# Intermittent versus continuous energy restriction: differential effects on postprandial glucose and lipid metabolism following matched weight-loss in overweight/obese subjects

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**ABSTRACT**

The intermittent energy restriction (IER) approach to weight-loss involves short periods of substantial (>70%) energy restriction interspersed with normal eating. Studies to date comparing IER to continuous energy restriction (CER) have predominantly measured fasting indices of cardiometabolic risk. This study aimed to compare the effects of IER and CER on postprandial glucose and lipid metabolism following matched weight-loss. 27 (13 male) overweight/obese participants (46±3y, 30.1±1.0kg/$m^2$) were randomised to either an IER (2638 kJ for two days/week with an overall ER of 22±0.3%, n=15) or CER (2510kJ below requirements with overall ER of 23±0.8%) intervention. Six-hour postprandial responses to a test meal and changes in anthropometry (fat mass, fat-free mass, circumferences) were assessed at baseline and upon attainment of 5% weight-loss, following a 7 day period of weight stabilisation. The study found no significant difference in the time to attain a 5% weight loss between groups (median 59 [41-70] days and 73 [48-128] days respectively, p=0.246), or in body composition (p≥0.430). For postprandial measures, neither diet significantly altered glycaemia (p=0.226), whereas insulinaemia was reduced comparatively (p=0.903). The reduction in c-peptide tended (p=0.057) to be greater following IER (309128±23268 to 247781±20709 pmol.360min.$L^{-1}$) versus CER (297204±25112 to 301655±32714 pmol.360min.$L^{-1}$). The relative reduction in triacylglycerol responses was greater (p=0.045) following IER (106±30 to 68±15 mmol.360min.$L^{-1}$) compared to CER (117±43 to 130±31 mmol.360min.$L^{-1}$). In conclusion, these preliminary findings highlight underlying differences between IER and CER, including a superiority of IER in reducing postprandial lipaemia, which now warrant targeted mechanistic evaluation within larger study cohorts.

## Introduction

The development of overweight/obesity is closely associated with numerous inter-related metabolic complications including insulin resistance and dyslipidaemia. These in turn increase an individual's risk of type 2 diabetes and cardiovascular disease (CVD), prevalence rates of which are rising in congruence with weight trends (1). Glucose and lipid homeostasis can be improved through weight-loss (2) which is most commonly advised via a modest (daily) continuous energy restriction (CER) (3). Intermittent energy restriction (IER) has received considerable recent interest as an alternative dietary strategy for weight-loss as IER entails intermittent periods of substantial energy restriction interspersed with periods of normal eating (4).

Previous studies comparing the effects of IER to CER on cardiometabolic risk factors have found them to have equivalent effects on most metabolic outcomes (5-7). There is some suggestion that IER (two consecutive days of 70% ER) may elicit greater benefits than CER on proxies of hepatic insulin sensitivity (5, 6), however, no study to date has controlled for the extent of weight-loss; a confounding factor from the perspective of metabolic comparisons. In addition, the majority of studies have conducted steady-state assessments, with only fasting blood measurements taken which is not truly representative as humans spend most of their day in a postprandial state; a dynamic, non-steady state condition. Furthermore, impairments in postprandial glucose and lipid handling are widely regarded as clinically significant cardiovascular disease risk factors (8, 9) and as such must also be considered within metabolic comparisons. One uncontrolled study by Heilbronn et al (10) demonstrated a decline in glucose tolerance after three weeks of IER (alternate days of total ER) among healthy and overweight women. However, baseline and post-treatment postprandial assessments were conducted following 12 hour and 36 hour fasting periods respectively. Prolonged (36 hour) fasting intervals are known to impair glucose tolerance (11), and as such, the observed decline in glucose tolerance may not reflect a true chronic treatment effect. In sum, there is very little known about the effects of IER on postprandial metabolism.

The present study, which was conducted as a randomised controlled dietary intervention in overweight/obese men and women, aimed to compare the effects of IER vs. CER on postprandial glucose and lipid responses to a liquid mixed test meal challenge following

matched 5% weight-loss. Changes in fasting cardiometabolic disease risk factors, resting energy expenditure (REE) and substrate oxidation were also assessed.

## Participants and methods

### Participants

Overweight and obese participants (BMI > 25 kg/m$^2$) aged 18-65 years were recruited to the study from Surrey (UK). All participants had an elevated waist circumference of >94 cm for men and >80 cm for women. Participants were weight-stable (±2 kg) over the preceding three months and had no significant medical history. To control for the potential influence of the menstrual cycle between visits, female participants were either post-menopausal (defined as absence of menses for ≥1 year) or taking oral contraceptives. The study obtained a favourable opinion from the University of Surrey ethics committee (UEC/2014/140/FHMS) and was conducted in accordance with the guidelines laid down in the Declaration of Helsinki. ISRCT registry number: ISRCTN13687043. The study ran between May 2015 and August 2016.

### Sample size considerations

On the basis of our previous acute observations (11), changes in postprandial lipaemia was selected the primary outcome, with the *a priori* hypothesis that the relative improvement in lipaemia would be greater following weight-loss via IER. As no comparable study has been performed, comparting the effects of IER vs. CER following matched weight-loss, prospective power calculations were not possible. To assess the possibility of type two error, retrospective power calculations were conducted for a secondary outcome measure, postprandial glucose. For the iAUC for plasma glucose, retrospective power calculations determined that at a two-sided 0.05 significance level, the study had 80% power to detect a mean difference of 120 mmol.360min.L$^{-1}$ between treatment groups (IER vs. CER), based on a pooled standard deviation of 105.0 mmol.360min.L-$^{1}$.

## Study design

The study was a randomised, parallel-armed, comparison between IER and CER. Participants were stratified by age (<42/≥42 years; mid-point of the recruitment range), BMI (<30/≥30kg/m$^2$), gender, ethnicity and Homeostasis model assessment–insulin resistance (HOMA-IR;<1/≥1) to ensure balanced group allocation, with matched pairs randomly assigned 1:1 to the interventions. The CER intervention served as the "standard treatment" control, compliant with UK National Institute of Clinical Excellence (NICE) obesity guidelines (3).

To control for the degree of weight-loss, study measurements were taken at baseline and after participants had attained a 5% weight-loss, a threshold adjudged to have a clinically significant impact on cardiometabolic risk factors (12).

**Dietary interventions**

Estimated energy requirements were calculated using the Henry predictive equation (13) for basal metabolic rate multiplied by an appropriate physical activity factor based on self-reported occupational and leisure activity levels (14). Healthy eating advice (compliant with UK guidelines) and individualised food portions lists were provided by an appropriately trained study investigator (RA). Participants were only informed of the comparison diet once they had completed the study.

*Intermittent energy restriction diet*

On two consecutive days of the week, participants consumed four commercially available LighterLife$^{TM}$ very-low energy formula-based Food Packs (2638kJ: 38%, 36% and 26% of total energy as carbohydrate, protein and fat) which delivered ~25% of their estimated euenergetic needs. Consecutive days were chosen to mirror that of previously published work by Harvie et al (5,6). On the remaining five days ("feed days"), participants' food intake was self-selected, but they were asked to consume an euenergetic healthy diet. Averaged overall prescribed ER was 22±0.3%.

*Continuous energy restriction diet*

Participants assigned to the CER diet were advised to consume a daily hypoenergetic diet of 2510kJ below their estimated energy requirements (3). All foods were self-selected by participants. Averaged overall prescribed ER was 23±0.8%, comparable to the IER intervention.

**Laboratory visits**

All participants initially undertook a one-week baseline period during which time they were required to record habitual dietary intakes. At the end of this baseline, participants attended the Surrey Clinical Research Centre (Guildford, UK) for initial measurements. Participants were instructed to abstain from alcohol and strenuous exercise for 48 hours before the visit, and were provided with a standardised pasta-based microwaveable meal (2377kJ, 75g

carbohydrate, 16g fat, 24g protein), which they consumed before 20:00 on the preceding evening as the macronutrient composition of an evening meal can affect metabolic responses on the following day (15). Participants arrived at the research unit following a 12-hour overnight, water only fast. Body weight and body composition (estimated by multi-frequency bioimpedance) were recorded using (Tanita BC420MA; Tanita Corp, Tokyo, Japan) alongside measures of waist and hip circumference. After a period of rest, blood pressure measurements were taken in duplicate (UA-767; AND, San Jose, USA) and the mean recorded. Following this, fasted resting measurements of energy expenditure and substrate utilisation were taken via indirect calorimetry. An indwelling cannula was then inserted following which the first (fasted) sample was taken. A liquid mixed test meal was provided (400ml Fortisip, Nutricia, Trowbridge, UK: 2510kJ, 74g carbohydrate [49% of total energy], 24g protein [16%] and 23g fat [35%]) which participants consumed within 5 minutes. This homogenous liquid meal was used for the purpose of standardisation, to minimise potential variance in postprandial response associated with factors such as cooking/food preparation and chewing rate. In addition, its composition is reflective of the macronutrient proportions of typical western dietary intakes. Serial blood samples were taken at regular intervals over the next 360 minutes (from the first mouthful) to assess postprandial changes in glucose, insulin, C-peptide, triacylglycerol (TAG), non-esterified fatty acids (NEFA) and 3-hydroxybutyrate (3-OHB). After the initial visit, both groups commenced their respective diets whilst maintaining habitual activity patterns. Participants returned to the research centre for repeated measurements once the 5% target was achieved. They consumed the same standardised evening meal and were given identical pre-visit instructions with regards to alcohol and exercise. Participants in both groups abstained from any form of ER for ≥7 days prior to the repeat study visit to mitigate the effects of acute ER on the metabolic outcomes. Participants did not complete diet diaries during this period however intake and weight were regularly reviewed during this period to ensure adherence.

**Monitoring and compliance**

Participants received fortnightly motivational contact from the study investigators via phone, email and/or texts in addition to monthly face-to-face clinic appointments, where weight was recorded. Every two weeks, participants were sent online questionnaires which asked them to self-report their morning fasted weight and, for IER participants, ER-day intakes with a compliant ER day defined as energy intake ≤3347kJ which corresponds to the very-low energy diet threshold defined by NICE (3). The frequency of weight monitoring increased as

participants approached their 5% target. All participants also completed seven-day diet diaries and self-reported physical activity levels mid-way (~ 2.5% weight-loss) and as they were approaching their 5% weight-loss target

**Blood biochemistry**

Blood samples were collected into potassium EDTA (for plasma lipid and insulin analysis) and sodium oxalate (for plasma glucose analysis). For the measurement of plasma C-peptide, blood was collected into EDTA containing 200 kallikrein inhibiting units of aprotinin per ml of whole blood. Samples were centrifuged for 15 minutes at 2500 rpm and separated; aliquots were then stored at -20°C or -80°C (for 3-OHB analysis). Plasma insulin was measured using radioimmunoassay (Millipore, Billerica, USA; intra/inter-assay CVs 8% and 4%); C-peptide by radioimmunoassay (Millipore; intra/inter-assay CVs 6% and 8%); glucose, TAG and NEFA using the ILAB 650 photometric auto-analyser (Instrumentation Laboratory, Warrington, UK; intra/inter-assay CV all <6 % and <6%); and 3-OHB using the Cobas MIRA photometric auto-analyser (Roche, Welwyn Garden City, UK; intra/inter-assay CVs <5% and <6%). All samples from an individual participant were included in the same assay.

**Indirect calorimetry**

REE and substrate utilisation were calculated using data obtained from a Gaseous Exchange Monitor ISGEM319 (GEM Nutrition, Cheshire, UK), an open-circuit indirect calorimeter based on the ventilated flow-through technique. Following a 30-minute period of rest, measurements were taken over 20 minutes and in accordance with methodological recommendations by Compher et al (16). REE was calculated utilising the modified Weir equation (17) and substrate utilisation implied from the respiratory quotient (RQ, $VC0_2/VO_2$). To permit comparisons between individuals of varying body masses, REE was also normalised for estimated metabolically active mass (REE/fat free mass+18kg; (18)).

**Dietary analyses**

All dietary analyses were carried out in Diet Plan Seven (Forestfield Software, Horsham, UK) using the McCance and Widdowson's composition of foods integrated dataset. Participants recorded food intake in validated diet diaries (19). Seven-day intakes were then averaged. Data for participants who did not complete a baseline seven-day food and/or at least one of their two diaries whilst dieting omitted completely from analyses.

Data manipulation and statistical analyses

Area under the curve (AUC, for NEFA and 3-OHB) and incremental AUC (iAUC, for all other metabolites) were calculated using the linear trapezoid method, subtracting the area below baseline for iAUC**.** Low density lipoprotein (LDL)-cholesterol was calculated using the Friedewald equation (20). HOMA-IR and %B were calculated using the HOMA2 online calculator (https://www.dtu.ox.ac.uk/homacalculator/) as proxies for insulin sensitivity and β-cell function respectively.

Data were statistically analysed using SPSS v23 (IBM, Chicago, USA). Data were first checked for normality using the Shapiro-Wilks test, with non-normally distributed data normalised via log transformation where possible to permit parametric testing. The primary analysis was a one-factor analysis of covariance (ANCOVA) between the dietary intervention groups with post-treatment values as the dependent variable, and baseline values of each parameter as the covariate. This is recommended statistical method (in terms of bias, precision and power) for the analysis of continuous outcomes in randomised studies with a single post-treatment measurement previously measured at baseline (21). Between-group factors which could have influenced study outcomes (age, gender, body fat and metabolic syndrome status) were entered systematically into the outcome models, but none were found to be statistically significant. To then enter all of them at once into the models would have invited spurious results and thus these factors were not included as covariates in the final models. The Mann Whitney U test was used as the non-parametric alternative to ANCOVA. Differences between intervention groups at baseline were assessed using independent t-tests for continuous variables or the Chi squared test for categorical variables. No significant baseline differences were found unless otherwise stated. A paired t-test (or non-parametric Wilcoxon signed-rank test) was used to assess the change between baseline and post intervention values within each dietary intervention group. Correlations between changes in metabolic and dietary intake variables were explored using Pearsons (parametric) or Spearmans (non-parametric) tests as appropriate. Statistical significance was accepted at $p<0.05$, and a statistical trend at $p=0.05$-1.0. Summary measures are presented as mean±SEM (for parametric data) or median and interquartile range (IQR, for non-parametric data).

## Results

### Participant baseline characteristics

Seven participants allocated to the CER intervention did not start the study. Of the 41 participants (IER=24, CER=17) who started the study, 27 (IER=15, CER=12) attained their 5% weight-loss target. The consort diagram is presented in **Figure 1**.

Baseline characteristics of the 27 study completers are presented in **Table 1**. The groups were matched for age, BMI, adiposity, gender, metabolic syndrome classification and were primarily Caucasian. Twelve participants withdrew from the study due to scheduling conflicts (IER=1), bereavement (IER=1), dental problems (IER=1), problems tolerating (IER=4) or adhering to (CER=2) their diet, or were lost to follow up (IER=2, CER=1). Two CER participants were unable to attain a 5% weight-loss within the maximum timeframe (nine months) and so were withdrawn from the study. Non-completers were significantly younger than completers (27±3 vs. 45±3 years; $p<0.001$, independent t-test), no other significant differences were noted.

### Changes in body composition and circumferences

Mean percentage weight-loss was 5.3±0.3% in the IER group and 5.0±0.3% in the CER group ($p=0.446$, ANCOVA). The accompanying changes in body composition were also comparable between the groups ($p\geq0.430$, ANCOVA) and are reported in **Table 2**. It took IER participants a median of 59 days (IQR: 41, 80) to attain their 5% weight-loss target and CER participants 73 days (IQR: 48, 128), which was not statistically different between groups ($p=0.246$, Mann Whitney U test).

### Dietary intakes and physical activity

Changes in dietary intake are reported in **Table 3**. By the end of the intervention the reductions in energy intake were significantly greater in the IER group (mean difference: 1081 kJ [95% confidence intervals: -1900, -263 kJ]; $p=0.012$ $d=1.21$, ANCOVA), with a similar tendency noted for total carbohydrate intake (mean difference: -28g [-57, 1 g]; $p=0.054$ $d=0.90$, ANCOVA). Adherence to the IER protocol (i.e. two substantial ER days/week) was high (93±4%), and were most frequently completed on consecutive days (86±7%). Physical activity levels remained stable in both groups across the study.

Fasting biochemistry and physiological markers

Changes in fasting biochemistry and physiological markers are reported in **Table 4**.

There were no significant between-group differences for changes in all fasting biochemical measures (all $p \geq 0.147$, ANCOVA). Within the IER group, there was a small increase in fasting glucose ($p=0.008$, paired t-test) post weight-loss, whilst a trend in favour of reduced plasma NEFA was also found ($p=0.056$, paired t-test).

The IER group exhibited a significantly greater reduction in systolic blood pressure (mean difference [95% confidence intervals]: -6 mmHg [-11, -1 mmHg]; $p=0.020$ $d=1.17$, ANCOVA), whereas the decreases in diastolic blood pressure were comparable between groups ($p=0.691$, ANCOVA). A positive relationship between the changes in energy intake and systolic blood pressure was found ($r=0.461$, $p=0.047$).

There were no significant differences between-groups for changes in REE ($p=0.205$, ANCOVA), although a trend in favour of a reduction was observed following IER ($p=0.058$, paired t-test). Similar within-group trends were noted when REE was normalised for metabolically active mass, whereas the between-group differences were strengthened (mean difference [95% confidence intervals]: -7.28 kJ/kg MAM/day [-15.07, 0.510 kJ/kg MAM/day; $p=0.067$ $d=0.97$, ANCOVA).

The relative change in RQ was not significantly different between the two diets ($p=0.148$, Mann Whitney U test) although a significant within-group decline in fasting RQ was noted in the IER group ($p=0.045$, Wilcoxon signed ranks test).

Postprandial lipid metabolism

Postprandial lipid parameters before and after the dietary interventions are presented in **Figure 2,** and as averaged hourly iAUC in **Supplementary Figure 1**. The relative reduction in postprandial TAG was significantly greater following IER vs. CER ($p=0.045$ $d=0.83$, ANCOVA). The log transformed mean difference between groups was -0.112 $mmol.360min.L^{-1}$ [-0.221, -0.003 $mmol.360min.L^{-1}$]. A trend in favour of a positive relationship between decreases in incremental TAG and RQ was found ($r=0.34$, $p=0.06$). For postprandial NEFA, there were no significant between-group differences ($p=0.410$, Mann-Whitney U test), although, a tendency for reduced NEFA AUC was observed within the CER

group (p=0.059, Wilcoxon signed ranks test). No significant within-group changes (p=0.618, ANCOVA) or between-group differences (p≥0.248, paired t-tests) in postprandial 3-OHB responses were found.

Postprandial glucose metabolism

Postprandial glycaemic indices before and after the dietary interventions are presented in **Figure 3.** For postprandial glucose responses, no significant between-group differences (p=0.226, ANCOVA) or within-group changes were observed. Postprandial insulinaemia was reduced comparatively in both groups (p=0.903, ANCOVA). On the other hand, postprandial c-peptide was reduced following IER but not CER (p=0.057 trend *d*=0.81, ANCOVA), with a mean difference (95% confidence intervals) between groups of -61769 pmol.360min.$L^{-1}$ [-127496, 3957 pmol.360min.$L^{-1}$].

## Discussion

Findings from the present study highlight underlying differences between IER and CER with respect to their effects on postprandial glucose and lipid metabolism following matched 5% weight-loss. These data are novel and as such, there are no directly comparative data in the literature.

In our previous work we have reported that acutely, one day of substantial 75% ER reduced incremental TAG responses by ~60% (11). Chronically, the present study found a ~40% reduction in incremental responses following 5% weight-loss achieved through IER. This finding has the potential to be of clinical importance based on evidence from large prospective cohort studies highlights an independent link between elevated postprandial TAG and CVD risk (22-24). Moreover, postprandial TAG responses has also been shown to predict the presence of coronary artery disease with one study in adult males finding that the magnitude of lipaemia was ~41% greater among cases versus controls (25), and has been positively correlated with markers of atherosclerotic progression (26). The mechanisms underlying these associations include the direct interaction between TAG-rich lipoprotein (TRL) remnants and the arterial wall, as well as indirect mechanisms, such as alterations in LDL particle size (27). There is

Postprandial assessments were limited to measuring changes in absolute substrate concentrations after a single meal, and which represent the balance but not the rate (or source) of TRL appearance or clearance, and as such their relative contributions cannot be ascertained. There were no significant differences between the dietary groups in changes in postprandial hepatic fatty acid partitioning (3-OHB) or NEFA which might have otherwise explained these findings. Reductions in waist circumference were also comparable between groups, but this cannot differentiate between changes in intra-hepatocellular or visceral stores which can augment postprandial lipaemia by driving increased very low density lipoprotein–TAG production (28, 29). Interestingly, a within-group increase in whole-body fat oxidation was observed following IER but not CER in the present study, although not statistically different between groups. It is perhaps not unreasonable to speculate that the repeated substantive periods of ER experienced during IER may have upregulated pathways associated with fatty acid metabolism and uptake in skeletal muscle and/or adipose tissues, manifesting as changes in basal substrate oxidation and postprandial lipaemia. These preliminary results

justify more detailed investigations into the kinetics of TAG metabolism, using targeted methodology.

Although insulin responses to the meal challenge were reduced comparatively following weight-loss via both IER and CER, however, using concurrent measurements of both insulin and C-peptide, the study does propose differences between the two weight-loss diets in terms of underlying mechanism. C-peptide undergoes negligible extraction by the liver and constant peripheral clearance, thus making it a more direct marker of insulin secretion than circulating insulin (30). Following CER, insulinaemia was reduced whereas postprandial C-peptide was unaltered, which suggest an increase in hepatic insulin clearance. By contrast, postprandial C-peptide responses following IER may reveal reduced insulin secretion over the first two hours of the six-hour postprandial period. Although this did not ultimately result in a significant alteration in glucose tolerance, the underlying mechanism and biological significance merits further evaluation.

At baseline, approximately half of IER participants were either pre-hypertensive (120-139/80-89 mmHg) or hypertensive (>140–159/90–99 mmHg). Following weight-loss, all but one IER participants became normotensive (<120/80 mmHg). In contrast, the proportion of participants who were pre- or hypertensive (~30%) did not change significantly following the CER diet. The shift observed in the IER group was largely driven by a reduction in systolic blood pressure, which was not significantly altered by CER. A positive relationship was found between the magnitude of the reduction in systolic blood pressure and the degree of ER, which as discussed in the next paragraph was greater in the IER group. It should be noted that the numerical trends in favour of higher baseline systolic blood pressures within the IER group would have been adjusted for by the ANCOVA statistical method. To date, previously published comparison studies have found no significant differences between the two diets (5-7); thus, these findings are unexpected and necessitate replication before any conclusions can be drawn and to exclude the possibility of type one error.

The time taken to achieve 5% weight-loss was not statistically different between groups, although, the IER group reported greater relative reductions in energy (~1081 kJ/day) driven by under-consumption on “feed” days (where an euenergetic diet was prescribed), which is in accordance with previous research (5-7). Numerically, IER participants attained their weight-loss target sooner (median 59 vs 73 days). Although type two error cannot be disregarded, on the alternate side of the energy balance equation, absolute REE was reduced by ~7% (~477

kJ) following IER, but not CER which may have contributed to these discrepancies between the dietary intake data and weight-loss trajectories. Food dietary records are susceptible to under-reporting (31), but this would have affected the validity of dietary records of both groups. These data may also be indicative of subtle alterations in physical activity thermogenesis which could not be captured by the factorial approach implemented by the study. Changes in body composition were comparable between groups, however, when normalised for metabolically active mass, the between-group differences in REE became more pronounced. In the context of the existing literature, our data contrast with Cattenaci's recent study (32) which found that weight-loss via IER (alternating days of total ER and *ad libitum* intake) mitigated the adaptive physiological reductions in REE that occur during weight-loss. However, the varying dietary protocols do not permit direct comparisons between studies, with one important distinction here being that participants under-consumed on "feed" days so most probably rarely attained energy balance.

There were some important caveats with IER, in that a higher attrition rate was reported among participants who started the intervention. Overall dropout rates were 34% in the study cohort as a whole, which exceeds that of previous studies utilising analogous 2-ER days per week protocols where rates have ranged from 21-23% (5, 6). This discrepancy can largely be attributed to the study design, whereby participants were assigned to the diet until a weight-loss target was achieved rather than fixed duration of time. More recently, a study by Trepanowski et al (7) of alternate day ER also reported a higher attrition rate among IER participants of 38% vs. 29% among CER participants. Put together, data from ours and Trepanowski's study do not support the popular notion that IER could prove "easier" to follow than CER, warranting further investigation of the factors that can influence the acceptability of IER amongst the public. Among the 24 participants assigned to the CER intervention, only 17 started with the majority (71%) declining to participate or contact was lost. Participants were blinded to the comparison diet which suggests that there was no bias to the IER diet *per se*, but, the perceived lack of novelty may have contributed to the drop outs in the CER group prior to commencing the diet.

The main strengths of the study were that weight-loss as an independent metabolic confounder, was controlled for, and the study conducted dynamic, concurrent, assessments of postprandial glucose and lipid metabolism in addition to static, steady-state measurements.

Limitations include the small sample of both overweight and obese participants which can increase the risk of type one and two error, use of bioimpedance, and that postprandial assessments were only conducted following a single meal. Correlation analyses found no relationship between the degree of ER to the degree of change in most outcome measures (with the exception of systolic blood pressure). It should be noted that the absence of a statistical relationship does not rule out the absence of a potential effect influenced by the greater overall ER during IER to study findings. Lastly, physical activity levels were only assessed via the factorial method, which is insensitive to small changes in activity and is unable to differentiate between the various components of energy expenditure.

In summary, our preliminary data suggests that mode of ER (intermittent but severe vs. modest continuous) may have different cardiometabolic effects in which may be important to long-term disease risk. Differences were observed between the diets, particularly with regards to postprandial lipaemia which was reduced to a greater extent following IER. In addition, these data also reveal distinctions between IER and CER with regards to their effects on insulin secretion dynamics, REE and blood pressure. These data now warrant further investigation utilising targeted methodology, and within distinct population groups such as individuals with morbid obesity and established metabolic disorders. Future studies should implement rigorous controls over energy intake and expenditure to minimise the influence that variances in these factors might have on study outcomes.

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**Conflict of Interest**

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

**Authors Contributions**: Designed the research (RA, ALC, MDR); Conducted research as study dietitian (RA); Provided essential materials (KLJ); Analyzed the data (RA); Wrote the paper (RA, KLJ, ALC, MDR); Primary responsibility for final content (MDR)

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| | IER (n=15) | CER (n=12) | IER vs CER |
|---|---|---|---|
| **Age** (years) | 42 sem 4 | 48 sem 3 | 0.289 |
| **Gender** (M/F) | 7 / 8 | 6 / 6 | 0.863 |
| **Ethnicity** | | | 0.255 |
| *Caucasian* | 15 | 11 | |
| *Black African* | 0 | 1 | |
| **BMI** ($kg/m^2$) | 29.8 sem 0.9 | 30.8 sem 1.1 | 0.482 |
| *Overweight/obese* | 9/6 | 6/6 | 0.707 |
| **Body fat** (%)[1] | 34.8 sem 2.2 | 37.5 sem 2.0 | 0.385 |
| **Metabolic Syndrome**[2] | 4 / 15 | 2 / 12 | 0.535 |

**Table 1 Baseline characteristics for study completers**

[1] Bioimpedance. [2] International Diabetes Federation criteria.

*Statistics and data presentation:* Between group comparisons conducted using unpaired t-test or Chi squared (for ethnicity, metabolic syndrome). Presented as mean ± SEM.

*Abbreviations*: CER – Continuous energy restriction; IER – Intermittent energy restriction; NS – Non-significant.

564

| | IER (n=15) | | | | CER (n=12) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Baseline** | | **Post** | | **Baseline** | | **Post** | | **IER vs CER** |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | |
| **Weight** (kg) | 88.8 | 3.4 | 84.1 | 3.2[a] | 89.3 | 4.5 | 84.9 | 4.3[a] | 0.430 |
| **Fat mass** (kg) | 30.8 | 2.3 | 27.1 | 2.3[a] | 33.8 | 2.9 | 30.0 | 2.7[a] | 0.821 |
| **Fat free mass** (kg) | 58.0 | 3.1 | 57.0 | 3.0[a] | 55.5 | 2.6 | 54.8 | 2.8[a] | 0.437 |
| **Waist** (cm) | 102 | 3.0 | 98 | 2.0[a] | 102 | 2.0 | 97 | 2.0[a] | 0.489 |
| **Hip** (cm) | 113 | 2.0 | 109 | 2.0[a] | 115 | 3.0 | 110 | 2.0[a] | 0.876 |

565

566 **Table 2 Body composition before and after 5% weight-loss via IER and CER**

567 *Abbreviations*: IER, Intermittent energy restriction; CER, Continuous energy restriction; NS,

568 Non-significant.

569 *Statistics and data presentation:* Between group comparisons conducted using analysis of

570 covariance. [a] Significant within-group change ($p<0.05$, paired t-test). Presented as mean ±

571 SEM.

572

| | IER | | | | | | CER | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Baseline (n=12)** | | **Midway (n=8)** | | **End (n=12)** | | **Baseline (n=11)** | | **Midway (n=6)** | | **End (n=11)** | | **IER vs CER** |
| | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | Mean | SEM | |
| **Energy intake** (kJ/day) | 8057 | 432 | 5771 | 358[a] | 5199 | 319[a] | 8437 | 594 | 6423 | 421[a] | 6396 | 300[a] | **0.012** |
| *Feed days only* | - | - | *7077* | *479* | *6236* | *447*[a] | - | - | - | - | - | | |
| **Carbohydrate** (g/day) | 224 | 20 | 147 | 10[a] | 141 | 9[a] | 258 | 38 | 177 | 16[(a)] | 175 | 12[a] | *0.054* |
| *Feed days only* | - | - | *183* | 13 | *170* | *14*[a] | - | - | - | - | - | - | - |
| **Fibre** (g/day) | 19 | 1 | 18 | 1 | 19 | 2 | 19 | 1 | 18 | 2 | 18 | 1 | 0.590 |
| *Feed days only* | - | - | *19* | 2 | *18* | *1* | - | - | - | - | - | - | - |
| **Sugars** (g/day) | 84 | 14 | 48 | 8[a] | 52 | 8[a] | 91 | 12 | 61 | 10[(a)] | 66 | 8[a] | 0.259 |
| *Feed days only* | - | - | *62* | *11* | *62* | *11*[(a)] | - | - | - | - | - | - | - |
| **Fat** (g/day) | 74 | 5 | 52 | 5[a] | 45 | 15[a] | 82 | 6 | 54 | 2[a(b)] | 57 | 4[a(b)] | 0.110 |
| *Feed days only* | - | - | *66* | *9*[a] | *56* | *6*[a] | - | - | - | - | - | - | - |
| **Saturated fat** (g/day) | 29 | 3 | 19 | 2[a] | 16 | 2[a] | 30 | 3 | 17 | 1[a(b)] | 19 | 1[a(b)] | 0.206 |
| *Feed days only* | - | - | *25* | *4* | *20* | *3*[(a)] | - | - | - | | - | - | - |
| **Protein** (g/day) | 83 | 5 | 73 | 3[a] | 67 | 5[a] | 80 | 5 | 80 | 7 | 69 | 7[(a)] | 0.523 |
| *Feed days only* | - | - | *78* | *4*[a] | *63* | *7*[a] | - | - | - | - | - | - | - |
| **Alcohol** (g/day) | 10 | 3 | 8 | 4[a] | 11 | 5[a] | 11 | 3 | 8 | 2 | 7 | 2[a] | 0.659 |
| **Salt** (g/day) | 5.4 | 0.6 | 4.9 | 0.5 | 4.0 | 1.3 | 5.5 | 0.5 | 5.5 | 1.0 | 3.8 | 0.2[a] | 0.544 |
| *Feed days only* | - | - | *4.4* | *0.5*[a] | *3.6* | *0.3*[a] | - | - | - | - | - | - | - |
| **Carbohydrate** (% total energy) | 43 | 2 | 40 | 2 | 42 | 2 | 42 | 2 | 43 | 1 | 42 | 2 | 0.830 |
| *Feed days only* | - | - | *40* | *2* | *43* | *2* | - | - | - | - | - | - | - |
| **Fat** (% total energy) | 34 | 1 | 33 | 2 | 32 | 1 | 36 | 1 | 32 | 2[a] | 33 | 2 | 0.872 |
| *Feed days only* | - | - | *35* | *3* | *33* | *2* | - | 1 | - | - | - | - | - |
| **Protein** (% total energy) | 18 | 1 | 21 | 2[(a)] | 22 | 1[a] | 16 | 1 | 20 | 1 | 19 | 1[(a)] | 0.302 |
| *Feed days only* | - | - | *19* | *2* | *19* | *2* | - | - | - | - | - | - | - |
| **Alcohol** (% total energy) | 4 | 1 | 4 | 1 | 2 | 1 | 4 | 1 | 3 | 1 | 3 | 1 | 0.326 |
| **Physical activity levels** | 1.56 | 0.02 | 1.57 | 0.01 | 1.57 | 0.01 | 1.54 | 0.01 | 1.54 | 0.01 | 1.54 | 0.01 | 0.885 |

**Table 3 Dietary intakes and physical activity levels at baseline, midway through (2.5% weight-loss) and at the end (nearing 5% weight loss) of the IER and CER dietary interventions.**

*Abbreviations*: IER, Intermittent energy restriction; CER, Continuous energy restriction; NS, Not significant.

*Statistics and data presentation:* Between group comparisons conducted using analysis of covariance. **[a-b]** Significant within-group change: [a] vs baseline or [b] between mid-way vs end time-points ($p<0.05$, paired t-test). **[(a-b)]** Within-group trend ($p=0.05$-$0.1$). Presented as mean ± SEM of seven days or five feed days.

| | IER (n=14) | | | | CER (n=12) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Baseline | | Post | | Baseline | | Post | | |
| | Mean/ median | SEM/ [IQR] | Mean/ median | SEM/ [IQR] | Mean/ median | SEM/ [IQR] | Mean/ median | SEM/ [IQR] | IER vs CER |
| **Glucose (**mmol/L) | 4.4 | 0.1 | 4.6 | 0.1[a] | 4.4 | 0.2 | 4.4 | 0.2 | 0.158 |
| **Insulin (**pmol/l) | 78 | 8 | 71 | 5 | 64 | 8 | 59 | 7 | 0.324 |
| **C-Peptide (**pmol/l) | 527 | 36 | 504 | 38 | 504 | 45 | 475 | 35 | 0.818 |
| **HOMA-IR** | 1.6 | 0.2 | 1.5 | 0.1 | 1.3 | 0.1 | 1.2 | 0.1 | 0.282 |
| **HOMA-%B** | 139 | 10 | 126 | 8 | 138 | 12 | 130 | 8 | 0.676 |
| **TOTC (**mmol/L) | 4.2 | 0.3 | 4.0 | 0.2 | 4.2 | 0.3 | 4.0 | 0.3 | 0.948 |
| **LDL (**mmol/L) | 2.6 | 0.3 | 2.4 | 0.2 | 2.7 | 0.2 | 2.6 | 0.2 | 0.837 |
| **HDL (**mmol/L) | 1.1 | 0.1 | 1.1 | 0.1 | 1.0 | 0.1 | 1.0 | 0.1 | 0.723 |
| **TAG (**mmol/L) | 1.1 | 0.1 | 1.0 | 0.1 | 0.9 | 0.1 | 0.8 | 0.1 | 0.147 |
| **NEFA (**µmol/L)[*] | 637 | 63 | 491 | 50[(a)] | 517 | 56 | 536 | 70 | 0.454 |
| **3-OHB (**µmol/L)[*] | 144 | 37 | 112 | 33 | 84 | 19 | 115 | 33 | 0.351 |
| **Systolic BP (**mm/Hg) | 123 | 3 | 111 | 3[a] | 115 | 3 | 113 | 3 | **0.020** |
| **Diastolic BP (**mm/Hg) | 74 | 3 | 69 | 3[(a)] | 75 | 3 | 70 | 4[a] | 0.691 |
| **REE** (kJ/day) [†] | 6617 | 257 | 6139 | 256[(a)] | 6190 | 309 | 6259 | 332 | 0.205 |
| **REE** (kJ/kg MAM/day)[†] | 87 | 4 | 81 | 2[(a)] | 83 | 2 | 85 | 2 | *0.067* |
| **RQ (**$VC0_2/VO_2$)[†] | 0.86 | [0.84,0.88] | 0.83 | [0.77,0.89][a] | 0.87 | [0.84,0.9] | 0.86 | [0.83,0.90] | 0.148 |

**Table 4 Fasting biochemistry and physiological markers before and after 5% weight-loss via IER and CER**

*Abbreviations*: IER, Intermittent energy restriction; CER, Continuous energy restriction; NS, Not significant; HOMA, Homeostasis model assessment; IR, Insulin resistance; TOTC, Total cholesterol; LDL, Low density lipoprotein; HDL, High density lipoprotein; TAG, Triacylglycerol; NEFA, Non-esterified fatty acids; 3-OHB, 3-hydroxybutyrate; BP, blood pressure; REE, Resting energy expenditure; MAM, Metabolically active mass; RQ, Respiratory quotient.

*Statistics and data presentation:* Between group comparisons conducted using analysis of covariance (parametric) or Mann Whitney U test (non-parametric). [a] Significant within-group change ($p<0.05$, paired t-test or Wilcoxon signed ranks). [(a)] Within-group trend ($p=0.05$-$0.1$). Presented as mean ± SEM or as median [interquartile range, IQR]. [*]n=24 (IER=13, CER=11. [†] n=23 (IER=13, CER=10).

**Figure 1**

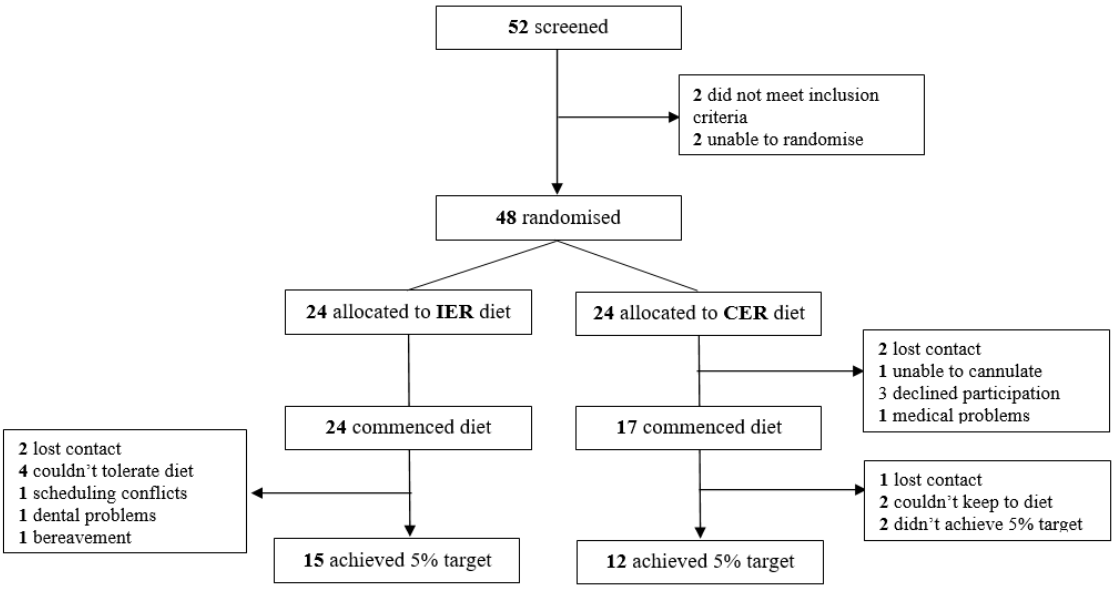

52 screened
2 did not meet inclusion criteria
2 unable to randomise
48 randomised
24 allocated to IER diet
24 allocated to CER diet
2 lost contact
1 unable to cannulate
3 declined participation
1 medical problems
24 commenced diet
17 commenced diet
2 lost contact
4 couldn't tolerate diet
1 scheduling conflicts
1 dental problems
1 bereavement
1 lost contact
2 couldn't keep to diet
2 didn't achieve 5% target
15 achieved 5% target
12 achieved 5% target

**Fig 2**

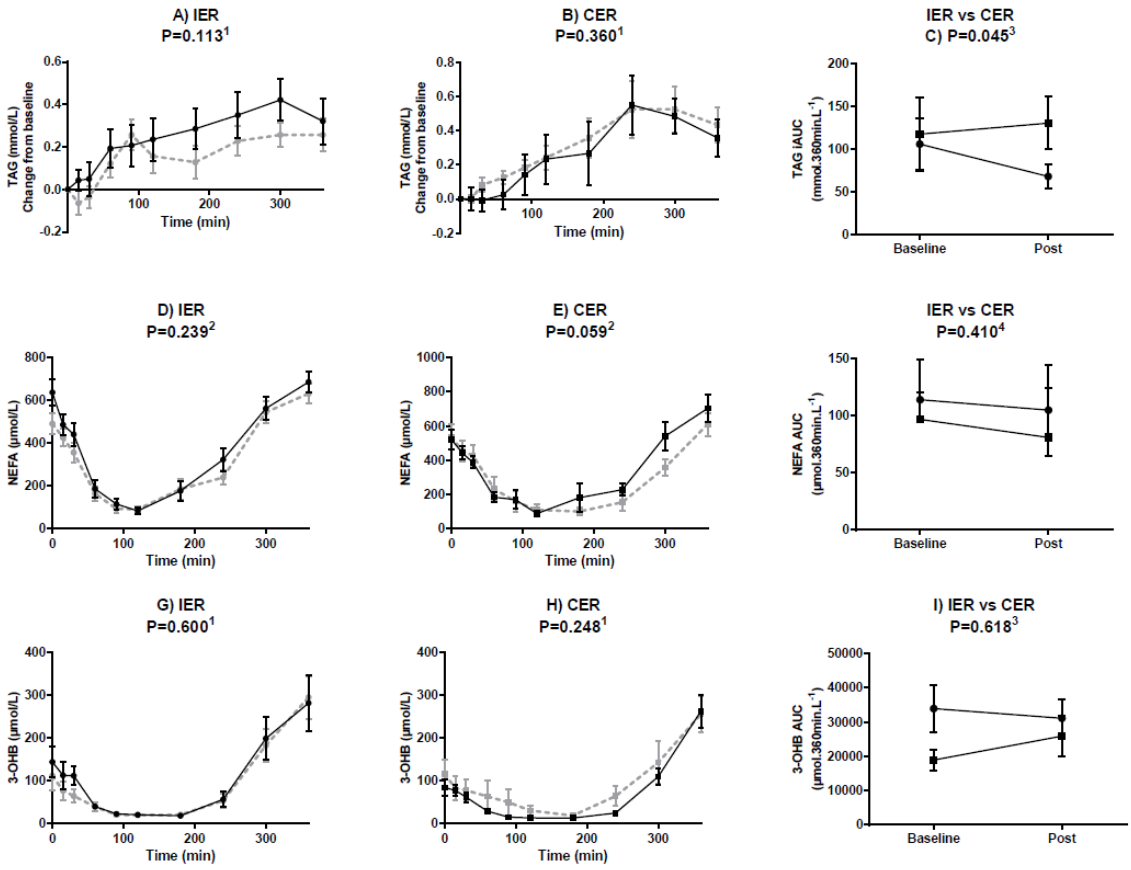
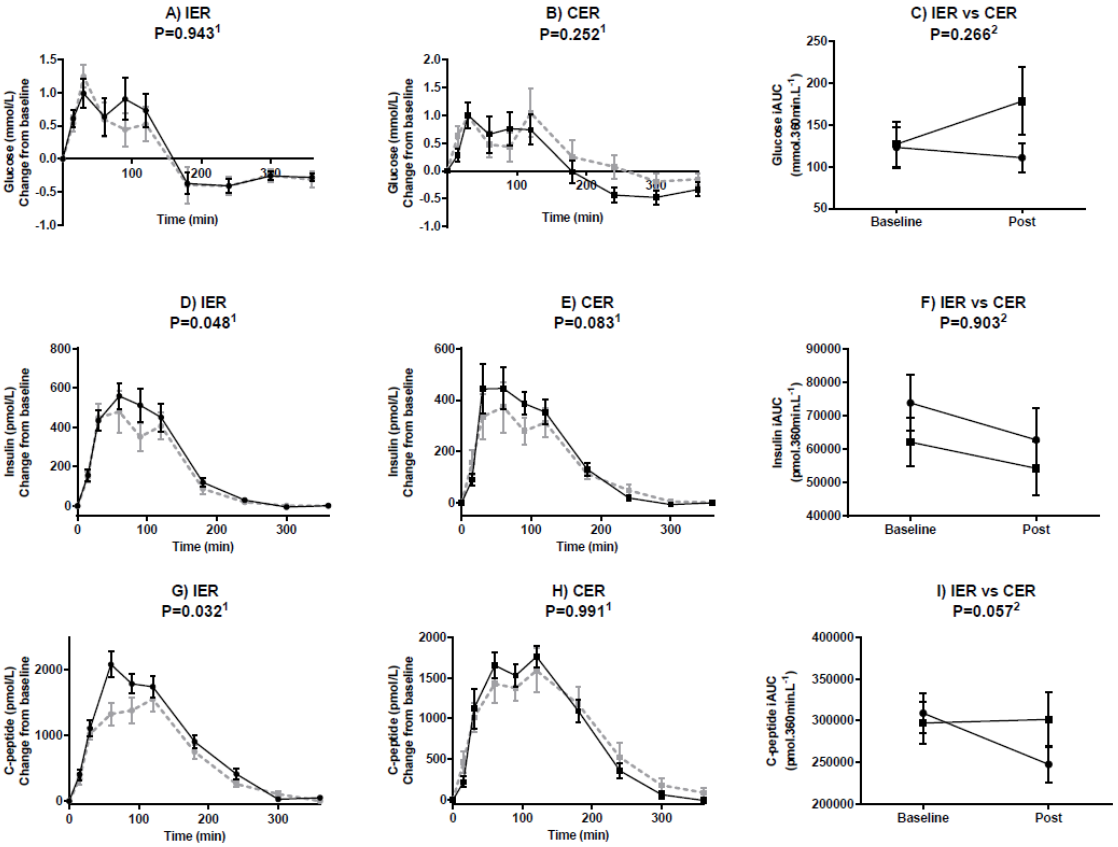

A) IER
P=0.113[1]
TAG (mmol/L)
Change from baseline
Time (min)
B) CER
P=0.360[1]
TAG (mmol/L)
Change from baseline
Time (min)
IER vs CER
C) P=0.045[3]
TAG iAUC
(mmol.360min.L-1)
Baseline
Post
D) IER
P=0.239[2]
NEFA (µmol/L)
Time (min)
E) CER
P=0.059[2]
NEFA (µmol/L)
Time (min)
IER vs CER
P=0.410[4]
NEFA AUC
(µmol.360min.L-1)
Baseline
Post
G) IER
P=0.600[1]
3-OHB (µmol/L)
Time (min)
H) CER
P=0.248[1]
3-OHB (µmol/L)
Time (min)
I) IER vs CER
P=0.618[3]
3-OHB AUC
(µmol.360min.L-1)
Baseline
Post

**Fig 3**


A) IER
P=0.943[1]
Glucose (mmol/L) Change from baseline
Time (min)
B) CER
P=0.252[1]
Glucose (mmol/L) Change from baseline
Time (min)
C) IER vs CER
P=0.266[2]
Glucose iAUC (mmol.360min.L-1)
Baseline
Post
D) IER
P=0.048[1]
Insulin (pmol/L) Change from baseline
Time (min)
E) CER
P=0.083[1]
Insulin (pmol/L) Change from baseline
Time (min)
F) IER vs CER
P=0.903[2]
Insulin iAUC (pmol.360min.L-1)
Baseline
Post
G) IER
P=0.032[1]
C-peptide (pmol/L) Change from baseline
Time (min)
H) CER
P=0.991[1]
C-peptide (pmol/L) Change from baseline
Time (min)
I) IER vs CER
P=0.057[2]
C-peptide iAUC (pmol.360min.L-1)
Baseline
Post

**Legends for figures**

**Figure 1: Consort diagram.** NB: Matched pairs could not be found for two participants to ensure balanced group allocation and so these individuals were not randomised to an intervention.

**Figure 2 A-I Postprandial lipid indices before and after 5% weight-loss via IER and CER**

IER (filled circles), CER (filled squares). For postprandial graphs: Baseline (black) and post-treatment (grey). Liquid test meal provided: 2510kJ, 74g carbohydrate, 24g protein and 23g fat.

*Abbreviations:* AUC, Area under the curve; CER, Continuous energy restriction; iAUC, Incremental area under curve; IER, Intermittent energy restriction; NEFA, non-esterified fatty acids; 3-OHB, 3-hydroxybutyrate.

*Statistics and data presentation:* [1]Paired t-tests or [2]Wilcoxon signed ranks test. [3]Analysis of covariance or [4]Mann Whitney U test. Figure 2F presented as median (interquartile range), all other data as mean ± SEM. TAG: n=26 (IER=14, CER=12). NEFA and 3-OHB: n=24 (IER=13, CER=11).

**Figure 3 A-I) Postprandial glycaemic indices before and after 5% weight-loss via IER and CER**

IER (filled circles), CER (filled squares). For postprandial graphs: Baseline (black) and post-treatment (grey). Liquid test meal provided: 2510kJ, 74g carbohydrate, 24g protein and 23g fat.

*Abbreviations:* AUC, Area under the curve; CER, Continuous energy restriction; IER, Intermittent energy restriction; iAUC, Incremental area under curve.

*Statistics and data presentation:* [1]Paired t-tests. [2]Analysis of covariance. Presented as mean ± SEM. n=26 (IER=14, CER=12).