



Citation for published version:

Templeman, I, Gonzalez, J, Thompson, D & Betts, JA 2020, 'The Role of Intermittent Fasting and Meal Timing in Weight Management and Metabolic Health', *Proceedings of the Nutrition Society*, vol. 79, no. 1, pp. 76-87.
<https://doi.org/10.1017/S0029665119000636>

DOI:

[10.1017/S0029665119000636](https://doi.org/10.1017/S0029665119000636)

Publication date:

2020

Document Version

Peer reviewed version

[Link to publication](#)

Publisher Rights

Unspecified

This article has been published in [Journal] [<http://doi.org/XXX>]. This version is free to view and download for private research and study only. Not for re-distribution, re-sale or use in derivative works. © copyright holder.

University of Bath

Alternative formats

If you require this document in an alternative format, please contact:
openaccess@bath.ac.uk

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Title The Role of Intermittent Fasting and Meal Timing in Weight Management and Metabolic Health

Authors Iain Templeman ¹

Javier T. Gonzalez ¹

Dylan Thompson ¹

James A. Betts ¹

Affiliations 1 – Department for Health, University of Bath, BA2 7AY, UK.

Corresponding Author James A. Betts (j.betts@bath.ac.uk)

Brief Title Intermittent Fasting & Health

Keywords Eating Pattern, Circadian Rhythms, Time Restricted Feeding, Weight Loss

Word Count 6936

1 **Acknowledgements** None

2 **Financial Support** I.T. was supported in writing this review by a PhD studentship
3 awarded by the University of Bath.

4 **Conflicts of Interest** J.T.G has received funding from The European Society for
5 Clinical Nutrition & Metabolism (ESPEN), The Rank Prize

6
7
8
9
10
11
12
13
14

Funds, Kenniscentrum Suiker & Voeding, Arla Foods
Ingredients, the Medical Research Council (MRC), the
Biotechnology & Biological Sciences Research Council
(BBSRC), PepsiCo and Lucozade Ribena Suntory. D.T. has
received funding from Unilever. J.A.B. has received funding
from the BBSRC, GlaxoSmithKline, Lucozade Ribena Suntory,
Kellogg's, Nestlé and PepsiCo and is a scientific advisor to the
International Life Sciences Institute (ILSI).

15 **Abstract**

16 Obesity remains a major public health concern and intermittent fasting is a popular strategy
17 for weight-loss, which may present independent health benefits. However, the number of diet
18 books advising *how* fasting *can* be incorporated into our daily lives is several orders of
19 magnitude greater than the number of trials examining *whether* fasting *should* be encouraged
20 at all. This review will consider the state of current understanding regarding various forms of
21 intermittent fasting (e.g. 5:2, time-restricted feeding and alternate-day fasting). The efficacy
22 of these temporally defined approaches appears broadly equivalent to that of standard daily
23 calorie restriction, although many of these models of intermittent fasting do not involve fed-
24 fasted cycles every other 24-h sleep-wake cycle and/or permit some limited energy intake
25 outside of prescribed feeding times. Accordingly, the intervention period therefore may not
26 regularly *alternate*, may not span all or even most of any given *day*, and may not even
27 involve absolute *fasting*. This is important because potentially advantageous physiological
28 mechanisms may only be initiated if a post-absorptive state is sustained by uninterrupted
29 fasting for a more prolonged duration than applied in many trials. Indeed, promising effects
30 on fat mass and insulin sensitivity have been reported when fasting duration is routinely
31 extended beyond 16 consecutive hours. Further progress will require such models to be tested
32 with appropriate controls to isolate whether any possible health effects of intermittent fasting
33 are primarily attributable to regularly protracted post-absorptive periods, or simply to the net
34 negative energy balance indirectly elicited by any form of dietary restriction.

35 Background

36 Obesity is a prevalent health concern throughout the world (1,2), which arises due to chronic
37 positive energy balance (3–5). Any energy surplus is stored primarily in the form of
38 triglycerides within adipocytes, thus leading to adipose tissue expansion (6,7) predominantly
39 as a result of adipocyte hypertrophy (8). If sustained over time, this hypertrophic expansion
40 can lead to adipocyte dysfunction, hyperglycaemia, hyperlipidaemia, ectopic lipid deposition,
41 chronic low-grade systemic inflammation and insulin resistance (9–15), thereby fostering
42 comorbidities such as type 2 diabetes and cardiovascular disease (16,17). To remedy this
43 metabolic dysfunction, interventions often seek to redress the underlying energy imbalance
44 by reducing energy intake and/or increasing expenditure, which can improve health outcomes
45 (18,19). However, these improvements are hampered by compensatory changes in appetite
46 and energy use (4,20–22), as well as poor adherence (23,24), resulting in poor long-term
47 success rates (4,25,26).

48 Strategies that exploit nutrient timing as a means of achieving weight loss and/or improving
49 metabolic health have been the subject of considerable public interest in recent years (27).
50 Intermittent fasting is an umbrella term that may be used to describe these approaches, which
51 involve a complete or partial restriction of energy within defined temporal windows on a
52 recurrent basis (27,28). Thus far, the therapeutic potential of intermittent fasting has been
53 largely overshadowed by direct manipulation of the principal components of the energy
54 balance equation (29). However, advances in the understanding of circadian rhythms suggest
55 that this could be a particularly effective approach for tackling obesity and the accompanying
56 dysfunction (30,31), in addition to arguably being more acceptable in practice than
57 conventional alternatives (32–36). To explore this notion, this review will consider the
58 literature on meal timing and intermittent fasting as it relates to metabolic health.

59 Meal Timing

60 In Western cultures, a pattern of three or more meals per day is generally accepted as a
61 societal norm (37,38). However, this typically results in an anabolic state predominating each
62 day (39,40). The postprandial metabolic response to a mixed-macronutrient meal in
63 metabolically healthy participants is characterised by a peak in glycaemia within the first
64 hour followed by a steady return to fasted glycaemia over the ensuing two hours (41,42). This
65 is paralleled by an accompanying peak in insulin secretion within the first hour followed by a

66 decrease over the next 4 hours (42). Conversely, plasma triglyceride concentrations rise
67 steadily to a peak after 3-5 hours and generally remain 50% higher than baseline even after 6
68 hours (41). When a subsequent meal is ingested approximately five hours after the first (as is
69 common in Western diets), glucose peaks at a similar time after feeding, albeit an attenuated
70 absolute peak (43). However, glucose then takes slightly longer to return to baseline as the
71 day progresses, a pattern that is largely mirrored by insulin concentrations (44). Plasma
72 triglycerides on the other hand do not reach their peak following the first meal until shortly
73 after the second meal is ingested, then fall rapidly due to the insulinaemic response to the
74 second meal, before peaking again around 5 hours after the second meal (44).

75 These responses suggest that, even with just two meals per day, plasma triglycerides are
76 elevated continuously for 12 hours, with this pattern then propagated when further extended
77 to include a third meal. This is well-demonstrated by Ruge *et al.* (45), who examined the 24-
78 hour circulating profiles of glucose, triglycerides and insulin in response to three successive
79 meals at 10:00, 15:00 and 20:00. Within this model, triglycerides remained elevated until
80 02:00, along with insulin and glucose concentrations. Similarly, McQuaid *et al.* (46) showed
81 that triglyceride extraction by adipose tissue in response to three meals per day is elevated for
82 over 16 hours. The net effect of this is that the majority of each 24-h day is spent in a
83 postprandial and lipogenic state, which is conducive to fat accretion (39,40). By extension,
84 this provides fewer opportunities for net lipolysis and the predominance of lipid-derived
85 substrates in energy metabolism, thereby favouring positive fat balance.

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

95 Further to this, the routine extension of fasting periods has been associated with metabolic
96 benefits which are independent of net energy balance (27,28,47), constituting a secondary

97 therapeutic dimension to these strategies. Specifically, Anton et al. (28) argue that the
98 depletion of hepatic glycogen reserves and the ensuing transition toward metabolism of
99 endogenous, lipid-derived substrates (i.e. non-esterified fatty acids, glycerol, ketone bodies),
100 prompts a series of adaptive processes conducive to improved health outcomes, including
101 improvements in body composition and insulin sensitivity. Considering that this transition
102 does not take place in most instances until the uninterrupted fasting duration proceeds beyond
103 12-14 hours (28,48), these adaptive processes are not often invoked by the conventional meal
104 patterns described above.

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

109 Eating Frequency

110 Perhaps the most widely researched dimension of nutrient timing within the context of
111 obesity in humans is eating frequency. Early work by Fabry *et al.* (49) deployed a cross-
112 sectional approach to explore the relationship between intake frequency and metabolic health.
113 Interestingly, in a cohort of 440 men, higher eating frequency broadly corresponded to a
114 healthier profile of body mass index (BMI), cholesterol concentrations and fasting glucose.
115 Contrary to this, using data from NHANES, Murakami and Livingstone (50) observed that
116 those eating on more than four occasions per day were approximately 50% more likely to be
117 overweight or obese by BMI relative to those eating on less than three occasions per day.
118 Such discrepancies are a consistent theme throughout these cross-sectional studies; a recent
119 systematic review by Canuto *et al.* (51) analysed data from 31 such studies containing a
120 collective sample of over 130,000 participants. Of these 31 studies, 14 established an inverse
121 association, 10 showed no association, and 7 revealed a positive association, which the
122 authors ascribe to the spectrum of approaches employed.

123 Upon shifting to prospective methodologies, the pattern appears to be largely the same; two
124 recent systematic reviews conclude that the majority of studies reveal no association between
125 eating frequency and subsequent obesity (52,53). The review of Raynor *et al.* (52) makes a
126 particularly strong case, given that these authors only included human studies in which food

127 was provided or intake monitored in a laboratory setting. However, of the studies covered in
128 these reviews, most evaluated the impact of increased meal frequency on metabolic health,
129 wherein three meals per day is used as the reference for lower frequency. Therefore, upon
130 framing these studies within the context of the 24-hour metabolite profiles discussed
131 previously, the lack of a consensus is perhaps not surprising. In fact, only one of the studies
132 reported is likely to have resulted in the predominance of a fasting state over the course of 24
133 hours (54).

134 Specifically, the study of Stote *et al.* (54) explored the impact of reducing meal frequency to
135 one meal per day under conditions of energy balance. Briefly, 15 normal-weight participants
136 completed two 8-week intervention periods in a randomised crossover design with an 11-
137 week washout interval. In one treatment, all calories were consumed in a single meal between
138 17:00 and 21:00, whilst the other treatment separated the same foods into a conventional
139 breakfast, lunch and dinner format. To facilitate compliance, the dinner in both conditions
140 was consumed under supervision and all foods were provided. The diets were matched for
141 both energy and macronutrient content and targeted weight maintenance, with daily
142 adjustment of prescribed intake based on body weight measurements, which were then
143 mirrored in the opposing trial. No differences in body mass, body composition or health
144 markers were apparent at the outset of each treatment and no differences in energy intake,
145 macronutrient balance or physical activity were noted between the two conditions. Despite
146 these null findings, body mass and fat mass (as assessed by bioelectrical impedance) were
147 reduced by 1.4 kg and 2.1 kg, respectively, following the one meal per day condition but not
148 the three meals per day condition. However, the reduction in adiposity was not accompanied
149 by improvements in lipid profile or glycaemia (55). This is consistent with the prior
150 suggestion that extending the daily fasting period may result in increased utilisation of lipid-
151 derived substrates in energy metabolism and favourable effects on fat balance (28).

152 The above interpretation suggests that, in much the same way as a protracted daily feeding
153 window may be conducive to an energy surplus, prolonged fasting on a routine basis could be
154 an effective strategy to counter fat accretion. However, what is particularly interesting here is
155 that this observation was made under carefully matched conditions. Whilst this does not
156 exclude any possibility of some amalgamation of undetectable changes in the various
157 components of energy balance (56), it is also plausible that the protracted fasting period is
158 exerting impacts on energy metabolism that are independent of net energy balance (28,57).

159 The current literature on intermittent fasting provides a useful platform for exploring this
160 notion further.

161 Intermittent Fasting

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

170 **The 5:2 Diet** – Amongst the most coveted forms of intermittent fasting is the 5:2 diet,
171 wherein severe energy restriction is imposed on two days per week with *ad libitum*
172 consumption on the remaining five. The study of Carter, Clifton and Keogh (58) randomised
173 63 adults with overweight or obesity and type 2 diabetes to 12 weeks of either daily calorie
174 restriction or a 5:2 approach. The 5:2 group reduced their intake to 400-600 kcal for two non-
175 consecutive days per week and followed their habitual diet on the remaining five, whilst the
176 daily restriction group simply reduced their intake to 1200-1550 kcal everyday. Although the
177 extent to which prescriptions were achieved was not reported, main effects of time but not
178 group were seen for reductions in body mass, fat mass and fat-free mass, as well as
179 improvements in glycated haemoglobin concentration and the use of diabetic medications.
180 Similar conclusions were also drawn by two recent studies which compared this 5:2 approach
181 (i.e. 400-600 kcal·day⁻¹ on two non-consecutive days per week) against daily energy
182 restriction over 6 months (59,60).

183 This pattern of results indicates a broad equivalency between the metabolic impacts of the 5:2
184 diet *versus* daily calorie restriction, arguing against any special properties of the fasting
185 element *per se*. However, this is not a consistent finding throughout the literature. Upon
186 comparing the 5:2 approach (requiring two consecutive days of 75% calorie restriction per
187 week) against daily calorie restriction (requiring 25% calorie restriction everyday) over six
188 months, Harvie *et al.* (61) observed differential changes in fasting insulin and fasting indices

189 of insulin resistance. Despite similar reductions in body mass and fat mass, the modest
190 reductions in fasting insulin and insulin resistance seen in both groups were more pronounced
191 with the 5:2 method. Although this may reflect a more potent influence of using two
192 consecutive days of severe energy restriction (as opposed to non-consecutive), there were
193 also greater reductions in energy and carbohydrate intake in this group, which complicate the
194 interpretation.

195 Using a similar approach, Antoni *et al.* (62) sought to compare the effects of intermittent
196 energy restriction (implemented using the 5:2 approach) against daily calorie restriction when
197 matched for net energy balance and thus weight losses, in order to minimise the confounding
198 influence of such factors on metabolic health. Furthermore, this study featured dynamic
199 indices of metabolic control, building upon the prior studies which only featured fasted
200 measures. Briefly, 27 participants with overweight or obesity were randomised to undertake
201 either an intermittent or a continuous energy restriction diet. The 5:2 condition restricted
202 participants to 630 kcal·day⁻¹ for two consecutive days each week, with a self-selected
203 eucaloric diet on the remaining five. Comparatively, the continuous restriction implemented a
204 self-selected diet intended to reduce energy intake by 600 kcal·day⁻¹. As opposed to returning
205 to the laboratory after a fixed period, participants were reassessed upon achieving a 5%
206 weight loss. Despite larger reductions in energy intake in the intermittent condition, the
207 design meant that changes in body mass were similar between groups. Body composition and
208 fasting biochemical outcomes were also similarly affected by the two diets, showing good
209 agreement with previous studies. However, the intermittent diet resulted in significant
210 reductions in postprandial triglyceride concentrations relative to daily calorie restriction,
211 whilst postprandial C-peptide concentration also showed a tendency for greater reductions in
212 the intermittent feeding group. The authors concluded that this highlights a potential
213 superiority of intermittent relative to continuous energy restriction.

214 Based on the above studies of the 5:2 approach to intermittent fasting, it seems that the
215 manner in which the fast is applied is a key determinant of the impacts on metabolic health.
216 When the fast is undertaken on consecutive days, there is an apparent superiority relative to
217 daily calorie restriction (61,62), whilst applying the fast on non-consecutive days results in
218 broadly equivalent effects (58–60). Upon considering this in terms of the resultant
219 uninterrupted fasting duration, this would appear to fit with the proposition of Anton *et al.*
220 (28), as fasting on consecutive days is more likely to result in an uninterrupted fast of over

221 12-14 hours when compared to fasting on non-consecutive days. However, as these
222 interventions do not confine the permitted intake during fasting to a specific time window
223 (e.g. 400-600 kcal consumed between 12:00 and 14:00 on fasting days), this makes it difficult
224 to establish the exact duration of absolute fasting achieved.

225 **Modified Alternate-Day Fasting** – The majority of human studies which examine
226 intermittent fasting have centred upon a strategy referred to as modified alternate-day fasting
227 (27). It differs from the 5:2 diet in two key regards: the severe restriction is applied during
228 alternating days (nominally 24 hours, although practically more varied to accommodate
229 sleep); and any permitted calories during ‘fasting’ are provided as a single meal (thereby
230 ensuring a tangible extension of the typical overnight fast). Much of the work undertaken in
231 this field originates from pioneering experiments by Varady and colleagues, in which
232 participants were required to alternate between 24-hour periods of fasting and *ad libitum*
233 feeding, with a single 600-800 kcal meal permitted between 12:00 and 14:00 on non-feeding
234 days.

235 The effects of this approach on body mass were initially explored by Varady *et al.* (32) in a
236 single-arm trial, where 12 obese participants completed 8 weeks of modified alternate-day
237 fasting. Reported adherence to the fasting protocol remained high throughout, with energy
238 intake averaging 26% of habitual (32,35). Comparatively, intake on feeding days reached
239 95% of the habitual level, resulting in a 37% net calorie restriction on average. This led to
240 body mass losses of 5.6 kg, 5.4 kg of which was accounted for by decreases in fat mass (32).
241 Total cholesterol, LDL cholesterol and triglycerides were also reduced by at least 20%,
242 effects which were associated with improvements in adipokine profile (63). Subsequent work
243 by the same group neatly demonstrates that these outcomes are similar when applied to
244 cohorts of adults who are overweight (64), when meal timing on the fasting day can be varied
245 (65), and that concurrent macronutrient manipulation does not exert additive effects (66).

246 Collectively, these data suggest that modified alternate-day fasting may be a viable means of
247 improving cardiometabolic health in adults who are overweight or obese. However, without a
248 comparative daily calorie restriction group it is difficult to isolate any independent effects of
249 the fasting periods from the effects of energy restriction and/or associated weight loss. This
250 was addressed recently by a comparison of the two methods under isocaloric conditions
251 relative to a no intervention control group (67,68). Briefly, 69 adults with obesity were

252 randomised to undertake 6 months of modified alternate-day fasting or daily calorie
253 restriction. The alternate day fasting diet restricted participants to a single meal containing
254 25% of their measured energy requirements between 12:00 and 14:00 during fasting periods,
255 but prescribed 125% of energy requirements on feeding days. Conversely, the daily calorie
256 restriction diet prescribed a 25% reduction in energy intake every day, resulting in an
257 equivalent reduction in energy intake of 25% in both groups. Macronutrient balance was
258 preserved in both instances and the attained calorie restriction was 21% and 24% for
259 alternate-day fasting and daily calorie restriction, respectively. The observed body mass loss
260 of 6.8 % was also similar between the two groups, a pattern driven by changes in both fat
261 mass and lean mass. Fasted markers of metabolic health were also largely unaffected by
262 either intervention, including lipid profile, inflammatory markers, adipokines, glucose
263 concentration and insulin resistance (67,68). Furthermore, few differences emerged during an
264 ensuing 6-month weight maintenance period in which the feeding patterns were maintained
265 but the prescriptions modified to fulfil energy requirements (i.e. no energy deficit).

266 This once again indicates that intermittent fasting and daily calorie restriction exert similar
267 effects on most health outcomes, as concluded previously for the 5:2 approach. However,
268 during the modified alternate-day fasting intervention, participants consistently over-
269 consumed on fasting days and under-consumed on fed days, in what the authors describe as
270 *de facto* calorie restriction (67). Consequently, over the duration of the study the difference in
271 reported energy intake between feeding and fasting days was less than 500 kcal on average
272 (69). Yet when the 34 participants that undertook alternate-day fasting were stratified into
273 those who lost more *versus* less than 5% body mass, those closest to the prescribed intake
274 targets showed larger decreases in body mass despite consuming more calories overall (69).
275 Unfortunately, the mechanisms underpinning this are unclear. The observation could reflect
276 increased use of lipid-derived substrates or lower levels of adaptive thermogenesis with
277 intermittent methods, or perhaps it simply reflects poorer dietary reporting by those with
278 lower adherence.

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

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

291 Work in our laboratory explored the impact of extending the overnight fast on energy balance
292 and nutrient metabolism, thereby providing several insights regarding the effects of such
293 strategies (71–74). Initially, 33 adults who were of healthy weight were randomised to 6
294 weeks of either consuming breakfast, defined as at least 700 kcal before 11:00 daily (with
295 half consumed within 2 hours of waking), or extended morning fasting up until 12:00 (73).
296 Interestingly, improvements in anthropometric parameters and fasting health markers were
297 not meaningfully different between interventions. In agreement, a panel of hormones
298 implicated in the regulation of energy balance showed little change following the two
299 interventions, although specific measures of adipose tissue insulin sensitivity suggested an
300 improvement in the breakfast group only (71).

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

313 Other studies have applied time-restricted feeding under eucaloric conditions, much alike the
314 study of Stote *et al.* (54). Focusing on energy metabolism, Moro *et al.* (75) randomised 34
315 men to 8 weeks of time-restricted feeding or a control diet. Diets were matched for energy
316 and macronutrient content and aimed to provide 100% of energy requirements across three
317 meals in both conditions. In the control condition, meals were consumed at 08:00, 13:00 and
318 20:00, whilst in the experimental condition meals were consumed at 13:00, 16:00 and 20:00
319 to give a 16-hour fast. The time-restricted approach resulted in reductions in fat mass relative
320 to controls, which were partnered by decreases in respiratory exchange ratio, indicating a
321 shift toward fat oxidation. Interestingly, however, despite accompanying reductions in leptin
322 and hypothalamic-pituitary-thyroid signalling, resting energy expenditure was maintained.
323 This reinforces the notion that nutrient timing impacts upon nutrient metabolism, whilst also
324 highlighting that this appears to occur to a greater degree with a 16-hour fast relative to a 12-
325 hour fast. Considering this in light of the typical postprandial nutrient profile discussed
326 previously, the increase in fasting duration may provide more opportunities for metabolism of
327 substrates derived from endogenous lipids. This again points to the possibility that routine
328 extension of the fasting period beyond 12-14 hours may be key to these benefits, which was
329 not necessarily achieved by the 5:2 or modified alternate-day methods discussed earlier. The
330 pivotal question is whether these improvements are enhanced with even longer durations of
331 complete fasting.

332 More prolonged and complete fasting was recently examined by Sutton *et al.* (70), who
333 hypothesised that circadian rhythms in energy metabolism would potentiate the effects of
334 time-restricted feeding when eating times are confined to earlier stages of the waking phase.
335 Using a repeated-measures crossover design, they compared the effect of consuming all daily
336 calories within a 6-hour window and a 12-hour window over 5 weeks in men with pre-
337 diabetes. The diets were prescribed based on energy requirements to maintain energy balance
338 and were also matched for energy and macronutrient content. Compliance to the two
339 conditions was high and the extended fasting period was accompanied by reductions in
340 fasting insulin, peak insulin and insulin resistance during an oral glucose tolerance test.
341 However, it appears the magnitude and persistence of any treatment effects may have
342 required a longer wash-out interval between repeated treatments, as the impacts on
343 insulinaemia were seemingly affected by baseline differences arising from a trial order effect.
344 Combined with the fact that the fasting duration preceding post-intervention measurements

345 was not standardised across trials, further investigations are warranted to verify these
346 intriguing possibilities.

347 Based on all the above findings, evidence does point to an effect of extended fasting intervals
348 on fat mass independent of energy balance, particularly when the fasting interval is extended
349 to at least 16 hours, as shown by Stote *et al.* (54) and Moro *et al.* (75). In both cases, this
350 produced significant reductions in fat mass relative to a routine 12 hour fast, which implicates
351 extended fasting beyond 12 hours as a key factor. However, the importance of such changes
352 for metabolic health are less clear due to a series of confounding influences.

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

367 Building upon this premise, Halberg *et al.* (47) applied a 20-hour fast on alternate days from
368 22:00 to 18:00, representing an integration of the strategies employed by Stote *et al.* (54) and
369 Varady *et al.* (32). Fasting prohibited all intake with the exception of water, whilst during the
370 intervening feeding periods participants were told to double their habitual intake to maintain
371 body mass. Although dietary intake was not monitored, blood samples collected in a subset
372 of fasting periods confirmed compliance with the fasting protocol, with corresponding
373 changes in systemic concentrations of glucose, non-esterified fatty acids, glycerol,
374 adiponectin and leptin. Although both body mass and fat mass were unchanged, the glucose
375 infusion rate during a euglycaemic-hyperinsulinemic clamp increased in the final 30 minutes

376 of the sampling period, suggesting enhanced insulin sensitivity following complete alternate-
377 day fasting. Accordingly, this was accompanied by more rapid suppression of adipose tissue
378 lipolysis during the insulin infusion. While the lack of an effect on body mass and fat mass
379 relative to prior studies may reflect the disparity in cumulative fasting time, the authors were
380 nonetheless able to conclude that this approach to intermittent fasting can improve metabolic
381 health even in the absence of detectable changes in body mass.

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

392 To the contrary of Halberg *et al.* (47) and Stote *et al.* (54), the above findings suggest that
393 recurrent extension of the fasting period exerts no influence on energy or nutrient
394 metabolism, aside from a possible decline in resting energy use. Whilst there are some
395 discrepancies in terms of the approach to feeding cycles and assessment of nutrient
396 metabolism under dynamic conditions, attributing to such factors would suggest the effect is
397 unlikely to be clinically meaningful. However, work by Heilbronn and colleagues provides
398 interesting insights that could explain such stark contrasts between ostensibly similar
399 approaches (34,78). Their study applied an intermittent fasting intervention to a cohort of 16
400 adults who were not obese which involved fasting from midnight to midnight on alternating
401 days for 3 weeks, with fasting periods only permitting energy-free drinks and sugar-free gum
402 (fed periods were *ad libitum*). Assessments of body composition, a mixed-meal test and
403 muscle biopsies were carried out at baseline and follow-up, with an additional set of
404 measurements collected after a 36-hour fast to explore the physiological impact of individual
405 fasting periods on energy metabolism.

406 Although energy intake was not reported, the intervention reduced body mass by 2.5%,
407 approximately two thirds of which was accounted for by reduced fat mass. However, the
408 majority of fasting parameters, including plasma glucose concentration, resting metabolic
409 rate, substrate oxidation and muscle GLUT4 content showed no notable change (34,78). Key
410 exceptions were sex-specific alterations in cholesterol profile, with women experiencing an
411 increase in HDL cholesterol concentration and men exhibiting reductions in fasting
412 triglycerides. Values collected after 36 hours of fasting confirmed increased fatty acid
413 oxidation, raising the question of why the routine upregulation of fat metabolism combined
414 with body mass losses resulted in no consistent changes in metabolic health. However, this
415 pattern of sexual dimorphism continued into postprandial outcomes, with increases in glucose
416 area under curve for females and reductions in insulin area under curve for males (78).

417 It might then be suggested that males and females respond differently to complete alternate-
418 day fasting. However, there were a number of baseline differences between men and women
419 in that study which should be considered in this interpretation, with men exhibiting higher
420 glucose, insulin and triglyceride concentrations in the fasted state (34). Upon contextualising
421 this in the physiology of insulin resistance (9–14), it seems plausible that the metabolic state
422 of male participants at baseline may stand to benefit more from the routine extension of
423 fasting (notwithstanding the possibility of statistical regression). In these individuals, the shift
424 toward fat oxidation seen in response to prolonged fasting could help to clear lipid
425 intermediaries from non-adipose tissues, thereby enhancing insulin sensitivity. This is
426 supported by the reported increase in CPT1 protein content in muscle tissue after the
427 intervention (78,79).

428 Extending this premise to the studies of Halberg *et al.* (47) and Soeters *et al.* (77), the
429 average body fat percentage of their cohorts was 20.1% and 14.8%, respectively. This may
430 therefore support the notion that those with lower levels of adiposity may not benefit from
431 such interventions. Consequently, it is imperative to consider the seemingly distinct
432 responses seen between leaner and more overweight cohorts when interpreting the results of
433 similar studies. This is not only because the potential for weight loss and health gain may
434 vary, but also because the presentation as lean or obese at baseline may be symptomatic of a
435 predisposition towards various compensatory adjustments that predict responsiveness to
436 treatment (5, 72–74,80).

437 Furthering this line of enquiry, Catenacci *et al.* (81) undertook a randomised controlled trial
438 of complete alternate-day fasting in a sample of adults with obesity. Briefly, 26 participants
439 were randomised to undertake 8 weeks of either daily calorie restriction (requiring a
440 reduction in energy intake of 400 kcal·day⁻¹) or a complete alternate-day fast. The
441 intermittent fasting condition imposed a fast on every other day and provided a diet to meet
442 estimated daily energy requirements during feeding periods, with a series of 200 kcal
443 optional food modules to permit *ad libitum* intake. All foods were provided and diets were
444 matched for macronutrient balance rather than energy intake. Consequently, energy intake
445 across the intervention was lower with the intermittent fasting approach, averaging 53% of
446 weight maintenance requirements compared to 72% for daily calorie restriction. This was
447 accompanied by a trend for greater reductions in body mass with intermittent fasting relative
448 to calorie restriction, with 8.8% and 6.2% reductions seen in the respective conditions.
449 Despite this, fat mass and lean mass decreased to a similar degree in both groups, a pattern
450 mirrored by improvements in fasted lipid profile. Only intermittent fasting produced
451 improvements in fasted glucose concentration from baseline to follow-up, yet responses to a
452 dynamic test of insulin sensitivity were unaltered. Conversely, resting metabolic rate was
453 reduced by daily calorie restriction only, following correction for body composition changes,
454 with a trend for a between-group difference. However, between-group comparisons were
455 compromised by baseline differences, with those in the daily calorie restriction group
456 presenting with higher body mass and fasting insulin concentrations on average.

457 Summary

458 Intermittent fasting clearly encompasses a broad spectrum of dietary interventions. The
459 defining characteristic is the confinement of calorie restriction to a specified temporal
460 window, be that 16 hours each day (75), every other day (32,34), or just two days per week
461 (61,62). Across these various models, intermittent fasting can elicit reductions in body mass
462 and improvements in metabolic health, effects which appear broadly comparable to standard
463 daily calorie restriction (82). However, because the therapeutic potential of these temporal
464 strategies may lie in routinely extending catabolic periods, thereby increasing reliance on
465 lipid-derived substrates (28), the similar efficacy in relation to standard approaches could
466 instead reflect a failure to meaningfully extend the post-absorptive period. The 5:2 diet and
467 modified alternate-day fasting rarely omit more than one meal in sequence and therefore this
468 transition to lipid-derived substrates may scarcely be made (58–60,67,68). Conversely, if

469 applying approaches that extend the fasting interval toward 20 hours and beyond (e.g.
470 consecutive fasting days in the 5:2 diet or time-restricted feeding), this transition to lipid-
471 derived substrates is likely to be made more frequently, perhaps explaining the proposed
472 superiority of these approaches (54, 61,62,75). Unfortunately, whilst the latter studies of
473 complete alternate-day fasting offer amongst the longest uninterrupted fasting periods, the
474 true effects of this are difficult to isolate due to metabolically diverse samples and the use of
475 single-arm trials. Consequently, there remains an urgent need for well-designed, randomised-
476 controlled trials of this commonly adopted approach.

477 Future Directions

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

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

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

509 **Energy Expenditure** – A key (but often overlooked) issue with conventional obesity
510 management approaches is compensatory changes in other dimensions of energy balance,
511 particularly decreased energy expenditure with daily calorie restriction (21,22,94). It is not
512 clear from the existing body of intermittent fasting research whether such compensatory
513 changes may be invoked. Focusing initially on resting metabolic rate, both Heilbronn *et al.*
514 (34) and Catenacci *et al.* (81) reported no detectable change in response to complete
515 alternate-day fasting, whilst Soeters *et al.* (77) suggest a decline in resting energy use of 59
516 kcal·day⁻¹. Conversely, physical activity energy expenditure has not been thoroughly and
517 objectively examined in response to complete alternate-day fasting. Klempel *et al.* (35)
518 observed no changes in daily step counts during 8-weeks of modified alternate-day fasting,
519 despite clinically meaningful weight losses, and ensuing studies employing accelerometers
520 have verified this outcome (95,96). However, it should be noted that these studies all
521 employed modified alternate-day fasting approaches, which can reasonably be expected to
522 differ in their effects on voluntary behaviour relative to complete alternate-day methods.

523 In the absence of objective measures of energy expenditure, Sutton *et al.* (70) argue that
524 energy expenditure is not affected by temporal restrictions of energy intake based on the
525 absence of significant differences in body mass in their eucaloric time-restricted feeding
526 study. However, Dhurandhar *et al.* (56) highlight that accurate determination of energy
527 balance necessitates measurement of all aspects of the equation. While it does then remain a
528 distinct possibility that typical compensatory responses to an energy deficit are blunted when
529 intermittent fasting, a lack of evidence about isolated dimensions of energy expenditure

530 currently prevents reliable conclusions being drawn. There is therefore definite need to
531 examine the cumulative impact of intermittent fasting on the components of energy balance
532 in a reliable and well-controlled manner, not least physical activity thermogenesis.

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

544 The same inconsistencies emerge when examining postprandial glycaemia, whilst
545 postprandial lipaemia has been largely ignored. On an acute basis, Antoni *et al.* (98)
546 demonstrate that a day of 100% caloric restriction results in enhanced suppression of
547 postprandial triglyceride and non-esterified fatty acid concentrations, relative to habitual
548 intake and partial energy restriction. Extending this to the 5:2 approach, a similar pattern
549 emerged with improvements in postprandial triglyceride levels with the intermittent condition
550 relative to continuous restriction (62). Such effects are also consistent with the enhanced
551 suppression of adipose tissue lipolysis reported by Halberg *et al.* (47). Given the importance
552 of these outcomes in the context of obesity and the associated comorbidities, closer
553 examination is warranted.

554 **Comparative Designs** – Despite being proposed as an alternative approach to weight loss,
555 few human trials to date have directly compared complete alternate-day fasting against
556 standard daily calorie restriction. Although it is generally reported that the outcomes are
557 similar, the broad spectrum of cohorts and experimental protocols employed confounds
558 reliable comparisons against the pre-existing literature (89). The study of Catenacci *et al.* (81)
559 is certainly an exception to this pattern, as they directly compared complete alternate-day
560 fasting and daily calorie restriction; however, the two conditions were not matched for the

561 degree of calorie restriction imposed. For this reason, reaching a consensus on the relative
562 merits of intermittent fasting is not possible without further studies with appropriate controls.

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

575 In simple terms, nutritional considerations can be broadly classified under the three headings
576 of type, quantity and timing, with current dietary guidelines such as the Eatwell guide
577 (99,100) providing a very clear and evidenced illustration of the first two categories (i.e. *what*
578 *foods we should eat* and *how much we should eat*). Further research is needed to complete the
579 picture and include recommendations about *when* we should eat – or choose not to.

580

581 References

- 582 1. World Health Organization. (2000). *Obesity: preventing and managing the global*
583 *epidemic*. Report of a World Health Organization consultation. World Health
584 Organization Technical Report Series 894. Geneva: World Health Organization.
- 585 2. Ng M, Fleming T, Robinson M *et al.* (2014). Global, regional, and national prevalence
586 of overweight and obesity in children and adults during 1980-2013: a systematic
587 analysis for the Global Burden of Disease Study 2013. *Lancet*. **384**(9945):766–81.
- 588 3. Wells JCK, Siervo M. (2011). Obesity and energy balance: is the tail wagging the dog?
589 *Eur J Clin Nutr*. **65**(11):1173–89.
- 590 4. Hall KD, Heymsfield SB, Kemnitz JW *et al.* (2012). Energy balance and its
591 components: implications for body weight regulation. *Am J Clin Nutr*. **95**(4):989–94.
- 592 5. Hill JO, Wyatt HR, Peters JC. (2012). Energy balance and obesity. *Circulation*.
593 **126**(1):126–32.
- 594 6. Abbott WG, Howard B V, Christin L *et al.* (1988). Short-term energy balance:
595 relationship with protein, carbohydrate, and fat balances. *Am J Physiol*. **255**(3):332–7.
- 596 7. Galgani J, Ravussin E. (2008). Energy metabolism, fuel selection and body weight
597 regulation. *Int J Obes*. **32**(Suppl 7):S109–19.
- 598 8. Sun K, Kusminski CM, Scherer PE. (2011). Adipose tissue remodeling and obesity. *J*
599 *Clin Invest*. **121**(6):2094–101.
- 600 9. Cho KW, Lumeng CN. (2011). SIRT1: A Guardian at the Gates of Adipose Tissue
601 Inflammation. *Diabetes*. **60**(12):3100–2.
- 602 10. Rutkowski JM, Stern JH, Scherer PE. (2015). The cell biology of fat expansion. *J Cell*
603 *Biol*. **208**(5):501–12.
- 604 11. Jung UJ, Choi M-S. (2014). Obesity and its metabolic complications: the role of

- 605 adipokines and the relationship between obesity, inflammation, insulin resistance,
606 dyslipidemia and nonalcoholic fatty liver disease. *Int J Mol Sci.* **15**(4):6184–223.
- 607 12. Martyn JAJ, Kaneki M, Yasuhara S. (2008). Obesity-induced insulin resistance and
608 hyperglycemia: etiologic factors and molecular mechanisms. *Anesthesiology.*
609 **109**(1):137–48.
- 610 13. Wensveen FM, Valentić S, Šestan M *et al.* (2015). The “Big Bang” in obese fat:
611 Events initiating obesity-induced adipose tissue inflammation. *Eur J Immunol.*
612 **45**(9):2446–56.
- 613 14. McArdle MA, Finucane OM, Connaughton RM *et al.* (2013). Mechanisms of obesity-
614 induced inflammation and insulin resistance: insights into the emerging role of
615 nutritional strategies. *Front Endocrinol.* **4**:52.
- 616 15. Hill AA, Reid Bolus W, Hasty AH. (2014). A decade of progress in adipose tissue
617 macrophage biology. *Immunol Rev.* **262**(1):134–52.
- 618 16. Alberti KG, Zimmet PZ. (1998). Definition, diagnosis and classification of diabetes
619 mellitus and its complications - Part 1: diagnosis and classification of diabetes mellitus
620 provisional report of a WHO consultation. *Diabet Med.* **15**(7):539–53.
- 621 17. Rocha VZ, Libby P. (2009). Obesity, inflammation, and atherosclerosis. *Nat Rev*
622 *Cardiol.* **6**(6):399–409.
- 623 18. Hall KD, Chung ST. (2018). Low-carbohydrate diets for the treatment of obesity and
624 type 2 diabetes. *Curr Opin Clin Nutr Metab Care.* **21**(4):308–12.
- 625 19. Most J, Tosti V, Redman LM. (2017). Calorie restriction in humans: An update.
626 *Ageing Res Rev.* **39**:36–45.
- 627 20. Westerterp KR. (2018). Exercise, energy balance and body composition. *Eur J Clin*
628 *Nutr.* **72**(9):1246–50.
- 629 21. Muller MJ, Enderle J, Pourhassan M *et al.* (2015). Metabolic adaptation to caloric

- 630 restriction and subsequent refeeding: the Minnesota Starvation Experiment revisited.
631 *Am J Clin Nutr.* **102**(4):807–19.
- 632 22. Martin CK, Das SK, Lindblad L *et al.* (2011). Effect of calorie restriction on the free-
633 living physical activity levels of nonobese humans: results of three randomized trials. *J*
634 *Appl Physiol.* **110**(4):956–63.
- 635 23. Dansinger ML, Gleason JA, Griffith JL *et al.* (2005). Comparison of the Atkins,
636 Ornish, Weight Watchers, and Zone diets for weight loss and heart disease risk
637 reduction: a randomized trial. *JAMA.* **293**(1):43–53.
- 638 24. Ahern AL, Olson AD, Aston LM *et al.* (2011). Weight Watchers on prescription: An
639 observational study of weight change among adults referred to Weight Watchers by
640 the NHS. *BMC Public Health.* **11**(1):434.
- 641 25. Franz MJ, VanWormer JJ, Crain AL *et al.* (2007). Weight-loss outcomes: a systematic
642 review and meta-analysis of weight-loss clinical trials with a minimum 1-year follow-
643 up. *J Am Diet Assoc.* **107**(10):1755–67.
- 644 26. Barte JCM, Ter Bogt NCW, Bogers RP *et al.* (2010). Maintenance of weight loss after
645 lifestyle interventions for overweight and obesity, a systematic review. *Obes Rev.*
646 **11**(12):899–906.
- 647 27. Patterson RE, Sears DD. (2017). Metabolic Effects of Intermittent Fasting. *Annu Rev*
648 *Nutr.* **37**(1):371–93.
- 649 28. Anton SD, Moehl K, Donahoo WT *et al.* (2018). Flipping the Metabolic Switch:
650 Understanding and Applying the Health Benefits of Fasting. *Obesity.* **26**(2):254–68.
- 651 29. Arble DM, Bass J, Laposky AD *et al.* (2009). Circadian timing of food intake
652 contributes to weight gain. *Obesity.* **17**(11):2100–2.
- 653 30. Ekmekcioglu C, Touitou Y. (2011). Chronobiological aspects of food intake and
654 metabolism and their relevance on energy balance and weight regulation. *Obes Rev.*
655 **12**(1):14–25.

- 656 31. Dibner C, Schibler U. (2018). Body clocks: Time for the Nobel Prize. *Acta Physiol.*
657 **222**(2):e13024.
- 658 32. Varady KA, Bhutani S, Church EC *et al.* (2009). Short-term modified alternate-day
659 fasting: a novel dietary strategy for weight loss and cardioprotection in obese adults.
660 *Am J Clin Nutr.* **90**(5):1138–43.
- 661 33. Scheer FAJL, Morris CJ, Shea SA. (2013). The internal circadian clock increases
662 hunger and appetite in the evening independent of food intake and other behaviors.
663 *Obesity.* **21**(3):421–3.
- 664 34. Heilbronn LK, Smith SR, Martin CK *et al.* (2005). Alternate-day fasting in nonobese
665 subjects: effects on body weight, body composition, and energy metabolism. *Am J Clin*
666 *Nutr.* **81**(1):69–73.
- 667 35. Klempel MC, Bhutani S, Fitzgibbon M *et al.* (2010). Dietary and physical activity
668 adaptations to alternate day modified fasting: implications for optimal weight loss.
669 *Nutr J.* **9**:35.
- 670 36. Johnstone A. (2015). Fasting for weight loss: an effective strategy or latest dieting
671 trend? *Int J Obes.* **39**:727–33.
- 672 37. Yates L, Warde A. (2015). The evolving content of meals in Great Britain. Results of a
673 survey in 2012 in comparison with the 1950s. *Appetite.* **84**:299–308.
- 674 38. Lhuissier A, Tichit C, Caillavet F *et al.* (2013). Who still eats three meals a day?
675 Findings from a quantitative survey in the Paris area. *Appetite.* **63**:59–69.
- 676 39. Frayn KN. (2016). Living with lipids: Reflections of a fat physiologist. *Nutr Bull.*
677 **41**(2):104–7.
- 678 40. Travers RL, Motta AC, Betts JA *et al.* (2017). Adipose tissue metabolic and
679 inflammatory responses to a mixed meal in lean, overweight and obese men. *Eur J*
680 *Nutr.* **56**(1):375–85.

- 681 41. Coppack SW, Fisher RM, Gibbons GF *et al.* (1990). Postprandial substrate deposition
682 in human forearm and adipose tissues in vivo. *Clin Sci.* **79**(4):339–48.
- 683 42. Frayn KN, Coppack SW, Humphreys SM *et al.* (1993). Periprandial regulation of lipid
684 metabolism in insulin-treated diabetes mellitus. *Metabolism.* **42**(4):504–10.
- 685 43. Gonzalez JT. (2014). Paradoxical second-meal phenomenon in the acute postexercise
686 period. *Nutrition.* **30**(9):961–7.
- 687 44. Ahmed M, Gannon MC, Nuttall FQ. (1976). Postprandial plasma glucose, insulin,
688 glucagon and triglyceride responses to a standard diet in normal subjects.
689 *Diabetologia.* **12**(1):61–7.
- 690 45. Ruge T, Hodson L, Cheeseman J *et al.* (2009). Fasted to Fed Trafficking of Fatty
691 Acids in Human Adipose Tissue Reveals a Novel Regulatory Step for Enhanced Fat
692 Storage. *J Clin Endocrinol Metab.* **94**(5):1781–8.
- 693 46. McQuaid SE, Hodson L, Neville MJ *et al.* (2011). Downregulation of Adipose Tissue
694 Fatty Acid Trafficking in Obesity: A Driver for Ectopic Fat Deposition? *Diabetes.*
695 **60**(1):47–55.
- 696 47. Halberg N, Henriksen M, Söderhamn N *et al.* (2005). Effect of intermittent fasting and
697 refeeding on insulin action in healthy men. *J Appl Physiol.* **99**(6):2128–36.
- 698 48. Soeters MR, Soeters PB, Schooneman MG *et al.* (2012). Adaptive reciprocity of lipid
699 and glucose metabolism in human short-term starvation. *Am J Physiol Metab.*
700 **303**(12):E1397–407.
- 701 49. Fabry P, Fodor J, Hejl Z *et al.* (1964). The frequency of meals. Its relation to
702 overweight, hypercholesterolaemia, and decreased glucose-tolerance. *Lancet.* **2**:614–5.
- 703 50. Murakami K, Livingstone MBE. (2015). Eating Frequency Is Positively Associated
704 with Overweight and Central Obesity in US Adults. *J Nutr.* **145**(12):2715–24.
- 705 51. Canuto R, da Silva Garcez A, Kac G *et al.* (2017). Eating frequency and weight and

- 706 body composition: a systematic review of observational studies. *Public Health Nutr.*
707 **20**(12):2079–95.
- 708 52. Raynor HA, Goff MR, Poole SA *et al.* (2015). Eating Frequency, Food Intake, and
709 Weight: A Systematic Review of Human and Animal Experimental Studies. *Front*
710 *Nutr.* **2**:38.
- 711 53. St-Onge M-P, Ard J, Baskin ML *et al.* (2017). Meal Timing and Frequency:
712 Implications for Cardiovascular Disease Prevention: A Scientific Statement From the
713 American Heart Association. *Circulation.* **135**(9):e96–121.
- 714 54. Stote KS, Baer DJ, Spears K *et al.* (2007). A controlled trial of reduced meal
715 frequency without caloric restriction in healthy, normal-weight, middle-aged adults.
716 *Am J Clin Nutr.* **85**(4):981–8.
- 717 55. Carlson O, Martin B, Stote KS *et al.* (2007). Impact of reduced meal frequency
718 without caloric restriction on glucose regulation in healthy, normal-weight middle-
719 aged men and women. *Metab - Clin Exp.* **56**(12):1729–34.
- 720 56. Dhurandhar N V, Schoeller D, Brown AW *et al.* (2015). Energy balance measurement:
721 when something is not better than nothing. *Int J Obes.* **39**(7):1109–13.
- 722 57. Mattson MP, Allison DB, Fontana L *et al.* (2014). Meal frequency and timing in health
723 and disease. *Proc Natl Acad Sci U S A.* **111**(47):16647–53.
- 724 58. Carter S, Clifton PM, Keogh JB. (2016). The effects of intermittent compared to
725 continuous energy restriction on glycaemic control in type 2 diabetes; a pragmatic
726 pilot trial. *Diabetes Res Clin Pract.* **122**:106–12.
- 727 59. Conley M, Le Fevre L, Haywood C *et al.* (2018). Is two days of intermittent energy
728 restriction per week a feasible weight loss approach in obese males? A randomised
729 pilot study. *Nutr Diet.* **75**(1):65–72.
- 730 60. Sundfør TM, Svendsen M, Tonstad S. (2018). Effect of intermittent versus continuous
731 energy restriction on weight loss, maintenance and cardiometabolic risk: a randomized

- 732 1-year trial. *Nutr Metab Cardiovasc Dis.* **28**(7): 698–706.
- 733 61. Harvie MN, Pegington M, Mattson MP *et al.* (2011). The effects of intermittent or
734 continuous energy restriction on weight loss and metabolic disease risk markers: a
735 randomized trial in young overweight women. *Int J Obes.* **35**(5):714–27.
- 736 62. Antoni R, Johnston KL, Collins AL *et al.* (2018). Intermittent v. continuous energy
737 restriction: differential effects on postprandial glucose and lipid metabolism following
738 matched weight loss in overweight/obese participants. *Br J Nutr.* **119**(5):507–16.
- 739 63. Bhutani S, Klempel MC, Berger RA *et al.* (2010). Improvements in Coronary Heart
740 Disease Risk Indicators by Alternate-Day Fasting Involve Adipose Tissue
741 Modulations. *Obesity.* **18**(11):2152–9.
- 742 64. Varady KA, Bhutani S, Klempel MC *et al.* (2013). Alternate day fasting for weight
743 loss in normal weight and overweight subjects: a randomized controlled trial. *Nutr J.*
744 **12**(1):146.
- 745 65. Hoddy KK, Kroeger CM, Trepanowski JF *et al.* (2014). Meal timing during alternate
746 day fasting: Impact on body weight and cardiovascular disease risk in obese adults.
747 *Obesity.* **22**(12):2524–31.
- 748 66. Klempel MC, Kroeger CM, Varady KA. (2013). Alternate day fasting (ADF) with a
749 high-fat diet produces similar weight loss and cardio-protection as ADF with a low-fat
750 diet. *Metab - Clin Exp.* **62**(1):137–43.
- 751 67. Trepanowski J, Kroeger C, Barnosky A *et al.* (2017). Effect of Alternate-Day Fasting
752 on Weight Loss, Weight Maintenance, and Cardioprotection Among Metabolically
753 Healthy Obese Adults. *JAMA - Intern Med.* **177**(7):930–8.
- 754 68. Trepanowski J, Kroeger C, Barnosky A *et al.* (2018). Effects of alternate-day fasting
755 or daily calorie restriction on body composition, fat distribution, and circulating
756 adipokines: Secondary analysis of a randomized controlled trial. *Clin Nutr.* **37**(6):
757 1871–78.

- 758 69. Kroeger CM, Trepanowski JF, Klempel MC *et al.* (2018). Eating behavior traits of
759 successful weight losers during 12 months of alternate-day fasting: An exploratory
760 analysis of a randomized controlled trial. *Nutr Health*. **24**(1):5–10.
- 761 70. Sutton EF, Beyl R, Early KS *et al.* (2018). Early Time-Restricted Feeding Improves
762 Insulin Sensitivity, Blood Pressure, and Oxidative Stress Even without Weight Loss in
763 Men with Prediabetes. *Cell Metab*. **27**(6):1212–21.
- 764 71. Gonzalez JT, Richardson JD, Chowdhury EA *et al.* (2018). Molecular adaptations of
765 adipose tissue to 6 weeks of morning fasting vs. daily breakfast consumption in lean
766 and obese adults. *J Physiol*. **596**(4):609–22.
- 767 72. Chowdhury EA, Richardson JD, Holman GD *et al.* (2016). The causal role of breakfast
768 in energy balance and health: a randomized controlled trial in obese adults. *Am J Clin*
769 *Nutr*. **103**(3):747–56.
- 770 73. Betts JA, Richardson JD, Chowdhury EA *et al.* (2014). The causal role of breakfast in
771 energy balance and health: a randomized controlled trial in lean adults. *Am J Clin*
772 *Nutr*. **100**(2):539–47.
- 773 74. Betts JA, Chowdhury EA, Gonzalez JT *et al.* (2016). Is breakfast the most important
774 meal of the day? *Proc Nutr Soc*. **75**(4):464–74.
- 775 75. Moro T, Tinsley G, Bianco A *et al.* (2016). Effects of eight weeks of time-restricted
776 feeding (16/8) on basal metabolism, maximal strength, body composition,
777 inflammation, and cardiovascular risk factors in resistance-trained males. *J Transl*
778 *Med*. **14**(1):290.
- 779 76. Gabel K, Hoddy KK, Haggerty N *et al.* (2018). Effects of 8-hour time restricted
780 feeding on body weight and metabolic disease risk factors in obese adults: A pilot
781 study. *Nutr Heal aging*. **4**(4):345–53.
- 782 77. Soeters MR, Lammers NM, Dubbelhuis PF *et al.* (2009). Intermittent fasting does not
783 affect whole-body glucose, lipid, or protein metabolism. *Am J Clin Nutr*. **90**(5):1244–
784 51.

- 785 78. Heilbronn LK, Civitarese AE, Bogacka I *et al.* (2005). Glucose Tolerance and Skeletal
786 Muscle Gene Expression in Response to Alternate Day Fasting. *Obes Res.* **13**(3):574–
787 81.
- 788 79. Henique C, Mansouri A, Fumey G *et al.* (2010). Increased mitochondrial fatty acid
789 oxidation is sufficient to protect skeletal muscle cells from palmitate-induced
790 apoptosis. *J Biol Chem.* **285**(47):36818–27.
- 791 80. Church T, Martin CK. (2018). The Obesity Epidemic: A Consequence of Reduced
792 Energy Expenditure and the Uncoupling of Energy Intake? *Obesity.* **26**(1):14–6.
- 793 81. Catenacci VA, Pan Z, Ostendorf D *et al.* (2016). A randomized pilot study comparing
794 zero-calorie alternate-day fasting to daily caloric restriction in adults with obesity.
795 *Obesity.* **24**(9):1874–83.
- 796 82. Barnosky AR, Hoddy KK, Unterman TG *et al.* (2014). Intermittent fasting vs daily
797 calorie restriction for type 2 diabetes prevention: a review of human findings. *Transl*
798 *Res.* **164**(4):302–11.
- 799 83. Rostom H, Shine B. (2018). Basic metabolism: proteins. *Surg.* **36**(4):153–8.
- 800 84. Felig P. (1975). Amino Acid Metabolism in Man. *Annu Rev Biochem.* **44**(1):933–55.
- 801 85. Atherton PJ, Smith K. (2012). Muscle protein synthesis in response to nutrition and
802 exercise. *J Physiol.* **590**(5):1049–57.
- 803 86. Dickinson JM, Rasmussen BB. (2013). Amino acid transporters in the regulation of
804 human skeletal muscle protein metabolism. *Curr Opin Clin Nutr Metab Care.*
805 **16**(6):638–44.
- 806 87. Tipton KD, Hamilton DL, Gallagher IJ. (2018). Assessing the Role of Muscle Protein
807 Breakdown in Response to Nutrition and Exercise in Humans. *Sport Med.* **48**(Suppl
808 1):53–64.
- 809 88. Vendelbo MH, Møller AB, Christensen B *et al.* (2014). Fasting Increases Human

- 810 Skeletal Muscle Net Phenylalanine Release and This Is Associated with Decreased
811 mTOR Signaling. *PLoS One*. **9**(7):e102031.
- 812 89. Varady KA. (2011). Intermittent versus daily calorie restriction: which diet regimen is
813 more effective for weight loss? *Obes Rev*. **12**(7):E593–601.
- 814 90. Alhamdan BA, Garcia-Alvarez A, Alzahrnai AH *et al.* (2016). Alternate-day versus
815 daily energy restriction diets: which is more effective for weight loss? A systematic
816 review and meta-analysis. *Obes Sci Pract*. **2**(3):293–302.
- 817 91. Johnstone AM, Murison SD, Duncan JS *et al.* (2005). Factors influencing variation in
818 basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but
819 not sex, circulating leptin, or triiodothyronine. *Am J Clin Nutr*. **82**(5):941–8.
- 820 92. Hopkins M, Finlayson G, Duarte C *et al.* (2016). Modelling the associations between
821 fat-free mass, resting metabolic rate and energy intake in the context of total energy
822 balance. *Int J Obes*. **40**(2):312–8.
- 823 93. Lam YY, Ravussin E. (2016). Analysis of energy metabolism in humans: A review of
824 methodologies. *Mol Metab*. **5**(11):1057–71.
- 825 94. Sumithran P, Prendergast LA, Delbridge E *et al.* (2011). Long-Term Persistence of
826 Hormonal Adaptations to Weight Loss. *N Engl J Med*. **365**(17):1597–604.
- 827 95. Varady KA, Hoddy KK, Kroeger CM *et al.* (2016). Determinants of weight loss
828 success with alternate day fasting. *Obes Res Clin Pract*. **10**:476–80.
- 829 96. Klempel MC, Kroeger CM, Bhutani S *et al.* (2012). Intermittent fasting combined with
830 calorie restriction is effective for weight loss and cardio-protection in obese women.
831 *Nutr J*. **11**(1):98.
- 832 97. Borai A, Livingstone C, Kaddam I *et al.* (2011). Selection of the appropriate method
833 for the assessment of insulin resistance. *BMC Med Res Methodol*. **11**(1):158.
- 834 98. Antoni R, Johnston KL, Collins AL *et al.* (2017). Effects of intermittent fasting on

- 835 glucose and lipid metabolism. *Proc Nutr Soc.* **76**(3):361–8.
- 836 99. Public Health England. (2016). *Government Dietary Recommendations: Government*
837 *recommendations for energy and nutrients for males and females aged 1-18 years and*
838 *19+ years.* London: Public Health England.
- 839 100. Public Health England. (2018). *The Eatwell Guide: Helping you eat a healthy,*
840 *balanced diet.* London: Public Health England.
- 841