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Beneficial metabolic effects of regular meal frequency on dietary thermogenesis, insulin sensitivity, and fasting lipid profiles in healthy obese women[1–3]

Hamid R Farshchi, Moira A Taylor, and Ian A Macdonald

ABSTRACT

Background: Although a regular meal pattern is recommended for obese people, its effects on energy metabolism have not been examined.

Objective: We investigated whether a regular meal frequency affects energy intake (EI), energy expenditure, or circulating insulin, glucose, and lipid concentrations in healthy obese women.

Design: Ten women [$\bar{x} \pm$ SD body mass index (in kg/m^2): 37.1 $\pm$ 4.8] participated in a randomized crossover trial. In phase 1 (14 d), the subjects consumed their normal diet on 6 occasions/d (regular meal pattern) or followed a variable meal frequency (3–9 meals/d, irregular meal pattern). In phase 2 (14 d), the subjects followed the alternative pattern. At the start and end of each phase, a test meal was fed, and blood glucose, lipid, and insulin concentrations were determined before and for 3 h after (glucose and insulin only) the test meal. Subjects recorded their food intake on 3 d during each phase. The thermogenic response to the test meal was ascertained by indirect calorimetry.

Results: Regular eating was associated with lower EI ($P < 0.01$), greater postprandial thermogenesis ($P < 0.01$), and lower fasting total (4.16 compared with 4.30 mmol/L; $P < 0.01$) and LDL (2.46 compared with 2.60 mmol/L; $P < 0.02$) cholesterol. Fasting glucose and insulin values were not affected by meal pattern, but peak insulin concentrations and area under the curve of insulin responses to the test meal were lower after the regular than after the irregular meal pattern ($P < 0.01$ and 0.02, respectively).

Conclusion: Regular eating has beneficial effects on fasting lipid and postprandial insulin profiles and thermogenesis. *Am J Clin Nutr* 2005;81:16–24.

KEY WORDS Regular meal pattern, energy intake, energy expenditure, insulin, lipids

INTRODUCTION

The prevalence of obesity continues to rise, and cardiovascular disease (CVD) remains a major health problem throughout the world. Moreover, obesity can increase the risk of CVD. Meal frequency is one of a number of factors that may contribute to the development of obesity and CVD. In the 1960s, Fabry et al (1) suggested a negative association between meal frequency and body weight, and many investigators have subsequently attempted a further evaluation of this association (2–7). The results of studies evaluating the effect of meal frequency on energy intake (EI) were inconclusive (2, 4, 8–13). There appears to be no association between meal frequency and total energy expenditure (EE; 14–17), whereas the results of studies of the effect of meal frequency on the thermic effect of foods (TEF) were contradictory (18–21). However, no studies have considered the effects of day-to-day variations in meal frequency on the components of energy balance.

Altered circulating total, LDL-, and HDL-cholesterol; triacylglycerol (22–24); and insulin (25) concentrations are recognized as risk factors for CVD. Meal frequency may influence these factors, and an increased meal frequency is associated with lower fasting total and LDL-cholesterol concentrations (26–28). However, studies of the response of glucose and insulin to variations in meal frequency were inconclusive (1, 2). No studies have investigated the effect of irregular meal frequency on lipid metabolism.

It seems that Western populations increasingly are moving away from regular meals, perhaps because more meals are being eaten outside the home and because the tradition of families dining together has been eroded by hectic schedules. The prevalence of irregular meal patterns is greater among adolescents than it was during previous decades (29, 30). Japanese studies (31–33) also found that irregular snacking has become more common in children and may have contributed to both the increasing prevalence of obesity in children and the elevated serum cholesterol concentrations in adolescents during the past few decades.

We recently showed that an irregular meal frequency disturbs energy metabolism in healthy lean women (34). This irregular meal frequency led to a lower postprandial EE than was seen with regular eating, whereas mean EI did not differ significantly. We

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also found a higher degree of insulin resistance and higher fasting lipid profiles in these lean subjects after a period of irregular meal frequency, which may indicate a deleterious effect on these CVD risk factors (35). It is not known whether an irregular meal pattern has similar effects in obese subjects. The purpose of the current study was to examine the effect of irregular meal frequency on EI, EE, and lipid and carbohydrate metabolism in healthy obese women.

SUBJECTS AND METHODS

Subjects

Ten healthy obese women aged 32–47 y ($\bar{x}$ ± SD: 39.9 ± 5.7 y) whose menstruation is regular or who are taking oral contraceptive pills, who are neither pregnant nor lactating, and who have no self-reported history of hypercholesterolemia, hyperglycemia, or any serious medical conditions were recruited from the general public by a notice in the local newspaper. Subjects were excluded if they reported that they were dieting [a score of >30 on The Eating Inventory (36)] or experiencing depression [score of >10 on the Beck Depression Inventory (37)]. Mean body mass index (in kg/m^2) was 37.1 ± 4.8.

Ethical permission for the study was obtained from The University of Nottingham Medical School Research Ethics Committee. Written informed consent was obtained from all subjects.

Design

The randomized crossover trial consisted of 2 phases spanning a total of 42 d. Over the course of the study, the subjects made 4 laboratory visits and attended the screening session. In phase 1 (14 d), the subjects were asked to eat and drink items from their normal diet but either to consume the food and beverages on 6 occasions/d (regular meal pattern) with regular intervals between meals or to follow a chaotic meal plan (irregular meal pattern). To achieve the irregular meal pattern, the subjects were asked to observe a predetermined meal frequency consisting of 3–9 meals/d for 14 d, during which each number of meals per day was repeated twice (average: 6 meals/d). To achieve this, the subjects were asked to consume their usual diet on 7, 4, 9, 3, 5, 8, 6, 5, 9, 8, 3, 4, 7, and 6 occasions/d on days 1 through 14, respectively, of the irregular meal pattern. This irregular meal pattern is unlikely to precisely reflect free-living, chaotic eating, but standardization between subjects had to be ensured so that an effective comparison with the period of regular eating could be made. After phase 1, subjects were asked to consume their habitual diet and to follow their normal meal pattern for 14 d as a washout period. In phase 2 (14 d), the subjects were asked to cross over to the alternative meal pattern from phase 1. Five of the subjects started with the regular meal pattern, and 5 started with the irregular one. In both phases, a "meal" was defined as a food or snack (solid or liquid) containing energy, with an interval of >1 h between 2 eating occasions. The participants remained free-living during the study, and the diet content was self-selected. Semiquantitative food intake diaries were kept on 2 weekdays and one weekend day before the start of the interventions. The subjects also recorded their food intake on the 3rd, 11th, and 14th days of both the regular and the irregular meal pattern periods. Each volunteer came to the laboratory on the first and the last days of phase 1 and 2, for a total of 4 visits. Each visit lasted up to 4 h.

Free-living food intake assessment

The participants were given training in keeping a food intake record by using a semiquantitative method based on household measurements. An instruction booklet with an example of a 1-d food intake record was given to each subject before each recording.

Before the start of phase 1, the subjects recorded their habitual dietary intake on 1 weekend day and 2 weekdays. The subjects were asked to provide a food intake record for 3 of the 14 d of the regular meal pattern phase to measure their adherence to this diet. They were also asked to record their dietary intake on the 3 corresponding days during the irregular meal pattern period, when they were eating 9, 6, and 3 meals/d. Food diaries of the subjects' habitual, regular, and irregular meal patterns were analyzed by using MICRODIET software (version 1.2; Downlee Systems Limited, Salford, United Kingdom).

Protocol for laboratory visits

The subjects were asked to fast overnight (for ≥10 h) and to take no exercise other than the walking required for the activities of daily living for 48 h before the laboratory visit. On the subjects' arrival at the laboratory, their weight, height, and waist and hip circumferences were measured. Then, a 20-gauge cannula was inserted retrogradely into a dorsal hand vein for collection of arterialized venous blood. Two baseline blood samples were taken in the fasting state, a milkshake test meal was given, and blood samples were taken at 15-min intervals for 3 h. Resting metabolic rate (RMR) was measured in the fasting state and then for 3 h after the test meal. Subjects also completed visual analogue scales for hunger-related factors before and for 3 h after consuming the test meal. All visits were undertaken in the morning.

Laboratory procedures

Anthropometric measurements

Weight and waist and hip circumference measurements were made at each laboratory visit. Weight was measured to the nearest 0.1 kg on a Seca electronic scale (model no. 882; Vogel and Halke, Hamburg, Germany) when subjects were fasting, had an empty bladder, were wearing light clothing with empty pockets, and were not wearing shoes. Height was measured to the nearest 0.1 cm by using a stadiometer during the screening visit. Waist circumference was measured to the nearest 0.1 cm in a horizontal plane at the midpoint between the lower margin of the last rib and the crest of the ileum when the subject stood with her feet 25–30 cm apart (38). Hip circumference was also measured to the nearest 0.1 cm in a horizontal plane at the maximum point over the buttock at the level of the femoral greater trochanter by using a flexible nonstretch nylon tape (38). Body composition was measured by using bioelectrical impedance analysis (QuadScan 4000; Bodystat Ltd, Douglas, United Kingdom) while the subject lay on a nonconductive couch with the arms and legs abducted.

Blood sampling

On each subject's arrival at the laboratory, her hand was warmed in a heated, ventilated perspex box (50–55 °C) for 15–20 min to open the arteriovenous anastomoses (39). A 20-gauge cannula was inserted retrogradely into a dorsal hand vein, and a slow-running infusion of saline (154 mmol sodium chloride/L) was begun to keep the cannula patent. Blood samples were withdrawn via a 3-way tap; the first 2 mL was discarded to

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avoid contamination with saline. Two baseline blood samples were tested for fasting blood glucose, serum insulin, and plasma cholesterol (total, HDL, and LDL), triacylglycerol, and catecholamines.

After the test meal, blood samples were taken every 15 min for 3 h. These blood samples were analyzed for blood glucose, serum insulin, and plasma catecholamines.

Test meal consumption

The milkshake test meal was given as a breakfast. Subjects were given a volume of test meal on the basis of their weight [12.8 kcal (53.5 kJ)/kg lean body mass]. Of the total energy from the macronutrients, 50% was as carbohydrate, 35% was as fat, and 15% was as protein. The test meal contained 2%-fat milk, a milkshake powder (Build-up; Nestlé SA, Lausanne, Switzerland), double cream (containing 1831 kJ energy and 47.5 g fat/100 mL, of which 29.7 g is saturated fat; Sainsbury's, London, United Kingdom), and Polycal glucose polymer (Nutricia Clinical Care, Trowbridge, United Kingdom) in either strawberry or vanilla flavor. The test meal was served at a temperature of 18–20 °C in an open glass. Subjects were asked to consume the drink over 10 min.

Visual analogue scales

Each subject completed visual analogue scale questionnaires to assess subjective hunger, satiety, fullness, and prospective food consumption (ie, desire to eat). Subjects completed these questionnaires just before and every 30 min after the test meal for 3 h. Ratings were made on 100-mm visual analogue scales with words at each end that expressed the most extreme rating (40).

Energy expenditure and substrate oxidation

RMR was measured after a 10-h fast by using an open-circuit indirect calorimeter [Gas Exchange Measurement (GEM) system; Nutren Technology Ltd, Manchester, United Kingdom]. After a warm-up period of 30 min, a reference gas (5% CO_2 and 95% O_2) was used to calibrate the oxygen and carbon dioxide analyzers. Ingoing and outgoing air were analyzed for oxygen and carbon dioxide every minute during each period of measurement. Readings from the metabolic monitor were collected every minute with a personal computer.

The subjects rested on a bed in a room maintained at 18–20 °C for ≈20 min. Fasting RMR was then measured for 30 min with the subject lying in the supine position. A transparent ventilated hood was positioned over the subject's head with Collins tubing connecting the hood to the monitor, and expired gases were continuously collected. The subjects then drank the milkshake test meal over a period of 10 min. Starting immediately after consumption of the test meal, postprandial metabolic rate (PPMR) was measured for 2 periods of 15 min every hour for 3 h. The subjects rested on the bed but were not allowed to sleep during the EE measurements. In the intervals between the measurements, subjects remained on the bed but were permitted to read. The TEF was measured by using the trapezoidal method to calculate the area under the curve (AUC) of PPMR above the baseline RMR for all of the visits.

Processing of blood samples

Immediately after blood samples were drawn, blood glucose was measured by using a B-Glucose analyzer (Hemocue AB, Angelholm, Sweden). After collection, blood samples for insulin analysis were left to clot for at least 30 min after collection before being centrifuged for 10 min at 3000 RPM (Minifuge RF; Heraeus Equipment Ltd, Brentwood, United Kingdom). The serum samples were then sealed and stored at −80 °C for future analysis. Blood samples for cholesterol, triacylglycerol, and catecholamine analyses were taken into tubes containing lithium heparin. The samples were kept in an icebox until the end of each visit before being centrifuged for 10 min at 3000 RPM (Minifuge RF; Heraeus Equipment Ltd). The plasma was transferred into a fresh tube containing 25 μL EGTA. The tube was then sealed and stored at −80 °C for later analysis of lipids, uric acid, and catecholamines.

Insulin measurements were performed by using a solid-phase [^{125}I]-radioimmunoassay method and coated-tube technology (Count-A-Count; Diagnostic Products Corp, Los Angeles). The intraassay CV was 2.8% and 3.3% for blood glucose and serum insulin, respectively.

Plasma total cholesterol and triacylglycerol were measured enzymatically by using kits and standards supplied by VITROS (Ortho-Clinical Diagnostics, Rochester, NY). HDL-cholesterol concentrations were measured after precipitation of apolipoprotein B–containing lipoproteins with heparin and manganese chloride (41) by using the EZ HDL cholesterol kit (Sigma Diagnostics, St Louis). LDL-cholesterol concentrations were calculated by using the formula of Friedewald et al (42). Plasma uric acid concentrations were measured enzymatically (43, 44) by using kits supplied by VITROS. The intraassay CV was 2.2%, 2.6%, 1.7%, and 1.6% for total cholesterol, HDL cholesterol, triacylglycerol, and uric acid, respectively.

Homeostasis model assessment was used to assess insulin resistance (HOMA-IR) when the subjects were in the fasting state. The HOMA-IR values for the subjects were calculated by using the following formula (45):

$$\text{HOMA-IR} = \text{fasting serum insulin } (\mu\text{IU/mL}) \times \text{fasting blood glucose (mmol/L)}/22.5 \quad (1)$$

HPLC with electrochemical detection was used to measure plasma epinephrine and norepinephrine concentrations (46). AUCs above the baseline for blood glucose, serum insulin, and plasma norepinephrine concentrations were measured during the 3-h posttest meal period by using the trapezoidal method.

Statistical analysis

SPSS software (version 10; SPSS, Chicago) was used for data entry and analysis. All data are reported as means ± SDs unless otherwise indicated. Data were tested for normality (Kolmogorov-Smirnov statistic with Lillefors correction). Comparisons of the preintervention data were performed by using Student's paired *t* test (two-tailed) to investigate possible differences before the meal pattern interventions. Statistical analysis of the results was then performed by using repeated-measures analysis of variance (ANOVA) with 2 within-subjects factors (ie, visits before and after the interventions and the meal pattern) or with 3 within-subjects factors (ie, meal pattern, values before and after the interventions, and the time after the test meal) as appropriate. When ANOVA indicated an interaction ($P < 0.10$) between the factors, paired *t* tests were undertaken between the postintervention results and between the preintervention and

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TABLE 1
Physical characteristics of the 10 subjects during the experiment[1]

	Regular meal pattern		Irregular meal pattern	
	Before intervention	After intervention	Before intervention	After intervention
Body weight (kg)	98.5 ± 15.6	98.1 ± 15.4	98.1 ± 15.3	98.4 ± 15.8
BMI (kg/m^2)	37.1 ± 4.8	36.9 ± 4.8	36.9 ± 4.9	37.0 ± 4.9
Waist (cm)	102.8 ± 12.1	103.8 ± 12.9	103.1 ± 10.6	104.1 ± 12.1
Waist-hip ratio	0.85 ± 0.04	0.84 ± 0.03	0.85 ± 0.05	0.84 ± 0.04
Body fat (%)	45.0 ± 3.3	44.7 ± 3.0	45.1 ± 3.0	45.3 ± 2.9

[1] All values are $\bar{x}$ ± SD. There were no significant differences in physical characteristics during the experiment (ANOVA).

postintervention values. In the absence of an interaction, significant main effects (detected by ANOVA) are reported. Pearson correlation analysis was performed between differences in energy intake and the observed metabolic changes. Significance was set at $P < 0.05$ for all statistical tests.

RESULTS

There were no differences between the preintervention and postintervention body weights under either meal pattern (ANOVA). There were also no significant differences between preintervention and postintervention anthropometric measurements or body-fat composition under either meal pattern period (ANOVA; **Table 1**).

Energy intake

The preexperiment food diaries did not show any significant difference in EI between the average of the 2 weekdays (8.43 ± 1.04 MJ/d) and the 1 weekend day (8.54 ± 1.02 MJ/d). Furthermore, there were no significant differences in the macronutrient composition of the food between weekdays and weekends.

All subjects reported having adhered to the appropriate meal patterns during the 2 interventions. Mean energy intake recorded over 3 d was significantly lower during the regular meal pattern (7.98 ± 0.49 MJ/d) than during the irregular meal pattern (8.32 ± 0.35 MJ/d; $P < 0.01$). EI did not differ significantly between the 3 d of the regular meal pattern, but EI values did differ significantly between all of the days with 9, 6, and 3 meals/d during the irregular meal pattern (ANOVA; $P = 0.017$). EI was significantly higher with 9 meals/d than with 3 meals/d (paired *t* test; $P = 0.012$), but there was no significant difference between 9 and 6 meals/d or between 6 and 3 meals/d (**Table 2**). There were no significant differences in macronutrient composition (ie, percentage of energy from protein, fat, and carbohydrate) between the 2 meal patterns or between the days within each meal pattern period (Table 2).

Appetite measurement

The response curves for the 4 appetite sensations (ie, hunger, satiety, fullness, and prospective food consumption) are shown in **Figure 1**. Fasting values for all variables and the profiles after the test meal did not differ significantly over the course of the experiment.

Energy expenditure and thermic effect of food

There was no significant difference in the fasting RMR values between the visits before the regular and the irregular meal pattern periods (paired *t* test), and there was no significant effect of either meal pattern on fasting RMR (by ANOVA; **Figure 2**). Metabolic rate increased significantly above fasting values after the test meal at all visits. The PPMR response showed a significant 3-way meal pattern–by–visit (before and after intervention)–by–time after the test meal interaction (ANOVA; $P = 0.03$). PPMR showed no significant difference between the preintervention visits; however, PPMR was significantly lower after the irregular meal pattern than before it and significantly higher after the regular meal pattern than before it (Figure 2). There was a significant meal pattern–by–visit (before and after intervention) interaction for the TEF response (ANOVA; $P = 0.002$; **Figure 3**). The TEF at the visits before the 2 periods did not differ significantly, but the change in TEF differed significantly between the intervention periods: TEF fell significantly after the irregular meal pattern (paired *t* test; $P = 0.008$), but it rose significantly after the regular meal pattern (paired *t* test; $P = 0.018$).

TABLE 2
Daily nutrient intakes of the 10 subjects during the regular and irregular meal patterns[1]

	Regular meal pattern				Irregular meal pattern			
	Day 1 (6 meals)	Day 2 (6 meals)	Day 3 (6 meals)	Overall	Day 1 (9 meals)	Day 2 (3 meals)	Day 3 (6 meals)	Overall
Energy (MJ)	7.97 ± 0.61	7.95 ± 0.63	8.01 ± 0.52	7.98 ± 0.49	8.82[2] ± 0.65	7.77[2] ± 0.56	8.37[2] ± 0.84	8.32[3] ± 0.35
Protein (%)	17.6 ± 4.9	16.2 ± 3.5	15.8 ± 5.1	16.5 ± 2.5	15.7 ± 2.1	16.9 ± 2.9	14.8 ± 3.8	15.8 ± 2
Fat (%)	38.5 ± 4.1	38.3 ± 6.3	36.9 ± 6.4	37.9 ± 3.2	35.7 ± 5.9	38.7 ± 5.9	40.9 ± 6.5	38.4 ± 4.4
Carbohydrate (%)	43.7 ± 4.5	43.9 ± 6.5	46.3 ± 8.1	44.6 ± 2.8	46.0 ± 4.7	42.0 ± 6.1	43.7 ± 8.2	43.9 ± 4.4

[1] $P = 0.07$ for interaction of pattern and days based on ANOVA.
[2] Significantly different between 9, 3, and 6 meals/d, $P = 0.017$ (ANOVA); significantly higher with 9 meals/d than with 3 meals/d, $P = 0.012$ (paired *t* test); no significant difference between 6 and 9 meals/d or between 6 and 3 meals/d.
[3] Significantly different from regular meal pattern, $P = 0.005$ (paired *t* test).

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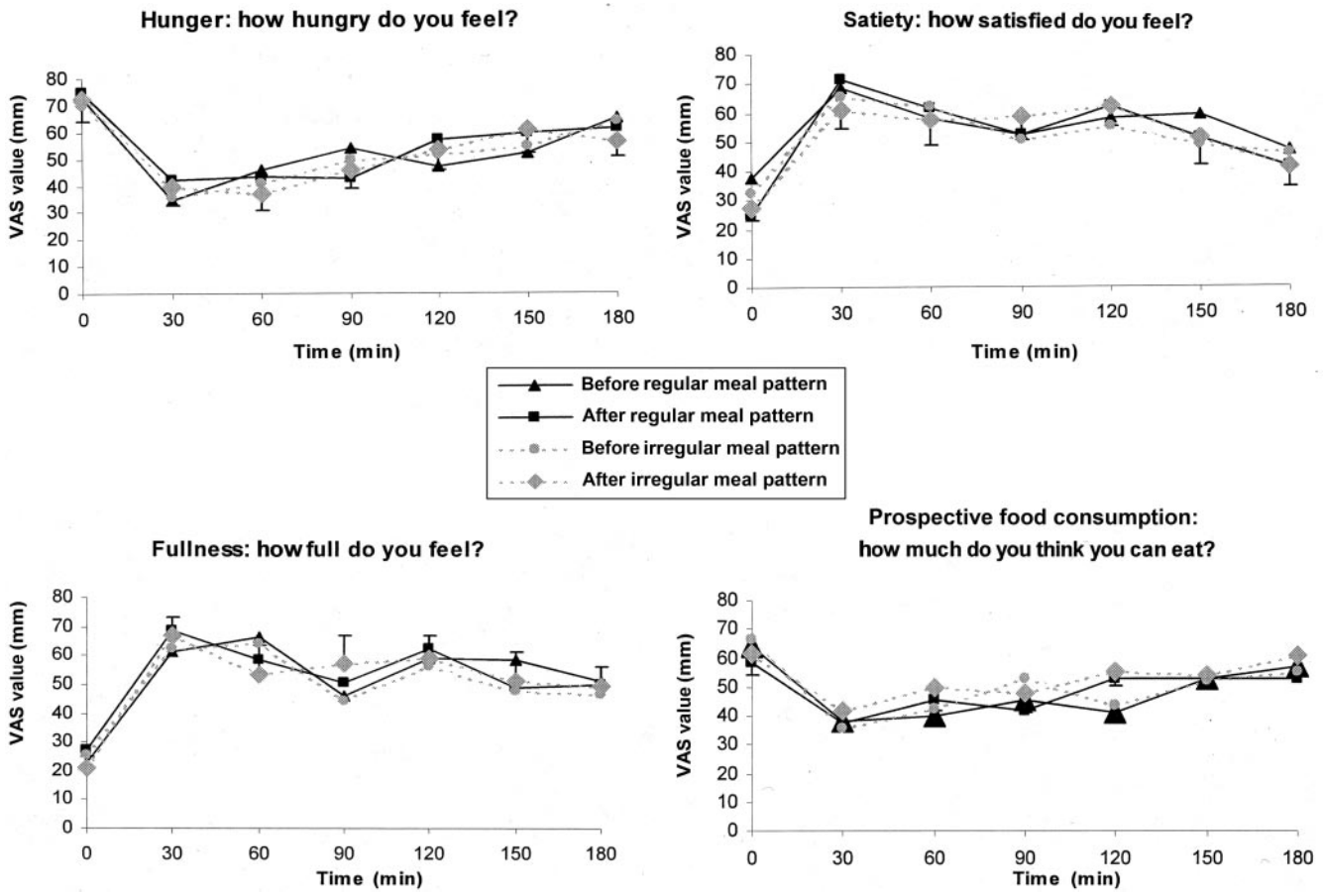

FIGURE 1. Mean (±SEM) subjective appetite scores (ie, hunger, satiety, fullness, and prospective food consumption) in 10 healthy obese women before and after the test meal at the visits before and after the regular and irregular meal pattern interventions. For clarity, only a few SEM values are shown. VAS, visual analogue scales. No significant differences were observed (ANOVA).

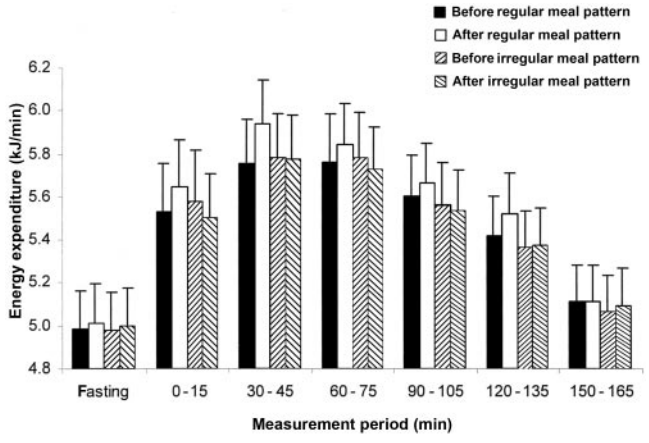

FIGURE 2. Mean (±SEM) energy expenditure in 10 healthy obese women before and after the test meal fed at the visits before and after the regular and irregular meal pattern interventions. Subjects drank the milkshake test meal within 10 min. The time zero value shown on the *x* axis refers to the end of the test meal consumption. There was a significant meal pattern–by–visit (before and after intervention)–by–time after the test meal interaction (ANOVA; $P = 0.03$). The regular meal pattern preintervention profiles did not differ significantly, whereas the profile after the irregular meal pattern intervention was significantly smaller than that after the regular meal pattern intervention.

Blood glucose and serum insulin

There was no significant difference in fasting blood glucose between the visits before the regular and irregular meal pattern periods (paired *t* test). There was no significant effect of either meal pattern on fasting blood glucose (ANOVA; **Table 3**). Blood glucose concentrations rose significantly after the test meal at all visits (**Figure 4**). The preintervention peak in blood glucose concentrations did not differ between meal pattern interventions

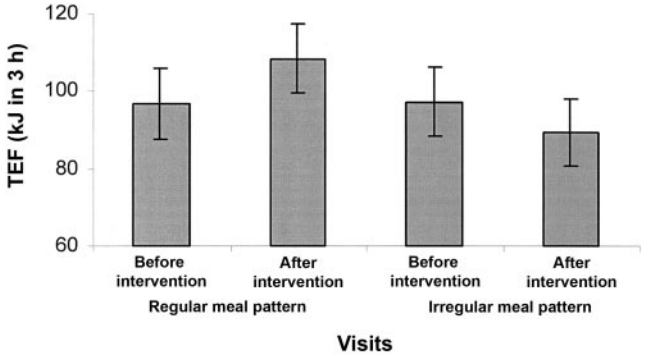

FIGURE 3. Mean (±SEM) thermic effect of food (TEF) in 10 healthy obese women in the visits before and after the regular and irregular meal pattern interventions. There were significant differences between the 2 interventions (ANOVA; $P = 0.002$).

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TABLE 3
Fasting glucose, insulin, lipid, uric acid, and norepinephrine concentrations and peak postprandial glucose and insulin concentrations in the 10 subjects during the experiment[1]

	Regular meal pattern		Irregular meal pattern	
	Before intervention	After intervention	Before intervention	After intervention
Fasting blood glucose (mmol/L)	5.06 ± 0.15	5.02 ± 0.17	5.13 ± 0.20	5.15 ± 0.19
Fasting serum insulin (pmol/L)	130 ± 54	122 ± 42	127 ± 45	128 ± 45
HOMA-IR	4.91 ± 2.16	4.55 ± 1.60	4.79 ± 1.66	4.89 ± 1.70
Plasma cholesterol (mmol/L)[2]				
Total	4.28 ± 0.50	4.16 ± 0.46	4.27 ± 0.52	4.29 ± 0.52[3]
HDL	1.45 ± 0.25	1.46 ± 0.26	1.47 ± 0.24	1.47 ± 0.28
LDL[4]	2.59 ± 0.64	2.43 ± 0.63	2.57 ± 0.61	2.60 ± 0.70[5]
Plasma triacylglycerol (mmol/L)	1.21 ± 0.34	1.16 ± 0.51	1.12 ± 0.29	1.13 ± 0.36
Plasma uric acid (mg/L)	239 ± 29	237 ± 31	238 ± 41	243 ± 38
Plasma norepinephrine (nmol/L)	1.18 ± 0.43	1.20 ± 0.38	1.17 ± 0.33	1.14 ± 0.29
Peak of postprandial blood glucose (mmol/L)	7.50 ± 0.41	7.44 ± 0.40	7.51 ± 0.31	7.51 ± 0.44
Peak of postprandial serum insulin (pmol/L)[6]	843 ± 274	807 ± 275	811 ± 258	885 ± 277

[1] All values are $\bar{x}$ ± SD. HOMA-IR, homeostasis model assessment of insulin resistance.
[2] A significant meal pattern–by–visit (before and after intervention) interaction was observed for plasma total cholesterol concentrations, $P = 0.002$ (ANOVA).
[3] Significantly different from the regular meal pattern, $P = 0.003$ (paired *t* test).
[4] A significant meal pattern–by–visit (before and after intervention) interaction was observed for plasma LDL concentrations, $P = 0.013$ (ANOVA).
[5] Significantly different from the regular meal pattern, $P = 0.038$ (paired *t* test).
[6] A significant meal pattern–by–visit (before and after intervention) interaction was observed for peak serum insulin concentrations, $P = 0.005$ (ANOVA). Peak of serum insulin fell significantly after the regular meal pattern intervention, $P = 0.001$ (paired *t* test), but it rose significantly after the irregular meal pattern intervention, $P = 0.021$ (paired *t* test).

(paired *t* test; Table 3), and there was no significant effect of either meal pattern intervention on peak blood glucose concentration (ANOVA). There was no significant difference in the AUC of glucose profiles above the baseline between the visits before the regular and irregular meal pattern interventions and no significant effect of either meal pattern on the AUC of glucose profiles over the course of the study (ANOVA).

There was no significant difference in fasting serum insulin between the visits before the regular and irregular meal pattern interventions and no significant change in fasting insulin with the 2 meal pattern interventions. There was no significant difference in HOMA-IR values between the visits before the regular and irregular meal pattern interventions, and HOMA-IR did not change significantly after either meal pattern intervention (Table 3).

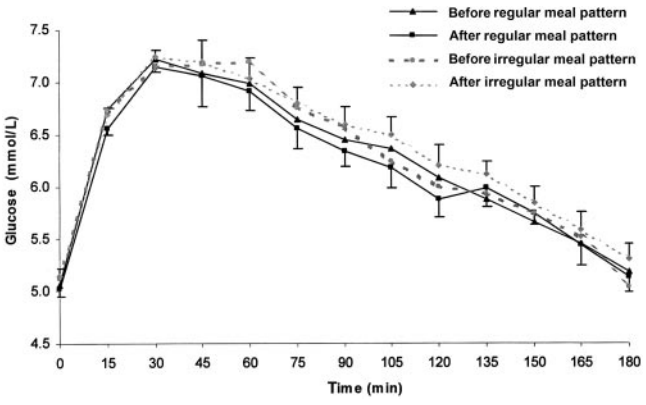

FIGURE 4. Mean (±SEM) blood glucose concentrations in 10 healthy obese women in the fasting state and after the test meal fed at the visits before and after the regular and irregular meal pattern interventions. No significant differences were observed in blood glucose profiles over the course of the experiment. For clarity, SEM values are presented for only 2 of the profiles.

Serum insulin concentrations rose significantly in response to the test meal at all visits. There was no significant difference in the peak insulin values after the test meal between the visits before the regular and irregular meal pattern interventions. There was a significant meal pattern–by–visit (before and after intervention) interaction for the peak serum insulin concentrations (ANOVA; $P = 0.005$). Peak of serum insulin fell significantly after the regular meal pattern (paired *t* test; $P = 0.001$) compared with a significant rise after the irregular meal pattern (paired *t* test; $P = 0.021$; Table 3). There was no significant difference in the AUC of postprandial serum insulin between the visits before the regular and irregular meal pattern interventions, but there was a significant meal pattern–by–visit (before and after intervention) interaction (ANOVA; $P = 0.017$) for the AUC of insulin response (**Figure 5**). The AUC of serum insulin response fell significantly after the regular meal pattern intervention (paired *t* test; $P = 0.02$), whereas it rose significantly after the irregular meal pattern intervention (paired *t* test; $P = 0.022$).

Plasma lipids and uric acid

The mean values of fasting plasma total, LDL, and HDL cholesterol and triacylglycerol concentrations at all visits are also shown in Table 3. The lipid values did not differ significantly between the visits before the 2 meal pattern interventions. There was a significant meal pattern–by–visit (before and after intervention) interaction for plasma total cholesterol concentrations (ANOVA; $P = 0.002$). Plasma total cholesterol was also significantly lower after the regular meal pattern intervention than after the irregular meal pattern intervention (paired *t* test; $P = 0.003$). There was also a significant meal pattern–by–visit (before and

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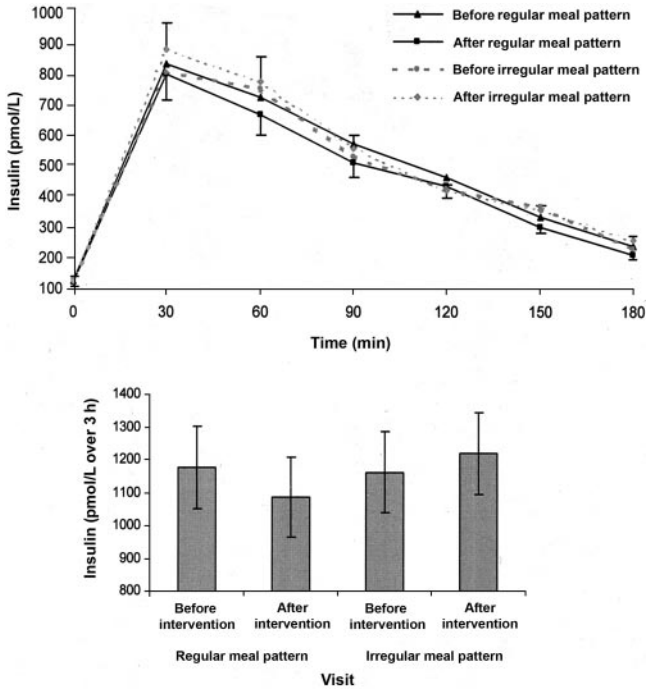

FIGURE 5. Mean (±SEM) serum insulin concentration and area under the curve (above the baseline) for the serum insulin profiles in 10 healthy obese women responding to the test meal at the visits before and after the regular and irregular meal pattern interventions. There was a significant premeal pattern–by–postmeal pattern condition interaction for serum insulin concentrations and AUC (ANOVA; $P = 0.002$), a significant reduction in both values after the regular meal pattern intervention (paired t test; $P = 0.02$), and a significant increase in both values after the irregular meal pattern intervention (paired t test; $P = 0.02$).

after intervention) interaction for plasma LDL-cholesterol concentrations (ANOVA; $P = 0.013$). In addition, the plasma LDL concentrations were significantly lower than after the regular meal pattern intervention than after the irregular meal pattern intervention (paired t test; $P = 0.038$). However, no significant differences were observed in plasma HDL concentrations during either meal pattern intervention. Plasma triacylglycerol concentrations also showed no significant difference over the course of the experiment.

There were no significant correlations between the changes in total or LDL cholesterol and the differences in mean energy intake between the regular and irregular meal pattern interventions. There were also no significant correlations between the differences in EI and the insulin and TEF responses. There were no significant changes in plasma uric acid over the course of the experiment (Table 3).

Plasma norepinephrine

There was no significant difference in fasting plasma norepinephrine concentrations between the 2 preintervention visits (paired t test). There was no effect of either meal pattern on fasting plasma norepinephrine (ANOVA; Table 3). Plasma norepinephrine concentration rose significantly after the test meal at all visits (**Figure 6**). The peak plasma norepinephrine concentrations before each intervention were not significantly different (paired t test) and were not significantly affected by meal pattern (ANOVA). There was no significant difference in the AUC of norepinephrine profiles above the baseline between the visits before

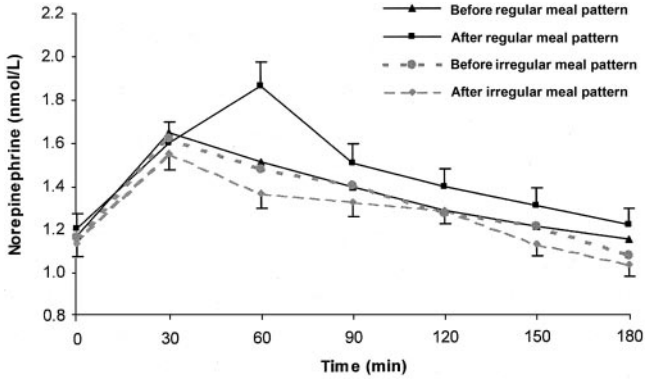

FIGURE 6. Mean (±SEM) plasma norepinephrine concentrations in 10 healthy obese women in the fasting state and after the test meal fed at the visits before and after the regular meal pattern intervention. No significant differences were observed in plasma norepinephrine profiles over the course of the experiment. For clarity, SEM values are presented for only 2 of the profiles.

the 2 meal pattern interventions. There was also no effect of either meal pattern intervention on the AUC of plasma norepinephrine profiles over the course of the study (ANOVA; Figure 6).

Plasma epinephrine concentrations were 0.05–0.21 nmol/L at baseline, and there was no significant difference between the 2 preintervention visits. Epinephrine concentrations did not change significantly during the postprandial periods (data not shown).

DISCUSSION

The aim of this study was to investigate the effect of irregular meal frequency on EI, resting EE, and indexes of carbohydrate and lipid metabolism and plasma uric acid concentration. We found that irregular meal frequency led to a lower postprandial EE and impaired TEF, higher total and LDL cholesterol, and lower postprandial insulin sensitivity than were seen with regular meal frequency in healthy obese women; these findings were in agreement with those of our earlier study in lean women (34, 35). We did not find any significant difference in fasting RMR across the 4 visits. As explained earlier, many studies since the 1960s have evaluated the effect of meal frequency on EI and EE. The poor definition of key variables and the lack of the management of the intervention factors have weakened their interpretations. In the current study, we clearly defined a meal as providing some energy, and the interval between any 2 consecutive meals was to be >1 h. Fluctuations have been reported in women's food intake (47) and RMR (48) through the menstrual cycle. To overcome this possible factor, each subject began the interventions at the same point in her menstrual cycle.

Previous studies showed that impaired thermogenesis is associated with insulin resistance in obesity (49, 50), which is consistent with the current finding of lower TEF and postprandial insulin insensitivity after the irregular meal pattern intervention. Another study (51) found an independent effect of insulin resistance and obesity in producing a blunted TEF. In the current study, the irregular meal pattern intervention failed to produce any significant differences in body weight, despite reduced dietary thermogenesis, but this was to be expected because of the short duration of the intervention. However, insulin insensitivity and the low TEF resulting from the irregular meal pattern intervention are in agreement with the findings of previous studies of

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an association between insulin resistance and blunted thermogenesis. There is some evidence of a link between sympathetic nervous system activation and TEF response to a meal in younger subjects but not in older subjects (52). Despite a significantly lower TEF after the irregular meal pattern intervention than after the regular meal pattern intervention, we did not find any significant differences in plasma norepinephrine concentration (an index of sympathetic nervous system activity) over the course of the experiment, although there was a trend for a higher response after the regular meal pattern intervention. Further investigation is required to ascertain the mechanism of a lower TEF in response to an irregular meal pattern intervention.

Several studies (53–55) found that underreporting of EI, especially for snacks, was more common in obese persons. The current study may also have been affected by underreporting, but we assume that the degree of underreporting was similar in all phases. Despite instructions to maintain a normal food intake, the subjects reported a significantly higher mean EI during the irregular meal pattern intervention than during the regular meal pattern intervention. In our earlier study with the same experimental design, lean women showed no significant differences in EI between regular and irregular meal pattern interventions (34). That indicates that obese persons consuming self-selected diets may have found it more difficult to adjust to an irregular meal frequency and also maintain their normal intake of foods. There may also be some inaccuracy in extrapolating the EI record for 3 d to represent the entire 14-d intervention period. The EI of the subjects in the irregular meal pattern intervention differed significantly between the days with 9, 6, and 3 meals/d. EI was significantly higher with 9 meals/d than with 3 meals/d. This is in agreement with the findings of one earlier study (8), but not with those of other studies (9–12). An intervention study (13) also reported no association between meal frequency and EI. The current study showed no significant differences between the 2 meal pattern interventions in the appetite ratings after the test meal. Further investigation in a feeding study combined with appetite ratings would be essential to ascertain the effect of an irregular meal pattern on total EI and appetite control.

The effect of a meal pattern, and especially of meal frequency, on the biochemical risk factors for CVD has been of interest to many researchers (26–28), but the results are not conclusive. A study (56) also indicated an inverse relation between meal frequency and serum uric acid concentration. The HOMA-IR index showed no significant differences in the current study, which indicated no overall change in fasting insulin sensitivity in these obese subjects. This finding is somewhat different from the results of the HOMA-IR index seen previously in nonobese subjects (35). It is possible that, because the obese subjects are likely to already have some degree of insulin resistance, this metabolic factor may not be exacerbated in obese subjects during the 2-wk intervention as much as it would be in nonobese subjects with greater initial fasting insulin sensitivity. The peak postprandial insulin response and AUC of the insulin profile after the test meal in the obese subjects were significantly lower after the regular meal pattern intervention than after the irregular meal pattern intervention, which is consistent with findings of our earlier study of lean women (35). This finding leads us to propose that an irregular meal frequency may reduce insulin sensitivity and thus lead to a higher insulin response to a test meal, whereas a regular meal frequency may increase insulin sensitivity. The molecular basis of these changes remains to be determined. The effect of a particular meal pattern on postprandial insulin sensitivity may carry over to the fasting state. In the current study, plasma total and LDL-cholesterol concentrations were significantly higher after the irregular meal pattern intervention than after the regular meal pattern intervention. The differences in total and LDL-cholesterol concentrations (and in insulin sensitivity and TEF) were not correlated with the differences in EI. This lack of correlation may be due to a lack of statistical power, but it suggests that meal pattern is likely to have a greater metabolic effect than small differences in EI. Fasting plasma HDL-cholesterol, triacylglycerol, and uric acid concentrations did not differ significantly between the regular and irregular meal pattern interventions. These results are in agreement with those of our earlier study of nonobese women (35). These 2 studies indicate that irregular eating may lead to unhealthy total and LDL-cholesterol concentrations in both obese and nonobese women. Previous studies showed that a greater meal frequency is associated with lower fasting total and LDL-cholesterol concentrations. An epidemiologic study (33) claimed that an irregular meal pattern may be associated with an elevated serum total cholesterol concentration in adolescents. However, the report of that study did not provide an exact definition of an irregular meal pattern.

The current study showed the importance of meal pattern in addition to the amount and composition of food in influencing carbohydrate and lipid metabolism. Further studies measuring the above factors and other possible influences are required. The current study also showed lower TEF and higher EI with an irregular meal frequency. This indicates a potential mechanism by which an irregular meal pattern might affect EE and EI, which could lead to weight gain in the longer term. In addition, the irregular meal pattern had potentially deleterious effects on insulin sensitivity and plasma cholesterol, which are known risk factors of CVD.

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HRF produced the initial study design, performed the laboratory investigations and biochemical analysis, undertook the statistical analysis, and wrote the first draft of the manuscript. MAT and IAM refined the study design and supervised the data collection, contributed to the data interpretation and re-drafting of the manuscript. MAT provided clinical and dietetic oversight to the dietary aspects of the study. None of the authors had any conflicts of interest.

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