a complete alternate-day fast. The intermittent fasting condition imposed a fast on every other day and provided a diet to meet estimated daily energy requirements during feeding periods, with a series of 837 kJ (200 kcal) optional food modules to permit *ad libitum* intake. All foods were provided and diets were matched for macronutrient balance rather than energy intake. Consequently, energy intake across the intervention was lower with the intermittent fasting approach, averaging 53 % of weight maintenance requirements compared with 72 % for daily energy restriction. This was accompanied by a trend for greater reductions in body mass with intermittent fasting relative to energy restriction, with 8·8 and 6·2 % reductions seen in the respective conditions. Despite this, fat mass and lean mass decreased to a similar degree in both groups, a pattern mirrored by improvements in fasted lipid profile. Only intermittent fasting produced improvements in fasted glucose concentration from baseline to follow-up, yet responses to a dynamic test of insulin sensitivity were unaltered. Conversely, RMR was reduced by daily energy restriction only, following correction for body composition changes, with a trend for a between-group difference. However, between-group comparisons were compromised by baseline differences, with those in the daily energy restriction group presenting with higher body mass and fasting insulin concentrations on average.

Summary

Intermittent fasting clearly encompasses a broad spectrum of dietary interventions. The defining characteristic is the confinement of energy restriction to a specified temporal window, be that 16 h each day[75], every other day[32,34] or just 2 d per week[61,62]. Across these various models, intermittent fasting can elicit reductions in body mass and improvements in metabolic health, effects which appear broadly comparable to standard daily energy restriction[82]. However, because the therapeutic potential of these temporal strategies may lie in routinely extending catabolic periods, thereby increasing reliance on lipid-derived substrates[28], the similar efficacy in relation to standard approaches could instead reflect a failure to meaningfully extend the post-absorptive period. The 5:2 diet and modified alternate-day fasting rarely omit more than one meal in sequence and therefore this transition to lipid-derived substrates may scarcely be made[58–60,67,68]. Conversely, if applying approaches that extend the fasting interval towards 20 h and beyond (e.g. consecutive fasting days in the 5:2 diet or time-restricted feeding), this transition to lipid-derived substrates is likely to be made more frequently, perhaps explaining the proposed superiority of these approaches[54,61,62,75]. Unfortunately, whilst the latter studies of complete alternate-day fasting offer amongst the longest uninterrupted fasting periods, the true effects of this are difficult to isolate due to metabolically diverse samples and the use of single-arm trials. Consequently, there remains an urgent need for well-designed, randomised-controlled trials of this commonly adopted approach.

Future directions

Identifying more effective strategies for managing obesity and associated metabolic disorders remains a public health challenge and intermittent fasting may represent a potent tool. However, research to support this is scarce and a number of important facets have been overlooked. Further research is therefore warranted to establish whether intermittent fasting is simply an alternative means of achieving energy restriction[76], or a dietary strategy which offers a favourable method for maintaining/improving metabolic health.

Body composition

Whilst much investigation has been devoted to the effects intermittent fasting exerts on fat balance, routinely extending catabolic periods also carries implications for fat-free mass. Anton *et al.*[28] argue that the increased reliance on lipid-derived substrates during prolonged fasting serves to minimise deteriorations in muscle mass and function, although this does not negate these deteriorations all together. Mechanistically, net protein balance is a product of constant interactions between protein synthesis and breakdown[83]. Following an overnight fast (approximately 8–12 h), there is an increase in amino acid efflux from muscle tissue[84], suggesting a shift in favour of net muscle protein breakdown[85–87]. Whilst there are limited data to support an exaggeration of this catabolic state when the fasting duration is extended to 24 h, a recent study by Vendelbo *et al.*[88] showed that fasting for 72 h doubled the rate of amino acid efflux from skeletal muscle when compared with a 10 h fast. Accordingly, it would be reasonable to anticipate a greater decline in fat-free mass in response to intermittent fasting when compared with daily energy restriction.

Contrary to this mechanistic perspective, however, a systematic review of randomised-controlled trials by Varady[89] concluded that intermittent fasting may in fact offer enhanced retention of fat-free mass when compared with daily energy restriction. A similar conclusion was also drawn by a more recent review comparing intermittent approaches with very-low energy dieting[90]. Whilst the predominance of modified alternate-day fasting studies in the former review may help to explain this, it is worthy of note that the complete alternate-day studies of both Halberg *et al.*[47] and Heilbronn *et al.*[34] were included. If verified, enhanced retention of fat-free mass relative to daily energy restriction would be a potent asset considering its association with RMR[91–93]. Consequently, clarifying the effect of complete alternate-day fasting on fat-free mass should be a central research priority.

Energy expenditure

A key (but often overlooked) issue with conventional obesity management approaches is compensatory changes in other dimensions of energy balance, particularly decreased energy expenditure with daily energy restriction[21,22,94]. It is not clear from the existing body of intermittent fasting research whether such compensatory changes may be invoked. Focusing initially on RMR, both Heilbronn *et al.*[34] and Catenacci *et al.*[81] reported no detectable change in response to complete alternate-day fasting, whilst Soeters *et al.*[77] suggest a decline in resting energy use of 247 kJ (59 kcal)/d. Conversely, physical activity energy expenditure has not been thoroughly and objectively examined in response to complete alternate-day fasting. Klempel *et al.*[35] observed no changes in daily step counts during 8 weeks of modified alternate-day fasting, despite clinically meaningful weight losses, and ensuing studies employing accelerometers have verified this outcome[95,96]. However, it should be noted that these studies all employed modified alternate-day fasting approaches, which can reasonably be expected to differ in their effects on voluntary behaviour relative to complete alternate-day methods.

In the absence of objective measures of energy expenditure, Sutton *et al.*[70] argue that energy expenditure is not affected by temporal restrictions of energy intake based on the absence of significant differences in body mass in their euenergetic time-restricted feeding study. However, Dhurandhar *et al.*[56] highlight that accurate determination of energy balance necessitates measurement of all aspects of the equation. While it does then remain a distinct possibility that typical compensatory responses to an energy deficit are blunted when intermittent fasting, a lack of evidence about isolated dimensions of energy expenditure currently prevents reliable conclusions being drawn. There is therefore a definite need to examine the cumulative impact of intermittent fasting on the components of energy balance in a reliable and well-controlled manner, not least physical activity thermogenesis.

Postprandial nutrient metabolism

An opportunity arising from the pre-existing literature stems from the fact that the majority of studies have focused on fasting measures of glucose, insulin and TAG, with very few studies employing dynamic tests. The relevance of this is well-illustrated by the impact of intermittent fasting on insulin; improvements in fasting insulin have been consistently shown in a number of studies as reviewed by Barnosky *et al.*[82]. They also show that in a subset of these studies, fasting indices of insulin resistance such as the homeostasis model generally improve following a period of intermittent fasting. However, it is important to note that while these fasting indices are useful in easing experimental demands, there are several limitations. For instance, Borai *et al.*[97] suggest it is possible for a participant to be insulin resistant without demonstrating fasting hyperinsulinaemia.

The same inconsistencies emerge when examining postprandial glycaemia, whilst postprandial lipaemia has been largely ignored. On an acute basis, Antoni *et al.*[98] demonstrate that a day of 100 % energetic restriction results in enhanced suppression of postprandial TAG and NEFA concentrations, relative to habitual intake and partial energy restriction. Extending this to the 5:2 approach, a similar pattern emerged for improvements in postprandial TAG concentrations with the intermittent condition relative to continuous restriction[62]. Such effects are also consistent with the enhanced suppression of adipose tissue lipolysis reported by Halberg *et al.*[47]. Given the importance of these outcomes in the context of obesity and the associated comorbidities, closer examination is warranted.

Comparative designs

Despite being proposed as an alternative approach to weight loss, few human trials to date have directly compared complete alternate-day fasting against standard daily energy restriction. Although it is generally reported that the outcomes are similar, the broad spectrum of cohorts and experimental protocols employed confounds reliable comparisons against the pre-existing literature[89]. The study of Catenacci *et al.*[81] is certainly an exception to this pattern, as they directly compared complete alternate-day fasting and daily energy restriction; however, the two conditions were not matched for the degree of energy restriction imposed. For this reason, reaching a consensus on the relative merits of intermittent fasting is not possible without further studies with appropriate controls.

Fasting-dependent effects

Lastly, and perhaps most importantly, is the possibility that remaining in a post-absorptive state for prolonged periods (i.e. fasting) may impart independent health benefits beyond the established effects of the net negative energy balance *per se* (and thus weight-loss). This is supported by Halberg *et al.*[47], who propose significant improvements in insulin sensitivity in response to complete alternate-day fasting; yet the failure of Soeters *et al.*[77] to replicate this finding with a near identical fasting protocol renders current data equivocal. This conflict may be driven by methodological contrasts in baseline adiposity and the refeeding protocol employed, but it leaves a pertinent question nonetheless. If fasting-dependent effects on health do exist, are conventional meal patterns contributing to metabolic disturbances irrespective of energy content? This would mean that changes in feeding times could constitute a novel dimension of what is considered a healthy diet, as opposed to simply being a vehicle for energy restriction.

In simple terms, nutritional considerations can be broadly classified under the three headings of type, quantity and timing, with current dietary guidelines such as the Eatwell guide[99,100] providing a very clear and evidenced illustration of the first two categories (i.e. what foods we should eat and how much we should eat). Further research is needed to complete the picture and include recommendations about when we should eat; or choose not to.

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Authorship

I. T. led on drafting the manuscript, J. T. G., J. A. B. and I. T. made edits and advised on revised versions, and all authors take responsibility for the final version.

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