Impact of isoenergetic intake of irregular meal patterns on thermogenesis, glucose metabolism and appetite: a randomized controlled trial

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Names for PubMed indexing: Alhussain, Macdonald, Taylor

Sources of support

Researchers Supporting Project number (RSP-2021/338), King Saud University, Riyadh, Saudi Arabia and the University of Nottingham, Nottingham, UK

Short running head

Meal pattern; thermogenesis and insulin resistance

Abbreviation used

BMI, body mass index; CGM, continuous interstitial glucose monitor; COMA, Committee on Medical Aspects of Food Policy; CONGA-1, continuous overall net glycemic action; GLP-1, glucagon-like peptide 1; High-density lipoprotein, HDL; HOMA-IR, homeostatic model assessment of insulin resistance; Net iAUC, net incremental area under the curve ; IPAQ, International Physical Activity Questionnaire; Low-density lipoprotein, LDL; Max, maximum; Min, minimum; Post, post-intervention; Pre, pre-intervention; PYY, peptide YY;

SD, standard deviation; SEM, standard error of the mean; TEF, thermic effect of food; VAS, visual analog scale.

www.clinicaltrials.gov (ID number; NCT02582606)

Data Share Statement:

Data described in the manuscript, code book, and analytic code will be made available upon request pending application and approval.

ABSTRACT

Background: Evidence is emerging that inter-daily meal pattern variability potentially impacts on response such as thermic effect of food (TEF), macronutrient metabolism and appetite.

Objectives: To investigate the effect of irregular ~~eating~~ meal pattern on TEF, glucose, insulin, lipid profile and appetite regulation in females with overweight or obesity and confirmed insulin resistance.

Design: In a randomized crossover trial, 9 females [mean±SD BMI: 33.~~-~~3±3.~~-~~1 kg/m^2) with confirmed insulin resistance consumed a regular (14 days; 6 meals/d) and an irregular (14 days; 3-9 meals/d) meal pattern separated by a 14-d wash-out interval. Identical foods were provided during the interventions and at the start and end of each meal pattern participants attended the laboratory after an overnight fast. Energy expenditure, glucose, insulin, lipids, adiponectin, leptin, glucagon-like peptide 1 (GLP-1), peptide YY (PYY), and ghrelin were measured at baseline and for three hours after consumption of a test drink, after which an a*d libitum* test meal was offered. Subjective appetite ratings were recorded before and after the test drink, after the *ad libitum* meal, and during the intervention. Continuous interstitial glucose monitoring was undertaken for 7 consecutive days during each intervention.

Results: TEF (over 3 h) was significantly lower post-irregular intervention compared with post-regular (97.7±19.2 kJ*3h in post-regular visit, and 76.7±35.2 kJ*3h in post-irregular visit (Paired T-test, p=0.048). Differences in HOMA-IR between the two interventions (3.3±1.7 and 3.6±1.6 in post-regular and post-irregular ~~eating~~ meal pattern, respectively) were not significant. Net iAUC for GLP-1 concentrations (over 3 h) post-regular meal pattern ~~eating~~ were higher (864.9±456.1 pmol/L*3h) than post-irregular meal pattern ~~eating~~ (487.6±271.7 pmol/L*3h), (Paired T-test; P=0.005).

Conclusion: Following a 14-d period of irregular meal pattern, TEF was significantly less than following regular meal pattern potentially compromising weight management if sustained long term.

Key words: meal pattern, thermogenesis, metabolism, appetite.

INTRODUCTION

Greater availability of foods requiring minimal home preparation and increased access to foods for immediate consumption outside the home, facilitate a more marked inter-daily variation in meal frequency and timing. Concurrently rates of obesity, and associated diseases, increased [1-3] potentially due to more irregular eating driving dysregulation of energy balance and poorer metabolic health.

A regular meal pattern may contribute to better health outcomes [4, 5] including glycemic control [6] and an irregular meal pattern has been associated with metabolic syndrome in observational studies [7-9]. In intervention studies, we demonstrate that an irregular meal pattern has potentially deleterious effects on the thermic effect of food (TEF) (suppression), energy intake (increased), carbohydrate metabolism and lipid profiles in females with a healthy weight, self- selecting their diet over 14 days [10, 11]. Similarly, in females with healthy weight provided with their food for 14-day periods, greater TEF and better glucose tolerance, in response to a test drink, were noted following a regular meal pattern compared with an irregular one [12]. In addition, potentially beneficial effects were seen during the intervention period using continuous glucose monitoring and visual analog scale (VAS) measurement of appetite.

In those with obesity, TEF after a test drink was significantly higher following a regular meal pattern and a reduced insulin response was observed, with no difference in circulating glucose [13], when participants self- selected foods according to a regular and irregular schedule. Over the longer term, these effects might result in a decreased risk of positive energy balance and ameliorate the elevated risk of insulin resistance and type 2 diabetes. However, those who are overweight or obese, with confirmed insulin resistance, have not been studied using our improved protocol, in which all food is provided, nor has glycemic response and appetite, during the intervention period been considered. Clearly, modifying

meal pattern regularity, would be an attractive, cost- effective public health strategy to reduce the risk of type 2 diabetes in this important group.

The primary aim of this study was thus to investigate the effect of meal pattern regularity, over two weeks, on TEF in females with overweight/obesity and insulin resistance.

Secondary outcome measures considered were as follows: circulating glucose, insulin, insulin resistant, lipids, appetite hormones and subjective appetite ratings measured when fasting and after the test drink; before and after each intervention period (regular; irregular) as well as anthropometry. Subjective appetite ratings and *ad libitum* food intake at a subsequent test lunch were also measured. Free living total energy expenditure estimation, subjective appetite assessment and continuous interstitial glucose monitoring were also undertaken during the intervention periods. All food was provided, to optimise adherence to the protocol whilst participants were otherwise free living.

METHODS

Participants

The present study was approved by the University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee (A16012014 SoL). The study took place in the David Greenfield Human Physiology Unit, School of Life Sciences, Queen's Medical Centre, University of Nottingham, between February 2014 and January 2015. The study was registered at clinicaltrials.gov as NCT02582606. The present analysis was a secondary analysis of a subset of participants from a completed RCT, and TEF was the primary outcome for this secondary analysis.

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Participants were recruited through poster advertisements placed at the University of Nottingham and via an advertisement in a local newspaper. Inclusion criteria were: healthy female with overweight or obesity [BMI (in kg/m^2): 28-40]; age: 18–45 y; normoglycemic but insulin resistant as assessed by homeostatic model assessment of insulin resistance

(HOMA-IR [14]) ≥ 1.5; non-smokers; and non–high-alcohol consumers (< 2 units/d); no history of a serious disease or currently taking any medications other than oral contraceptives; not pregnant or lactating and with regular menstrual cycles; not dieting or seeking to lose weight; and weight stable during the past 3 months (self-reported weight change less than ±2 kg).

Participants were excluded on the basis of the following criteria: individuals with symptoms of clinical depression [defined by a score >10 on the Beck Depression Inventory [15]]; eating disorders [defined by a score >20 on the Eating Attitudes Test (EAT-26) [16]]; or an allergy or intolerance to any of the foods provided during the study. Informed written consent was obtained from all participants after the experimental protocol had been described to them in writing and orally.

The power calculation was based on our previous work [12], where the net incremental area under the curve (net iAUC) for TEF (following a test drink) after the regular meal pattern was 25.8 ± 6.8 kcal and after the irregular meal pattern was 14.8 ± 11.7 kcal. Using a one sample model and statistical power at the level of 0.8, the number of participants with a cross-over design was 10, based on a two- sided alpha of 0.05.

Screening

Females who responded to the advertisements were invited for a screening visit. In this visit, height was measured to the nearest 0.1 cm with the use of a stadiometer (Seca, Germany). Body weight was measured with the use of an electronic scale (Seca, Germany) to the nearest 0.1 kg while participants were wearing light clothing with no shoes and with an empty bladder. BMI was calculated as weight divided by the square of height. A blood sample was taken for routine tests to confirm the general health of participants. Eligible participants were asked to complete a weighed 7-day food diary, which was used to characterize their habitual

diet. They were instructed to consume their normal diets and maintain their normal pattern of activity before the study.

Study design

The study followed a randomized, crossover design with two 14-d intervention periods that were separated by a washout period of 14 days. Participants were assigned to the randomization scheme in the order of recruitment. The randomization scheme was generated by the investigator with the use of the Second Generator Plan from randomization.com [17] before the study began.

Participants were free living except that, during each intervention period, they were required to consume only food that was provided by the experimenter. They were advised not to change their physical activity patterns during the study.

Participants attended the laboratory before and after each intervention period for a total of 4 visits. Each visit lasted about 5 h. Participants started each intervention period during the early phase of the menstrual cycle (days 1–7) in order to avoid the potential impact on outcome measures of the stage in the menstrual cycle [18-20].

Dietary intervention periods

During the regular and irregular meal pattern intervention periods, identical foods were provided in amounts designed to keep body weight constant over the study period based on estimated individual energy requirement (± 418.4 kJ) [12]. Menus were designed for 8577 kJ/d, 9832 kJ/d, 10669 kJ/d and 12134 kJ/d according to a 4-day menu cycle in order to avoid monotony and boredom during the study period.

Details of the procedure of dietary intervention periods including diet composition, number of meals and meal times have been described in detail previously [12]. In brief, diet composition (as a percentage of total energy per day) was 50% carbohydrate, 35% fat, and 15% protein. The number of meals during the regular meal pattern was 6 meals/d whilst the

number of meals during the irregular meal pattern varied from 3 to 9 meals/d (i.e. 7, 4, 9, 3, 5, 8, 6, 5, 9, 8, 3, 4, 7, and 6 meals/d) with a mean of 6 meals per day. Participants were instructed to eat their meals and snacks at specific times, between 0800 and 2100 during both interventions to remove the potential confounding impact of the time period over which the food was consumed. The only exception was when 3 meals/d were consumed, and the last meal was at 1800h.

Measurements made during intervention periods

Energy-expenditure assessment

During the two intervention periods, participants wore a multi-sensor armband device (SenseWear, BodyMedia Inc., Pittsburgh, PA, USA)[21], to estimate total energy expenditure continuously [12].

Continuous glucose monitoring

Glucose concentrations were monitored under free living conditions using an ambulatory continuous interstitial glucose monitor (CGM) device (iPro™2, Medtronic, Northridge, CA, USA) for 7 consecutive days. CGM was placed subcutaneously over the participant's anterior abdominal wall on day 6 and removed on day 13 of each intervention period. Finger prick glucose readings were taken four times a day, by the participants, using a portable monitor (Accu-Chek Aviva System, Roche Diagnostics, Switzerland) to calibrate the CGM.

The data obtained were analysed per 24 h, during the day (7:00–midnight) and during the night (midnight-7:00). Within day glycemic variability, considered to reflect greater blood glucose swings occurring as a consequence of diminished or absent autoregulation, can be characterised by a method described by McDonnell et al [22]. The method, Continuous Overlapping Net Glycemic Action (CONGAn), is based on comparing current measurements, with measurements made, n hours previously [22]. The standard deviation (SD) of the summated differences between the current observations and the observations made n hours

previously is calculated. CONGA-1 was calculated in the morning (each current observation from 9:00-10:00) and night (each current observation from 22:00-23:00). Postprandial (meal + 90 min) net iAUC for glucose was analysed following each meal on day 7 (6 meals/day in both regular and irregular periods). Postprandial (meal + 90 min) net iAUC on day 8 (6 meals/d vs 5 meals/d in regular and irregular interventions, respectively), day 9 (6 meal/d vs. 9 meals/d in regular and irregular periods, respectively) and day 10 (6 meals/d vs. 8 meals/d in regular and irregular periods, respectively) was analysed following each meal in which the identical type and amount of food was consumed in both regular and irregular interventions. However, on day 11 (6 meals/d vs. 3 meals/d in regular and irregular periods, respectively) and 12 (6 meals/d vs. 4 meals/d in regular and irregular periods, respectively), postprandial (meal + 90 min) net iAUC was not calculated for any meals because there were no identical meals (type and amount of food) consumed during those days for the regular and irregular periods.

Appetite assessment

Subjective appetite ratings were collected using paper-based VAS. Horizontal line scales were displayed with the rating questions presented above the line. The questions were in the form of "How (rating) do you feel?" (with ratings of hungry, satisfied, and full), "How much of a desire to eat?" and "How much do you think you can eat?" [23]. Participants were instructed to place a vertical mark through the horizontal line describing their current feeling. Quantification was made by measuring the distance (mm) on the horizontal line from the positive rating to the negative rating, providing a score between 0 and 100 mm. Participants were provided with a booklet (consisting of several sets of VAS) in which to record subjective appetite before and after each single meal when they were consuming 6 meals/d (day 7 and day 14) during both intervention periods.

Laboratory-visit protocol

Participants were instructed to fast overnight (≥ 12 h) and to take no exercise other than the walking related to carrying out their normal activities of daily living for 48 h before the laboratory visit. Participants consumed 6 meals/d on the day before the final laboratory visit in both interventions to eliminate an acute effect of the meal frequency on the day immediately preceding the laboratory visit. All visits were undertaken in the morning. On the participants' arrival at the laboratory, baseline measurements were taken, then participants were served a test drink at ~0900. Additional measurements were taken over a 3-h period, and an ad libitum test lunch was given at 12:30. Subjective appetite ratings were measured with the use of VAS before and over a 1-h period after the *ad libitum* test meal.

Anthropometric measurements

On arrival, weight and circumference measurements for waist and hip were taken. Waist circumference was measured to the nearest 0.5 cm in a horizontal plane at a point midway between the lower margin of the last rib and the top of the iliac crest with the use of a stretch resistant tape while the participant was standing with feet ~25–30 cm apart [24]. Hip circumference was measured to the nearest 0.5 cm in a horizontal plane at the point yielding the maximum circumference over the buttocks [24]. Skinfold-thickness measurements were made in triplicate by the same investigator at 4 sites (triceps, biceps, subscapular, and suprailiac) to assess the body composition of participants [25].

Blood sampling

Once the anthropometric measurements were taken, participants were asked to rest in a semi supine position in a temperature-controlled (23–24º C) room for ≥ 20 min. A 20-G cannula (Venflon) was inserted into a dorsal hand vein under local anesthetic (1% lignocaine; B Braun Melsungen AG) for subsequent blood sampling. The participant's hand was placed in a hot, air-warmed, ventilated perspex box (50–55ºC) to allow arterialized venous blood sampling [26]. Blood samples were drawn from a 3-way tap, and the first 2 mL of each

sample was discarded to avoid contamination with the saline (Baxter Healthcare Ltd.) that was used to maintain patency. Two blood samples were taken, with a 5-min interval, just before ingestion of the test drink to assess the mean of fasting total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), triacylglycerol, blood glucose, insulin, adiponectin, leptin, plasma glucagon-like peptide 1 (GLP-1), peptide YY (PYY), and ghrelin. After test drink ingestion, blood samples were taken every 15 min for glucose and every 30 min for 3 h to assess all of the markers mentioned except lipids, adiponectin and leptin for which only a fasting measurement was made.

Blood was dispensed into serum-separating tubes (allowed to clot for 30 min at room temperature before centrifugation) and into EDTA-coated tubes. EDTA-coated tubes contained either 20 µL dipeptidyl peptidase IV inhibitor (Millipore) for GLP-1 measurements or 50 µL aprotinin (Nordic Pharma) for PYY and ghrelin measurements. All samples were centrifuged (5702 R; Eppendorf) for 10 min at 3000 x g at 4ºC. The supernatant fluid was transferred into plastic tubes and kept at -80ºC until further analysis.

Blood analysis

Analyses were carried out at the University of Nottingham. Serum total cholesterol, HDL, LDL, and triacylglycerol concentrations were quantified with the use of an enzymatic photometric method (HORIBA ABX, Montpellier, France). Blood glucose was measured immediately with the use of a HemoCue analyser (AB, Angelholm, Sweden). Serum insulin, adiponectin and leptin, plasma PYY and ghrelin concentrations were measured with commercially available radioimmunoassays (Millipore, Billerica, MA, USA). Fasting insulin sensitivity was calculated with the use of homeostatic model assessment [14]. Plasma GLP-1 concentrations were measured with the use of an ELISA kit (Linco Research, St Charles, MO, USA).

Test-drink consumption

The standardized test drink (vanilla flavour milkshake) was served at room temperature in an open glass as a breakfast. The milkshake test drink comprised of 50, 35 and 15 % of energy as carbohydrate, fat, and protein, respectively (see Alhussain et al. [12] for more details). Participants were instructed to drink it over a period of 10 min. Participants were given a volume of 41.8 kJ/kg healthy body weight (equivalent to a BMI of 22.5 kg/m^2). A BMI of 22.5 kg/m^2 was selected following the precedent of the calculation of the DRV for energy by the Scientific Advisory Committee on Nutrition [27]. The mean energy provided by the test drink was 2431 ± 213 kJ, which provided a mean of 23.7 ± 1.8% of the estimated energy requirement.

Energy expenditure measurement

Energy expenditure and TEF were measured using an indirect calorimetry system (GEM system; Europa Scientific Ltd, England) for which alcohol burns are regularly undertaken. The percentage of relative error calculated using the method proposed by Kayiani et al. [28] is 0.1% which is less than the cut off of 2%, hence acceptable. Two cylinders of pressurised gas of known composition were used to calibrate the gas analysers in the indirect calorimetry system before the start of the experiment. Energy expenditure was measured in the fasted state for 20 min. TEF was then measured for periods of 15 min at 30 min intervals during the 3 h following the milkshake consumption. During the measurements, participants rested on the bed and relaxed but were not permitted to sleep. In the intervals between the measurements, they also rested on the bed, but they were allowed to read. Room air was measured at the start and both before and after each 15 min measurement period.

Ad libitum test meal

A pasta-based test meal (providing 699 kJ/100 g with 53, 34 and 13 % energy provided by carbohydrate, fat, and protein, respectively) was served at lunchtime to assess *ad libitum* food intake (see Alhussain et al. [12] for details). Participants were given portions of ~500 g and

instructed to consume as much as they wanted until they felt 'comfortably full'. The plate of pasta was continually topped up, when it was approximately ¾ empty. This ensured that there was always ample hot food available to participants and they were not cued to stop eating by having emptied their plate. Any left-over was removed and energy intake was calculated from the weight of food consumed. Duration and speed (g/min) of eating were also calculated.

Subjective appetite ratings

Participants completed the VAS for subjective appetite ratings just before, just after, and every 30 min after consumption of the test drink for 3 h. Additional VASs were completed before and immediately after consumption of the lunch test meal and at 15, 30, 45, and 60 min. VASs were constructed as described above. To avoid participants' responses to each set of VASs being biased by their responses to the previous set, each paper sheet was taken from the participant before the next one was provided. During this period of time, participants were asked to stay in the laboratory, but they were free to read.

Statistical analyses

All statistical analyses were performed by using SPSS 22.0 for Windows (SPSS Inc.). All data are reported as means ± SDs unless otherwise stated. Data were tested for normality with the use of the Shapiro-Wilks Test. The net iAUC was calculated by applying the trapezoid rule to data from which the baseline had been subtracted. Values greater than baseline were considered to be positive, and values that were below the baseline were considered to be negative. Comparisons of baseline variables at the pre-intervention visit were made with the use of Student's paired t test (2 tailed) as were measurements of mean 24 hour energy intake, total energy expenditure, VAS, and continuous glucose monitoring during the intervention period. Two-factor repeated-measure ANOVAs (factor 1: meal pattern, regular and irregular meal pattern; factor 2: visit, before and after each 14-d intervention) were conducted to assess the impact of the 14-d meal-pattern intervention on a range of dependent variables (e.g.,

weight, the net iAUC for the TEF, and the weight of pasta consumed) having established that there were no violations with respect to the use of an ANOVA. When an interaction was identified, simple main effects were explored with the use of pairwise comparisons. When no interaction was identified but significant main effects were shown, pairwise comparisons were made for the effect of the meal pattern or visit. No adjustment was made for multiple comparisons in the post hoc tests.

Order and sequence effects were explored by applying Paired T-tests to pre-intervention data to confirm that difference was not significant. An ANOVA was then undertaken, for primary outcome variable TEF, in which the two factors were Factor 1- Order (Level 1: before washout, Level 2: after washout) and Factor 2-Time (Level 1: pre-intervention; Level 2: post-intervention) confirming that there were neither main effect nor interactions relating to order. Differences were considered significant at $P < 0.05$ for all statistical tests.

RESULTS

Of the 35 females who responded to the advertisements, 10 participants, who met the study requirements, were recruited. 10 females were excluded because their HOMA-IR value did not meet the inclusion criteria. 15 females were ineligible because of BMI (4 females), age (2 females), medication (3 females), weight not stable (1 female), smoking (2 females) and not subsequently responding to correspondence (3 females). For the eligible participants, five were scheduled to start with the regular meal pattern and five with the irregular one. However, one participant withdrew from the study after she completed the irregular period due to lack of time. Therefore, of the ten females initially randomised, nine participants completed the study (**Figure 1)**.

Anthropometric measurements

Anthropometric measurements for the participants over the study are presented in **Table 1**. Bodyweight, body composition, waist circumference and waist-to-hip ratio did not show

303 significant differences, either at the pre-intervention visits or across the two intervention
304 periods.

305 **Dietary intervention compliance and comparison with the habitual diet**

306 Food diaries completed by participants during the two intervention periods showed that 99 ±
307 1.4 % and 99 ± 1.0 % of the energy from the food provided was consumed in the regular and
308 irregular intervention periods, respectively. There were no significant differences between
309 energy intake during the regular intervention period (10334 ± 1489 kJ/d) and the irregular
310 one (10355 ± 1485 kJ/d), nor were significant differences seen in the food composition
311 consumed in the regular intervention period (53 ± 0.3 % carbohydrate, 33 ± 0.3 % fat and 14
312 ± 0.4 % protein) compared with the irregular intervention period (53 ± 0.8 % carbohydrate,
313 33 ± 0.6 % fat and 14 ± 0.3 % protein).
314 Self-reported daily energy intake before the start of the study (8037 ± 1615 kJ/d) was
315 significantly lower than the estimated energy requirement for weight maintenance (10347 ±
316 1423 kJ/d) (Paired T-test, $p = 0.018$).

317 **Free-living energy expenditure during the intervention periods**

318 In both the regular and irregular intervention periods, the mean proportion of time spent
319 wearing the armband device was 96.83 ± 4.25 and 95.58 ± 5.80%, respectively. There were
320 no significant differences in the estimated total energy expenditure (10636 ± 1887 and 10297
321 ± 1251 kJ/d for regular and irregular intervention periods, respectively).
322 The estimated total energy expenditure, measured using the armband device, during the
323 regular intervention period (10636 ± 1887 kJ/d) was not significantly different from energy
324 intake consumed in the same period (10334 ± 1489 kJ/d). In the irregular intervention period,
325 there was also no significant difference between the estimated total energy expenditure
326 obtained by armband (10297 ± 1251 kJ/d) and energy intake consumed (10355 ± 1485 kJ/d).

There were also no significant differences for physical activity level between the two intervention periods (1.22 ± 0.07 and 1.23 ± 0.09 METs for regular and irregular intervention periods, respectively).

Post prandial energy expenditure in response to the test drink (indirect calorimetry data)

Figure 2 shows mean energy expenditure (mean of measurements taken over the 15-minute measurement periods) ± SEM at baseline and for three hours after consumption of a test drink in all study visits. The differences between fasting energy expenditure at the pre-intervention visits were not significant. Fasting energy expenditure did not show a significant meal pattern by visit interaction or main effect of meal pattern or visit (5904.5 ± 781.6, 5539.6 ± 538.1, 5750.9 ± 538.1 and 5665.6 ± 1013.6 kJ/d in pre, post-regular and pre and post-irregular visits, respectively).

Following the test drink consumption, energy expenditure increased above the fasting values at all visits (**Figure 2).** For TEF, the difference between the pre-intervention visits (74.9 ± 52.1 kJ*3h in pre-regular visits; and 98.5 ± 35.8 kJ*3h in pre-irregular visit) was not significant (Paired T-test, $p > 0.05$). However, comparison of the TEF values across the study showed a significant meal pattern by visit interaction (ANOVA; $p = 0.016$). TEF was significantly lower post-irregular intervention period compared with the post-regular one (97.7±19.2 kJ*3h in post-regular visit, and 76.7±35.2 kJ*3h in post-irregular visit) (Paired T-test, $p=0.048$). TEF post-regular visit was 20.9 ± 27.2 kJ (22%) higher than the post-irregular visit.

Glucose metabolism

Circulating glucose during the intervention period

Nine participants collected CGM data on day 7, 8, 9, 10, 11 and 12 of each intervention periods. One of these failed, providing inadequate data, therefore data from eight participants were used for analysis.

The 24 h mean, minimum, maximum and net iAUC values for glucose concentrations during the two intervention periods are shown in **Table 2** and **Table 3**. No significant differences were observed between the regular and irregular intervention periods for any of these values, comparing the equivalent day (e.g. day 7) on each intervention. There were also no significant differences in the mean, minimum, maximum, net iAUC values during the day (7:00–midnight) or night (midnight-7:00) or for glycemic variability (CONGA-1) comparing the equivalent day (eg. day 7) on each intervention period (Table 2 and 3).

Postprandial (meal +90 min) net iAUC analyses on day 7, 8, 9 and 10 are shown, for meals when intake was identical on the two interventions, in **Table 4**. The values during the regular intervention period did not differ significantly from the values during the irregular intervention period.

Circulating glucose in response to the test drink

Glucose concentrations before and over a 3-h period following the test drink consumption in all study visits are shown in **Figure 3**. Fasting, peak and net iAUC for blood glucose concentrations did not differ significantly between the two pre-intervention visits. There was also no significant interaction between meal pattern and visit or main effect of meal pattern or visit in all blood glucose variables (fasting, peak and net iAUC, **Table 5**).

Insulin response to the test drink

Insulin concentrations at all visits are shown in **Figure 4.** The difference in fasting insulin between the pre-intervention visits was not significant. No significant meal pattern by visit interaction or main effects of meal pattern or visit were observed in fasting and peak insulin values (Table 5) across the study visits.

Net iAUC for insulin (Table 5) demonstrated neither significant interaction between meal pattern and visit, nor significant main effect of visit. A significant main effect of meal pattern was noted; however, this was attributed to the difference at baseline which although not significant were markedly greater than the differences seen post intervention.

HOMA-IR

HOMA-IR values from all visits are shown in Table 5. HOMA-IR was not significantly different at the pre-intervention visits. There was also no meal pattern by visit interaction, or main effect of meal pattern or visit for HOMA-IR.

Lipids

Fasting lipid concentrations from all visits are shown in Table 5. There were no significant differences at the pre-intervention visits in any lipid's variables.

Fasting total and LDL showed no significant interaction between meal pattern and visit, or main effect of meal pattern or visit over the study visits. There was no significant meal pattern by visit interaction or main effect of meal pattern for fasting HDL and triglycerides. However, a significant main effect of visit was seen in these variables (ANOVA, $p = 0.027$ for HDL and $p = 0.028$ for triglycerides). Mean fasting HDL concentrations decreased approximately by 11% and 3% post-regular and post-irregular visits, respectively, compared with pre-intervention visits, whilst mean fasting triglycerides concentrations increased approximately by 19% and 23% post-regular and post-irregular visits, respectively, compared with pre-intervention visits, with no significant differences between the meal patterns.

Appetite regulation

Responses to the meal pattern during the intervention period-VAS

Participants completed the VAS before and after each meal on day 7 and 14 (6 meals/d) of each intervention period. For both day 7 and 14 comparison of mean pre-meal values for all VAS ratings (average of the 6 pre-meal ratings on the day) did not demonstrate significant

differences between the regular and irregular intervention periods (data were not shown). No significant differences were seen in the mean of post-meal VAS ratings (average of the 6 post-meal ratings on the day) between the regular and irregular intervention periods. The mean of the differences between pre and post-meal with respect to the VAS ratings did not demonstrate significant differences between the two intervention periods.

Response to the test drink- VAS

Fasting VAS ratings (hunger, satiety, fullness, desire to eat and prospective food consumption) were not significantly different between the pre-intervention visits, nor were there significant meal pattern by visit interactions or main effects of meal pattern or visit (data not shown).

Net iAUC responses for VAS ratings over the 3 h postprandial period did not show significant differences between pre-regular and pre-irregular intervention visits. Net iAUC responses did not show significant differences across the study visits, either (data not shown).

Response to the test drink- Regulatory gut peptides

Table 5 shows gut peptides values from all visits. Fasting adiponectin, leptin, GLP-1, PYY and ghrelin concentrations were not significantly different between the pre-intervention visits. No significant meal pattern by visit interaction or main effects for meal pattern or visit in fasting adiponectin, leptin, PYY and ghrelin concentrations was observed across the study visits. However, a significant main effect of visit (ANOVA, $p = 0.029$) was seen in fasting GLP-1 concentrations. Mean fasting GLP-1 concentrations increased by 5% and 28% post-regular and post-irregular visits, respectively, compared with pre-intervention visits.

Figure 5 shows GLP-1, PYY and ghrelin concentrations in all study visits. There was a significant interaction between meal pattern and visits in net iAUC for GLP-1 concentrations across the study (ANOVA; $p < 0.05$), GLP-1 concentrations net iAUC tended to be higher post-regular visit compared with pre-regular visit but (Paired T-test $p = 0.091$), unlike at the

irregular visits, where there was no significant difference between pre and post-intervention visits. GLP-1 concentrations post-regular visit were significantly higher (43%) than post-irregular visit (Paired T-test p = 0.005).

Net iAUC for PYY concentrations showed no significant interaction between meal pattern and visits, or main effect on meal pattern or visit. Net iAUC for ghrelin concentrations showed a trend for an interaction between meal pattern and visits (ANOVA, p = 0.08), but no significant main effect of meal pattern or visit was observed in ghrelin net iAUC.

Response to the ad-libitum test meal- VAS

There were no significant differences in net iAUC responses for VAS ratings over the 1 h postprandial period between pre-intervention visits. There was also no significant meal pattern by visit interaction or main effect of meal pattern or visit with net iAUC responses (data not shown).

Intake at the ad libitum test meal

Participants' energy intake during the ad libitum test meal, duration of eating and speed of consuming the meal did not show significant differences between the pre-intervention visits. There was no meal pattern by visit interaction or main effect of meal pattern or visit for participants' energy intake across the study visits (3573.6 ± 1136.0, 3589.9 ± 903.3, 3443.9 ± 1143.5 and 3767.3 ± 902.5 kJ in pre and post-regular and irregular visits, respectively). The duration of eating did not show a significant interaction between the meal pattern and visit or main effect of meal pattern or visit (12.1 ± 5.7, 12.3 ± 7.4, 11.3 ± 5.9 and 11.0 ± 3.6 min in pre and post-regular and irregular visits, respectively). There was also no significant interaction between the meal pattern and visit or main effect of meal pattern or visit on speed of eating (46.2 ± 12.4, 48.2 ± 15.2, 46.9 ± 11.5 and 51.8 ± 14.4 g/min in pre and post-regular and irregular visits, respectively).

DISCUSSION

Our findings show that a 14-day period of irregular eating was accompanied by a lower TEF measured for 3h following a breakfast, test drink. A higher net iAUC of GLP-1 concentrations, following the test drink, was observed in response to the regular compared with the irregular eating. Fasting GLP-1 concentrations were higher after the interventions than before. There was a tendency for the suppression of ghrelin in response to the test drink to be greater after the regular eating.

A higher TEF after the regular meal pattern, compared with the irregular meal pattern is in accordance with findings from our previous work [10, 12, 13] suggesting that this effect is robust across participants with healthy weight and obesity and participants with obesity and insulin resistance. Although there was no significant change in body weight or other anthropometric measurements, almost certainly because of the short duration for the study and in common with our previous studies [10, 12, 13], in the longer term regular eating might have a beneficial effect on weight regulation. In common with our previous work, TEF was measured over a 3-h~~our~~ period which it has been suggested may capture 60% of TEF [29]. The liquid test drink may result in a more rapid and shorter thermic response given more rapid absorption of liquids. Resting metabolic rate was assumed to be constant across the day, when calculating TEF despite it showing circadian variation [30, 31]. However, the impact of this on comparisons may have been ameliorated by careful standardisation of the time when the test drink was consumed.

Increases in GLP-1 concentrations suppress subjective appetite and reduce subsequent energy intake in humans [32]. In the present study, fasting GLP-1 concentrations were higher after both intervention periods which may reflect the macronutrient differences between the intervention and habitual diet. GLP-1 concentrations can be affected by the phases of the menstrual cycle [33]. Participants started the meal pattern interventions in the same phase of

the menstrual cycle so finished in a different phase which may in part explain why fasting GLP-1 concentrations were higher post-interventions compared with pre-interventions. Following the regular meal pattern intervention there were higher GLP-1 concentrations in response to the test drink compared with following the irregular intervention but no significant differences in VAS or *ad libitum* intake at the pasta meal between the two interventions. This may reflect the differences in GLP-1 were insufficient to result in differences in behaviour.

In our previous studies, with self-selected food, we noted a trend for lower intake when following the regular meal pattern in females with healthy weight [10], and a lower intake that was statistically significant in females with obesity [13] compared with the irregular period. Providing food to participants in this and our previous study [12], may explain why no significant difference in intake was observed.

Ghrelin stimulates appetite and food intake in humans [34]. In the current study, ghrelin concentrations, in response to the test drink, tended to be suppressed to a greater extent following the regular intervention compared with pre-intervention, and in fact, when considering the irregular meal pattern, there appeared to be a smaller suppression post intervention. However, a significant interaction was not seen, potentially because the study was insufficiently powered for this outcome. In the present study, differences in VAS ratings between the two intervention periods were not significant either in the laboratory or free-living conditions.

The net iAUC of glucose responses to the test drink did not show significant differences between the regular and irregular intervention periods, in contrast to our earlier study in females with healthy weight [12] where the net iAUC for glucose was lower after the test drink and at some time points during the intervention period with the regular intervention. Nor were differences seen in insulin responses that we had noted previously in females with

healthy weight and obesity [11, 13]. In the current study a significant main effect of meal pattern for net iAUC for insulin is considered to be a consequence of numerical differences at baseline, which were maintained post intervention, rather than a true effect of meal pattern. Previously the irregular meal pattern had had a higher post meal peak insulin, higher net iAUC for insulin (females with healthy weight and obesity) and higher HOMA-IR (females with healthy weight) [11, 13] than following the regular meal pattern. It would thus seem that in those who are already demonstrating insulin resistance, meal pattern regularity is ineffective at improving insulin sensitivity.

Given that blunted TEF is associated with insulin resistance [35], we have proposed previously that this could be the mechanism behind the impaired TEF that was observed after the irregular meal pattern [11-13]. However, this does not appear to be a plausible explanation in these females with overweight or obesity and insulin resistance given the lack of difference in insulin/glucose response to the test meal, or difference in fasting HOMA-IR. More work is warranted in this area to establish the mechanism behind the blunted TEF response including the potential role of an irregular meal pattern in disrupting circadian rhythms controlled and generated via the 'biological clock' located in the suprachiasmatic nuclei and via other peripheral clocks sensitive to substrate availability [36].

No significant differences were found in fasting total or LDL over the study, findings that are not consistent with those of the previous study [13] who reported that the irregular meal pattern was associated with higher fasting total and LDL compared with the regular one. Part of the explanation may be that the higher energy intake during the irregular intervention compared with the regular intervention [13]. In the present study the same type and amount of food was consumed in both intervention periods, whilst the food was self-selected in the previous study, which may mean the type and amount of food consumed varied between the two interventions.

No meal pattern effect was found on fasting HDL and triglycerides concentrations, but fasting HDL concentrations were lower and fasting triglycerides concentrations were higher following the intervention periods compared with before. This might have been due to the differences in carbohydrate percentage between self-reported habitual diet and consumed intervention diet. Moreover, it might be that in the day prior to post regular and irregular visits participants consumed the same food type and composition, whilst the food that was self-selected in the day prior to the pre-intervention visits may have differed.

The relatively small sample size used in the present study is acknowledged as a potential limitation, particularly with respect to the secondary outcome measures and may increase the probability of type 2 errors. Although there is a risk of type 2 errors in secondary analyses, the primary analysis was positive so there is ~~it is~~ not at risk of a false negative. The risk of type 1 errors is increased by testing several study outcomes, especially with the secondary outcome measures. Data obtained will however support pre-priori calculation of appropriate numbers to achieve power in future work, in these novel areas. Stable body weight was successfully maintained during the two intervention periods which suggests that the methods of estimating energy requirement used in the current study were appropriate. The estimate of total energy expenditure was obtained by using the armband device during the two intervention periods indicated a compliance with requested instructions to maintain similar levels of activity during the intervention periods. These instructions were intended to reduce the potentially confounding effect of physical activity level on the key outcomes of interest. However, this protocol does preclude any potential differences in physical activity as a result of the differences in meal pattern.

In conclusion, in females with obesity and insulin resistance, a regular meal pattern is associated with a greater TEF and postprandial GLP-1 compared with an irregular meal pattern. This demonstrates that the constancy of daily meal pattern may be a contributory

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549 factor to weight control. However, it would be of interest to determine these effects in further
550 long-term studies not only in females but also in men and in patients with type 2 diabetes to
551 produce more reliable and relevant findings for public health. The interaction between
552 regularity of meal pattern and circadian rhythms should be considered in further studies.

ACKNOWLEDGMENTS

We thank Michael Rittig and Tariq Taylor for providing medical supervision and Sally Cordon and Karen Swift for the analysis of blood samples. In addition, we thank Liz Simpson for her assistance provided throughout the study.

The authors' responsibilities were as follows—MHA: contributed to the design of the study, conducted the study, performed the statistical analysis, interpreted the results, wrote the manuscript, and was responsible for the final content of the manuscript; IAM and MAT: contributed to the design of the study, supervised the data collection and analysis, had input into the interpretation of the results, and helped produce a final draft of the manuscript; and all authors: read and approved the final version of the manuscript.

The authors have no direct financial or personal competing interests to declare. Full list of potential conflicts for IAM over the past 5 years: Scientific Advisory Boards: Nestle Research, Novozymes, AIJN (European Fruit Juice Consortium), ILSI Europe Dietary Carbohydrate Task Force, Mars Inc., Waltham Pet Healthcare Research Institute. Government Committees: Scientific Advisory Committee on Nutrition-including Working Group with NHS-E and Diabetes UK on High Fat Diets in Diabetes management. MRC Nutrition Grants panel. Editorial duties: Joint Editor of International Journal of Obesity

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TABLE 1

Anthropometric measurements of participants over the study[1]

	Regular meal pattern		Irregular meal pattern	
	Pre	**Post**	**Pre**	**Post**
Body weight (kg)	86.5±13.6	86.1±13.5	85.3±12.9	85.7±13.4
BMI (kg/m^2)	33.7±3.3	33.5±3.3	33.2±3.1	33.3±3.2
Body fat (%)	40.8±7.7	40.9±8.8	40.9±8.3	41.2±8.3
Waist (cm)	91.4±11.7	90.6±11.9	90.9±11.6	91.1±11.6
Waist/hip ratio	0.78±0.1	0.78±0.1	0.78±0.1	0.78±0.1

[1]Data are presented as mean ± SD. n= 9. There were no significant differences in the characteristics of the study participants across the study for the comparison of regular and irregular meal patterns (ANOVA).
BMI, body mass index; Post, post-intervention; Pre, pre-intervention. SD, standard deviation.

TABLE 2

Analyses of the CGM data compared between the two meal pattern interventions on day 7, 8 and 9[1]

Glucose (mmol/L)	Regular meal pattern			Irregular meal pattern		
	Day 7 (6 meals)	Day 8 (6 meals)	Day 9 (6 meals)	Day 7 (6 meals)	Day 8 (5 meals)	Day 9 (9 meals)
Fasting	5.3±1.5	5.7±0.6	5.7±0.6	5.1±0.5	5.6±0.5	5.5±0.7
Mean 24 h	5.6±0.5	5.6±0.4	5.9±0.6	5.6±0.6	5.7±0.7	6.2±0.6
Mean day h	5.6±0.6	5.7±0.4	6.1±0.6	5.6±0.5	5.8±0.8	6.2±0.7
Mean night h	5.6±0.6	5.6±0.6	5.5±0.7	5.6±0.6	5.5±0.7	5.8±0.5
Max 24 h	7.8±1.8	7.7±1.7	8.3±1.7	7.1±0.7	7.7±1.4	8.1±1.3
Max day h	7.8±1.8	7.9±1.9	8.3±1.7	7.0±0.7	7.6±1.4	8.1±1.3
Max night h	6.1±0.9	6.2±0.7	6.2±1.2	6.2±0.7	6.2±0.7	6.7±0.7
Min 24 h	4.0±0.8	4.1±0.9	4.5±0.4	4.3±0.6	4.6±0.6	4.8±0.4
Min day h	4.0±0.8	4.0±0.9	4.8±0.3	4.3±0.6	4.7±0.6	4.9±0.4
Min night h	5.0±0.6	4.9±0.4	4.8±0.6	5.0±0.7	5.0±.09	5.4±0.5
Net iAUC 24h	744.8±686.3	301.9±235.6	603.3±367.9	994.9±778.5	355.3±211.5	759.9±798.8
Net iAUC day h	550.0±454.5	269.1±234.2	618.0±352.2	685.9±597.2	335.5±278.7	661.1±532.5
Net iAUC night h	-96.6±140.2	-107.7±176.9	-41.3±60.3	-53.0±197.9	-180.7±174.0	-90.5±185.9
CONGA-1(9:00-10:00)	0.89±1.2	0.72±0.6	0.83±1.1	0.83±0.6	0.53±0.3	0.69±0.6
CONGA-1 (22:00-23:00)	0.29±0.2	0.48±0.3	0.46±0.3	0.39±0.3	0.49±0.2	0.44±0.3

[1] Data are presented as mean± SD, n= 8. There were no significant differences when comparing the equivalent day for the regular and irregular meal pattern (Paired T-test).

CGM, continuous glucose monitoring; CONGA, continuous overlapping net glycemic action; Net iAUC, net incremental area under the curve; Max, maximum; Min, minimum. SD, standard deviation.

Table 3

Analyses of the CGM data compared between the two meal pattern interventions on day 10, 11 and 12[1]

Glucose (mmol/L)	Regular meal pattern			Irregular meal pattern		
	Day 10 (6 meals)	Day 11 (6 meals)	Day 12 (6 meals)	Day 10 (8 meals)	Day 11 (3 meals)	Day 12 (4 meals)
Fasting	5.3±0.7	5.7±0.6	5.9±0.9	5.5±0.5	5.6±0.6	5.6±0.5
Mean 24 h	5.9±0.6	6.3±0.6	6.1±0.7	5.9±0.5	6.0±0.3	5.9±0.7
Mean day h	5.9±0.6	6.4±0.6	6.2±0.6	5.9±0.4	6.0±0.3	5.9±0.7
Mean night h	5.7±0.7	6.1±0.9	5.9±0.8	5.7±0.6	5.8±0.2	5.8±0.7
Max 24 h	8.2±1.4	7.7±1.4	8.6±1.6	7.9±1.4	8.2±1.7	8.2±1.9
Max day h	8.2±1.4	8.1±1.3	8.6±1.6	7.9±1.4	8.2±1.7	8.2±1.9
Max night h	6.3±1.1	7.1±1.2	6.4±0.9	6.4±1.0	6.4±0.4	6.4±1.0
Min 24 h	4.2±0.3	4.9±0.2	4.3±0.6	4.5±0.7	4.6±0.7	4.5±0.7
Min day h	4.2±0.3	5.0±0.2	4.4±0.7	4.7±0.8	4.7±0.7	4.4±0.6
Min night h	5.2±0.6	5.5±0.9	5.4±0.5	5.1±0.6	5.3±0.2	5.3±0.7
Net iAUC 24h	797.6±982.8	684.6±770.8	356.2±432.4	624.5±266.1	624.6±433.1	363.4±482.6
Net iAUC day h	670.2±738.3	548.6±601.9	342.5±408.8	490.1±186.6	514.3±313.7	301.2±344.0
Net iAUC night h	-118.5±345.4	-180.7±142.5	-91.7±112.8	-193.7±311.2	-162.9±211.1	-48.0±90.9
CONGA-1(9:00-10:00)	0.89±0.6	0.82±0.9	0.48±0.7	0.64±0.5	0.78±1.1	0.72±1.1
CONGA-1 (22:00-23:00)	0.39±0.3	0.27±0.2	0.99±0.7	0.54±0.3	0.80±1.0	0.59±0.6

[1] Data are presented as mean± SD, n= 8. There were no significant differences when comparing the equivalent day for the regular and irregular meal pattern (Paired T-test).

CGM, continuous glucose monitoring; CONGA, continuous overlapping net glycemic action; Net iAUC, net incremental area under the curve; Max, maximum; Min, minimum**;** SD, standard deviation.

Table 4

Analyses of the CGM data (postprandial, meal +90 min, net iAUC) compared between the two meal pattern interventions on day 7, 8, 9 and 10[1]

Glucose (mmol/L)	Regular meal pattern				Irregular meal pattern			
	Day 7 (6 meals)	Day 8 (6 meals)	Day 9 (6 meals)	Day 10 (6 meals)	Day 7 (6 meals)	Day 8 (5 meals)	Day 9 (9 meals)	Day 10 (8 meals)
Net **iAUC-breakfast +90**	75.9±98.9	64.7±89.8	-	88.9±60.9	77.2±46.1	61.3±20.0	-	68.0±41.5
Net **iAUC-mid-morning snack +90**	47.9±31.1	38.7±31.0	-	-	40.8±23.2	54.8±44.2	-	-
Net **iAUC-lunch +90**	46.7±52.5	-	83.9±114.3	36.9±18.1	45.5±39.3	-	91.2±58.5	54.1±16.5
Net **iAUC-afternoon snack +90**	34.1±27.2	-	-	-	30.9±11.6	-	-	-
Net **iAUC-dinner +90**	49.8±49.7	-	78.1±72.2	-	38.1±27.6	-	104.3±84.4	-
Net **iAUC-night snack +90**	35.5±22.6	58.2±45.0	36.4±20.3	80.33±44.8	36.1±26.3	45.9±28.4	67.4±49.5	44.3±27.8

[1] Data are presented as mean± SD, n= 8. There were no significant differences when comparing the equivalent day for the regular and irregular meal pattern (Paired T-test).

CGM, continuous glucose monitoring; Net iAUC, net incremental area under the curve; SD, standard deviation.

TABLE 5

Fasting and post-test drink blood measurements across the study for regular and irregular meal patterns[1]

Variables	Regular meal pattern		Irregular meal pattern	
	Pre	**Post**	**Pre**	**Post**
Fasting glucose (mmol/L)	4.77±0.4	4.66±0.3	4.90±0.5	4.70±0.4
Glucose Peak (mmol/L)	7.39±1.3	7.40±1.4	7.49±1.2	7.48±1.8
Net iAUC for glucose (mmol/L over 3h)	204.0±98.4	252.7±125.4	183.6±76.3	242.4±126.5
Fasting insulin (pmol/L)	98.5±37.8	93.8±46.1	108.3±58.2	102.0±46.2
Insulin peak (pmol/L)	864.3±312.7	842.5±278.0	904.3±348.1	831.2±276.0
Net iAUC for insulin (pmol/L over 3h)	66334.0 ± 30966.7	70128.8 ± 24315.9	58356.1 ± 33521.4	59704.9 ± 23614.5
HOMA-IR	3.5±1.5	3.3±1.7	4.1±2.8	3.6±1.6
Fasting total cholesterol (mmol/L)	4.33±0.47	4.30 ± 0.38	4.25±0.39	4.44±0.38
Fasting LDL (mmol/L)	2.49±0.46	2.55±0.44	2.47±0.30	2.69±0.38
Fasting HDL[2] (mmol/L)	1.35±0.23	1.22±0.24	1.31±0.20	1.27±0.22
Fasting Triglycerides[2] (mmol/L)	0.98±0.41	1.17±0.38	0.86±0.25	1.12±0.31
Fasting Adiponectin (µg/mL)	7.67±1.99	7.19±2.64	7.94±2.26	7.61±1.96
Fasting Leptin (µg/L)	56.70±24.58	49.26±15.64	50.10±21.88	49.23±21.97
Fasting GLP-1[2] (pmol/L)	5.03±2.31	5.28±1.04	4.87 ± 1.92	6.22±2.28
Net iAUC for GLP-1 (pmol/L over 3h)[3]	697.8 ± 345.1	864.9 ± 456.1	663.6 ± 350.4	487.6 ± 271.7
Fasting PYY (pg/mL)	98.92±22.73	96.03±34.61	94.06±22.68	84.71±16.38
Net iAUC for PYY (pg/mL over 3h)	3219.7 ± 2129.0	3905.4 ± 1591.8	2870.9 ± 2851.9	3357.1 ± 2750.3
Fasting Ghrelin (pg/mL)	1004.95±366.08	1025.67±310.95	1024.06±319.08	1005.25±299.67
Net iAUC for ghrelin (pg/mL over 3h)	-38669.2 ± 24668.0	-45720.4 ± 29180.6	-41143.4 ± 23430.6	-32856.4 ± 15775.2

[1]Data are presented as mean± SD. n= 9.
[2]A significant main effect of visit was observed with HDL, triglyceride and fasting GLP-1 concentrations (ANOVA, $p < 0.05$).

[3]There was a significant meal pattern by visit interaction for net iAUC GLP-1 between the regular and irregular meal pattern periods (ANOVA; $p < 0.05$). Net iAUC GLP-1 concentration was significantly higher post-regular compared with post-irregular meal pattern (Paired T-test $p < 0.05$).
There were no significant differences in all other values across the study for the comparison of regular and irregular meal patterns (ANOVA).

GLP-1, glucagon-like peptide 1; High-density lipoprotein, HDL; HOMA-IR, homeostatic model assessment of insulin resistance; Net iAUC, net incremental area under the curve; Low-density lipoprotein, LDL; Post, post-intervention; Pre, pre-intervention; PYY, peptide YY; SD, standard deviation.

FIGURE 1 Study participant flow diagram.

FIGURE 2 Mean (of 15-minute periods) ± SEM energy expenditure, which were measured with Indirect calorimetry, at baseline and for three hours after consumption of a test drink in all study visits. n= 9.

The differences between fasting energy expenditure at the pre-intervention visits was not significant. Fasting energy expenditure did not show a significant meal pattern by visit interaction or main effect of meal pattern or visit.

SEM, standard error of the mean.

FIGURE 3 Mean ± SEM glucose concentrations at baseline and for three hours after consumption of a test drink in all study visits. n= 9.

The difference between fasting glucose for the pre- intervention visits was not significant. There was no significant interaction between meal pattern and visit or main effect of meal pattern or visit for fasting or peak blood glucose values.

FIGURE 4 Mean ± SEM insulin concentrations at baseline and for three hours after consumption of a test drink in all study visits. n= 9.

The difference in fasting insulin between the pre-intervention visits was not significant. No significant meal pattern by visit interaction or main effects of meal pattern or visit were observed in fasting and peak insulin values across the study visits.

SEM, standard error of the mean.

FIGURE 5 Mean ± SEM for GLP-1, PYY, and ghrelin concentrations at baseline and for three hours after consumption of a test drink in all study visits. n= 9.

The difference between fasting GLP-1, PYY and ghrelin concentrations were not significantly different between the pre-intervention visits in each case. No significant meal pattern by visit interaction or main effects for meal pattern or visit were seen in fasting PYY and ghrelin concentrations across the study visits. However, a significant main effect of visit (ANOVA, $p = 0.029$) was seen in fasting GLP-1 concentrations.

GLP-1, glucagon-like peptide 1; PYY, peptide YY; SEM, standard error of the mean.