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Irregular meal pattern- effect on energy expenditure, metabolism and appetite regulation; a randomised controlled trial in healthy normal-weight women

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Irregular meal pattern- effect on energy expenditure, metabolism and appetite regulation; a randomised controlled trial in healthy normal-weight women

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Short running head

Regular eating, thermogenesis and appetite

Abbreviations

Ambulatory energy expenditure estimation (AEEE); Analysis of variance (ANOVA); Body mass index (BMI); Continuous glucose monitor (CGM); Continuous overlapping net glycemic action (CONGA); Hour (h); Incremental area under the curve (iAUC); International

Physical Activity Questionnaire (IPAQ); kilocalories per day (kcal/day); Minute (min); Maximum (max); Minimum (min); Minute (min); Physical activity level (PAL); Resting energy expenditure (REE); Standard deviation (SD); Standard error of the mean (SEM); Thermic effect of food (TEF); Visual analogue scale (VAS)

Clinical trial registry number and website

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ABSTRACT

Background: Obesity is increasing in parallel with greater all day food availability. The latter may promote meal irregularity, dysregulation of energy balance and poor metabolic health.

Objective: To investigate the effect of meal irregularity on the thermic effect of food (TEF), lipid levels, carbohydrate metabolism, subjective appetite and gut hormones in healthy women.

Design: 11 normal-weight women (18–40y) were recruited to a randomized crossover trial with two, 14-day isoenergetic diet periods (identical foods provided/ free-living), separated by a 14-day habitual diet wash-out period. In period 1, participants followed a regular (6meals/day) or an irregular meal pattern (3-9meals/day) and in period 2, the alternative meal pattern. Before and after each period, when fasting and for 3h following a test drink, measurements were made of energy expenditure, circulating glucose, lipids (fasting only), insulin, GLP-1, PYY and ghrelin. An *ad libitum* test meal was offered**.** Subjective appetite ratings were assessed fasted, following the test drink, following the *ad libitum* meal and during the intervention. Continuous interstitial glucose monitoring (CGM) was undertaken for 3 consecutive days during each intervention and ambulatory activity pattern was recorded (AEEE).

Results: Regularity was associated with greater TEF ($P<0.05$) and a lower incremental area under the curve (iAUC) for glucose following the test drink (over 3h) and, for some identical meals on the two interventions (over 90min) (Day 7: post-breakfast; Day 9: post-lunch and dinner). There was no difference between-treatments for test drink gut hormone response. A time effect for fasting GLP-1, fasting PYY, PYY responses and hunger rating responses to the test drink ($P<0.05$) was noted. Lower hunger and higher fullness ratings were seen, pre and post meal, during the regular period, whilst free living.

Conclusion: Meal regularity appears to be associated with greater TEF and lower glucose responses, which may favour weight management, and metabolic health.

Key words: normal-weight women, meal regularity, thermic effect of food, metabolism, appetite.

INTRODUCTION

Obesity, an abnormally large accumulation of adipose tissue, occurs as a result of long term positive energy balance, and is associated with impaired metabolic function and poor health (1). A rapid increase in obesity prevalence over recent decades has occurred concurrently with greater availability of food requiring minimal preparation, inside and outside the home, and throughout the day. This environment offers greater individual choice with respect to time of eating, and potentially facilitates greater inter-daily variation in meal pattern. Meal pattern research, initiated in the 1960's, was based on the premise that meal pattern is a stable characteristic for an individual, with inter-daily repetition of, for example, meal frequency (2-5). Few studies have evaluated the impact of meal pattern irregularity (i.e. between day variations) on energy metabolism and health in adults.

We have previously undertaken14-day feeding studies comparing a regular meal pattern with an irregular meal pattern in normal-weight and obese participants (6-8). The thermic effect of food (TEF), in response to a test drink, in normal-weight and obese women was significantly lower following an irregular meal pattern compared with regular (6, 8). In addition, irregular meal pattern was associated with a lower fasting insulin sensitivity (7), a greater insulin response to a test meal (7, 8) and higher fasting levels of total and LDL cholesterol (7, 8). These results were consistent with a negative association between irregular meal pattern and metabolic health found in observational studies (9, 10).

Food intake in our intervention studies was self-selected and the obese participants, interestingly, reported a lower energy intake during the regular period (8). Differences in subjective appetite might have mediated this with the potential involvement of gut hormones associated with appetite (11-14). These however were not measured.

The present study aims to compare the impact of 14 days of more highly controlled regular and irregular eating (all food provided) on TEF, metabolic, appetitive and gut hormone

responses to a test drink and *ad libitum* intake of a test meal. The term 'meal' was used for both prescribed eating incidents at traditional 'meal times', and those that occurred at traditional 'snack times'. Measures were made during the free-living intervention periods of physical activity (AEEE), continuous interstitial glucose monitoring (CGM) and subjective appetite.

SUBJECTS AND METHODS

Participants

The study was conducted at David Greenfield Human Physiology Unit, School of Life Sciences, Queen's Medical Centre, University of Nottingham between January 2013 and July 2013. The study was approved by the University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee (J14082012 BMS). Participants were recruited from the student and staff population of the University of Nottingham via poster advertisement. Inclusion criteria for participants were: normal weight women (BMI 18.5 and 25 kg/m^2), aged 18-40 years, non-smoker, non-high alcohol consumers (< 3 units/day), no history of serious disease or currently taking any medications other than oral contraceptives, not pregnant/lactating and with regular menstrual cycles, not dieting/seeking to lose weight and weight stable during the last 3 months (self-reported weight change < ± 2 kg). Exclusion criteria were: participants with symptoms of clinical depression (defined by a score > 10 on the Beck Depression Inventory (15)), with eating disorders (defined by a score > 20 on the EAT- 26 (16)), with an allergy or intolerance to any of the foods provided during the study. Of the 19 healthy normal-weight participants who responded to the advertisement 11 were recruited to the study (**Figure 1**). These 11 participants were the ones that met the study requirements. Values that were outside the inclusion criteria resulted in exclusion of four and two subjects respectively for BMI and EAT- 26 score. Two women were ineligible because they were anaemic. The remaining 11 participants gave written consent, and then 5

participants were scheduled to start with the regular meal pattern and 6 others with the irregular one. Blood sampling could not be performed on one participant due to problems associated with venous cannulation. Thus data from 10 participants were available for the intention-to-treat blood analysis. Two subjects were excluded from the analysis of CGM data because inadequate data were obtained. Informed, written consent was obtained from all participants after the experimental protocol had been described to them in writing and orally. The study is registered at clinical Trials.gov with the identification number: NCT02052076.

Screening

All potential participants attended a screening visit in order to establish that they met the inclusion criteria for the study. Height was measured to the nearest 0.1 cm using a stadiometer (Seca, Germany). Body weight was measured using an electronic scale to the nearest 0.1 kg (Seca, Germany) whilst participants were wearing light clothing with no shoes and with an empty bladder. BMI was calculated from their height and weight as kg/m^2. A blood sample was taken for routine tests to confirm their general health.

Eligible participants then 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 diet and participate in their usual level of activity before the study.

Study design

The study followed a randomized crossover design with two, 14-day intervention periods, separated by a wash-out period of 14 days. Participants consumed their habitual diet during the wash out period which was included to avoid interaction between the two interventions. The randomization scheme was generated using the Second Generator Plan from randomization.com (17) before the study began. Participants were assigned to the randomization scheme in the order of recruitment. The study investigator generated the randomization scheme, enrolled participants, and assigned participants to interventions.

Participants were free-living except that during each intervention period they were required to consume food provided by the experimenter. Participants attended the laboratory pre and post each intervention period, for a total of 4 visits. Each laboratory visit lasted up to 5 h. In order to avoid the potential impact on outcome measures of the stage in the menstrual cycle (18-20), participants started each intervention period during the early phase of their menstrual cycle (days 1-7).

Dietary intervention periods

Each participant was provided with, free of charge, all their food during each of the intervention periods. An individual had identical foods during each of the intervention periods and differences between participant food provision were minimised, but were sometimes necessary to meet the different energy requirements of participants. The food was supplied in a 4 day cycle of menus consisting of a variety of items commonly consumed in the British diet. The menu was designed to cover participants' energy requirement for weight maintenance (± 100 kcal). Menus were designed for 1900 kcal/day, 2050 kcal/day and 2350 kcal/day to meet the different estimated energy requirements of participants. Energy requirements were based on the Oxford-Henry equations (21) multiplied by physical activity level. This equation was chosen following the precedent of the calculation of the DRV for energy by the Scientific Advisory Committee on Nutrition (22). Physical activity was estimated by using the International Physical Activity Questionnaire (IPAQ) (23). The level ascribed by the IPAQ was then translated to a PAL level using the Committee on Medical Aspects of Food Policy (COMA) classifications (24) (IPAQ score low = non active, moderate = moderately active, high = very active) and taking into account occupational activity which was classified according to COMA as light, moderate or heavy.

The macronutrient composition of the diet, as a percentage of total energy for the day, was approximately 50 % carbohydrates, 35 % fat and 15 % protein. These macronutrient

percentages were based on the Report of the Panel on Dietary Reference Values of COMA (24).

Participants were reassured that the amount of food provided was designed to ensure a stable body weight over the course of the study. All participants declared an intention to consume the entire amount of food supplied. However, they were asked to record any left-over food in the diary provided. Participants were instructed to avoid alcohol consumption, limit caffeine-containing drinks to two cups of tea per day (without sugar/milk). They were advised not to change physical activity patterns during the study.

Following the design of previous studies in our laboratory (6-8), the number of meals during the regular meal pattern was 6 meals/day which was based upon three 'meals' providing approximately 70 % of energy requirements (breakfast, lunch and dinner) and three 'snacks' (mid-morning, afternoon and evening snack) providing a total of approximately 30 % of energy requirements (**Supplemental Table 1**).

The number of meals (including eating incidences labelled as snacks on the menu) during the irregular meal pattern varied from 3 to 9 meals/day. The average was 6 meals/day during the 14-day period (i.e. 7, 4, 9, 3, 5, 8, 6, 5, 9, 8, 3, 4, 7, 6 meals/day). Participants were asked to eat their meals at specific times between 8:00 am and 9:00 pm, during both interventions, to remove the potentially confounding impact of the time period over which food was consumed. The only deviation from this instruction was that when they had 3 meals/day, during the irregular period, their last meal was at 6:00 pm (instead of 9:00 pm) as it was anticipated that this was when they would consume a meal with others in their household.

Measurements made during the intervention periods

Energy expenditure assessment

Participants wore a SenseWear™ armband (SWA, BodyMedia Inc, Pittsburgh, PA, USA) to obtain an ambulatory estimate of their energy expenditure (AEEE) continuously during the

intervention periods. The armband was worn over the left triceps muscle, halfway between the acromion process of the scapula and the olecranon process of ulna. Participants were instructed to wear it continuously, including while sleeping and to remove it only for brief periods for bathing, showering or swimming.

Energy expenditure data were derived from, a skin temperature sensor, a near body temperature sensor, a galvanic skin response sensor, a heat flux sensor, and accelerometer (25). These data were used in combination with demographic characteristics including age, sex, weight and height, to estimate energy expenditure using a proprietary equation developed by the manufacturer (SenseWear Software, version 7) which was not published.

Continuous Glucose monitoring (CGM)

CGM (Medtronic Minimed, Northridge, USA) provided continuous glucose profiles for up to 72 h. Subcutaneous interstitial fluid glucose concentrations were measured every 10 seconds and the average glucose value for each 5 min period was stored (up to 288 measurements daily).

The CGM was placed subcutaneously over the participant's anterior abdominal wall on day 6 and removed on day 10 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. A 24 h contact number was available for any inquiries or if any problems arose. Data from CGM were downloaded and glucose profiles were evaluated based on data collected on day 7 (6 meals/day in both regular and irregular periods), day 8 (6 meals/day vs. 5 meals/day in regular and irregular periods respectively) and day 9 (6 meals/day vs. 9 meals/day in regular and irregular periods respectively). Data were analysed per 24 h, during the day (7:00–midnight) and during the night (midnight-7:00) with respect to 24 h mean, max, min and iAUC for glucose for each time period.

On day 7 (6 meals/day in both regular and irregular interventions) postprandial iAUC for 90 min was analysed following each meal (breakfast, mid-morning snack, lunch, afternoon snack, dinner and evening snack). However on day 8 (6 meals/day vs 5 meals/day in regular and irregular interventions respectively) analysis was restricted to the points in the day when participants consumed identical meals on the two interventions (breakfast, mid-morning snack and evening snack).The afternoon snack was omitted during the irregular period and the food distributed between lunch and dinner. On day 9 (6 meal/day vs. 9 meals/day in regular and irregular periods respectively), analysis similarly was restricted to lunch, dinner and evening snack. The breakfast was divided into two meals during the irregular period. The mid-morning and afternoon snacks were also divided into two small meals in order to achieve 9 meals/day.

Intra-day glycemic variability was computed by an approach described by McDonnell et al (2005) specifically for CGM data, known as continuous overlapping net glycemic action (CONGA-n) (26). CONGA-n is calculated as the standard deviation of the summed differences in glucose concentration between current observation and the observation n hours previous. CONGA-1 was calculated in the morning (current observation from 9:00-10:00) and night (current observation from 22:00-23:00). CONGA-1 indicated intra-day glycemic variability based on one hour time periods.

Appetite assessment

Subjective appetite ratings were assessed by using paper based visual analogue scales (VAS) with words anchored at each end of a 100-mm horizontal line that expressed the most positive and the most negative rating for a question (**Supplemental Figure 1**). The questions were in the form ‘How (rating) do you feel?’ and the ratings were ‘hungry’, ‘satisfied’, ‘full’, ‘how much of a desire to eat?’ and ‘how much do you think you can eat?’ (27).

Participants were provided with a booklet in which to record subjective appetite before and after each single meal on days 7 and 14 during both intervention periods, when they were consuming 6 meals/day on each intervention.

Laboratory visit protocol and procedures

Participants were asked to attend the laboratory at 8:00 am after a minimum 12 h overnight fast and were required to take no exercise other than walking related to carrying out their normal activities of daily living, for 48 h before the laboratory visit. Participants consumed 6 meals/day on the day prior to the final laboratory visit on both interventions in order to eliminate an acute effect of the meal frequency on the day immediately preceding the laboratory visit. Once baseline measurements were completed, participants were served a test drink at approximately 9:00 am. Further measurements were then taken over a 3 h period, and then an *ad libitum* test lunch was given at 12:30 pm. Subjective appetite ratings were measured using VAS before and over a 1 h period after the *ad libitum* test meal

Anthropometric measurements

Immediately after arrival, participants were weighed on an electronic scale (Seca, Germany) to the nearest 0.1 kg with an empty bladder, wearing similar light clothes on each visit and without shoes. 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 using a stretch-resistant tape, while the participant was standing with feet about 25–30 cm apart (28). Hip circumference was measured to the nearest 0.5 cm in a horizontal plane at the point yielding the maximum circumference over the buttocks (28). Skinfold thickness measurements were made by the same investigator, in triplicate, at four sites (triceps, biceps, subscapular and suprailiac) in order to assess participants' body composition (29).

Blood sampling

Following the anthropometric measurements, participants rested in a semi-supine position in a temperature-controlled (23-24 ℃) room for a minimum of 20 min. Then a 20 G cannula (Venflon) was inserted into a dorsal hand vein under local anaesthetic (1 % lignocaine: B.Braun Melsungen AG. Melsungen, Germany) for subsequent blood sampling. The hand was placed into a hot air-warmed, ventilated perspex box (50–55 ºC) to allow arterialised venous blood sampling (30). Blood samples were drawn from a 3-way tap, the first 2 mL of each sample was discarded to avoid contamination with the saline (Baxter Healthcare Ltd., Thetford, UK) 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 serum total, HDL, LDL-cholesterol, triacylglycerol, blood glucose, serum insulin, plasma glucagon-like peptide-1 (GLP-1), plasma Peptide YY (PYY), and plasma ghrelin. After the test drink ingestion, blood samples were taken every 15 min for glucose and every 30 min for 3 h to assess all the markers mentioned above except lipids, 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 EDTA tubes. EDTA tubes containing either 20 µl dipeptidyl peptidase IV (DPP-IV) inhibitor (Millipore, Billerica, MA, USA) for GLP-1 measurement or 50 µl aprotinin (Nordic Pharma, Reading, UK) for PYY and ghrelin measurements. All samples were centrifuged (5702 R, Eppendorf, Germany) for 10min at 3000 r.p.m at 4 ºC. The supernatant was transferred into plastic tubes and kept at -80 ºC until further analysis.

Blood analysis

The analyses were carried out at the University of Nottingham. Serum total, HDL, LDL-cholesterol and triacylglycerol concentrations were quantified by an enzymatic photometric method (HORIBA ABX, Montpellier, France). Blood glucose was measured immediately

using a HemoCue analyser (AB, Angelholm, Sweden). Serum insulin concentrations were measured with commercially available radioimmunoassays (Millipore, Billerica, MA, USA). Fasting insulin sensitivity was calculated using the homeostatic model assessment (HOMA model) (31). Plasma GLP-1 concentrations were measured using an ELISA kit (Linco Research, St Charles, MO, USA). Plasma PYY and ghrelin concentrations were measured with commercially available radioimmuno assays (Millipore, Billerica, MA, USA).

Test drink consumption

The standardized test drink (vanilla flavour milkshake) was served at room temperature in an open glass as a breakfast. Participants were instructed to drink it over a period of 10 min. The test drink provided 10 kcal/kg body weight and comprised 50 % of energy as carbohydrate, 35 % as fat, and 15 % as protein. All participants consumed all of the test drink. The mean energy provided by the test drink was 584.3 ± 51.8 kcal which provided a mean of 27.9 ± 1.1% of the estimated energy requirement.

The test drink contained skimmed milk (Sainsbury's, London, UK), Build-up (Nestle SA, Lausanne, Switzerland), Polycal (Nutricia Clinical Care, Trowbridge, UK) and double cream (Sainsbury's, London, UK).

Energy expenditure measurement

Indirect calorimetry (GEM system; Europa Scientific Ltd, England) was used to determine REE and TEF by measuring the volume of oxygen uptake and carbon dioxide expired. An open-circuit flow-through canopy, with a mass flow meter, mixing chamber and a vacuum pump, was used to draw room air over the participants' face at a rate of 50-60 l/min. This is considered to be the most convenient way for measuring energy expenditure in human studies at rest (32). The system was connected to a computer, and data from the mass flow meter and gas analysers were used to calculate the VO_2 and VCO_2 using the software provided by the manufacturer. The indirect calorimetry system was turned on for half an hour prior to use, to

278 warm up. Two cylinders of pressurised gas of known composition were used to calibrate the
279 gas analysers in the indirect calorimetry system before the start of the experiment. REE was
280 measured in the fasted state for 20 min. TEF was then measured for periods of 15 min at 30
281 min intervals during the 3 h following the milkshake consumption. During the measurements,
282 participants rested on the bed and relaxed but were not permitted to sleep. In the intervals
283 between the measurements, they also rested on the bed, but they were allowed to read. Room
284 air was measured at the start and both before and after each 15 min measurement period.
285 *Ad libitum test meal*
286 A pasta-based test meal (providing 167 kcal/100 g with 13, 34 and 53 % energy provided by
287 protein, fat and carbohydrate, respectively) was served at lunchtime to assess *ad libitum* food
288 intake. This meal had a homogeneous nature, so energy intake could be assessed from the
289 weight of food consumed. The meal consisted of pasta (Sainsbury's, London, UK: 125 g
290 cooked in 800 ml boiling water on full power in a microwave (900 W) for 13 min- stirred mid
291 period). The pasta was then drained, cooled rapidly using cold water and then mixed with
292 cheddar cheese (Sainsbury's: 40 g), olive oil (Sainsbury's: 15 g), and tomato and basil pasta
293 sauce (Dolmio, Mars food, UK: 170 g; macronutrient composition of sauce in **Supplemental**
294 **Table 2**). The mixture was then chilled until required and heated in the microwave for 2 min
295 before being served to the participants. Participants were given portions of ~500 g and
296 instructed to consume as much as they wanted until they felt 'comfortably full'. The plate of
297 pasta was continually topped up, when it was approximately ¾ empty. This ensured that there
298 was always ample hot food available to participants and they were not cued to stop eating by
299 having emptied their plate. Any left-over was removed and energy intake was calculated from
300 the weight of food consumed. Duration and speed (g/min) of eating were also calculated.
301 *Subjective appetite ratings*
302 Participants completed the VAS for subjective appetite ratings just before, after and then

every 30 min after consumption of the test drink for 3 h. Further VAS were completed before and immediately after consuming the lunch test meal, and then at 15, 30, 45 and 60 min. The VAS were as described above. To avoid participants' response to each set of VAS 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

SPSS software (version 21 for windows; SPSS) was used for data entry and analysis. All data are presented as means± standard deviation (SD), unless otherwise stated. Data were tested for normality with the Kolmogorov-Smirnov test to inform whether parametric or non-parametric analysis should be used.

Values for the incremental area under the curve (iAUC) of the TEF, postprandial glucose, insulin, appetite ratings and gut hormone responses were calculated using differences from the baseline. Values above baseline were considered positive, and below baseline negative. The area above or below baseline was calculated using the trapezoid rule.

Comparisons of the baseline data at the pre intervention visit were made using Student's paired t test (two-tailed) as were measurements of energy intake, AEEE, VAS and CGM during the intervention period.

Two-way repeated measure ANOVAs (Factor 1: meal pattern, regular and irregular meal pattern; Factor 2: visit - pre and post each 14-day intervention) were conducted to assess the impact of the 14-day meal pattern intervention on a range of dependant variables (e.g. weight, iAUC for TEF, weight of pasta consumed). Where an interaction was identified, simple main effects were explored by pairwise comparisons. Where no interaction was identified, but significant main effects were found, pairwise comparisons were made for the effect of meal

pattern or the effect of visit. Differences were considered significant at $P < 0.05$ for all statistical tests.

Results obtained from a previous study (6) indicated that the iAUC TEF after a regular meal pattern was 0.74 ± 0.37 kJ/min and after an irregular meal pattern was 0.39 ± 0.26 kJ/min. Therefore, with a cross-over design, eleven participants in each group would be required to detect a difference in TEF (~ 0.35 kJ/min) with the power of 80 % at the significance level of 0.05.

TEF (kcal/min) over 3 h (following the test drink), as assessed by indirect calorimetry, was the primary outcome for comparison between the two intervention periods. Responses for lipids, glucose, insulin, gut hormones, subjective appetite ratings and *ad libitum* food intake of the test meal were considered as secondary outcomes.

RESULTS

In this study, the effect of meal irregularity on thermic effect of food (TEF), lipid concentrations, carbohydrate metabolism, subjective appetite and gut hormones were investigated in 11 healthy normal-weight women. Participants undertook either a regular meal pattern (14 days, 6 meals/day) an irregular meal pattern (14 days,varying from 3 to 9 meals/day) or in a randomised crossover design, separated by a 14-day wash out period. Participants attended the laboratory after an overnight fast at the start and end of each intervention period.

Anthropometric measurements

There were no significant differences in bodyweight, body composition, or other anthropometric measurements at the pre intervention visits or across the study visits (**Table 1**).

Energy Intake

Self-reported daily energy intake before the start of the study (2081 ± 214 kcal/day) was similar to the estimated energy requirement for weight maintenance (2104 ± 204 kcal/day). However self-reported carbohydrate percentage (47 ± 4.1 %) was significantly lower and self-reported fat percentage (38 ± 3.7 %) was significantly higher compared with the consumed intervention diet (53 ± 0.2 % carbohydrate and 33 ± 0.6 % fat) (paired T-test, $p < 0.01$). There were no significant differences in the protein percentage between the self-reported and the prescribed diet (14 ± 2.5 vs 14 ± 0.4 % respectively).

During the study, food intake was designed to be the same by type, and amount in each intervention period, hence provide the same amount of energy and have the same macronutrient composition. The food intake diaries completed to check compliance showed that 98 ± 6 % and 100 ± 2 % of the energy given was consumed in the regular and irregular intervention periods respectively indicating good compliance. There were no significant differences in energy intake between the two intervention periods (2043 ± 248 kcal/day regular vs. 2098 ± 195 kcal/day irregular intervention period) as intended by the design of the study. The composition of consumed foods also did not differ significantly between the two intervention periods being (53 ± 0.9 % carbohydrate, 14 ± 0.4 % protein and 33 ± 0.8 % fat in regular and 53 ± 0.3 % carbohydrate; 14 ± 0.5 % protein and 33 ± 0.7 % fat in irregular intervention period).

Free-living energy expenditure

On average, the SWA device was worn 96.8 ± 5.5 and 95.1 ± 7.7 % of the regular and irregular intervention periods respectively. There were no significant differences between mean values of AEEE during the intervention period for both regular and irregular meal pattern (2241± 360 kcal/day and 2305 ± 399 kcal/day for regular and irregular intervention periods respectively). There were no significant differences between the mean of the physical activity level during the regular and irregular intervention period (1.60 ± 0.2 and 1.64 ± 0.2

376 METs for regular and irregular intervention periods respectively). In both conditions the
377 estimated energy expenditure was approximately 200 kcal greater than the prescribed energy
378 requirement.

379 **Free-living CGM**

380 For the nine participants for whom CGM data were available, analyses (mean, max, min,
381 CONGA-1 and iAUC) were done for each meal pattern on day 7 (6 meals consumed in both
382 intervention periods), day 8 (6 meals and 5 meals consumed in regular and irregular period
383 respectively), and day 9 (6 meals and 9 meals consumed in regular and irregular period
384 respectively) (**Table 2**). Twenty-four hour mean, max, min and iAUC for glucose
385 concentrations showed no significant differences between the two intervention periods. There
386 were also no significant differences in the day period and the night period between the two
387 intervention periods for these variables. CONGA-1 with current observation period 9:00 to
388 10:00 and 22:00 to 23:00 also showed no significant differences between the two intervention
389 periods.

390 On day 7 of the intervention (6 meals/day both interventions), there was a significantly higher
391 glucose concentration for the postprandial (breakfast +90 min) iAUC analysis (Table 2) in the
392 irregular meal pattern intervention compared with the regular meal pattern intervention
393 (paired T-test, $p < 0.05$). On day 9 (6 meals v 9 meals), for the meals that were identical on
394 the two interventions, postprandial (lunch +90 min) and (dinner +90 min) iAUC analysis
395 showed a similar difference in that the iAUC in the irregular intervention was significantly
396 higher compared with the regular intervention (paired T-test, $p < 0.05$). No significant
397 differences were seen in the other postprandial iAUC analysis.

398 **Energy expenditure (indirect calorimetry data)**

399 Fasting REE was not significantly different at the pre intervention visits. There was also no
400 meal pattern by visit interaction, or main effect of meal pattern or visit for fasting REE (1167

401 ± 134, 1207 ± 89, 1183 ± 171 and 1188 ± 149 kcal/day in pre, post regular and pre, post
402 irregular visits respectively).
403 REE increased above the fasting values, after the test drink, at all visits. The overall TEF for
404 the 3 h postprandial period is shown in **Figure 2**. There was no significant difference in
405 overall 3 h TEF at the pre intervention visits. There was a significant meal pattern by visit
406 interaction for the 3 h TEF (ANOVA; $p < 0.05$). TEF post regular visit was increased
407 significantly compared with pre regular visit (paired T-test $p < 0.01$) unlike in the irregular
408 visits, where there was no significant difference between pre and post intervention visits. TEF
409 post regular visit was 11.1 ± 15.8 kcal higher than post irregular visit (paired T-test $p < 0.05$).
410 **Blood variables**
411 There were no significant differences at the pre intervention visits for all blood variables.
412 *Lipids*
413 The results for fasting serum total, LDL, HDL-cholesterol, serum triglycerides are shown in
414 **Table 3**. There were no significant interactions for meal pattern by visit or main effects of
415 meal pattern or visit in fasting serum total, LDL, HDL-cholesterol, serum triglycerides.
416 *Glucose*
417 No significant meal pattern by visit interaction or main effects of meal pattern or visit were
418 observed in fasting blood glucose across the study (Table 3). Blood glucose responses to the
419 test drink reached a maximum level 30 and 45 min after the test drink and remained above
420 fasting levels at the last sampling time-point (180 min after the test drink) in all visits. The
421 peak values (Table 3) did not show a significant interaction for meal pattern by visit or main
422 effects for these two factors. Blood glucose iAUC response to the test drink **(Figure 3)**
423 showed a significant interaction between meal pattern and visit (ANOVA; $p < 0.05$). A larger
424 area was seen at the post irregular visit compared with post regular visit ($p < 0.05$). Post
425 irregular visit, blood glucose iAUC was significantly higher than pre irregular visit ($p <$

0.05), unlike in the regular intervention, where there was no significant difference between pre and post regular visits.

Insulin

Table 3 shows fasting serum insulin in all visits. There were no significant interactions for meal pattern by visit or main effects of meal pattern or visit. Serum insulin concentrations increased rapidly from 15 min after consuming the test drink in all visits. Following peak values, concentrations declined to some extent but remained above fasting values for the reminder of the sampling period. The peak values of insulin (Table 3) did not show a significant meal pattern by visit interaction or main effects of meal pattern or visit. There was no significant interaction between meal pattern and visit on iAUC for serum insulin nor were there significant main effects for meal pattern or visit (5826.2 ± 2150.5 mIU/L in 3h pre regular visit, 5719.4 ± 3326.6 mIU/L in 3 h post regular visit, 5842.6 ± 3775.2 mIU/L in 3 h pre irregular visit and 5268.9 ± 2248.0 mIU/L in 3 h post irregular visit).

GLP-1

There was no significant interaction for meal pattern by visit or main effect of meal pattern for fasting plasma GLP-1 concentrations (Table 3). However, a significant main effect of visit was observed (ANOVA, $p < 0.05$). Mean fasting plasma GLP-1 concentrations decreased by approximately 16 % and 20 % post regular and irregular visits respectively compared with pre intervention visits. Following consumption of the test drink, plasma GLP-1 concentrations increased in all visits. iAUC for plasma GLP-1 concentrations (**Figure 4**), showed no significant interaction between meal pattern and visits, or main effects for meal pattern or visit.

PYY

No significant meal pattern by visit interaction or main effects of meal pattern were observed in fasting plasma PYY concentrations (Table 3). However, there was a significant main effect

of visit (ANOVA, $p < 0.05$). Mean fasting plasma PYY concentrations decreased about 9 % and 23 % post regular and irregular visits respectively compared with pre intervention visits. Plasma PYY concentrations increased rapidly above the fasting values after consuming the test drink and remained at a plateau until the last sampling time point in all visits. iAUC for the 3 h postprandial period in all visits (Figure 4) showed no significant interaction between meal pattern and visit or main effect for meal pattern. However, there was a significant main effect of visit (ANOVA, $p < 0.05$). Mean iAUC for plasma PYY concentrations increased by approximately 57 % post regular compared with pre regular visit, and by 70 % post irregular compared with pre irregular visit.

Ghrelin

No significant meal pattern by visit interaction or main effects of meal pattern or visit were observed in fasting plasma ghrelin (Table 3). Following consumption of the test drink, plasma ghrelin concentrations declined in all visits. iAUC for plasma ghrelin (Figure 4) showed no significant interaction between meal pattern and visits, or main effects for meal pattern or visit.

Subjective appetite ratings

Responses to the test drink

There were no significant differences between the pre intervention visits for any of the iAUC for subjective appetite ratings collected in the fasting state (**Supplemental Table 3**). There was also no meal pattern by visit interaction, or main effect of meal pattern or visit for fasting VAS ratings (Supplemental Table 3). The assessments of subjective hunger for the 3 h postprandial period in all visits showed no significant interaction between meal pattern and visit or main effect for meal pattern, but a significant main effect of visit (ANOVA, $p < 0.05$) was found. Mean hunger ratings decreased by 195 % and 104 % post regular and irregular visits respectively compared with pre intervention visits (Supplemental Table 3). The

response for the other VAS ratings showed no significant differences between the intervention periods (Supplemental Table 3).

Responses to the ad libitum test meal

The response (for hunger, fullness, satiety, desire to eat and prospective food consumption) for the 1 h postprandial period in all visits showed no significant interaction between meal pattern and visit or main effect for meal pattern or visits (Supplemental Table 3).

Responses to the meal pattern during the intervention

Subjective appetite ratings were assessed pre and post meals during day 7 and 14, when 6 meals/day were consumed in both regular and irregular intervention periods. On day 7, there were no significant differences between mean pre meal ratings (average of the 6 pre-meal ratings on the day) (**Table 4**). However, mean post meal ratings for hunger and fullness showed significant differences between the interventions. Higher post meal ratings for hunger and lower for fullness (paired T- test, $p < 0.01$) were observed in irregular compared with the regular intervention period (Table 4).

On day 14 (the final day of intervention), the ratings of pre meals hunger was significantly greater in irregular compared with regular intervention period (**Table 5**, paired T- test, $p < 0.05$,). Furthermore, the ratings of post meal hunger were significantly greater in the irregular period (Table 5, paired T- test, $p < 0.05$). There were no significant differences in the pre and post meal values for the other VAS appetite ratings.

Intake at the *ad libitum* test meal

There was no significant difference between participants' energy intake at the *ad libitum* test meal 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 (778.8 ± 272.8, 745.7 ± 214.7, 722.4 ± 324.0 and 764.3 ± 246.6 kcal in pre and post regular and irregular visits respectively).

The duration of eating and speed of consuming the *ad libitum* test meal were not significantly different pre intervention visits. The duration of eating did not show a significant interaction between the meal pattern and visit or main effect of meal pattern or visit (9.6 ± 3.9, 9.8 ± 3.8, 9.5 ± 3.1 and 9.1 ± 2.3 min in pre and post regular and irregular visits respectively). Speed of eating also showed the same result (51.1 ± 13.2, 47.9 ± 10.1, 45.1 ± 13.4 and 50.6 ± 11.1 g/min in pre and post regular and irregular visits respectively).

DISCUSSION

The aim of this study was to investigate the metabolic, endocrine and appetite related effects of a regular compared with an irregular meal pattern, in healthy normal-weight women consuming identical, isoenergetic diets and undertaking comparable activity. We also assessed activity using AEEE, continuous interstitial glucose monitoring (CGM) and appetite in the free-living state.

No differences were found in body weight between the two interventions, suggesting that the aim to match intake and activity were met. With the regular meal pattern, TEF was greater, whilst post prandial glucose response was smaller both in response to a test drink, and in response to some identical meals, whilst free-living. No differences were found in fasting lipid values. PYY showed a greater postprandial response after both interventions, concurrently with anticipated differences in hunger and fullness. Pre and post appetite ratings during the regular intervention suggested greater fullness and reduced hunger.

The differences in TEF are compatible with our previous findings (6, 8). Compensation in other components of energy expenditure might explain the similar body weights seen after the two interventions, despite differences in TEF. However, there was no difference in REE, and although the estimate of ambulatory energy expenditure made using the SWA device has limitations, for example, the absence of published validated equations for this population group and inconsistent findings when compared with indirect calorimetry (25, 33-35), it gives

an indication of comparable activity patterns. The short duration of the study is a more likely explanation, as over a longer time period, the greater TEF with a regular meal pattern, if repeated at all meals and in the longer term, could have beneficial effects on weight control. The range of published values for the TEF of diets containing comparable macronutrient composition makes estimating the expected TEF from the test drink problematic (36). However using a generally accepted figure for TEF of 10 % of total energy consumed, and a mean test drink dose of 584 kcal, a TEF of approximately 60 kcal might be expected. The smaller values seen (over 3 h) may reflect that the full metabolic rate response had not occurred in 3h. It has been estimated that weight gain in 90 percent of the adult population could be prevented by reducing positive energy balance by 100 kcal/day (37) and Brown et al. found that over 5 years a 10 kcal/day excess in energy intake resulted in a 0.5 kg gain in weight per year (38). Future work should assess energy expenditure over 24 h, in order to capture the full response to each meal, and the accumulative effect of more than one meal in the day.

Insulin resistance has been shown to be associated with blunted TEF (39-41), and may contribute to the differences we have seen. In this study, a lower postprandial glucose response to the test meal was seen after the regular compared with the irregular meal pattern. In our previous studies (7, 8), there was no difference in glucose response, but a greater post prandial insulin response was seen after the irregular meal pattern period. Both of these patterns of results are consistent with the regular meal pattern resulting in greater insulin sensitivity. The novel addition to the present study of continuous interstitial glucose measurements on three days during the intervention periods (each preceded by the same last meal on the previous day) further corroborates reduced insulin sensitivity with an irregular pattern. Day 7 allowed direct comparison of six meals per day and showed a beneficial response to breakfast with regular eating. On Day 8 however, despite having several

identical meals, no differences were found, perhaps because of an acute effect of the preceding day being identical for both patterns (6 meals per day). On Day 9, for those meals that were identical, a beneficial reduction in post prandial response at lunch and dinner (but not the night snack) was seen for the regular pattern. Further work is needed to establish whether, under laboratory conditions, a comparable difference in blood glucose response occurs throughout the day, how quickly differences are seen in response to dietary differences, and whether the differences are sustained over a longer time period.

Fasting Triglyceride and HDL cholesterol concentrations showed no significant differences between the two meal patterns in the present study, in agreement with previous studies in normal-weight and obese women (7, 8). However previously differences were found between fasting total and LDL cholesterol (7) in contrast to this study. This is perhaps because the food intake was better controlled in this study. The participants in the current study were similar to those in the previous study with respect to age, BMI and body fat, however their ethnicity may have been different, possibly resulting in differences in sensitivity to meal pattern.

Greater post-meal ratings for hunger and lower ratings of fullness on day 7 (6 meals/day on both interventions), during the irregular meal pattern period suggest a reduction in the satiation experienced. Additionally, greater pre and post-meal ratings for hunger were observed on the final day of the irregular meal pattern when again 6 meals were consumed in both interventions, suggesting that by the end of the study satiety was reduced as well. However there was no difference by intervention for subjective appetite in response to the test meal (although there was a time effect), or the pasta meal. The energy intake of pasta consumed at the *ad libitum* test meal in the laboratory was decreased by 4 % post regular visit and increased by 6 % post irregular visit. This did not reach significance, possibly because the study was insufficiently powered for this secondary outcome.

Whilst no meal pattern effect was found for fasting plasma GLP-1 and PYY concentrations, a main effect of time was seen, and in response to the test meal for PYY. The explanation for these differences, in common with the time effect reported above for subjective appetite, may be the differences in composition of habitual diet and the intervention diet. The 7 day food record would suggest that the habitual diet contained a lower percentage of carbohydrate and a higher percentage of fat. In addition, on day 14, before the final visit, the number of meals and amount of food was the same on both occasions, in contrast to the first visits when the habitual diet was consumed the preceding day. The stage in the menstrual cycle was also different as the study started in the early phase of the follicular phase, which may have impacted on appetite (42, 43) and GLP-1 (42). The differences observed in PYY in response to the test drink were consistent with the differences in VAS hunger responses, confirming the inverse relationship between PYY and subjective hunger (44). Given, that the differences in subjective appetite noted whilst free-living in this study, might offer an explanation for the higher energy intake previously noted in obese participants eating *ad libitum* while following an irregular meal pattern (8), this aspect warrants further work, with a larger sample size. As demonstrated with respect to TEF, small differences in energy intake, sustained over the long term, can have a major impact on weight regulation. It is also of interest that associations have been found between TEF and satiety (45) suggesting that there may be some inter-relation between differences in subjective appetite, and the blunted TEF measured in this study.

In conclusion, the results of this study show that a regular meal pattern compared with an irregular meal pattern results in greater TEF, greater insulin sensitivity, and potentially beneficial subjective appetite changes. These desirable effects could support weight control and metabolic health, in the general population. Future studies should include overweight and

600 obese participants, with and without type II diabetes, and should include 24 hour

601 measurement, and longer term interventions.

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The authors' contributions are as follows: M.H.A contributed to the design of the study, conducted the study, performed the statistical analysis, interpreted the results, wrote the manuscript and was responsible for its final content. M.A.T. and I.A.M. 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. All authors read and approved the final version of the manuscript.

I.A.M is a member of the UK Government Scientific Advisory Committee on Nutrition, Treasurer of the Federation of European Nutrition Societies, Treasurer of the World Obesity Federation, a member of the Mars Scientific Advisory Council, the Mars Europe Nutrition Advisory Board, Scientific Adviser to the Waltham Centre for Pet Nutrition, and has a UK Government Research Grant (from Innovate UK) for a project which is led by Mars UK. He is also the Academic lead for the University of Nottingham's strategic research partnership with Unilever. M.H.A and M.A.T have no conflicts of interest.

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TABLE 1. Participants' characteristics over the study [1]

	Regular meal pattern		Irregular meal pattern	
	Pre	**Post**	**Pre**	**Post**
Body weight (kg)	58.7 ± 6.1	58.3 ± 6.2	58.6 ± 6.6	58.2 ± 6.1
BMI (kg/m^2)	22.0 ± 2.0	21.8 ± 1.9	21.9 ± 1.9	21.8 ± 2.0
Body fat (%)	22.2 ± 3.0	22.1 ± 3.6	22.3 ± 3.5	22.7 ± 3.8
Waist (cm)	69.5 ± 5.5	69.5 ± 5.1	70.5 ± 5.7	69.9 ± 5.1
Waist/hip	0.7 ± 0.6	0.7 ± 0.6	0.7 ± 0.6	0.7 ± 0.6

[1] mean ± SD, n=11.

There were no significant differences in the characteristics of the ten participants across the study comparing a regular and irregular meal pattern (Two-way ANOVA).

TABLE 2. Analyses of the CGM data compared between the two meal pattern interventions [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	4.7±0.8	4.9±0.4	4.9±0.4	5.0±0.6	4.9±0.6	5.1±0.4
Mean 24 h	5.2±0.5	5.3±0.4	5.4±0.6	5.2±0.4	5.2±0.4	5.5±0.3
Mean day h [2]	5.3±0.7	5.4±0.5	5.5±0.5	5.3±0.4	5.3±0.4	5.6±0.3
Mean night h [2]	4.9±0.3	5.2±0.6	5.2±0.8	4.9±0.6	5.0±0.4	5.1±0.5
Max [3] **24 h**	7.1±1.0	7.1±1.4	7.9±1.5	7.5±1.4	7.2±0.8	7.9±1.2
Max day h	7.1±1.0	7.1±1.4	7.9±1.5	7.5±1.3	7.2±0.8	7.9±1.2
Max night h	5.5±0.4	5.8±0.8	5.9±0.7	5.8±1.0	5.5±0.5	5.8±0.6
Min [3] **24 h**	4.1±0.8	4.3±0.5	4.1±0.5	3.8±0.4	3.9±0.5	4.1±0.5
Min day h	4.1±0.8	4.3±0.5	4.1±0.6	4.3±0.4	4.1±0.6	4.3±0.4
Min night h	4.5±0.4	4.7±0.6	4.8±0.8	4.2±0.6	4.4±0.6	4.5±0.6
iAUC 24h	566.9±935.2	464.8±756.9	625.7±633.4	473.2±760.0	659.3±834.9	969.0±808.8
iAUC day h	553.3±723.0	376.7±610.4	515.0±591.7	500.8±547.1	629.9±637.6	850.5±685.5
iAUC night h	-95.0±226.8	-74.1±169.4	-186.4±209.8	-69.5±138.4	-75.5±199.7	-136.2±145.9
CONGA-1 [3] **(9:00-10:00)**	0.67±0.6	0.68±0.4	1.13±0.8	1.14±0.7	0.59±0.3	0.72±0.3
CONGA-1 (22:00-23:00)	0.38±0.22	0.36±0.1	0.60±0.4	0.32±0.2	0.32±0.2	0.52±0.2
iAUC [3] **-breakfast +90**	50.3±54.4 [4]	56.3±52.0	-	95.7±70.8 [4]	66.6±42.2	-
iAUC-mid-morning snack +90	25.3±29.3	29.9±40.4	-	31.8±42.3	43.2±25.9	-
iAUC-lunch +90	34.6±40.0	-	51.4±43.9 [5]	21.5±45.0	-	102.8±74.7 [5]
iAUC-afternoon snack +90	36.8±61.0	-	-	41.7±43.1	-	-
iAUC-dinner +90	46.0±58.9	-	50.5±43.3 [6]	56.3±53.0	-	90.3±54.7 [6]
iAUC-night snack +90	17.2±21.7	25.3±26.7	9.4±45.0	35.7±32.1	21.3±33.0	23.1±21.9

[1] mean ± SD, n=9.

[2] Day h (7:00-midnight), Night h (midnight-7:00).

[3] Max (maximum), Min (minimum), CONGA-1 (continuous overall net glycemic action), iAUC (incremental area under the curve).

[4, 5, 6] There were significant differences in iAUC-breakfast + 90 on day 7, iAUC-lunch + 90 iAUC-dinner + 90 on day 9, between the regular and irregular intervention periods (paired T-test, $p < 0.05$).

No significant differences were observed in the other measurements (paired T-test).

TABLE 3. Fasting blood measurements and peak postprandial glucose and insulin concentrations over the study comparing regular and irregular meal pattern [1]

	Regular meal pattern		Irregular meal pattern	
	Pre	**Post**	**Pre**	**Post**
Total cholesterol (mmol/L)	4.22 ± 1.13	4.34 ± 1.07	4.14 ± 1.25	4.15 ± 0.92
LDL (mmol/L)	2.48 ± 1.01	2.60 ± 1.04	2.44 ± 0.97	2.48 ± 0.82
HDL (mmol/L)	1.41 ± 0.21	1.39 ± 0.23	1.31 ± 0.30	1.31 ± 0.24
Triglycerides (mmol/L)	0.74 ± 0.23	0.80 ± 0.31	0.81 ± 0.55	0.83 ± 0.32
Glucose (mmol/L)	4.6 ± 0.40	4.4 ± 0.24	4.5 ± 0.52	4.3 ± 0.55
Insulin (mIU/L)	9.64 ± 2.87	8.97 ± 2.55	10.28 ± 4.14	8.52 ± 2.95
HOMA-IR	1.98 ± 0.96	1.77 ± 0.52	2.04 ± 0.91	1.60 ± 0.57
Glucose Peak (mmol/L)	7.4 ± 0.57	6.7 ± 0.65	6.8 ± 0.55	6.9 ± 0.80
Insulin peak (mIU/L)	83.1 ± 46.49	83.1 ± 54.94	103.8 ± 78.41	71.6 ± 32.25
GLP-1 (pmol/L) [2]	3.70 ± 2.66	3.12 ± 2.63	3.95 ± 3.05	3.16 ± 2.67
PYY (pg/mL) [3]	103.46 ± 25.80	94.20 ± 21.11	117.31 ± 41.20	90.10 ± 19.51
Ghrelin (pg/mL)	1012.5 ± 174.3	1017.9 ± 177.2	985.9 ± 227.4	1041.3 ± 208.0

[1] mean ± SD, n=10.

[2] There was a significant main effect of visit on fasting plasma GLP-1 concentrations (Two-way ANOVA, $p < 0.05$).

[3] There was a significant main effect of visit on fasting plasma PYY concentrations (Two-way ANOVA, $p < 0.05$).

There were no significant differences in fasting serum lipids, blood glucose, serum insulin, HOMA-IR and plasma ghrelin concentrations across the study comparing regular and irregular meal pattern (Two-way ANOVA).

TABLE 4. Comparison of mean appetite ratings (all day points combined) on day 7 (6 meals per day) of regular and irregular meal patterns [1]

	Regular meal pattern		Irregular meal pattern	
	Pre meals	**Post meals**	**Pre meals**	**Post meals**
Hunger (mm)	46.5± 10.2	14.5± 7.0 [2]	48.8± 10.0	23.4 ±6.0 [2]
Satiety (mm)	42.2± 12.0	74.9 ±5.1	40.4± 13.1	74.6 ±5.8
Fullness (mm)	39.5± 12.2	80.6 ±4.4 [3]	40.2± 13.0	73.6 ±5.3 [3]
Desire to eat (mm)	51.8± 10.2	22.3 ±7.1	49.6± 9.8	26.0 ±6.0
Prospective food consumption (mm)	56.5± 7.7	24.9 ±8.3	54.4± 8.3	29.9 ±8.1

[1] mean ± SD, n=11.

[2] There was a significant difference in post meals hunger ratings between the regular and irregular intervention periods (paired T-test, $p < 0.05$).

[3] There was a significant difference in post meals fullness ratings between the two intervention periods (paired T-test, $p < 0.05$).

No significant differences were observed in the other VAS ratings between the two intervention periods (paired T-test).

TABLE 5. Comparison of mean appetite ratings (all day points combined) on day 14 (6 meals per day) of regular and irregular meal patterns [1]

	Regular meal pattern		Irregular meal pattern	
	Pre meals	**Post meals**	**Pre meals**	**Post meals**
Hunger (mm)	51.0± 11.5 [2]	18.9± 4.5 [3]	58.0± 8.7 [2]	22.8± 5.0 [3]
Satiety (mm)	40.7± 7.4	77.2± 2.6	44.0± 13.3	75.3± 4.7
Fullness (mm)	44.6± 13.1	75.6± 3.5	37.2± 9.0	76.0± 3.6
Desire to eat (mm)	51.3± 11.9	26.5± 4.3	58.2± 5.9	24.9± 3.9
Prospective food consumption (mm)	58.6± 9.3	30.9± 4.5	55.6± 9.3	27.9± 3.3

[1] mean ± SD, n=11.

[2] There was a significant difference in pre meals hunger ratings between the regular and irregular intervention periods (paired T-test, $p < 0.05$).

[3] There was a significant difference in post meals hunger ratings between the two intervention periods (paired T-test, $p < 0.05$).

No significant differences were observed in the other VAS ratings between the two intervention periods (paired T-test).

FIGURE 1. Study participant flow diagram.

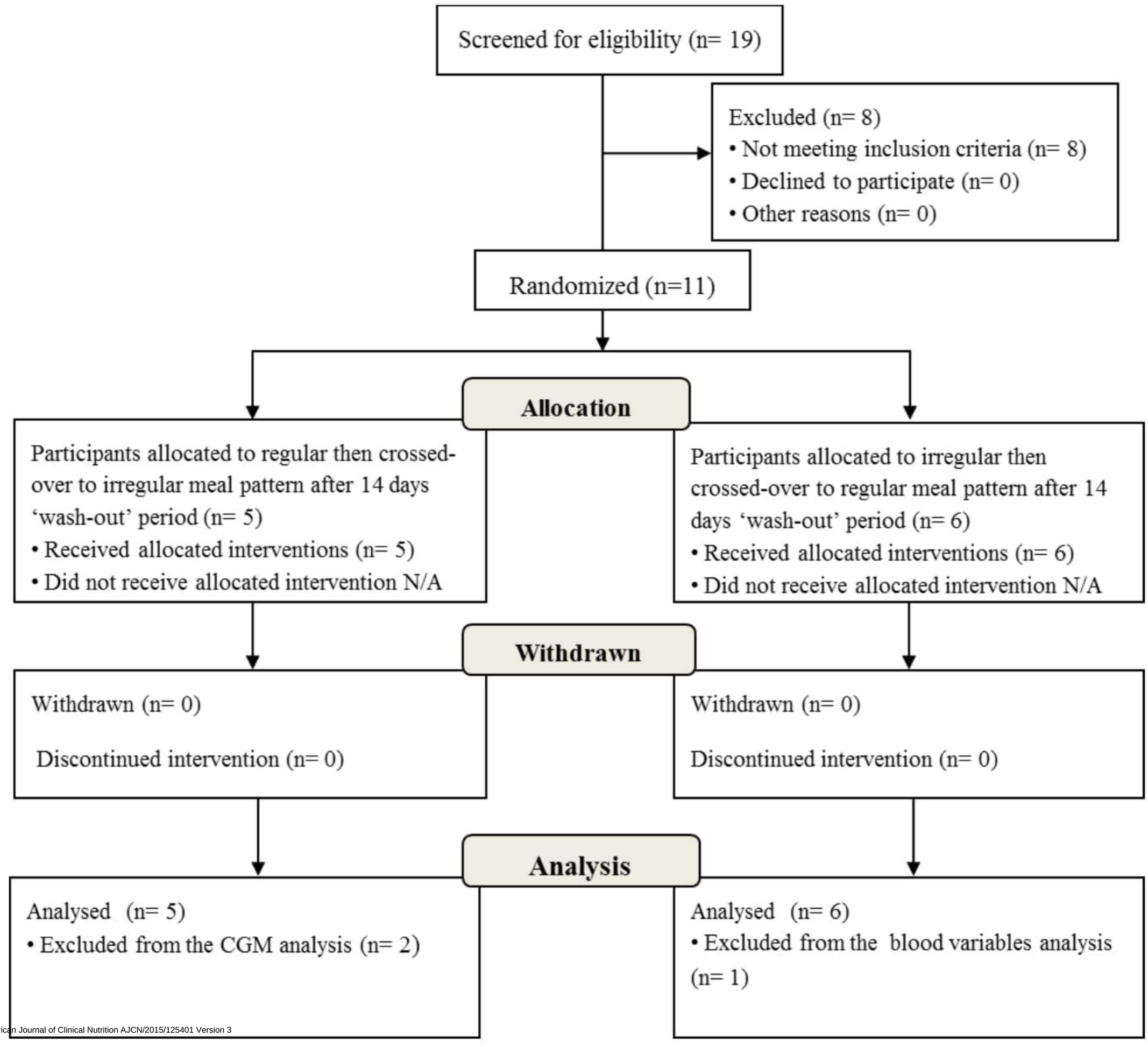
FIGURE 2. Mean (± SEM) iAUC for TEF in eleven healthy women in the visits pre and post regular and irregular meal pattern, measured by the trapezoidal method.

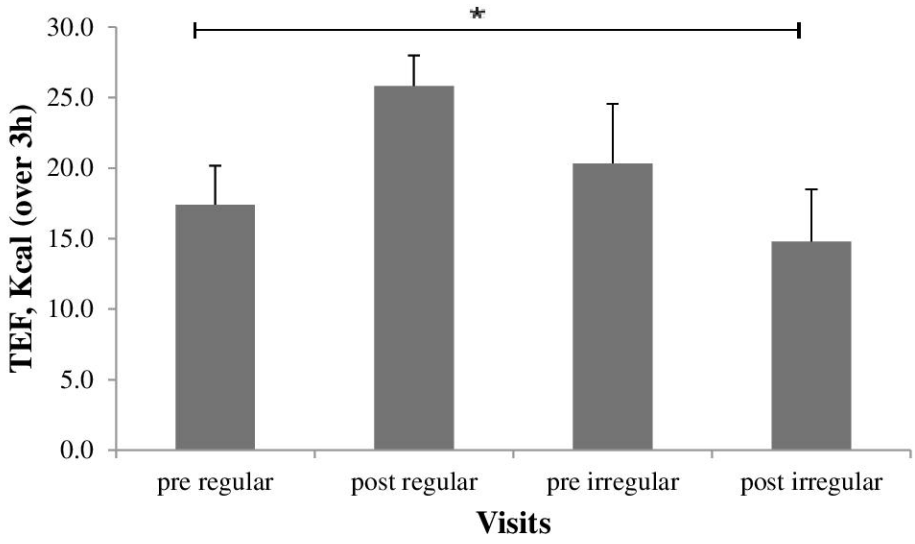
* There was a significant meal pattern by visit interaction between the regular and irregular meal pattern periods (Two-way ANOVA, $p < 0.05$). iAUC for TEF was significantly higher post-regular compared with post-irregular meal pattern ($p < 0.05$). iAUC for TEF was significantly higher post-regular compared with pre-regular meal pattern ($p < 0.05$). There was no significant difference for TEF iAUC between pre-irregular and post-irregular intervention visits.

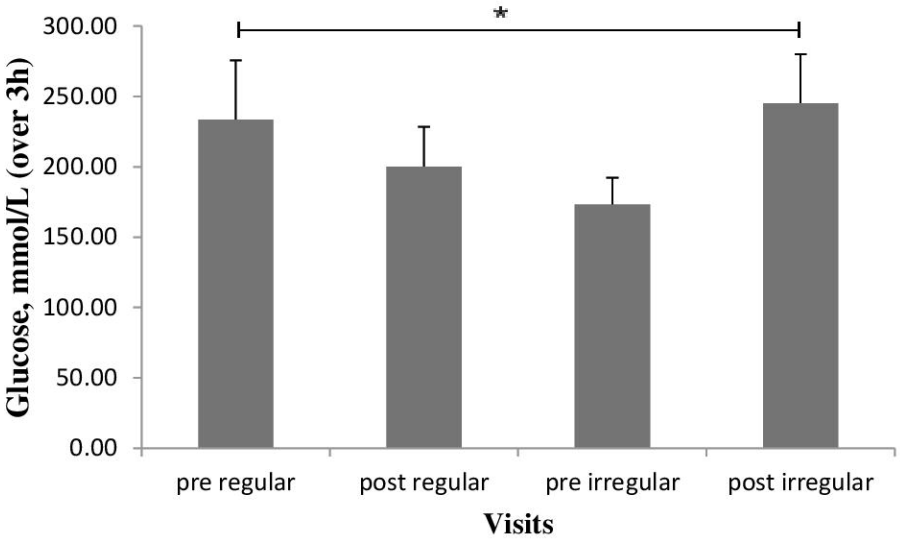
FIGURE 3. Mean iAUC for (± SEM) blood glucose concentration in ten healthy women in the visits pre and post regular and irregular intervention period, measured by the trapezoidal method.

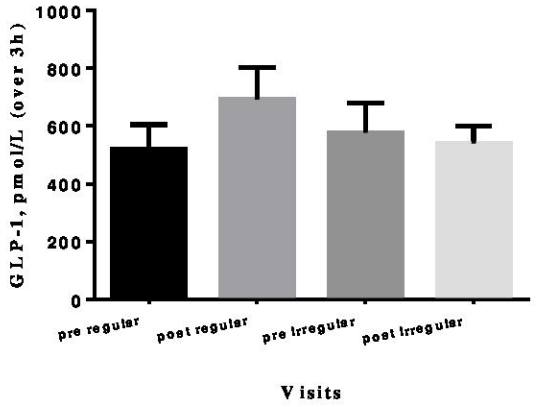
* There was a significant meal pattern by visit interaction between the regular and irregular meal pattern periods (Two-way ANOVA, $p < 0.05$). iAUC for blood glucose concentration was significantly lower post-regular compared with post-irregular meal pattern ($p < 0.05$). iAUC for blood glucose concentration was significantly higher post-irregular compared with pre-irregular meal pattern ($p < 0.05$).

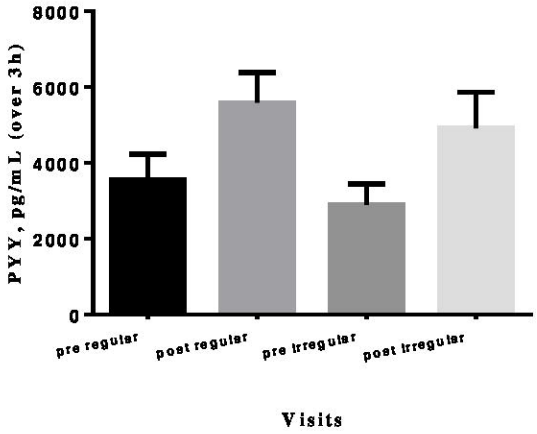
FIGURE 4. Mean (± SEM) iAUC plasma GLP-1, PYY and ghrelin concentrations in ten healthy women in the visits pre and post regular and irregular meal pattern, measured by the trapezoidal method. A significant main effect of visit was observed for iAUC plasma PYY (Two-way ANOVA, $p < 0.05$).

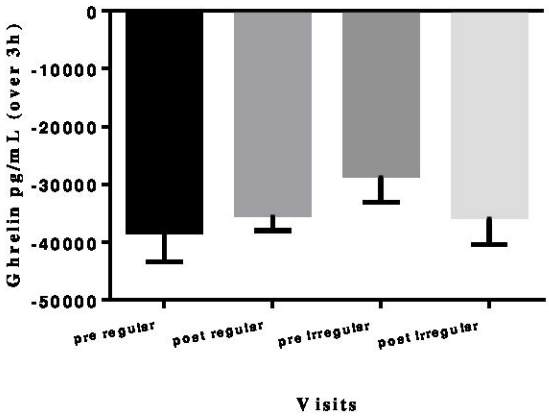

Screened for eligibility (n= 19)
Excluded (n= 8)
• Not meeting inclusion criteria (n= 8)
• Declined to participate (n= 0)
• Other reasons (n= 0)
Randomized (n=11)
Allocation
Participants allocated to regular then crossed-over to irregular meal pattern after 14 days 'wash-out' period (n= 5)
• Received allocated interventions (n= 5)
• Did not receive allocated intervention N/A
Participants allocated to irregular then crossed-over to regular meal pattern after 14 days 'wash-out' period (n= 6)
• Received allocated interventions (n= 6)
• Did not receive allocated intervention N/A
Withdrawn
Withdrawn (n= 0)
Discontinued intervention (n= 0)
Withdrawn (n= 0)
Discontinued intervention (n= 0)
Analysis
Analysed (n= 5)
• Excluded from the CGM analysis (n= 2)
Analysed (n= 6)
• Excluded from the blood variables analysis (n= 1)


*
30.0
25.0
20.0
15.0
10.0
5.0
0.0
TEF, Kcal (over 3h)
pre regular
post regular
pre irregular
post irregular
Visits


*
300.00
250.00
200.00
150.00
100.00
50.00
0.00
Glucose, mmol/L (over 3h)
pre regular
post regular
pre irregular
post irregular
Visits


GLP-1, pmol/L (over 3h)
1000
800
600
400
200
0
pre regular
post regular
pre irregular
post irregular
Visits


PYY, pg/mL (over 3h)
8000
6000
4000
2000
0
pre regular
post regular
pre irregular
post irregular
Visits


Ghrelin pg/mL (over 3h)
0
-10000
-20000
-30000
-40000
-50000
pre regular
post regular
pre irregular
post irregular
Visits