Investigation into the acute effects of total and partial energy restriction on postprandial metabolism among overweight/obese...

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Investigation into the acute effects of total and partial energy restriction on postprandial metabolism among overweight/obese participants

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Abstract
The intermittent energy restriction (IER) approach to weight loss involves short periods of substantial (75–100 %) energy restriction (ER) interspersed with normal eating. This study aimed to characterise the early metabolic response to these varying degrees of ER, which occurs acutely and prior to weight loss. Ten (three female) healthy, overweight/obese participants (36 (SEM 5) years; 29.0 (SEM 1.1) kg/m2) took part in this acute three-way crossover study. Participants completed three 1-d dietary interventions in a randomised order with a 1-week washout period: isonenergetic intake, partial 75 % ER and total 100 % ER. Fasting and postprandial (6-h) metabolic responses to a liquid test meal were assessed the following morning via serial blood sampling and indirect calorimetry. Food intake was also recorded for two subsequent days of ad libitum intake. Relative to the isonenergetic control, postprandial glucose responses were increased following total ER (+142 %; P=0.015) and to a lesser extent after partial ER (+76 %; P=0.051). There was also a delay in the glucose time to peak after total ER only (P=0.024). Both total and partial ER interventions produced comparable reductions in postprandial TAG responses (~75 and ~59 %, respectively; both P<0.05) and 3-d energy intake deficits of approximately 30 % (both P=0.015). Resting and meal-induced thermogenesis were not significantly affected by either ER intervention. In conclusion, our data demonstrate the ability of substantial ER to acutely alter postprandial glucose-lipid metabolism (with partial ER producing the more favourable overall response), as well as incomplete energy-intake compensation amongst overweight/obese participants. Further investigations are required to establish how metabolism adapts over time to the repeated perturbations experienced during IER, as well as the implications for long-term health.

Key words: Intermittent energy restriction: Intermittent fasting: Alternate-day fasting: Type 2 diabetes: CVD: Cardiometabolic risk

In recent years, intermittent energy restriction (IER) has become the subject of considerable research interest as an alternative to the more conventional continuous energy restriction (ER) approaches to weight loss. An array of IER protocols have been studied; each protocol involves intermittent periods of very low (or no) energy intake followed by normal eating, most commonly for 1(2) or 2 d/week(3,4), or on alternate days(5,6). Consequently, dieters undergo repeated ER-re-feed cycles over the course of the week. However, little is known about the effects of this altered eating pattern on postprandial glucose-lipid metabolism, which is pertinent, given the growing evidence base implicating both as independent discriminators of CVD risk(7,8).

Additionally, studies of very low-energy diets demonstrate dramatic changes in metabolic control within overweight/obese cohorts, long before significant weight loss occurs(9,10). This acute timecourse suggests a primary role of dietary ER, or more specifically the creation of a profound negative energy balance, as a primary mediator underlying these early effects. Understanding the timecourse over which such metabolic changes occur during ER is important for our understanding of the metabolic adaptation which occurs during weight loss. It is well established that short-term fasting elicits a coordinated metabolic response to the accompanying energy deficit, resulting in increased fatty acid mobilisation from adipose tissue, as well as a shift in fuel utilisation towards fatty acid oxidation (FAO) and ketogenesis (reviewed by Soeters et al(11)). There is also a marked decline in peripheral insulin sensitivity and glucose tolerance(12–16). From an evolutionary standpoint, these reciprocal adaptations in glucose-lipid metabolism serve to conserve glucose, and thus limit the utilisation of protein stores. They are reversed through food re-introduction, but this may require upwards of 48 h of re-feeding(17).

The majority of acute research to date has either involved healthy-weight individuals or more prolonged (≥48 h) total fasting intervals, as this is the timecourse in which the short-term adaptations to fasting occur and become maximal(11). IER has predominantly been investigated as a weight-loss strategy within overweight/obese cohorts. The majority of dietary protocols used in these studies allow a small amount of food intake in an attempt to improve the tolerability and compliance to IER, such that energy is substantially (but not completely) restricted. This has the

Abbreviations: 3-OHB, 3-β-hydroxybutyrate; dAUC, decremental AUC; ER, energy restriction; iAUC, incremental AUC; IER, intermittent energy restriction; REE, resting energy expenditure.

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potential to alter the response which has been well defined for more prolonged fasting intervals, and thus the subsequent post-prandial response during re-feeding. Acutely, this is best assessed using a within-subject study design, which to our knowledge has not been performed.

With this in mind the present cross-over study, conducted in ten overweight or obese participants, aimed to characterise the early metabolic response to varying degrees of ER by assessing postprandial responses to a liquid mixed test meal after 1 d of total (100 %) and partial (75 %) ER. Secondary outcomes included subsequent energy intake compensation and thermogenic responses, potential limiting factors for the long-term weight-loss efficacy of IER.

**Methods**

**Participants**

In total, fourteen (six female) healthy, overweight or obese participants aged 18–60 years were initially recruited to the study from the University of Surrey and wider community. Four (three female) participants did not complete the study because of non-compliance (n 1) or cannulation difficulties (n 3), culminating in ten study completers (see Table 1 for participant characteristics). Participants were weight stable (±2 kg) over the preceding 3 months and had no significant medical history. Health status was determined by medical questionnaire and screening blood sample. Restrained eaters were identified and excluded using the Dutch Eating Behaviour Questionnaire (cut-off: >4) as potential confounders for the secondary outcome (17). To control for the potential influence of the menstrual cycle between visits, recruited female participants were either post-menopausal or taking oral contraceptives. The study was approved by the University of Surrey Ethics Committee and conducted in accordance with the guidelines laid down in the Declaration of Helsinki. Written informed consent was obtained from all participants.

**Table 1. Participant characteristics taken at the pre-trial visit (Mean values with their standard errors)**

<table>
<thead>
<tr>
<th>All participants (n 10)</th>
<th>Males (n 7)</th>
<th>Females (n 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td>Age (years)</td>
<td>36 ± 4</td>
<td>42 ± 5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>91 ± 2</td>
<td>97 ± 4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29 ± 1</td>
<td>29 ± 1</td>
</tr>
<tr>
<td>Body fat (%)*</td>
<td>29 ± 2</td>
<td>26 ± 1</td>
</tr>
</tbody>
</table>

* Measured using bioimpedance (Tanita MC180A; Tanita Corp).

**General protocol**

The study was a three-way, randomised, cross-over study (Fig. 1), in which the participants completed three 1-d dietary interventions in a random order with a minimum 1-week washout: 1 d of isocaloric intake (0% ER), which served as the control; 1 d of total (100%) ER; and 1 d of partial (75%) ER. Metabolic assessments were conducted on the following day (Day 2). To assess for short-term energy compensation, dietary intakes were recorded over a 3-d period, which encompassed each controlled intake day (Day 1), while participants were at the research unit (Day 2) and upon resumption of ad libitum intake (Days 2 and 3). To ensure familiarity with study procedures, participants first completed a pre-study test run, which was identical in design to the isocaloric intervention.

**Day 1: experimental diets.** Energy requirements were calculated using the Henry predictive equation for BMR (18) as this forms part of the updated estimated average requirement for energy (19). Participants were asked to minimise their activity levels during each intervention period, and so the physical

![Fig. 1. Schematic overview of study. A randomised, cross-over study where participants completed three dietary interventions in a random order: 1 d of isocaloric intake (0% energy restriction (ER)), which served as the control; 1 d of total (100%) ER; and 1 d of partial (75%) ER. Metabolic assessments were conducted on the following day (Day 2). To assess for short-term energy compensation, dietary intakes were recorded over a 3-d period, which encompassed each controlled intake day (Day 1), while participants were at the research unit (Day 2) and upon resumption of ad libitum intake (Days 2 and 3). There was a minimum 1-week washout period. To ensure familiarity with study procedures, participants first completed a pre-study test run, which was identical in design to the isocaloric intervention. PP, postprandial.
activity levels used to calculate daily energy requirements were representative of sedentary activity levels\(^{19}\).

**Isocaloric control diet (0 % ER):** Each participant was supplied with an isocaloric diet comprised of commonly consumed food and drinks (11 040 (SEM 1482) kJ, 327 (SEM 12) g carbohydrate (55 % of total energy), 99 (SEM 3) g protein (15 % of total energy) and 97 (SEM 5) g fat (30 % of total energy)) providing 100 % of their estimated isocaloric needs.

**Total (100 %) ER:** Participants started their fast from 20.00 hours the night before their dietary intervention day until 08.00 hours on the morning of their study day, totalling 36 h.

**Partial (75 %) ER:** Participants consumed four commercially available LighterLife™ FoodPacks (2638 kJ, 58 g carbohydrate (37 % of total energy), 54 g protein (35 % of total energy), 17 g fat (28 % of total energy)), which provided approximately 25 % of their estimated isocaloric needs. The degree of ER chosen is comparable with that used by previously published IER weight-loss trials\(^{5,4,6}\).

During each dietary intervention, participants were advised to consume sufficient amounts of non-caloric fluids and to abstain from alcohol. During the isocaloric and partial ER interventions, participants ate their last meal no later than 20.00 hours.

**Day 2: metabolic studies.** Participants attended the Surrey Clinical Research Centre on the morning (08.00 hours) after each controlled energy intake day. Body weight and composition were measured by bioimpedance (Tanita MC180A; Tanita Corp.). Resting measurements of energy expenditure and substrate utilisation were then measured via indirect calorimetry (GEM Nutrition) after participants had rested for 30 min. An indwelling cannula was then inserted following which the first (fasting) sample was collected. A liquid, mixed test meal was provided (400 ml Fortisip; Nutricia: 2510 kJ, 74 g carbohydrate, 24 g protein, 23 g fat). Serial measurements of energy expenditure, substrate utilisation and blood samples were obtained at regular intervals from the start of the test meal over the next 360 min. Participants were then presented with a large pre-weighed ad libitum homogeneous pasta meal in excess of normal portions (whole derived from the Henry equation\(^{18}\). Fasting and postprandial substrate oxidation were calculated using the non-protein stoichiometric equations from Frayn\(^{23}\). These equations assume negligible contributions from gluconeogenesis, protein oxidation and ketogenesis; whilst these assumptions may not hold true following substantial ER, evidence from studies over longer (72-h) fasting durations suggest that the error introduced by not accounting for the latter two metabolic processes would be minimal\(^{14}\).

**Blood biochemistry and calculations.** Serial blood measurements were taken at baseline and then at regular intervals of 15, 30, 60, 90, 120, 180, 240, 300 and 360 min. Blood samples were collected into potassium EDTA tubes (for the analysis of TAG, NEFA, insulin and 3-β-hydroxybutyrate (3-OHB)) and sodium oxalate tubes (for glucose analysis). Samples were centrifuged for 15 min at 2500 rpm and separated; plasma aliquots were then stored at −20°C with a subset intended for 3-OHB analysis stored at −80°C. Samples were batch analysed upon study completion with all samples from an individual participant included in the same assay. Metabolites were analysed using the following methods: insulin using RIA (Millipore; intra/inter-assay CV 6 and 8 %); glucose, TAG and NEFA using commercially available kits (Instrumentation Laboratory) for the ILAB650 (Instrumentation Laboratory; intra/inter-assay CV all <4 and <7 %); and 3-OHB using commercially available kits (Randox) for the Cobas MIRA (Roche; intra/inter-assay CV 6 and 9 %). Incremental AUC (iAUC) was calculated to quantify postprandial metabolic responses\(^{24}\).

**Dietary analyses.** All diet diary analyses were carried out in Diet Plan 6 (Forestfield Software) using the McCance and Widdowson’s composition of foods integrated data set. Participants recorded intake in validated diet diaries which included pictorial guides to aid portion size estimations when exact weights could not be provided. Nutritional intake information recorded in diet diaries (Days 1–3), from the weighted ad libitum meal intake (Day 2) and test meal (Day 2) were aggregated. This information was then used to calculate daily and cumulative 3-d energy consumption, expressed as the percentage of estimated isocaloric needs derived from the Henry equation\(^{18}\).

**Statistical analyses.** Statistical analyses were carried out using IBM SPSS version 22 (SPSS Inc.). In view of the small sample size, non-parametric testing was deemed the most appropriate for the analysis of summary measures. Comparisons between the three experimental arms were conducted using the Friedman test with the Wilcoxon signed-ranks test used for subsequent pairwise comparisons. A Bonferroni correction was applied to correct for multiple post hoc testing. For timecourse data, where no robust non-parametric test is able to examine main and interaction effects, repeated-measures ANOVA was used with a Sidak correction applied to post hoc pairwise comparisons. Before this, any non-normally distributed data were log transformed. Diet and time point (for timecourse data) were used as within-subject factors. Statistical significance was accepted at the 5 % level. All results are presented as mean values with their standard errors.
Sample size

Based on the change in the postprandial TAG iAUC between isoenergetic and partial (75%) ER conditions: ten participants in a cross-over design study, α=0.05, two-sided, would give a 90% power of detecting a difference in response of 47 units assuming a standard deviation of the response to be 41 units. In retrospect, the actual mean change in TAG was 89 (SEM 13) units, resulting in a study power >99% for this primary outcome variable.

Results

Dietary intakes

Table 2 displays day-by-day and cumulative energy consumption across each 3-d study period. Reported dietary intakes during controlled energy intake days (Day 1) were close to prescribed amounts. On Day 2, participants over-consumed by 22·7 (SEM 7·5) % above estimated daily energy requirements after their total ER (fast) day (P=0·010 v. iso) and by +10·0 (SEM 5·7) % following their partial ER day (non-significant v. iso). On Day 3, no significant differences in energy intake were detected across the three dietary interventions when expressed either in kJ or relative to isoenergetic requirements, with participants seemingly under-consuming after all three interventions. Overall, cumulative 3-d intakes were significantly lower following both total and partial ER interventions relative to the isoenergetic control leg, with participants remaining in comparable net energy deficits of −28·4 (SEM 5·2)% and −30·3 (SEM 3·1) %, respectively (both P=0·015 v. iso). No significant differences in any dietary intake measure were noted between total and partial ER interventions.

Table 2. Daily and cumulative 3-d energy intakes during isoenergetic, total energy restriction (ER) and partial ER dietary interventions. Broken down into individual and cumulative energy consumption during the controlled energy intake day, 1 and 2 d later

<table>
<thead>
<tr>
<th>Isoenergetic</th>
<th>Partial (75%) ER</th>
<th>Total (100%) ER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td>Controlled intake (Day 1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake (kJ/d)</td>
<td>11 040</td>
<td>468</td>
</tr>
<tr>
<td>% ER achieved*</td>
<td>+0·4</td>
<td>0·3</td>
</tr>
<tr>
<td>1 day post (Day 2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ad libitum meal (kJ)</td>
<td>4929</td>
<td>354</td>
</tr>
<tr>
<td>Total 24 h intake (kJ/d)</td>
<td>11 816</td>
<td>1057</td>
</tr>
<tr>
<td>% 24 h energy balance*</td>
<td>+7·5</td>
<td>8·6</td>
</tr>
<tr>
<td>2 days post (Day 3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total 24 h intake (kJ/d)</td>
<td>9675</td>
<td>1089</td>
</tr>
<tr>
<td>% 24 h energy balance*</td>
<td>−12·6</td>
<td>8·6</td>
</tr>
<tr>
<td>Cumulative 3 d total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total daily intake (kJ/d)</td>
<td>32 532</td>
<td>2257</td>
</tr>
<tr>
<td>Net 3 d energy balance*</td>
<td>−1·5</td>
<td>5·1</td>
</tr>
</tbody>
</table>

* Expressed as the percentage relative to participants’ estimated daily requirement for weight maintenance. Comparisons made using the Friedman test, with post hoc Wilcoxon signed-ranks testing used for subsequent pairwise comparisons (Bonferroni correction applied).

Table 3. Fasting substrate levels and fuel oxidation the morning after 1 day of isoenergetic intake, total energy restriction (ER) and partial ER (i.e Day 2)

<table>
<thead>
<tr>
<th>Isoenergetic</th>
<th>Partial (75%) ER</th>
<th>Total (100%) ER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4·7</td>
<td>0·1</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>89·7</td>
<td>9·2</td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td>1·5</td>
<td>0·2</td>
</tr>
<tr>
<td>NEFA (mmol/l)</td>
<td>0·63</td>
<td>0·07</td>
</tr>
<tr>
<td>3-OHB (mmol/l)</td>
<td>0·05</td>
<td>0·02</td>
</tr>
<tr>
<td>NP RO (VO2/VCO2)*</td>
<td>0·89</td>
<td>0·03</td>
</tr>
</tbody>
</table>

* Expressed as the percentage relative to participants’ estimated daily requirement for weight maintenance. Comparisons made using the Friedman test, with post hoc Wilcoxon signed-ranks testing used for subsequent pairwise comparisons (Bonferroni correction applied).

Fasting metabolism

Fasting substrate levels and fuel oxidation, assessed on the morning after each intervention (Day 2), are presented in Table 3. Relative to the isoenergetic control, total ER led to a significant reduction in levels of fasting plasma glucose (P=0·022 v. iso) and TAG (P=0·039 v. iso), whereas NEFA levels and 3-OHB were higher (P=0·022 and 0·015, respectively, v. iso). Accordingly, fasting RQ was significantly lower following total ER v. iso only (P=0·024), indicative of higher rates of fat oxidation. Following
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Partial ER, similar trends were noted but the rise in plasma NEFA did not reach statistical significance v. iso. Plasma NEFA and 3-OHB were significantly higher after total ER when compared with partial ER (both \( P = 0.015 \)); no other statistical differences between the ER interventions were noted.

**Postprandial substrate metabolism**

Postprandial substrate and insulinaemic responses to the liquid test meal, given the morning after each ER intervention, are presented in Fig. 2(a–d) along with main and interaction effects for timecourse data.

**Glucose and insulin responses**

There were main effects of intervention on postprandial glucose iAUC (\( P = 0.001 \); Fig. 2(a)); glucose iAUC in response to the liquid test meal was significantly greater following total ER relative to iso (\( 402 \text{ (SEM 64)} \text{ mmol/min per litre}; P = 0.015 \)) and tended to be greater than the partial ER leg (\( 294 \text{ (SEM 64)} \text{ mmol/min per litre}; P = 0.051 \)). A non-significant trend in favour of greater glucose iAUC following partial ER v. iso was also found (\( P = 0.051 \)). Postprandial glucose time to peak was significantly delayed following total ER relative to both iso (\( P = 0.024 \)) and partial ER (\( P = 0.018 \)) interventions. No significant differences in plasma insulin iAUC were detected across the dietary interventions (Fig. 2(b)).

**TAG responses**

There were highly significant main effects of intervention on TAG iAUC (\( P = 0.001 \); Fig. 2(c)); compared with iso (\( 131 \text{ (SEM 20)} \text{ mmol/min per litre} \)), postprandial iAUC was significantly lower following both total (\( 32 \text{ (SEM 11)} \text{ mmol/min per litre}; P = 0.015 \) v. iso) and partial (\( 54 \pm 19 \text{ mmol/min per litre}; P = 0.039 \) v. iso) ER interventions. TAG iAUC did not differ between total and partial ER.

**NEFA**

There were significant main effects of intervention on NEFA postprandial decremental AUC (dAUC) (\( P = 0.002 \); Fig. 2(d)); following total ER, plasma NEFA dAUC was significantly different compared with iso (\( -195 \text{ (SEM 36)} \text{ mmol/min per litre}; P = 0.037 \)) and partial ER (\( -101 \text{ (SEM 18)} \text{ mmol/min per litre}; P = 0.015 \)) dAUC did not differ significantly between partial ER and iso interventions.

**Postprandial substrate oxidation and energy metabolism**

Postprandial substrate oxidation responses (\( n = 8 \)) following the liquid test meal are presented in Fig. 3(a–c). Indirect calorimetry data for two participants were excluded after data loss due to equipment failure. No significant main effects were observed for postprandial fat oxidation (Fig. 3(a)). A significant main effect of intervention was observed for postprandial carbohydrate oxidation (\( P = 0.011 \); Fig. 3(c)), which was lower following total ER v. iso only (\( P = 0.05 \)). Postprandial plasma 3-OHB concentrations (Fig. 3(b)) paralleled NEFA responses (Fig. 2(d)) with circulating levels suppressed to the same extent across the dietary interventions by approximately 180 min, before rising markedly thereafter following total and partial ER interventions only. Statistical analyses showed significant main effects of intervention (\( P < 0.001 \)) on postprandial 3-OHB dAUC; following total ER, plasma 3-OHB dAUC was significantly different compared with iso (\( -133 \text{ (SEM 36)} \text{ mmol/min per litre}; P = 0.001 \)).

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**Fig. 2.** Postprandial (6-h) substrate responses to liquid test meal (2510 kJ (600 kcal), 74 g carbohydrate, 24 g protein, 23 g fat) following 1 d of total 100% energy restriction (ER) (–) and partial 75% ER (–) v. an isoenergetic (iso) control diet (–). (a) Plasma glucose: there was a significant diet × time interaction (\( P < 0.001 \)). (b) Plasma insulin: there was a trend in favour of a diet × time interaction (\( P = 0.091 \)). (c) Plasma TAG: there were significant main effects of diet (\( P < 0.001 \)) and a diet × time interaction (\( P < 0.001 \)). Significant differences found between: iso v. total ER (\( P = 0.001 \)) and partial ER (\( P = 0.005 \)). (d) Plasma NEFA: there were significant main effects of diet (\( P = 0.008 \)) and a diet × time interaction (\( P < 0.001 \)). Differences found between total ER v. iso (\( P = 0.039 \)) and partial ER (\( P = 0.083 \); non-significant trend). Comparisons (using plasma concentration) made using repeated-measures ANOVA (Sidak correction applied). Values are mean change from baseline (\( n = 10 \)), with their standard errors.
(a) Fat oxidation (indirect calorimetry): there was a trend in favour of a main effect of diet ($P=0.080$). No significant pairwise differences found between iso ($17$ (SEM $2$) g), total ER ($26$ (SEM $4$) g; $P=0.184$) or partial ER ($24$ (SEM $4$) g; $P=0.233$) interventions. (b) Plasma 3-β-hydroxybutyrate: there were significant main effects of diet ($P<0.001$) and a diet × interaction ($P<0.001$) for postprandial 3-β-hydroxybutyrate responses. Significant differences found between iso v. total ER ($P=0.001$) and partial ER ($P=0.007$). (c) Carbohydrate oxidation (indirect calorimetry): there were significant main effects of diet ($P=0.023$). Trends found between iso ($59$ (SEM $5$) g) v. total ER ($32$ (SEM $6$) g; $P=0.051$) but not partial ER ($43$ (SEM $7$) g; $P=0.174$). Comparisons made using repeated-measures ANOVA (Sidak correction applied). Values are means ($n$ 8, indirect calorimetry data; $n$ 10, 3-β-hydroxybutyrate), with their standard errors.

**Discussion**

Our data demonstrate a number of distinct alterations to postprandial substrate metabolism, assessed on the morning after 1 d of total (100%) and partial (75%) ER, which were evident following both levels of ER. Very few studies have quantified the metabolic and physiological changes that occur in response to substantial ER. These acute changes in fuel management could lead to changes in so-called metabolic flexibility, a marker of metabolic health. As a result, regimens incorporating repeated bouts of substantial ER could lead to altered cardio-metabolic risk, independent of weight change.

**Postprandial glucose metabolism**

Our finding that oral glucose tolerance was impaired after 1 d (36 h) of total ER is in accordance with the existing research on prolonged fasting, which up to now has either involved lean participants or more prolonged ($\geq 48$ h) fasting intervals.$^{13,14,16}$ We additionally found a significant delay in the postprandial glucose curve, which, in the context of early type 2 diabetes mellitus, is associated with impairments in β-cell function and insulin secretion.$^{25}$ Indeed, postprandial insulin profiles following both ER interventions appeared flattened and prolonged; however, without measuring C-peptide levels, we were unable to assess the individual effects on insulin secretion $v$. hepatic clearance.

In the present study, the prolonged 36-h fast was accompanied by a significant elevation in circulating plasma NEFA, which has been implicated as a key driver behind our observations; directly, peripheral tissue accumulation of NEFA and/or its derivatives (ceramides, diacylglycerol) are linked to disruptions in peripheral insulin signalling and/or glucose transport$^{26,27}$, as well as the acute insulin response$^{16}$; indirectly, the associated rise in fat oxidation and ketosis are also capable of impairing peripheral glucose disposal$^{14,28}$. Previously, Salgin et al$^{16}$ showed that treatment with an antilipolytic agent (acipimox) could only partially reverse fast-induced effects on insulin secretion and peripheral insulin sensitivity, highlighting the role of other contributory mediators such as circulating counter-regulatory hormones, which are also elevated after prolonged fasts.$^{16,29}$

In an acute sense, our findings can be viewed as an adaptive physiological response to short-term starvation, one which allows for a shift in postprandial nutrient partitioning in favour of glucose conservation and glycogen repletion$^{11}$. Whether there may be tachyphylaxis of these effects or metabolic adaption with repeated
spells of ER re-feeding is unknown. The premise of IER is that there is
long-term adaptation to the repeated elevations in NEFA, leading
to the up-regulation of mitochondrial FAO in skeletal muscle. This
has the potential to improve insulin sensitivity by reducing the
accumulation of lipid intermediates over time. In lean or
overweight humans, one short-term study using 3 weeks of
alternate-day total fasting demonstrated trends for up-regulation
of the mitochondrial fatty acid transporter CPT-1 within skeletal
muscle, but conversely a decline in mitochondrial DNA copy
number (a marker of mitochondrial function)(35). Also observed by
this study was an impairment in glucose tolerance among women;
however, the uneven pre- and post-intervention fasting intervals
used in this study (12 v. 36 h respectively) make it difficult to
ascrbe this as a true chronic treatment effect. Animal models using
a similar alternate-day fasting protocol also report increases in
insulin receptor nitration in skeletal muscle during long-term IER,
which was accompanied by a reduction in glucose tolerance(30).

The partial (75%) ER diet was designed to place participants in
a severe negative energy balance while allowing some food
intake. This consequently blunted the progressive rise in plasma
NEFA seen with prolonged fasting intervals. Accordingly, observed
alterations in postprandial glucose tolerance and nutrient oxidation
effectively displayed a dose–response across the two levels of
substantial (75–100%) ER, with partial ER attenuating the impair-
ments to glycaemic control seen following a day of total ER.
Ultimately, in humans, the significance of short-term increases in
NEFA and impaired glucose tolerance with fasting is unknown;
whether repeated spells of total ER present too great a metabolic
perturbation; and if any potential long-term adverse health impli-
cations of this could be avoided through partial ER warrant further
investigation.

Postprandial TAG metabolism

We observed favourable alterations to TAG metabolism following
both degrees of substantial ER, which has not been shown
previously in overweight/obese participants. By combining lines of
evidence from our study and others, we propose a number of
major contributory mechanisms: first, the liver is highly sensitive
to changes in energy status. Profound negative energy deficits have
been shown to partition fatty acids derived from circulating TAG,
NEFA and intra-hepatocellular (IHCL) stores towards β-oxidation or
ketogenesis(31). Indeed, fasting levels of 3-OHB were elevated
after both ER interventions. Fatty acid availability for TAG re-
esterification, storage as IHCL and VLDL-TAG secretion is thus
limited, as evidenced by studies of regional glycerol turnover
during prolonged fasting, which have shown rates of hepatic
non-oxidative NEFA disposal to be comparatively lower than
that of peripheral tissues(32). Accordingly, we observed acute
reductions in fasting TAG, which predominantly reflects an
ER-induced decrease in fasting VLDL-TAG secretion(33). A major
regulator of ketogenesis in humans is the supply of fatty acids(34).
Our metabolic (NEFA and 3-OHB) and substrate oxidation data
closely parallel each other and are suggestive of a shift in
postprandial partitioning of fatty acids towards hepatic ketone
body synthesis (away from VLDL-TAG secretion) and FAO
(facilitating glycogen repletion). In the postprandial state,
chylomicron-TAG (derived from dietary sources) are preferentially
cleared, and thus VLDL-TAG make up approximately 90% of TAG
in the postprandial state(35). A reduction in fasting and postprandial
VLDL-TAG would be expected to reduce the overall magnitude of
the postprandial response via reduced competition between
endogenously and exogenously derived lipids for clearance via a
common, saturable lipolytic pathway(30,37).

Energy intake compensation and energy expenditure

To contextualise to a situation of weight management, our sec-
ondary aims were to study the acute effects of different degrees of
ER on acute compensation in energy intake and components
of energy expenditure. Our food intake data suggest that the
participants were still in negative energy balance after 2 d of
ad libitum intake, which is particularly interesting, given that
individuals following IER often undergo repeated ER/feed cycles
over the course of a week. We are the first to show that both
total and partial ER can produce comparable short-term energy
intake deficits of approximately 30% in overweight/obese partici-
pants, with no additional deficit achieved by fasting completely.
Anecdotally, it is likely that protocols that allow a small amount of
food intake are likely to be better tolerated over time, although this
has not been directly assessed. Our data are in line with other
studies that have similarly demonstrated an apparent lack of tight
physiological control in day-to-day energy balance following
substantial ER within lean participants with no/mild eating
restraint(38,39) and in overweight participants during chronic IER(37).

On the other side of the energy balance equation, we found
no compensatory declines in REE or MIT. To date, acute data on
energy expenditure have been equivocal(15,40–42), with fast-
induced increases in mitochondrial uncoupling(43), sympathetic
nervous system activation and catecholamine levels(42) thought
to contribute towards the paradoxical increases in REE
found by some studies(15,41,42). Although our assessments of
energy expenditure are limited to REE and MIT responses to a
single meal, others have reported ∼6% reductions in
24-h energy expenditure after prolonged fasts using whole-
body calorimeters(44,45). However, any small changes to energy
expenditure are unlikely to offset the larger changes in energy
intake.

Strengths and limitations

A particular strength of this study is the novel use of a within-
subject study design to compare individual responses to varying
levels of substantial ER, while allowing participants to act as their
own control. From a statistical standpoint, although this study
design somewhat mitigates the small heterogeneous group used
by removing the inter-individual variation element, the study
(originally powered on postprandial TAG) is likely to be
underpowered with respect to our indirect calorimetry data. This
is exemplified by the loss of statistical significance with our
substrate oxidation time-course data following multiple post hoc
comparisons. Owing to the sample size and relatively small
proportion of women, it was not possible to fully explore sex
differences. Sex dimorphism in fasting responses have previously
been shown, with women tending to display greater NEFA
mobilisation owing to differences in adiposity(46). Finally, our

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[45x399]perturbation; and if any potential long-term adverse health impli-
[45x411]whether repeated spells of total ER present too great a metabolic
impli-
cations of this could be avoided through partial ER warrant further
investigation.

Postprandial TAG metabolism

We observed favourable alterations to TAG metabolism following
both degrees of substantial ER, which has not been shown
previously in overweight/obese participants. By combining lines of
evidence from our study and others, we propose a number of
major contributory mechanisms: first, the liver is highly sensitive
to changes in energy status. Profound negative energy deficits have
been shown to partition fatty acids derived from circulating TAG,
NEFA and intra-hepatocellular (IHCL) stores towards β-oxidation or
ketogenesis(31). Indeed, fasting levels of 3-OHB were elevated
after both ER interventions. Fatty acid availability for TAG re-
esterification, storage as IHCL and VLDL-TAG secretion is thus
limited, as evidenced by studies of regional glycerol turnover
during prolonged fasting, which have shown rates of hepatic
non-oxidative NEFA disposal to be comparatively lower than
that of peripheral tissues(32). Accordingly, we observed acute
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been shown, with women tending to display greater NEFA
mobilisation owing to differences in adiposity(46). Finally, our
postprandial assessments were limited to measuring changes in absolute substrate concentrations after a single meal, which represents the balance but not the rate (or source) of substrate appearance or clearance.

Summary and future research directions

Put together, our data demonstrate the ability of substantial (75–100%) ER to acutely alter glucose–lipid metabolism, as well as incomplete short-term energy intake compensation amongst overweight/obese participants. Partial ER (as compared with total ER) reduced the fast-associated decline in oral glucose tolerance, whilst producing a comparable 3-d energy-intake deficit and improvement in postprandial TAG. The experimental partial ER diet was low in carbohydrate (37% of total energy); perhaps a greater carbohydrate supply on ER days, through further attenuation of this starvation response, may have ameliorated the negative short-term effects on glycaemia. It should be noted that our findings represent the acute postprandial changes after a single test meal in a group of healthy overweight/obese individuals. Whether there is tachyphylaxis or metabolic adaptation to duals. Whether there is tachyphylaxis or metabolic adaptation to single test meal in a group of healthy overweight/obese indivi-

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The authors’ contributions are as follows: R. A. was involved in the study conception, design, running of the laboratory work, statistical analysis and manuscript preparation; K. L. J. assisted with manuscript preparation; A. L. C. was involved in the study conception, design and manuscript preparation; and M. D. R. was involved in the study conception, design, statistical analysis and manuscript preparation.

References

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