Irregular meal-pattern effects on energy expenditure, metabolism, and appetite regulation: a randomized controlled trial in healthy normal-weight women[1,2]

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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 the energy balance, and poor metabolic health.

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

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

Results: Regularity was associated with a greater TEF ($P < 0.05$) and a lower incremental area under the curve (iAUC) for glucose after intake of the test drink (over 3 h) and, for some identical meals, during the 2 interventions (over 90 min) (day 7: after breakfast; day 9: after lunch and dinner). There was no difference between treatments for the test-drink gut hormone response. A time effect was noted for fasting GLP-1, fasting PYY, PYY responses, and hunger-rating responses to the test drink ($P < 0.05$). Lower hunger and higher fullness ratings were seen premeal and postmeal during the regular period while subjects were free living.

Conclusion: Meal regularity appears to be associated with greater TEF and lower glucose responses, which may favor weight management and metabolic health. This trial was registered at clinicaltrials.gov as NCT02052076. *Am J Clin Nutr* 2016;104:21–32.

Keywords: appetite, meal regularity, metabolism, normal-weight women, thermic effect of food

INTRODUCTION

Obesity, which is an abnormally large accumulation of adipose tissue, occurs as a result of a long-term positive energy balance and has been associated with impaired metabolic function and poor health (1). A rapid increase in obesity prevalence over recent decades has occurred concurrently with the greater availability of food that requires minimal preparation both inside and outside the home and throughout the day. This environment offers a greater individual choice with respect to the time of eating and potentially facilitates a greater interdaily variation in the meal pattern. Meal-pattern research, which was initiated in the 1960s, has been based on the premise that meal pattern is a stable characteristic for an individual with the interdaily repetition of, e.g., the 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 previously undertook 14-d feeding studies that compared a regular meal pattern with an irregular meal pattern in normal-weight and obese participants (6–8). In response to a test drink, the thermic effect of food (TEF)[5] in normal-weight and obese women was significantly lower ($P < 0.05$) after an irregular meal pattern than after a regular meal pattern (6, 8). In addition, an 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 concentrations of total cholesterol and LDL cholesterol (7, 8). These results were consistent with a negative association between an

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[2] Supplemental Figure 1 and Supplemental Tables 1–3 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[5] Abbreviations used: AEEE, ambulatory energy expenditure estimation; CGM, continuous glucose monitor; COMA, Committee on Medical Aspects of Food Policy; CONGA-1, continuous overall net glycemic action; GLP-1, glucagon-like peptide 1; iAUC, incremental AUC; IPAQ, International Physical Activity Questionnaire; PYY, peptide YY; REE, resting energy expenditure; TEF, thermic effect of food; VAS, visual analog scale.

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irregular meal pattern and metabolic health that was shown in observational studies (9, 10).

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

The current study aimed to compare the impact of 14 d of more–highly controlled regular and irregular eating (all food provided) on the 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 [ambulatory energy expenditure estimation (AEEE)], continuous interstitial glucose monitoring, and subjective appetite.

METHODS

Participants

The study was conducted at the 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 a poster advertisement. Inclusion criteria for participants were as follows: normal-weight women [BMI (in kg/m^2): 18.5–25]; age: 18–40 y; nonsmokers; 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 mo (self-reported weight change less than ±2 kg). Exclusion criteria were as follows: participants 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. Of the 19 healthy normal-weight individuals who responded to the advertisement, 11 subjects were recruited to the study (**Figure 1**). These 11 participants were the individuals who met the study requirements. Values that were outside the inclusion criteria resulted in the exclusion of 4 and 2 subjects for BMI and the Eating Attitudes Test score, respectively. Two women were ineligible because they were anemic. The remaining 11 participants gave written consent, and 5 participants were scheduled to start with the regular meal pattern, and the 6 other subjects were scheduled to start with the irregular meal pattern. Blood sampling could not be performed on one participant because of problems that were 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 continuous glucose monitoring 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. This trial was registered at clinicaltrials.gov as NCT02052076.

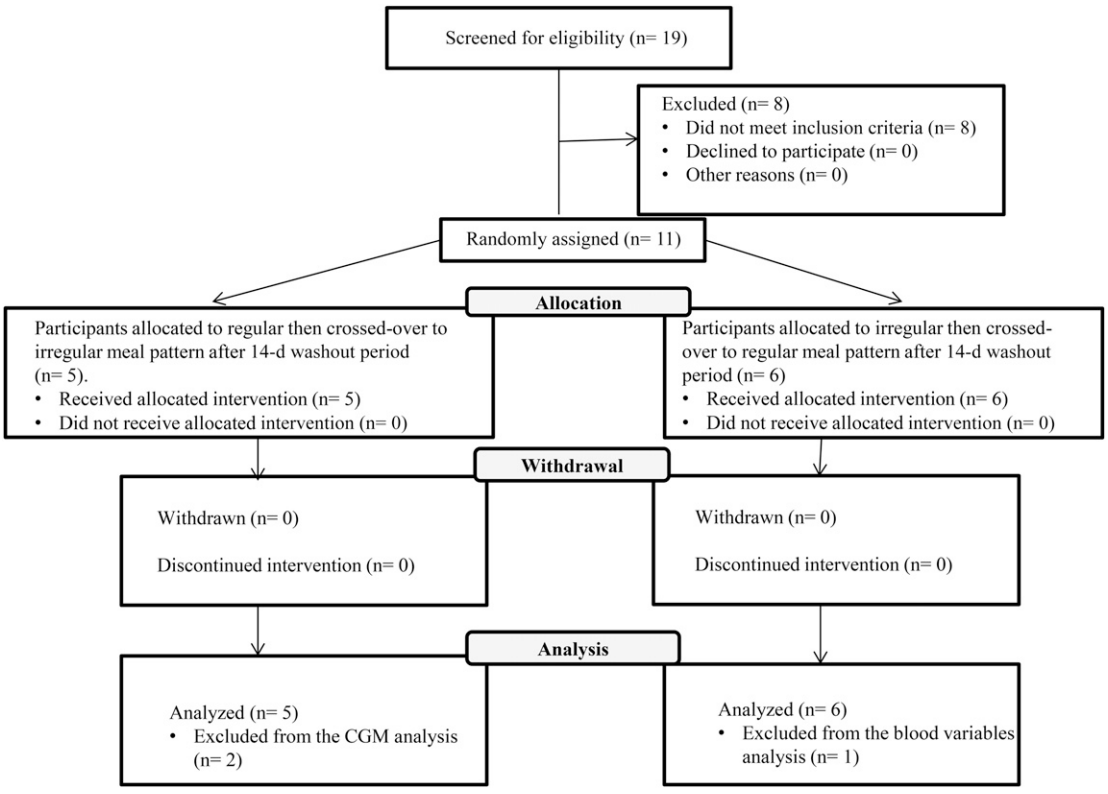

FIGURE 1 Study participant flow diagram. CGM, continuous glucose monitor.

Screening

All potential participants attended a screening visit to establish that they met the inclusion criteria for the study. Height was measured to the nearest 0.1 cm with the use of a stadiometer (Seca). Body weight was measured with the use of an electronic scale (Seca) 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 subjects.

Eligible participants were asked to complete a weighed 7-d food diary, which was used to characterize their habitual diet. They were instructed to consume their normal diets and participate at their usual levels 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 d. Participants consumed their habitual diets during the washout period, which was included to avoid an interaction between the 2 interventions. The randomization scheme was generated with the use of 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 that was provided by the experimenter. Participants attended the laboratory before and after each intervention period for a total of 4 visits. Each laboratory visit lasted ≤5 h. 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 the menstrual cycle (days 1–7).

Dietary intervention periods

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

The macronutrient composition of the diet (as a percentage of total energy per day) was ∼50% carbohydrate, 35% fat, and 15% protein. These macronutrient percentages were based on the Report of the Panel on Dietary Reference Values of the 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 leftover food in the diary that was provided. Participants were instructed to avoid alcohol consumption and to limit caffeine-containing drinks to 2 cups tea/d (without sugar or milk). Subjects were advised not to change their physical activity patterns during the study.

In accordance with the design of previous studies in our laboratory (6–8), the number of meals during the regular meal pattern was 6 meals/d, which was based on 3 meals providing ∼70% of energy requirements (breakfast, lunch, and dinner) and 3 snacks (midmorning, afternoon, and evening snacks) providing a total of ∼30% of energy requirements (**Supplemental Table 1**).

The number of meals (including eating incidences labeled as snacks on the menu) during the irregular meal pattern varied from 3 to 9 meals/d. The mean was 6 meals/d during the 14-d period (i.e., 7, 4, 9, 3, 5, 8, 6, 5, 9, 8, 3, 4, 7, and 6 meals/d, respectively). Participants were asked to eat their meals at specific times between 0800 and 2100 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 subjects consumed 3 meals/d during the irregular period, their last meal was at 1800 (instead of 2100) because it was anticipated that this time was when they would consume a meal with others in their households.

Measurements made during intervention periods

Energy-expenditure assessment

Participants wore a SenseWear Armband device (BodyMedia Inc.) to obtain an 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 the ulna. Participants were instructed to wear the device 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 an accelerometer (25). These data were used in combination with demographic characteristics including age, sex, weight, and height to estimate energy expenditure with the use of a proprietary equation developed by the manufacturer (SenseWear Software, version 7; BodyMedia Inc.), which was not published.

Continuous glucose monitoring

The continuous glucose monitor (CGM) (Medtronic Minimed) provided continuous glucose profiles for ≤72 h. Subcutaneous interstitial fluid glucose concentrations were measured every 10 s, and the average glucose value for each 5 min period was stored (≤288 measurements/d).

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 4 times/d by the participants with the use of a portable monitor (Accu-Chek Aviva System; Roche Diagnostics) to calibrate the continuous glucose monitoring. A 24-h contact number was available for any inquiries or if any problems arose. Data from the CGM were downloaded, and glucose profiles were evaluated on the basis of data collected on day 7 (6 meals/d in both regular and irregular periods), day 8 (6 compared with 5 meals/d in regular and irregular periods, respectively), and day 9 (6 compared with 9 meals/d in regular and irregular periods, respectively). Data were analyzed per 24 h during the day (0700–2359) and during the night (2400–0659) with respect to the 24-h mean, maximum, minimum, and incremental AUC (iAUC) of glucose for each time period.

On day 7 (6 meals/d in both regular and irregular interventions), the postprandial iAUC for 90 min was analyzed after each meal (breakfast, midmorning snack, lunch, afternoon snack, dinner, and evening snack). However, on day 8 (6 compared with 5 meals/d in regular and irregular interventions, respectively), the analysis was restricted to the points in the day when participants consumed identical meals during the 2 interventions (breakfast, midmorning snack, and evening snack).The afternoon snack was omitted during the irregular period, and the previously omitted foods, with respect to energy, were equally distributed between lunch and dinner. On day 9 (6 compared with 9 meals/d in regular and irregular periods, respectively), the analysis similarly was restricted to lunch, dinner, and the evening snack. The breakfast was divided into 2 meals during the irregular period. Midmorning and afternoon snacks were also divided into 2 small meals to achieve 9 meals/d.

The intraday glycemic variability was computed with the use of an approach described by McDonnell et al. (26) specifically for continuous glucose monitoring data that is known as the continuous overlapping net glycemic action. The continuous overlapping net glycemic action (CONGA-1) was calculated as the SD of the summed differences in glucose concentrations between each current observation and the observation n hours previous for a period of n hours. CONGA-1 was calculated in the morning (each current observation from 0900 to 1000) and night (each current observation from 2200 to 23:00). The CONGA-1 indicated the intraday glycemic variability on the basis of 1-h time periods.

Appetite assessment

Subjective appetite ratings were assessed with the use of paper-based visual analog scales (VASs) with words anchored at each end of a 100-mm horizontal line that expressed the most-positive rating and the most-negative rating for a question (**Supplemental Figure 1**). 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?" (27).

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

Laboratory-visit protocol and procedures

Participants were asked to attend the laboratory at 0800 after ≥12 h of an 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/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. Once baseline measurements were completed, 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 1230. Subjective appetite ratings were measured with the use of VASs 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) to the nearest 0.1 kg with an empty bladder, wearing similar light clothes at 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 with the use of a stretch-resistant tape while the participant was standing with feet ∼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 in triplicate by the same investigator at 4 sites (triceps, biceps, subscapular, and suprailiac) to assess the body composition of participants (29).

Blood sampling

After anthropometric measurements were taken, participants rested in a semisupine position in a temperature-controlled (23–24°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 subject's hand was placed in a hot, air-warmed, ventilated perspex box (50–55°C) to allow arterialized venous blood sampling (30). 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 serum total cholesterol, HDL cholesterol, LDL cholesterol, triacylglycerol, blood glucose, serum insulin, plasma glucagon-like peptide 1 (GLP-1), plasma peptide YY (PYY), and plasma 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, 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 × g at 4°C. The supernatant fluid was transferred into plastic tubes and kept at −80°C until further analysis.

Blood analysis

Analyses were carried out at the University of Nottingham. Serum total cholesterol, HDL-cholesterol, LDL-cholesterol, and triacylglycerol concentrations were quantified with the use of an

enzymatic photometric method (HORIBA ABX). Blood glucose was measured immediately with the use of a HemoCue analyzer (AB). Serum insulin concentrations were measured with commercially available radioimmunoassays (Millipore). Fasting insulin sensitivity was calculated with the use of homeostatic model assessment (31). Plasma GLP-1 concentrations were measured with the use of an ELISA kit (Linco Research). Plasma PYY and ghrelin concentrations were measured with commercially available radioimmunoassays (Millipore).

Test-drink consumption

The standardized test drink (vanilla-flavor 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% of energy as fat, and 15% of energy 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), Build-up (a milk-based sweet supplement fortified with vitamins and minerals; Nestle SA), Polycal (a nonsweet, unflavored carbohydrate supplement powder; Nutricia Clinical Care), and double cream (Sainsbury's).

Energy-expenditure measurement

Indirect calorimetry (GEM system; Europa Scientific Ltd.) was used to determine the resting energy expenditure (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 vacuum pump, was used to draw room air over the participant's face at a rate of 50–60 L/min. This method 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 analyzers were used to calculate the $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ with the use of the software provided by the manufacturer. The indirect calorimetry system was turned on for 0.5 h before use to warm up. Two cylinders of pressurized gas of a known composition were used to calibrate the gas analyzers in the indirect calorimetry system before the start of the experiment. REE was measured in the fasted state for 20 min. The TEF was measured for periods of 15 min at 30-min intervals during the 3 h after milkshake consumption. During the measurements, participants rested on beds and relaxed but were not permitted to sleep. In the intervals between measurements, subjects also rested on the beds, 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 167 kcal/100 g with 13%, 34%, and 53% of energy provided by protein, fat, and carbohydrate, respectively) was served at lunchtime to assess ad libitum food intake. The meal had a homogeneous nature, and thus, energy intake could be assessed from the weight of food consumed. The meal consisted of pasta (125 g; Sainsbury's) that was cooked in 800 mL boiling water at full power in a microwave (900 W) for 13 min and stirred during the midperiod. The pasta was drained, cooled rapidly with cold water, and mixed with cheddar cheese (40 g; Sainsbury's), olive oil (15 g; Sainsbury's), and tomato and basil pasta sauce (170 g, Dolmio; Mars Food) (the macronutrient composition of the sauce is shown in **Supplemental Table 2**). The mixture was kept chilled until required and heated in the microwave for 2 min before being served to the participants. Participants were given ∼500-g portions and were 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 three-quarters empty, which ensured that there was always ample hot food available to participants and that they were not cued to stop eating by having emptied their plate. Any leftover food was removed, and energy intake was calculated from the weight of food consumed. The 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 having 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

SPSS software (version 21 for Windows; SPSS) was used for data entry and analyses. All data are presented as means ± SDs unless otherwise stated. Data were tested for normality with the use of the Kolmogorov-Smirnov test to inform whether a parametric or nonparametric analysis should be used.

Values for the iAUC of the TEF, postprandial glucose, insulin, appetite ratings, and gut hormone responses were calculated with the use of differences from baseline. Values greater than baseline values were considered to be positive, and values less than baseline values were considered to be negative. The area above or below baseline was calculated with the use of the trapezoid rule.

Comparisons of baseline data at the preintervention visit were made with the use of Student's paired *t* test (2 tailed) as were measurements of energy intake, AEEE, 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 iAUC for the TEF, and the weight of pasta consumed). 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. Differences were considered significant at $P < 0.05$ for all statistical tests.

Results obtained from a previous study (6) indicated that the iAUC for the TEF after a regular meal pattern was 0.74 ± 0.37 kJ/min, and after an irregular meal pattern, it was 0.39 ± 0.26 kJ/min. Therefore, with a crossover design, 11 participants in

each group were required to detect a difference in the TEF (~0.35 kJ/min) with a power of 80% at the significance level of 0.05.

The TEF (kcal/min) over 3 h (after the test drink), as assessed by indirect calorimetry, was the primary outcome for the comparison between the 2 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 the 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 d; 6 meals/d) or an irregular meal pattern (14 d; varying from 3 to 9 meals/d) in a randomized crossover design that was separated by a 14-d washout 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 body weight, body composition, or other anthropometric measurements at preintervention visits or across study visits (**Table 1**).

Energy intake

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

During the study, food intake was designed to be the same by type and amount in each intervention period, hence providing the same amount of energy and having the same macronutrient composition. Food-intake diaries that were 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, which indicated good compliance. There were no significant differences in energy intake between the 2 intervention periods (2043 ± 248 kcal/d in the regular intervention period compared with 2098 ± 195 kcal/d in the irregular intervention period) as intended by the design of the study. The composition of consumed foods also did not differ significantly between the 2 intervention periods (53% ± 0.9% of carbohydrate, 14% ± 0.4% of protein, and 33% ± 0.8% of fat in the regular intervention period and 53% ± 0.3% of carbohydrate; 14% ± 0.5% of protein, and 33% ± 0.7% of fat in the irregular intervention period).

TABLE 1
Characteristics of participants 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 ratio	0.7 ± 0.6	0.7 ± 0.6	0.7 ± 0.6	0.7 ± 0.6

[1]All values are means ± SDs. $n = 11$. There were no significant differences in the characteristics of the 11 participants across the study for the comparison of regular and irregular meal patterns (2-factor ANOVA). Post, postintervention; Pre, pre-intervention.

Free-living energy expenditure

On average, the SenseWear Armband device was worn 96.8% ± 5.5% and 95.1% ± 7.7% of regular and irregular intervention periods, respectively. There were no significant differences between mean values of the AEEE during the intervention period for both regular and irregular meal patterns (2241 ± 360 and 2305 ± 399 kcal/d for regular and irregular intervention periods, respectively). There were no significant differences between the mean physical activity level during regular and irregular intervention periods (1.60 ± 0.2 and 1.64 ± 0.2 times REE for regular and irregular intervention periods, respectively). In both conditions, the estimated energy expenditure was ~200 kcal greater than the prescribed energy requirement.

Free-living continuous glucose monitoring

For 9 participants for whom continuous glucose monitoring data were available, analyses (mean, maximum, minimum, CONGA-1, and iAUC) were done for each meal pattern on day 7 (6 meals were consumed in both intervention periods), day 8 (6 and 5 meals were consumed in regular and irregular periods, respectively), and day 9 (6 and 9 meals were consumed in regular and irregular periods, respectively) (**Table 2**). The 24-h mean, maximum, minimum, and iAUC for glucose concentrations showed no significant differences between the 2 intervention periods. There were also no significant differences in the day and night periods between the 2 interventions for these variables. The CONGA-1 in the current observation period from 0900 to 1000 and from 2200 to 2300 also showed no significant differences between the 2 intervention periods.

On day 7 of the intervention (6 meals/d in both interventions), there was a significantly higher glucose concentration for the postprandial breakfast period (breakfast + 90 min) iAUC analysis (Table 2) in the irregular meal-pattern intervention than in the regular meal-pattern intervention (paired *t* test, $P < 0.05$). On day 9 (6 compared with 9 meals), for the meals that were identical in the 2 interventions, the postprandial lunch period (lunch + 90 min) and postprandial dinner period (dinner + 90 min) iAUC analyses showed a similar difference in that the iAUC in the irregular intervention was significantly higher than in the regular intervention (paired *t* test, $P < 0.05$). No significant differences were seen in the other postprandial iAUC analysis.

Energy expenditure (indirect calorimetry data)

The fasting REE was not significantly different at the preintervention visits. There was also no interaction of the meal pattern by visit or the main effect of the meal pattern or visit for the fasting REE (1167 ± 134, 1207 ± 89, 1183 ± 171, and 1188 ± 149 kcal/d in

TABLE 2
Analyses of continuous glucose monitoring data compared between the 2 meal-pattern interventions[1]

	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
Glucose, mmol/L						
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
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
Day hours	5.3 ± 0.7	5.4 ± 0.5	5.5 ± 0.5	5.3 ± 0.4	5.3 ± 0.4	5.6 ± 0.3
Night hours	4.9 ± 0.3	5.2 ± 0.6	5.2 ± 0.8	4.9 ± 0.6	5.0 ± 0.4	5.1 ± 0.5
Maximum, h						
24	7.1 ± 1.0	7.1 ± 1.4	7.9 ± 1.5	7.5 ± 1.4	7.2 ± 0.8	7.9 ± 1.2
Day	7.1 ± 1.0	7.1 ± 1.4	7.9 ± 1.5	7.5 ± 1.3	7.2 ± 0.8	7.9 ± 1.2
Night	5.5 ± 0.4	5.8 ± 0.8	5.9 ± 0.7	5.8 ± 1.0	5.5 ± 0.5	5.8 ± 0.6
Minimum, h						
24	4.1 ± 0.8	4.3 ± 0.5	4.1 ± 0.5	3.8 ± 0.4	3.9 ± 0.5	4.1 ± 0.5
Day	4.1 ± 0.8	4.3 ± 0.5	4.1 ± 0.6	4.3 ± 0.4	4.1 ± 0.6	4.3 ± 0.4
Night	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, h						
24	566.9 ± 935.2	464.8 ± 756.9	625.7 ± 633.4	473.2 ± 760.0	659.3 ± 834.9	969.0 ± 808.8
Day	553.3 ± 723.0	376.7 ± 610.4	515.0 ± 591.7	500.8 ± 547.1	629.9 ± 637.6	850.5 ± 685.5
Night	−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						
0900–1000	0.67 ± 0.6	0.68 ± 0.4	1.13 ± 0.8	1.14 ± 0.7	0.59 ± 0.3	0.72 ± 0.3
2200–2300	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, +90 min						
Breakfast	50.3 ± 54.4[2]	56.3 ± 52.0	—	95.7 ± 70.8[2]	66.6 ± 42.2	—
Midmorning snack	25.3 ± 29.3	29.9 ± 40.4	—	31.8 ± 42.3	43.2 ± 25.9	—
Lunch	34.6 ± 40.0	—	51.4 ± 43.9[2]	21.5 ± 45.0	—	102.8 ± 74.7[2]
Afternoon snack	36.8 ± 61.0	—	—	41.7 ± 43.1	—	—
Dinner	46.0 ± 58.9	—	50.5 ± 43.3[2]	56.3 ± 53.0	—	90.3 ± 54.7[2]
Night snack	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]All values are means ± SDs. $n = 9$. Day hours were from 0700 to 2359. Night hours were from 2400 to 0659. CONGA-1, continuous overall net glycemic action; iAUC, incremental AUC.

[2]Significant difference between regular and irregular intervention periods on the day indicated, $P < 0.05$ (paired t test). No significant differences were observed in the other measurements (paired t test).

the before and after regular intervention visits and the before and after irregular intervention visits, respectively).

The REE increased above fasting values after consumption of the test drink in all visits. The overall TEF for the 3-h postprandial period is shown in **Figure 2**. There was no significant difference in the overall 3-h TEF at preintervention visits. There was a significant interaction of the meal pattern by visit for the 3-h TEF (ANOVA, $P < 0.05$). The TEF after the regular intervention was increased significantly compared with that before the regular intervention (paired t test, $P < 0.01$). This result was unlike that for the irregular visits, for which there was no significant difference between preintervention and postintervention visits. The TEF after the regular intervention was 11.1 ± 15.8 kcal higher than after the irregular intervention (paired t test, $P < 0.05$).

Blood variables

There were no significant differences for preintervention visits for all blood variables.

Lipids

Results for fasting serum total cholesterol, LDL cholesterol, HDL cholesterol, and serum triglycerides are shown in **Table 3**. There were no significant interactions for the meal pattern by visit or for the main effect of the meal pattern or visit in fasting serum total cholesterol, LDL cholesterol, HDL cholesterol, or serum triglycerides.

Glucose

No significant interaction of the meal pattern by visit or main effect of the meal pattern or visit were observed in fasting blood glucose across the study (Table 3). Blood glucose concentrations reached a maximum at 30 and 45 min after the test drink and remained above fasting concentration at the last sampling time point (180 min after the test drink) in all visits. Peak values (Table 3) did not show a significant interaction for the meal pattern by visit or for the main effects for these 2 factors. The blood glucose iAUC response to the test drink (**Figure 3**) showed a significant interaction between the meal pattern and visit (ANOVA, $P < 0.05$). A larger area was seen at after the irregular intervention than after the regular intervention ($P < 0.05$). After the irregular intervention, the blood glucose iAUC was significantly higher than before the irregular intervention ($P < 0.05$), unlike for the regular intervention, whereby there was no significant difference between before and after the regular intervention.

Insulin

Table 3 shows fasting serum insulin concentrations in all visits. There were no significant interactions for the meal pattern by visit

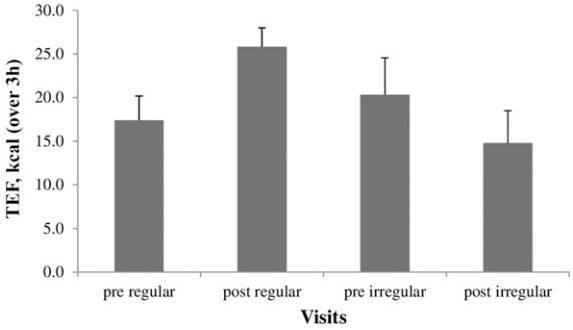

FIGURE 2 Mean ± SEM iAUCs for the TEF in 11 healthy women in the visits before and after regular and irregular meal pattern, which were measured with the use of the trapezoidal method. There was a significant meal pattern–by–visit interaction between regular and irregular meal-pattern periods (2-factor ANOVA, $P < 0.05$). The iAUC for the TEF was significantly higher after the regular meal pattern than after the irregular meal pattern ($P < 0.05$). The iAUC for the TEF was significantly higher after the regular meal pattern than before the regular meal pattern ($P < 0.05$). There was no significant difference for the TEF iAUC between pre–irregular and post–irregular intervention visits. iAUC, incremental AUC; TEF, thermic effect of food.

or for the main effect of the meal pattern or visit. Serum insulin concentrations increased rapidly from 15 min after consumption of the test drink in all visits. After peak values, concentrations declined to some extent but remained above fasting values for the reminder of the sampling period. Peak values of insulin (Table 3) did not show a significant meal pattern–by–visit interaction or main effect of the meal pattern or visit. There was no significant interaction between the meal pattern and visit for the iAUC of serum insulin, and there was no significant main effect for the meal pattern or visit (5826.2 ± 2150.5 mIU/L in 3 h before the regular intervention, 5719.4 ± 3326.6 mIU/L in 3 h after the regular intervention, 5842.6 ± 3775.2 mIU/L in 3 h before the irregular intervention, and 5268.9 ± 2248.0 mIU/L in 3 h after the irregular intervention).

GLP-1

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

PYY

No significant meal pattern–by–visit interaction or main effect of the meal pattern was observed in fasting plasma PYY concentrations (Table 3). However, there was a significant main effect of the visit (ANOVA, $P < 0.05$). Mean fasting plasma PYY concentrations decreased by ∼9% and ∼23% after the regular and irregular interventions, respectively, compared with before the interventions.

Plasma PYY concentrations increased rapidly above fasting values after the consumption of the test drink and remained at a plateau until the last sampling time point in all visits. The iAUC for the 3-h postprandial period in all visits (Figure 4) showed no significant interaction between the meal pattern and visit or for a main effect of the meal pattern. However, there was a significant main effect of the visit (ANOVA, $P < 0.05$). The mean iAUC for plasma PYY concentrations increased by ∼57% after the regular intervention compared with before the regular intervention and by 70% after the irregular intervention compared with before the irregular intervention.

Ghrelin

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

TABLE 3
Fasting blood measurements and peak postprandial glucose and insulin concentrations over the study for the comparison of regular and irregular meal patterns[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	3.70 ± 2.66	3.12 ± 2.63	3.95 ± 3.05	3.16 ± 2.67
PYY, pg/mL	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]All values are means ± SDs. $n = 10$. There was a significant main effect of the visit on fasting plasma GLP-1 and PYY concentrations, $P < 0.05$ (2-factor ANOVA). There were no significant differences in fasting serum lipids, blood glucose, serum insulin, HOMA-IR, and plasma ghrelin concentrations across the study for the comparison of regular and irregular meal patterns (2-factor ANOVA). Post, postintervention; Pre, pre-intervention.

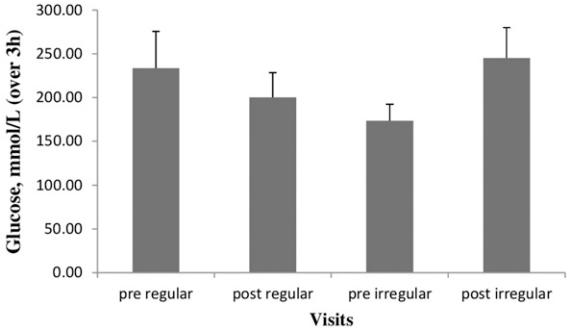

FIGURE 3 Mean ± SEM iAUCs for blood glucose concentration in 10 healthy women in the visits before and after regular and irregular intervention periods, which were measured with the use of the trapezoidal method. There was a significant meal pattern–by–visit interaction between regular and irregular meal-pattern periods (2-factor ANOVA, $P < 0.05$). The iAUC for the blood glucose concentration was significantly lower after the regular meal pattern than after the irregular meal pattern ($P < 0.05$). The iAUC for the blood glucose concentration was significantly higher after the irregular meal pattern than before the irregular meal pattern ($P < 0.05$). iAUC, incremental AUC.

between the meal pattern and visit or for main effect for the meal pattern or visit.

Subjective appetite ratings

Responses to the test drink

There were no significant differences between the preintervention visits for any of the iAUCs for subjective appetite ratings that were collected in the fasting state (**Supplemental Table 3**). There was also no meal pattern–by–visit interaction or main effect of the meal pattern or visit for fasting VAS ratings (Supplemental Table 3). Assessments of subjective hunger for the 3-h postprandial period in all visits showed no significant interaction between the meal pattern and visit or of a main effect for the meal pattern, but a significant main effect of the visit (ANOVA, $P < 0.05$) was shown. Mean hunger ratings decreased by 195% and 104% after regular and irregular interventions, respectively, compared with at preintervention visits (Supplemental Table 3). The response for the other VAS ratings showed no significant differences between 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 the meal pattern and visit or a main effect for the meal pattern or visit (Supplemental Table 3).

Responses to the meal pattern during the intervention

Subjective appetite ratings were assessed before and after the consumption of meals during days 7 and 14 when 6 meals/d were consumed in both regular and irregular intervention periods. On day 7, there were no significant differences between mean premeal ratings (average of the 6 premeal ratings on the day) (**Table 4**). However, mean postmeal ratings for hunger and fullness showed significant differences between interventions. Higher postmeal ratings for hunger and lower postmeal ratings for fullness (paired t test, $P < 0.01$) were observed in irregular compared with regular intervention periods (Table 4).

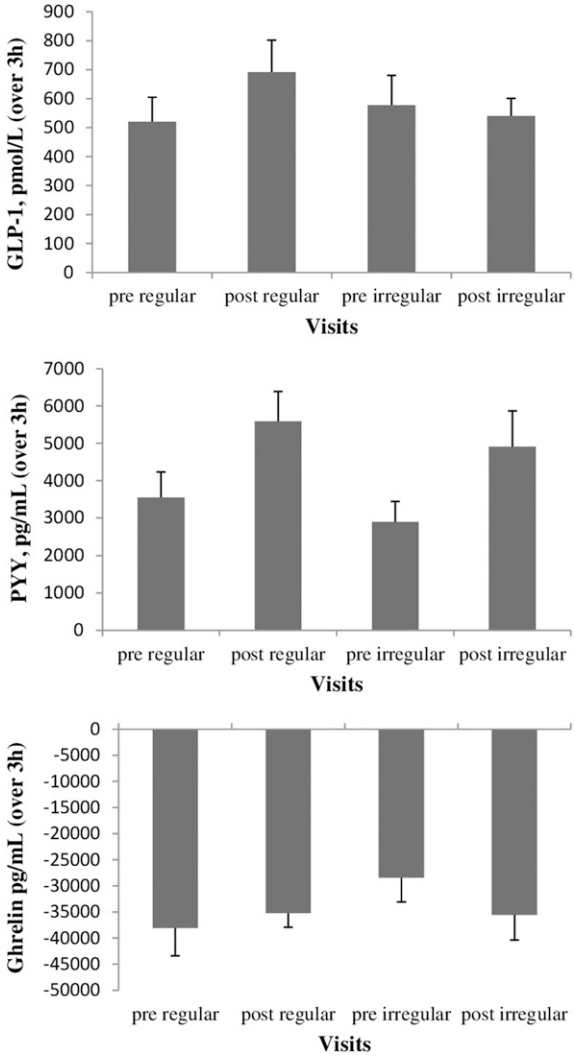

FIGURE 4 Mean ± SEM iAUCs for plasma GLP-1, PYY, and ghrelin concentrations in 10 healthy women in the visits before and after regular and irregular meal pattern, which were measured with the use of the trapezoidal method. A significant main effect of the visit was observed for the iAUC for plasma PYY (2-factor ANOVA, $P < 0.05$). GLP-1, glucagon-like peptide 1; iAUC, incremental AUC; PYY, peptide YY.

On day 14 (the final day of the intervention), ratings of premeal hunger were significantly greater in irregular than regular intervention periods (paired t test, $P < 0.05$) (**Table 5**). Furthermore, the ratings of postmeal hunger were significantly greater in the irregular period (paired t test, $P < 0.05$) (Table 5). There were no significant differences in premeal and postmeal values for the other VAS appetite ratings.

Intake of the ad libitum test meal

There was no significant difference between participants' energy intakes at the ad libitum test-meal preintervention visits. There was no meal pattern–by–visit interaction or a main effect of the meal pattern or visit for participants' energy intakes across study visits (778.8 ± 272.8, 745.7 ± 214.7, 722.4 ± 324.0, and 764.3 ± 246.6 kcal for before and after regular and irregular interventions, respectively).

The duration of eating and speed of consumption of the ad libitum test meal were not significantly different at preintervention

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

	Regular meal pattern		Irregular meal pattern	
	Premeal	Postmeal	Premeal	Postmeal
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[2]	40.2 ± 13.0	73.6 ± 5.3[2]
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]All values are means ± SDs. $n = 11$. Unless otherwise indicated, there were no significant differences observed in visual analog scale ratings between the 2 intervention periods (paired t test).
[2]Significant difference between regular and irregular intervention periods, $P < 0.05$ (paired t test).

visits. The duration of eating did not show a significant interaction between the meal pattern and visit or a main effect of the meal pattern or visit (9.6 ± 3.9, 9.8 ± 3.8, 9.5 ± 3.1, and 9.1 ± 2.3 min before and after regular and irregular interventions, respectively). The 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 before and after regular and irregular interventions, respectively).

DISCUSSION

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

No differences were shown in body weight between the 2 interventions, which suggested that the aim to match intake and activity was met. With the regular meal pattern, the TEF was greater, whereas the postprandial glucose response was smaller both in response to a test drink and in response to some identical meals while free-living. No differences were shown in fasting lipid values. PYY showed a greater postprandial response after both interventions concurrently with anticipated differences in hunger and fullness. Pre-appetite and postappetite ratings during the regular intervention suggested that participants experienced greater fullness and reduced hunger.

Differences in the TEF were compatible with our previous findings (6, 8). Compensation in other components of energy expenditure might explain the similar body weights seen after the 2 interventions despite differences in the TEF. However, there was no difference in the REE, and although the estimate of ambulatory energy expenditure that was made with the use of the SenseWear Armband device had limitations [e.g., the absence of published validated equations for this population group and inconsistent findings compared with the use of indirect calorimetry (25, 33–35)], it gave an indication of comparable activity patterns. The short duration of the study was a more likely explanation because, over a longer time period, the greater TEF with a regular meal pattern could have beneficial effects on weight control if repeated at all meals and in the longer term. The range of published values for the TEF of diets that contained comparable macronutrient compositions made estimating the expected TEF from the test drink problematic (36). However, with the use of a generally accepted figure for the TEF of 10% of total energy consumed and a mean test drink dose of 584 kcal, a TEF of ~60 kcal might be expected. The smaller values seen (over 3 h) may reflect that the full metabolic rate response had not occurred in 3 h. It has been estimated that weight gain in 90% of the adult population could be prevented by reducing the positive energy balance by 100 kcal/d (37), and Brown et al. (38) showed that, over 5 y, a 10-kcal/d excess in energy intake resulted in a 0.5-kg gain in weight per year. Future work should assess energy expenditure over 24 h to capture the full response to each meal and the accumulative effect of more than one meal during the day.

Insulin resistance has been shown to be associated with a blunted TEF (39–41) and may contribute to the differences we showed. In this study, a lower postprandial glucose response to the test meal was seen after the regular meal pattern than after the irregular meal pattern. In our previous studies (7, 8), there was

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

	Regular meal pattern		Irregular meal pattern	
	Premeal	Postmeal	Premeal	Postmeal
Hunger,[2] mm	51.0 ± 11.5	18.9 ± 4.5	58.0 ± 8.7	22.8 ± 5.0
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]All values are means ± SDs. $n = 11$. Unless otherwise indicated, there were no significant differences observed in visual analog scale ratings between the 2 intervention periods (paired t test).
[2]Significant differences between premeal regular and irregular intervention periods and between postmeal regular and irregular intervention periods, $P < 0.05$ (paired t test).

no difference in the glucose response, but a greater post prandial insulin response was seen after the irregular meal-pattern period. Both of these patterns of results were consistent with the regular meal pattern resulting in greater insulin sensitivity. To our knowledge, the novel addition to the current study of continuous interstitial glucose measurements on 3 d during the intervention periods (each of which were preceded by the same last meal on the previous day) further corroborated reduced insulin sensitivity with an irregular pattern. Day 7 allowed for the direct comparison of 6 meals/d and showed a beneficial response to breakfast with regular eating. However, on day 8, despite having several identical meals, no differences were shown, perhaps because of an acute effect of the preceding day that was identical for both patterns (6 meals/d). On day 9, for those meals that were identical, a beneficial reduction in the postprandial response at lunch and dinner (but not for the night snack) was seen for the regular pattern. Additional work is needed to establish whether, under laboratory conditions, a comparable difference in the 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 2 meal patterns in the current study in agreement with previous studies in normal-weight and obese women (7, 8). However, previous differences were shown between fasting total and LDL cholesterol (7) in contrast with the results of this study. These difference were perhaps due to better-controlled food intake 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, which possibly resulted in differences in sensitivity to the meal pattern.

Greater postmeal ratings for hunger and lower ratings of fullness on day 7 (6 meals/d in both interventions) during the irregular meal-pattern period suggested a reduction in the satiation experienced. In addition, greater premeal and postmeal ratings for hunger were observed on the final day of the irregular meal pattern when 6 meals were again consumed in both interventions, which suggested 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 in response to the pasta meal. The energy intake of pasta consumed at the ad libitum test meal in the laboratory was decreased by 4% after the regular intervention and increased by 6% after the irregular intervention. This did not reach significance, possibly because the study was insufficiently powered for this secondary outcome.

Although no meal-pattern effect was shown for fasting plasma GLP-1 and PYY concentrations, a main effect of time was seen in response to the test meal for PYY. The explanation for these differences, like the time effect previously noted for subjective appetite, may be the differences in the compositions of the habitual diet and the intervention diet. The 7-d food record suggested that the habitual diet contained a lower percentage of carbohydrate and a higher percentage of fat. In addition, before the final visit on day 14, the number of meals and amounts of food were the same in both interventions, in contrast with the first visits when habitual diets were consumed the preceding day. The stage in the menstrual cycle was also different because the study started in the early phase of the follicular phase, which may have had an effect on appetite (42, 43) and GLP-1 (42). Differences observed in PYY in response to the test drink were consistent with the differences in VAS hunger responses, which confirmed the inverse relation between PYY and subjective hunger (44). Because the differences in subjective appetite that were noted while subjects were free living in this study might offer an explanation for the higher energy intake previously noted in obese participants who ate ad libitum while following an irregular meal pattern (8), this aspect warrants further work with a larger sample size. As shown with respect to the TEF, small differences in energy intake, sustained over the long term, can have a major impact on weight regulation. In addition, associations have been shown between the TEF and satiety (45), suggesting that there may be some interrelation between differences in subjective appetite and the blunted TEF measured in this study.

In conclusion, the findings of this study show that a regular meal pattern compared with an irregular meal pattern results in a 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 obese participants with and without type II diabetes and should include 24-h measurements and longer-term interventions.

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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. IAM 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, member of the Mars Scientific Advisory Council, member of the Mars Europe Nutrition Advisory Board, and Scientific Adviser to the Waltham Centre for Pet Nutrition and has a UK Government Research Grant (from Innovate UK) for a project that is led by Mars UK. IAM is also the Academic lead for the University of Nottingham's strategic research partnership with Unilever. The remaining authors reported no conflicts of interest related to the study.

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