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

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Short running head

Regular eating, thermogenesis and appetite

Abbreviations

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

Physical Activity Questionnaire (IPAQ); kilocalories per day (kcal/day); Minute (min);

Maximum (max); Minimum (min); Minute (min); Physical activity level (PAL); Resting

energy expenditure (REE); Standard deviation (SD); Standard error of the mean (SEM);

Thermic effect of food (TEF); Visual analogue scale (VAS)

Clinical trial registry number and website

ID number NCT02052076, www.clinicaltrials.gov

1   **ABSTRACT**

2   **Background:** Obesity is increasing in parallel with greater all day food availability. The

3   latter may promote meal irregularity, dysregulation of energy balance and poor metabolic

4   health.

5   **Objective**: To investigate the effect of meal irregularity on the thermic effect of food (TEF),

6   lipid levels, carbohydrate metabolism, subjective appetite and gut hormones in healthy

7   women.

8   **Design:** 11 normal-weight women (18–40y) were recruited to a randomized crossover trial

9   with two, 14-day isoenergetic diet periods (identical foods provided/ free-living), separated

10   by a 14-day habitual diet wash-out period. In period 1, participants followed a regular

11   (6meals/day) or an irregular meal pattern (3-9meals/day) and in period 2, the alternative meal

12   pattern. Before and after each period, when fasting and for 3h following a test drink,

13   measurements were made of energy expenditure, circulating glucose, lipids (fasting only),

14   insulin, GLP-1, PYY and ghrelin. An *ad libitum* test meal was offered**.** Subjective appetite

15   ratings were assessed fasted, following the test drink, following the *ad libitum* meal and

16   during the intervention. Continuous interstitial glucose monitoring (CGM) was undertaken

17   for 3 consecutive days during each intervention and ambulatory activity pattern was recorded

18   (AEEE).

19   **Results:** Regularity was associated with greater TEF (P<0.05) and a lower incremental area

20   under the curve (iAUC) for glucose following the test drink (over 3h) and, for some identical

21   meals on the two interventions (over 90min) (Day 7: post-breakfast; Day 9: post-lunch and

22   dinner). There was no difference between-treatments for test drink gut hormone response. A

23   time effect for fasting GLP-1, fasting PYY, PYY responses and hunger rating responses to

24   the test drink (P<0.05) was noted. Lower hunger and higher fullness ratings were seen, pre

25   and post meal, during the regular period, whilst free living.

26   **Conclusion:** Meal regularity appears to be associated with greater TEF and lower glucose

27   responses, which may favour weight management, and metabolic health.

28   **Key words:** normal-weight women, meal regularity, thermic effect of food, metabolism,

29   appetite.

INTRODUCTION

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

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

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

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

55 responses to a test drink and *ad libitum* intake of a test meal. The term 'meal' was used for

56 both prescribed eating incidents at traditional 'meal times', and those that occurred at

57 traditional 'snack times'. Measures were made during the free-living intervention periods of

58 physical activity (AEEE), continuous interstitial glucose monitoring (CGM) and subjective

59 appetite.

60 **SUBJECTS AND METHODS**

61 **Participants**

62 The study was conducted at David Greenfield Human Physiology Unit, School of Life

63 Sciences, Queen's Medical Centre, University of Nottingham between January 2013 and July

64 2013. The study was approved by the University of Nottingham Faculty of Medicine and

65 Health Sciences Research Ethics Committee (J14082012 BMS). Participants were recruited

66 from the student and staff population of the University of Nottingham via poster

67 advertisement. Inclusion criteria for participants were: normal weight women (BMI 18.5 and

68 25 kg/m^2), aged 18-40 years, non-smoker, non-high alcohol consumers (< 3 units/day), no

69 history of serious disease or currently taking any medications other than oral contraceptives,

70 not pregnant/lactating and with regular menstrual cycles, not dieting/seeking to lose weight

71 and weight stable during the last 3 months (self-reported weight change $< \pm 2$ kg). Exclusion

72 criteria were: participants with symptoms of clinical depression (defined by a score > 10 on

73 the Beck Depression Inventory (15)), with eating disorders (defined by a score > 20 on the

74 EAT- 26 (16)), with an allergy or intolerance to any of the foods provided during the study.

75 Of the 19 healthy normal-weight participants who responded to the advertisement 11 were

76 recruited to the study (**Figure 1**). These 11 participants were the ones that met the study

77 requirements. Values that were outside the inclusion criteria resulted in exclusion of four and

78 two subjects respectively for BMI and EAT- 26 score. Two women were ineligible because

79 they were anaemic. The remaining 11 participants gave written consent, and then 5

80 participants were scheduled to start with the regular meal pattern and 6 others with the

81 irregular one. Blood sampling could not be performed on one participant due to problems

82 associated with venous cannulation. Thus data from 10 participants were available for the

83 intention-to-treat blood analysis. Two subjects were excluded from the analysis of CGM data

84 because inadequate data were obtained. Informed, written consent was obtained from all

85 participants after the experimental protocol had been described to them in writing and orally.

86 The study is registered at clinical Trials.gov with the identification number: NCT02052076.

87 **Screening**

88 All potential participants attended a screening visit in order to establish that they met the

89 inclusion criteria for the study. Height was measured to the nearest 0.1 cm using a

90 stadiometer (Seca, Germany). Body weight was measured using an electronic scale to the

91 nearest 0.1 kg (Seca, Germany) whilst participants were wearing light clothing with no shoes

92 and with an empty bladder. BMI was calculated from their height and weight as kg/m^2. A

93 blood sample was taken for routine tests to confirm their general health.

94 Eligible participants then were asked to complete a weighed 7-day food diary which was used

95 to characterize their habitual diet. They were instructed to consume their normal diet and

96 participate in their usual level of activity before the study.

97 **Study design**

98 The study followed a randomized crossover design with two, 14-day intervention periods,

99 separated by a wash-out period of 14 days. Participants consumed their habitual diet during

100 the wash out period which was included to avoid interaction between the two interventions.

101 The randomization scheme was generated using the Second Generator Plan from

102 randomization.com (17) before the study began. Participants were assigned to the

103 randomization scheme in the order of recruitment. The study investigator generated the

104 randomization scheme, enrolled participants, and assigned participants to interventions.

105   Participants were free-living except that during each intervention period they were required to

106   consume food provided by the experimenter. Participants attended the laboratory pre and post

107   each intervention period, for a total of 4 visits. Each laboratory visit lasted up to 5 h. In order

108   to avoid the potential impact on outcome measures of the stage in the menstrual cycle (18-

109   20), participants started each intervention period during the early phase of their menstrual

110   cycle (days 1-7).

111   **Dietary intervention periods**

112   Each participant was provided with, free of charge, all their food during each of the

113   intervention periods. An individual had identical foods during each of the intervention

114   periods and differences between participant food provision were minimised, but were

115   sometimes necessary to meet the different energy requirements of participants. The food was

116   supplied in a 4 day cycle of menus consisting of a variety of items commonly consumed in

117   the British diet. The menu was designed to cover participants' energy requirement for weight

118   maintenance (± 100 kcal). Menus were designed for 1900 kcal/day, 2050 kcal/day and 2350

119   kcal/day to meet the different estimated energy requirements of participants. Energy

120   requirements were based on the Oxford-Henry equations (21) multiplied by physical activity

121   level. This equation was chosen following the precedent of the calculation of the DRV for

122   energy by the Scientific Advisory Committee on Nutrition (22). Physical activity was

123   estimated by using the International Physical Activity Questionnaire (IPAQ) (23). The level

124   ascribed by the IPAQ was then translated to a PAL level using the Committee on Medical

125   Aspects of Food Policy (COMA) classifications (24) (IPAQ score low = non active, moderate

126   = moderately active, high = very active) and taking into account occupational activity which

127   was classified according to COMA as light, moderate or heavy.

128   The macronutrient composition of the diet, as a percentage of total energy for the day, was

129   approximately 50 % carbohydrates, 35 % fat and 15 % protein. These macronutrient

130   percentages were based on the Report of the Panel on Dietary Reference Values of COMA

131   (24).

132   Participants were reassured that the amount of food provided was designed to ensure a stable

133   body weight over the course of the study. All participants declared an intention to consume

134   the entire amount of food supplied. However, they were asked to record any left-over food in

135   the diary provided. Participants were instructed to avoid alcohol consumption, limit caffeine-

136   containing drinks to two cups of tea per day (without sugar/milk). They were advised not to

137   change physical activity patterns during the study.

138   Following the design of previous studies in our laboratory (6-8), the number of meals during

139   the regular meal pattern was 6 meals/day which was based upon three 'meals' providing

140   approximately 70 % of energy requirements (breakfast, lunch and dinner) and three 'snacks'

141   (mid-morning, afternoon and evening snack) providing a total of approximately 30 % of

142   energy requirements (**Supplemental Table 1**).

143   The number of meals (including eating incidences labelled as snacks on the menu) during the

144   irregular meal pattern varied from 3 to 9 meals/day. The average was 6 meals/day during the

145   14-day period (i.e. 7, 4, 9, 3, 5, 8, 6, 5, 9, 8, 3, 4, 7, 6 meals/day). Participants were asked to

146   eat their meals at specific times between 8:00 am and 9:00 pm, during both interventions, to

147   remove the potentially confounding impact of the time period over which food was

148   consumed. The only deviation from this instruction was that when they had 3 meals/day,

149   during the irregular period, their last meal was at 6:00 pm (instead of 9:00 pm) as it was

150   anticipated that this was when they would consume a meal with others in their household.

151   **Measurements made during the intervention periods**

152   *Energy expenditure assessment*

153   Participants wore a SenseWear™ armband (SWA, BodyMedia Inc, Pittsburgh, PA, USA) to

154   obtain an ambulatory estimate of their energy expenditure (AEEE) continuously during the

155   intervention periods. The armband was worn over the left triceps muscle, halfway between

156   the acromion process of the scapula and the olecranon process of ulna. Participants were

157   instructed to wear it continuously, including while sleeping and to remove it only for brief

158   periods for bathing, showering or swimming.

159   Energy expenditure data were derived from, a skin temperature sensor, a near body

160   temperature sensor, a galvanic skin response sensor, a heat flux sensor, and accelerometer

161   (25). These data were used in combination with demographic characteristics including age,

162   sex, weight and height, to estimate energy expenditure using a proprietary equation

163   developed by the manufacturer (SenseWear Software, version 7) which was not published.

164   *Continuous Glucose monitoring (CGM)*

165   CGM (Medtronic Minimed, Northridge, USA) provided continuous glucose profiles for up to

166   72 h. Subcutaneous interstitial fluid glucose concentrations were measured every 10 seconds

167   and the average glucose value for each 5 min period was stored (up to 288 measurements

168   daily).

169   The CGM was placed subcutaneously over the participant's anterior abdominal wall on day 6

170   and removed on day 10 of each intervention period. Finger prick glucose readings were taken

171   four times a day, by the participants, using a portable monitor (Accu-Chek Aviva System,

172   Roche Diagnostics, Switzerland) to calibrate the CGM. A 24 h contact number was available

173   for any inquiries or if any problems arose. Data from CGM were downloaded and glucose

174   profiles were evaluated based on data collected on day 7 (6 meals/day in both regular and

175   irregular periods), day 8 (6 meals/day vs. 5 meals/day in regular and irregular periods

176   respectively) and day 9 (6 meals/day vs. 9 meals/day in regular and irregular periods

177   respectively). Data were analysed per 24 h, during the day (7:00–midnight) and during the

178   night (midnight-7:00) with respect to 24 h mean, max, min and iAUC for glucose for each

179   time period.

180 On day 7 (6 meals/day in both regular and irregular interventions) postprandial iAUC for 90

181 min was analysed following each meal (breakfast, mid-morning snack, lunch, afternoon

182 snack, dinner and evening snack). However on day 8 (6 meals/day vs 5 meals/day in regular

183 and irregular interventions respectively) analysis was restricted to the points in the day when

184 participants consumed identical meals on the two interventions (breakfast, mid-morning

185 snack and evening snack).The afternoon snack was omitted during the irregular period and

186 the food distributed between lunch and dinner. On day 9 (6 meal/day vs. 9 meals/day in

187 regular and irregular periods respectively), analysis similarly was restricted to lunch, dinner

188 and evening snack. The breakfast was divided into two meals during the irregular period. The

189 mid-morning and afternoon snacks were also divided into two small meals in order to achieve

190 9 meals/day.

191 Intra-day glycemic variability was computed by an approach described by McDonnell et al

192 (2005) specifically for CGM data, known as continuous overlapping net glycemic action

193 (CONGA-n) (26). CONGA-n is calculated as the standard deviation of the summed

194 differences in glucose concentration between current observation and the observation n hours

195 previous. CONGA-1 was calculated in the morning (current observation from 9:00-10:00)

196 and night (current observation from 22:00-23:00). CONGA-1 indicated intra-day glycemic

197 variability based on one hour time periods.

198 *Appetite assessment*

199 Subjective appetite ratings were assessed by using paper based visual analogue scales (VAS)

200 with words anchored at each end of a 100-mm horizontal line that expressed the most

201 positive and the most negative rating for a question (**Supplemental Figure 1**). The questions

202 were in the form 'How (rating) do you feel?' and the ratings were 'hungry', 'satisfied', 'full',

203 'how much of a desire to eat?' and 'how much do you think you can eat?' (27).

204   Participants were provided with a booklet in which to record subjective appetite before and

205   after each single meal on days 7 and 14 during both intervention periods, when they were

206   consuming 6 meals/day on each intervention.

207   **Laboratory visit protocol and procedures**

208   Participants were asked to attend the laboratory at 8:00 am after a minimum 12 h overnight

209   fast and were required to take no exercise other than walking related to carrying out their

210   normal activities of daily living, for 48 h before the laboratory visit. Participants consumed 6

211   meals/day on the day prior to the final laboratory visit on both interventions in order to

212   eliminate an acute effect of the meal frequency on the day immediately preceding the

213   laboratory visit. Once baseline measurements were completed, participants were served a test

214   drink at approximately 9:00 am. Further measurements were then taken over a 3 h period, and

215   then an *ad libitum* test lunch was given at 12:30 pm. Subjective appetite ratings were

216   measured using VAS before and over a 1 h period after the *ad libitum* test meal

217   *Anthropometric measurements*

218   Immediately after arrival, participants were weighed on an electronic scale (Seca, Germany)

219   to the nearest 0.1 kg with an empty bladder, wearing similar light clothes on each visit and

220   without shoes. Waist circumference was measured to the nearest 0.5 cm in a horizontal plane

221   at a point midway between the lower margin of the last rib and the top of the iliac crest using

222   a stretch-resistant tape, while the participant was standing with feet about 25–30 cm apart

223   (28). Hip circumference was measured to the nearest 0.5 cm in a horizontal plane at the point

224   yielding the maximum circumference over the buttocks (28). Skinfold thickness

225   measurements were made by the same investigator, in triplicate, at four sites (triceps, biceps,

226   subscapular and suprailiac) in order to assess participants' body composition (29).

227   *Blood sampling*

228 Following the anthropometric measurements, participants rested in a semi-supine position in

229 a temperature-controlled (23-24 °C) room for a minimum of 20 min. Then a 20 G cannula

230 (Venflon) was inserted into a dorsal hand vein under local anaesthetic (1 % lignocaine:

231 B.Braun Melsungen AG. Melsungen, Germany) for subsequent blood sampling. The hand

232 was placed into a hot air-warmed, ventilated perspex box (50–55 ºC) to allow arterialised

233 venous blood sampling (30). Blood samples were drawn from a 3-way tap, the first 2 mL of

234 each sample was discarded to avoid contamination with the saline (Baxter Healthcare Ltd.,

235 Thetford, UK) used to maintain patency.

236 Two blood samples were taken with a 5 min interval just before ingestion of the test drink to

237 assess the mean of fasting serum total, HDL, LDL-cholesterol, triacylglycerol, blood glucose,

238 serum insulin, plasma glucagon-like peptide-1 (GLP-1), plasma Peptide YY (PYY), and

239 plasma ghrelin. After the test drink ingestion, blood samples were taken every 15 min for

240 glucose and every 30 min for 3 h to assess all the markers mentioned above except lipids, for

241 which only a fasting measurement was made.

242 Blood was dispensed into serum separating tubes (allowed to clot for 30 min at room

243 temperature before centrifugation) and EDTA tubes. EDTA tubes containing either 20 µl

244 dipeptidyl peptidase IV (DPP-IV) inhibitor (Millipore, Billerica, MA, USA) for GLP-1

245 measurement or 50 µl aprotinin (Nordic Pharma, Reading, UK) for PYY and ghrelin

246 measurements. All samples were centrifuged (5702 R, Eppendorf, Germany) for 10min at

247 3000 r.p.m at 4 ºC. The supernatant was transferred into plastic tubes and kept at -80 ºC until

248 further analysis.

249 *Blood analysis*

250 The analyses were carried out at the University of Nottingham. Serum total, HDL, LDL-

251 cholesterol and triacylglycerol concentrations were quantified by an enzymatic photometric

252 method (HORIBA ABX, Montpellier, France). Blood glucose was measured immediately

253   using a HemoCue analyser (AB, Angelholm, Sweden). Serum insulin concentrations were

254   measured with commercially available radioimmunoassays (Millipore, Billerica, MA, USA).

255   Fasting insulin sensitivity was calculated using the homeostatic model assessment (HOMA

256   model) (31). Plasma GLP-1 concentrations were measured using an ELISA kit (Linco

257   Research, St Charles, MO, USA). Plasma PYY and ghrelin concentrations were measured

258   with commercially available radioimmuno assays (Millipore, Billerica, MA, USA).

259   *Test drink consumption*

260   The standardized test drink (vanilla flavour milkshake) was served at room temperature in an

261   open glass as a breakfast. Participants were instructed to drink it over a period of 10 min. The

262   test drink provided 10 kcal/kg body weight and comprised 50 % of energy as carbohydrate,

263   35 % as fat, and 15 % as protein. All participants consumed all of the test drink. The mean

264   energy provided by the test drink was 584.3 ± 51.8 kcal which provided a mean of 27.9 ±

265   1.1% of the estimated energy requirement.

266   The test drink contained skimmed milk (Sainsbury's, London, UK), Build-up (Nestle SA,

267   Lausanne, Switzerland), Polycal (Nutricia Clinical Care, Trowbridge, UK) and double cream

268   (Sainsbury's, London, UK).

269   *Energy expenditure measurement*

270   Indirect calorimetry (GEM system; Europa Scientific Ltd, England) was used to determine

271   REE and TEF by measuring the volume of oxygen uptake and carbon dioxide expired. An

272   open-circuit flow-through canopy, with a mass flow meter, mixing chamber and a vacuum

273   pump, was used to draw room air over the participants' face at a rate of 50-60 l/min. This is

274   considered to be the most convenient way for measuring energy expenditure in human studies

275   at rest (32). The system was connected to a computer, and data from the mass flow meter and

276   gas analysers were used to calculate the VO_2 and VCO_2 using the software provided by the

277   manufacturer. The indirect calorimetry system was turned on for half an hour prior to use, to

278 warm up. Two cylinders of pressurised gas of known composition were used to calibrate the

279 gas analysers in the indirect calorimetry system before the start of the experiment. REE was

280 measured in the fasted state for 20 min. TEF was then measured for periods of 15 min at 30

281 min intervals during the 3 h following the milkshake consumption. During the measurements,

282 participants rested on the bed and relaxed but were not permitted to sleep. In the intervals

283 between the measurements, they also rested on the bed, but they were allowed to read. Room

284 air was measured at the start and both before and after each 15 min measurement period.

285 *Ad libitum test meal*

286 A pasta-based test meal (providing 167 kcal/100 g with 13, 34 and 53 % energy provided by

287 protein, fat and carbohydrate, respectively) was served at lunchtime to assess *ad libitum* food

288 intake. This meal had a homogeneous nature, so energy intake could be assessed from the

289 weight of food consumed. The meal consisted of pasta (Sainsbury's, London, UK: 125 g

290 cooked in 800 ml boiling water on full power in a microwave (900 W) for 13 min- stirred mid

291 period). The pasta was then drained, cooled rapidly using cold water and then mixed with

292 cheddar cheese (Sainsbury's: 40 g), olive oil (Sainsbury's: 15 g), and tomato and basil pasta

293 sauce (Dolmio, Mars food, UK: 170 g; macronutrient composition of sauce in **Supplemental**

294 **Table 2**). The mixture was then chilled until required and heated in the microwave for 2 min

295 before being served to the participants. Participants were given portions of ~500 g and

296 instructed to consume as much as they wanted until they felt 'comfortably full'. The plate of

297 pasta was continually topped up, when it was approximately ¾ empty. This ensured that there

298 was always ample hot food available to participants and they were not cued to stop eating by

299 having emptied their plate. Any left-over was removed and energy intake was calculated from

300 the weight of food consumed. Duration and speed (g/min) of eating were also calculated.

301 *Subjective appetite ratings*

302 Participants completed the VAS for subjective appetite ratings just before, after and then

303   every 30 min after consumption of the test drink for 3 h. Further VAS were completed before

304   and immediately after consuming the lunch test meal, and then at 15, 30, 45 and 60 min. The

305   VAS were as described above. To avoid participants' response to each set of VAS being

306   biased by their responses to the previous set each paper sheet was taken from the participant

307   before the next one was provided. During this period of time, participants were asked to stay

308   in the laboratory, but they were free to read.

309   **Statistical analyses**

310   SPSS software (version 21 for windows; SPSS) was used for data entry and analysis. All data

311   are presented as means± standard deviation (SD), unless otherwise stated. Data were tested

312   for normality with the Kolmogorov-Smirnov test to inform whether parametric or non-

313   parametric analysis should be used.

314   Values for the incremental area under the curve (iAUC) of the TEF, postprandial glucose,

315   insulin, appetite ratings and gut hormone responses were calculated using differences from

316   the baseline. Values above baseline were considered positive, and below baseline negative.

317   The area above or below baseline was calculated using the trapezoid rule.

318   Comparisons of the baseline data at the pre intervention visit were made using Student's

319   paired t test (two-tailed) as were measurements of energy intake, AEEE, VAS and CGM

320   during the intervention period.

321   Two-way repeated measure ANOVAs (Factor 1: meal pattern, regular and irregular meal

322   pattern; Factor 2: visit - pre and post each 14-day intervention) were conducted to assess the

323   impact of the 14-day meal pattern intervention on a range of dependant variables (e.g. weight,

324   iAUC for TEF, weight of pasta consumed). Where an interaction was identified, simple main

325   effects were explored by pairwise comparisons. Where no interaction was identified, but

326   significant main effects were found, pairwise comparisons were made for the effect of meal

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

328   statistical tests.

329   Results obtained from a previous study (6) indicated that the iAUC TEF after a regular meal

330   pattern was 0.74 ± 0.37 kJ/min and after an irregular meal pattern was 0.39 ± 0.26 kJ/min.

331   Therefore, with a cross-over design, eleven participants in each group would be required to

332   detect a difference in TEF (~ 0.35 kJ/min) with the power of 80 % at the significance level of

333   0.05.

334   TEF (kcal/min) over 3 h (following the test drink), as assessed by indirect calorimetry, was

335   the primary outcome for comparison between the two intervention periods. Responses for

336   lipids, glucose, insulin, gut hormones, subjective appetite ratings and *ad libitum* food intake

337   of the test meal were considered as secondary outcomes.

338   **RESULTS**

339   In this study, the effect of meal irregularity on thermic effect of food (TEF), lipid

340   concentrations, carbohydrate metabolism, subjective appetite and gut hormones were

341   investigated in 11 healthy normal-weight women. Participants undertook either a regular

342   meal pattern (14 days, 6 meals/day) an irregular meal pattern (14 days,varying from 3 to 9

343   meals/day) or in a randomised crossover design, separated by a 14-day wash out period.

344   Participants attended the laboratory after an overnight fast at the start and end of each

345   intervention period.

346   **Anthropometric measurements**

347   There were no significant differences in bodyweight, body composition, or other

348   anthropometric measurements at the pre intervention visits or across the study visits (**Table**

349   **1**).

350   **Energy Intake**

351   Self-reported daily energy intake before the start of the study (2081 ± 214 kcal/day) was

352   similar to the estimated energy requirement for weight maintenance (2104 ± 204 kcal/day).

353   However self-reported carbohydrate percentage (47 ± 4.1 %) was significantly lower and

354   self-reported fat percentage (38 ± 3.7 %) was significantly higher compared with the

355   consumed intervention diet (53 ± 0.2 % carbohydrate and 33 ± 0.6 % fat) (paired T-test, p <

356   0.01). There were no significant differences in the protein percentage between the self-

357   reported and the prescribed diet (14 ± 2.5 vs 14 ± 0.4 % respectively).

358   During the study, food intake was designed to be the same by type, and amount in each

359   intervention period, hence provide the same amount of energy and have the same

360   macronutrient composition. The food intake diaries completed to check compliance showed

361   that 98 ± 6 % and 100 ± 2 % of the energy given was consumed in the regular and irregular

362   intervention periods respectively indicating good compliance. There were no significant

363   differences in energy intake between the two intervention periods (2043 ± 248 kcal/day

364   regular vs. 2098 ± 195 kcal/day irregular intervention period) as intended by the design of the

365   study. The composition of consumed foods also did not differ significantly between the two

366   intervention periods being (53 ± 0.9 % carbohydrate, 14 ± 0.4 % protein and 33 ± 0.8 % fat in

367   regular and 53 ± 0.3 % carbohydrate; 14 ± 0.5 % protein and 33 ± 0.7 % fat in irregular

368   intervention period).

369   **Free-living energy expenditure**

370   On average, the SWA device was worn 96.8 ± 5.5 and 95.1 ± 7.7 % of the regular and

371   irregular intervention periods respectively. There were no significant differences between

372   mean values of AEEE during the intervention period for both regular and irregular meal

373   pattern (2241± 360 kcal/day and 2305 ± 399 kcal/day for regular and irregular intervention

374   periods respectively). There were no significant differences between the mean of the physical

375   activity level during the regular and irregular intervention period (1.60 ± 0.2 and 1.64 ± 0.2

376   METs for regular and irregular intervention periods respectively). In both conditions the

377   estimated energy expenditure was approximately 200 kcal greater than the prescribed energy

378   requirement.

379   **Free-living CGM**

380   For the nine participants for whom CGM data were available, analyses (mean, max, min,

381   CONGA-1 and iAUC) were done for each meal pattern on day 7 (6 meals consumed in both

382   intervention periods), day 8 (6 meals and 5 meals consumed in regular and irregular period

383   respectively), and day 9 (6 meals and 9 meals consumed in regular and irregular period

384   respectively) (**Table 2**). Twenty-four hour mean, max, min and iAUC for glucose

385   concentrations showed no significant differences between the two intervention periods. There

386   were also no significant differences in the day period and the night period between the two

387   intervention periods for these variables. CONGA-1 with current observation period 9:00 to

388   10:00 and 22:00 to 23:00 also showed no significant differences between the two intervention

389   periods.

390   On day 7 of the intervention (6 meals/day both interventions), there was a significantly higher

391   glucose concentration for the postprandial (breakfast +90 min) iAUC analysis (Table 2) in the

392   irregular meal pattern intervention compared with the regular meal pattern intervention

393   (paired T-test, $p < 0.05$). On day 9 (6 meals v 9 meals), for the meals that were identical on

394   the two interventions, postprandial (lunch +90 min) and (dinner +90 min) iAUC analysis

395   showed a similar difference in that the iAUC in the irregular intervention was significantly

396   higher compared with the regular intervention (paired T-test, $p < 0.05$). No significant

397   differences were seen in the other postprandial iAUC analysis.

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

399   Fasting REE was not significantly different at the pre intervention visits. There was also no

400   meal pattern by visit interaction, or main effect of meal pattern or visit for fasting REE (1167

401 $\pm$ 134, 1207 $\pm$ 89, 1183 $\pm$ 171 and 1188 $\pm$ 149 kcal/day in pre, post regular and pre, post

402 irregular visits respectively).

403 REE increased above the fasting values, after the test drink, at all visits. The overall TEF for

404 the 3 h postprandial period is shown in **Figure 2**. There was no significant difference in

405 overall 3 h TEF at the pre intervention visits. There was a significant meal pattern by visit

406 interaction for the 3 h TEF (ANOVA; $p < 0.05$). TEF post regular visit was increased

407 significantly compared with pre regular visit (paired T-test $p < 0.01$) unlike in the irregular

408 visits, where there was no significant difference between pre and post intervention visits. TEF

409 post regular visit was 11.1 $\pm$ 15.8 kcal higher than post irregular visit (paired T-test $p < 0.05$).

410 **Blood variables**

411 There were no significant differences at the pre intervention visits for all blood variables.

412 *Lipids*

413 The results for fasting serum total, LDL, HDL-cholesterol, serum triglycerides are shown in

414 **Table 3**. There were no significant interactions for meal pattern by visit or main effects of

415 meal pattern or visit in fasting serum total, LDL, HDL-cholesterol, serum triglycerides.

416 *Glucose*

417 No significant meal pattern by visit interaction or main effects of meal pattern or visit were

418 observed in fasting blood glucose across the study (Table 3). Blood glucose responses to the

419 test drink reached a maximum level 30 and 45 min after the test drink and remained above

420 fasting levels at the last sampling time-point (180 min after the test drink) in all visits. The

421 peak values (Table 3) did not show a significant interaction for meal pattern by visit or main

422 effects for these two factors. Blood glucose iAUC response to the test drink **(Figure 3)**

423 showed a significant interaction between meal pattern and visit (ANOVA; $p < 0.05$). A larger

424 area was seen at the post irregular visit compared with post regular visit ($p < 0.05$). Post

425 irregular visit, blood glucose iAUC was significantly higher than pre irregular visit ($p <$

426   0.05), unlike in the regular intervention, where there was no significant difference between

427   pre and post regular visits.

428   *Insulin*

429   Table 3 shows fasting serum insulin in all visits. There were no significant interactions for

430   meal pattern by visit or main effects of meal pattern or visit. Serum insulin concentrations

431   increased rapidly from 15 min after consuming the test drink in all visits. Following peak

432   values, concentrations declined to some extent but remained above fasting values for the

433   reminder of the sampling period. The peak values of insulin (Table 3) did not show a

434   significant meal pattern by visit interaction or main effects of meal pattern or visit. There was

435   no significant interaction between meal pattern and visit on iAUC for serum insulin nor were

436   there significant main effects for meal pattern or visit (5826.2 ± 2150.5 mIU/L in 3h pre

437   regular visit, 5719.4 ± 3326.6 mIU/L in 3 h post regular visit, 5842.6 ± 3775.2 mIU/L in 3 h

438   pre irregular visit and 5268.9 ± 2248.0 mIU/L in 3 h post irregular visit).

439   *GLP-1*

440   There was no significant interaction for meal pattern by visit or main effect of meal pattern

441   for fasting plasma GLP-1 concentrations (Table 3). However, a significant main effect of

442   visit was observed (ANOVA, $p < 0.05$). Mean fasting plasma GLP-1 concentrations

443   decreased by approximately 16 % and 20 % post regular and irregular visits respectively

444   compared with pre intervention visits. Following consumption of the test drink, plasma GLP-

445   1 concentrations increased in all visits. iAUC for plasma GLP-1 concentrations (**Figure 4**),

446   showed no significant interaction between meal pattern and visits, or main effects for meal

447   pattern or visit.

448   *PYY*

449   No significant meal pattern by visit interaction or main effects of meal pattern were observed

450   in fasting plasma PYY concentrations (Table 3). However, there was a significant main effect

451   of visit (ANOVA, p < 0.05). Mean fasting plasma PYY concentrations decreased about 9 %

452   and 23 % post regular and irregular visits respectively compared with pre intervention visits.

453   Plasma PYY concentrations increased rapidly above the fasting values after consuming the

454   test drink and remained at a plateau until the last sampling time point in all visits. iAUC for

455   the 3 h postprandial period in all visits (Figure 4) showed no significant interaction between

456   meal pattern and visit or main effect for meal pattern. However, there was a significant main

457   effect of visit (ANOVA, p < 0.05). Mean iAUC for plasma PYY concentrations increased by

458   approximately 57 % post regular compared with pre regular visit, and by 70 % post irregular

459   compared with pre irregular visit.

460   *Ghrelin*

461   No significant meal pattern by visit interaction or main effects of meal pattern or visit were

462   observed in fasting plasma ghrelin (Table 3). Following consumption of the test drink,

463   plasma ghrelin concentrations declined in all visits. iAUC for plasma ghrelin (Figure 4)

464   showed no significant interaction between meal pattern and visits, or main effects for meal

465   pattern or visit.

466   **Subjective appetite ratings**

467   *Responses to the test drink*

468   There were no significant differences between the pre intervention visits for any of the iAUC

469   for subjective appetite ratings collected in the fasting state (**Supplemental Table 3**). There

470   was also no meal pattern by visit interaction, or main effect of meal pattern or visit for fasting

471   VAS ratings (Supplemental Table 3). The assessments of subjective hunger for the 3 h

472   postprandial period in all visits showed no significant interaction between meal pattern and

473   visit or main effect for meal pattern, but a significant main effect of visit (ANOVA, p < 0.05)

474   was found. Mean hunger ratings decreased by 195 % and 104 % post regular and irregular

475   visits respectively compared with pre intervention visits (Supplemental Table 3). The

476 response for the other VAS ratings showed no significant differences between the

477 intervention periods (Supplemental Table 3).

478 *Responses to the ad libitum test meal*

479 The response (for hunger, fullness, satiety, desire to eat and prospective food consumption)

480 for the 1 h postprandial period in all visits showed no significant interaction between meal

481 pattern and visit or main effect for meal pattern or visits (Supplemental Table 3).

482 *Responses to the meal pattern during the intervention*

483 Subjective appetite ratings were assessed pre and post meals during day 7 and 14, when 6

484 meals/day were consumed in both regular and irregular intervention periods. On day 7, there

485 were no significant differences between mean pre meal ratings (average of the 6 pre-meal

486 ratings on the day) (**Table 4**). However, mean post meal ratings for hunger and fullness

487 showed significant differences between the interventions. Higher post meal ratings for hunger

488 and lower for fullness (paired T- test, $p < 0.01$) were observed in irregular compared with the

489 regular intervention period (Table 4).

490 On day 14 (the final day of intervention), the ratings of pre meals hunger was significantly

491 greater in irregular compared with regular intervention period (**Table 5**, paired T- test, $p <$

492 0.05,). Furthermore, the ratings of post meal hunger were significantly greater in the irregular

493 period (Table 5, paired T- test, $p < 0.05$). There were no significant differences in the pre and

494 post meal values for the other VAS appetite ratings.

495 **Intake at the *ad libitum* test meal**

496 There was no significant difference between participants' energy intake at the *ad libitum* test

497 meal pre intervention visits. There was no meal pattern by visit interaction or main effect of

498 meal pattern or visit for participants' energy intake across the study visits (778.8 ± 272.8,

499 745.7 ± 214.7, 722.4 ± 324.0 and 764.3 ± 246.6 kcal in pre and post regular and irregular

500 visits respectively).

501   The duration of eating and speed of consuming the *ad libitum* test meal were not significantly

502   different pre intervention visits. The duration of eating did not show a significant interaction

503   between the meal pattern and visit or main effect of meal pattern or visit (9.6 ± 3.9, 9.8 ± 3.8,

504   9.5 ± 3.1 and 9.1 ± 2.3 min in pre and post regular and irregular visits respectively). Speed of

505   eating also showed the same result (51.1 ± 13.2, 47.9 ± 10.1, 45.1 ± 13.4 and 50.6 ± 11.1

506   g/min in pre and post regular and irregular visits respectively).

507   **DISCUSSION**

508   The aim of this study was to investigate the metabolic, endocrine and appetite related effects

509   of a regular compared with an irregular meal pattern, in healthy normal-weight women

510   consuming identical, isoenergetic diets and undertaking comparable activity. We also

511   assessed activity using AEEE, continuous interstitial glucose monitoring (CGM) and appetite

512   in the free-living state.

513   No differences were found in body weight between the two interventions, suggesting that the

514   aim to match intake and activity were met. With the regular meal pattern, TEF was greater,

515   whilst post prandial glucose response was smaller both in response to a test drink, and in

516   response to some identical meals, whilst free-living. No differences were found in fasting

517   lipid values. PYY showed a greater postprandial response after both interventions,

518   concurrently with anticipated differences in hunger and fullness. Pre and post appetite ratings

519   during the regular intervention suggested greater fullness and reduced hunger.

520   The differences in TEF are compatible with our previous findings (6, 8). Compensation in

521   other components of energy expenditure might explain the similar body weights seen after

522   the two interventions, despite differences in TEF. However, there was no difference in REE,

523   and although the estimate of ambulatory energy expenditure made using the SWA device has

524   limitations, for example, the absence of published validated equations for this population

525   group and inconsistent findings when compared with indirect calorimetry (25, 33-35), it gives

526   an indication of comparable activity patterns. The short duration of the study is a more likely

527   explanation, as over a longer time period, the greater TEF with a regular meal pattern, if

528   repeated at all meals and in the longer term, could have beneficial effects on weight control.

529   The range of published values for the TEF of diets containing comparable macronutrient

530   composition makes estimating the expected TEF from the test drink problematic (36).

531   However using a generally accepted figure for TEF of 10 % of total energy consumed, and a

532   mean test drink dose of 584 kcal, a TEF of approximately 60 kcal might be expected. The

533   smaller values seen (over 3 h) may reflect that the full metabolic rate response had not

534   occurred in 3h. It has been estimated that weight gain in 90 percent of the adult population

535   could be prevented by reducing positive energy balance by 100 kcal/day (37) and Brown et

536   al. found that over 5 years a 10 kcal/day excess in energy intake resulted in a 0.5 kg gain in

537   weight per year (38). Future work should assess energy expenditure over 24 h, in order to

538   capture the full response to each meal, and the accumulative effect of more than one meal in

539   the day.

540   Insulin resistance has been shown to be associated with blunted TEF (39-41), and may

541   contribute to the differences we have seen. In this study, a lower postprandial glucose

542   response to the test meal was seen after the regular compared with the irregular meal pattern.

543   In our previous studies (7, 8), there was no difference in glucose response, but a greater post

544   prandial insulin response was seen after the irregular meal pattern period. Both of these

545   patterns of results are consistent with the regular meal pattern resulting in greater insulin

546   sensitivity. The novel addition to the present study of continuous interstitial glucose

547   measurements on three days during the intervention periods (each preceded by the same last

548   meal on the previous day) further corroborates reduced insulin sensitivity with an irregular

549   pattern. Day 7 allowed direct comparison of six meals per day and showed a beneficial

550   response to breakfast with regular eating. On Day 8 however, despite having several

551 identical meals, no differences were found, perhaps because of an acute effect of the

552 preceding day being identical for both patterns (6 meals per day). On Day 9, for those meals

553 that were identical, a beneficial reduction in post prandial response at lunch and dinner (but

554 not the night snack) was seen for the regular pattern. Further work is needed to establish

555 whether, under laboratory conditions, a comparable difference in blood glucose response

556 occurs throughout the day, how quickly differences are seen in response to dietary

557 differences, and whether the differences are sustained over a longer time period.

558 Fasting Triglyceride and HDL cholesterol concentrations showed no significant differences

559 between the two meal patterns in the present study, in agreement with previous studies in

560 normal-weight and obese women (7, 8). However previously differences were found between

561 fasting total and LDL cholesterol (7) in contrast to this study. This is perhaps because the

562 food intake was better controlled in this study. The participants in the current study were

563 similar to those in the previous study with respect to age, BMI and body fat, however their

564 ethnicity may have been different, possibly resulting in differences in sensitivity to meal

565 pattern.

566 Greater post-meal ratings for hunger and lower ratings of fullness on day 7 (6 meals/day on

567 both interventions), during the irregular meal pattern period suggest a reduction in the

568 satiation experienced. Additionally, greater pre and post-meal ratings for hunger were

569 observed on the final day of the irregular meal pattern when again 6 meals were consumed in

570 both interventions, suggesting that by the end of the study satiety was reduced as well.

571 However there was no difference by intervention for subjective appetite in response to the

572 test meal (although there was a time effect), or the pasta meal. The energy intake of pasta

573 consumed at the *ad libitum* test meal in the laboratory was decreased by 4 % post regular visit

574 and increased by 6 % post irregular visit. This did not reach significance, possibly because

575 the study was insufficiently powered for this secondary outcome.

576   Whilst no meal pattern effect was found for fasting plasma GLP-1 and PYY concentrations, a

577   main effect of time was seen, and in response to the test meal for PYY. The explanation for

578   these differences, in common with the time effect reported above for subjective appetite, may

579   be the differences in composition of habitual diet and the intervention diet. The 7 day food

580   record would suggest that the habitual diet contained a lower percentage of carbohydrate and

581   a higher percentage of fat. In addition, on day 14, before the final visit, the number of meals

582   and amount of food was the same on both occasions, in contrast to the first visits when the

583   habitual diet was consumed the preceding day. The stage in the menstrual cycle was also

584   different as the study started in the early phase of the follicular phase, which may have

585   impacted on appetite (42, 43) and GLP-1 (42). The differences observed in PYY in response

586   to the test drink were consistent with the differences in VAS hunger responses, confirming

587   the inverse relationship between PYY and subjective hunger (44). Given, that the differences

588   in subjective appetite noted whilst free-living in this study, might offer an explanation for the

589   higher energy intake previously noted in obese participants eating *ad libitum* while following

590   an irregular meal pattern (8), this aspect warrants further work, with a larger sample size. As

591   demonstrated with respect to TEF, small differences in energy intake, sustained over the long

592   term, can have a major impact on weight regulation. It is also of interest that associations

593   have been found between TEF and satiety (45) suggesting that there may be some inter-

594   relation between differences in subjective appetite, and the blunted TEF measured in this

595   study.

596   In conclusion, the results of this study show that a regular meal pattern compared with an

597   irregular meal pattern results in greater TEF, greater insulin sensitivity, and potentially

598   beneficial subjective appetite changes. These desirable effects could support weight control

599   and metabolic health, in the general population. Future studies should include overweight and

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

601   measurement, and longer term interventions.

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

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

[1] mean $\pm$ SD, n=11.

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

TABLE 2. Analyses of the CGM data compared between the two meal pattern interventions [1]

Glucose (mmol/L)	Regular meal pattern			Irregular meal pattern		
	Day 7 6 meals	Day 8 6 meals	Day 9 6 meals	Day 7 6 meals	Day 8 5 meals	Day 9 9 meals
Fasting	4.7±0.8	4.9±0.4	4.9±0.4	5.0±0.6	4.9±0.6	5.1±0.4
Mean 24 h	5.2±0.5	5.3±0.4	5.4±0.6	5.2±0.4	5.2±0.4	5.5±0.3
Mean day h [2]	5.3±0.7	5.4±0.5	5.5±0.5	5.3±0.4	5.3±0.4	5.6±0.3
Mean night h [2]	4.9±0.3	5.2±0.6	5.2±0.8	4.9±0.6	5.0±0.4	5.1±0.5
Max [3] 24 h	7.1±1.0	7.1±1.4	7.9±1.5	7.5±1.4	7.2±0.8	7.9±1.2
Max day h	7.1±1.0	7.1±1.4	7.9±1.5	7.5±1.3	7.2±0.8	7.9±1.2
Max night h	5.5±0.4	5.8±0.8	5.9±0.7	5.8±1.0	5.5±0.5	5.8±0.6
Min [3] 24 h	4.1±0.8	4.3±0.5	4.1±0.5	3.8±0.4	3.9±0.5	4.1±0.5
Min day h	4.1±0.8	4.3±0.5	4.1±0.6	4.3±0.4	4.1±0.6	4.3±0.4
Min night h	4.5±0.4	4.7±0.6	4.8±0.8	4.2±0.6	4.4±0.6	4.5±0.6
iAUC 24h	566.9±935.2	464.8±756.9	625.7±633.4	473.2±760.0	659.3±834.9	969.0±808.8
iAUC day h	553.3±723.0	376.7±610.4	515.0±591.7	500.8±547.1	629.9±637.6	850.5±685.5
iAUC night h	-95.0±226.8	-74.1±169.4	-186.4±209.8	-69.5±138.4	-75.5±199.7	-136.2±145.9
CONGA-1 [3] (9:00-10:00)	0.67±0.6	0.68±0.4	1.13±0.8	1.14±0.7	0.59±0.3	0.72±0.3
CONGA-1 (22:00-23:00)	0.38±0.22	0.36±0.1	0.60±0.4	0.32±0.2	0.32±0.2	0.52±0.2
iAUC [3]-breakfast +90	50.3±54.4 [4]	56.3±52.0	-	95.7±70.8 [4]	66.6±42.2	-
iAUC-mid-morning snack +90	25.3±29.3	29.9±40.4	-	31.8±42.3	43.2±25.9	-
iAUC-lunch +90	34.6±40.0	-	51.4±43.9 [5]	21.5±45.0	-	102.8±74.7 [5]
iAUC-afternoon snack +90	36.8±61.0	-	-	41.7±43.1	-	-
iAUC-dinner +90	46.0±58.9	-	50.5±43.3 [6]	56.3±53.0	-	90.3±54.7 [6]
iAUC-night snack +90	17.2±21.7	25.3±26.7	9.4±45.0	35.7±32.1	21.3±33.0	23.1±21.9

[1] mean ± SD, n=9.

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

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

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

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

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

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

[1] mean $\pm$ SD, n=10.

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

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

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

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

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

[1] mean ± SD, n=11.

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

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

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

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

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

[1] mean ± SD, n=11.

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

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

No significant differences were observed in the other VAS ratings between the two intervention periods (paired T-test).
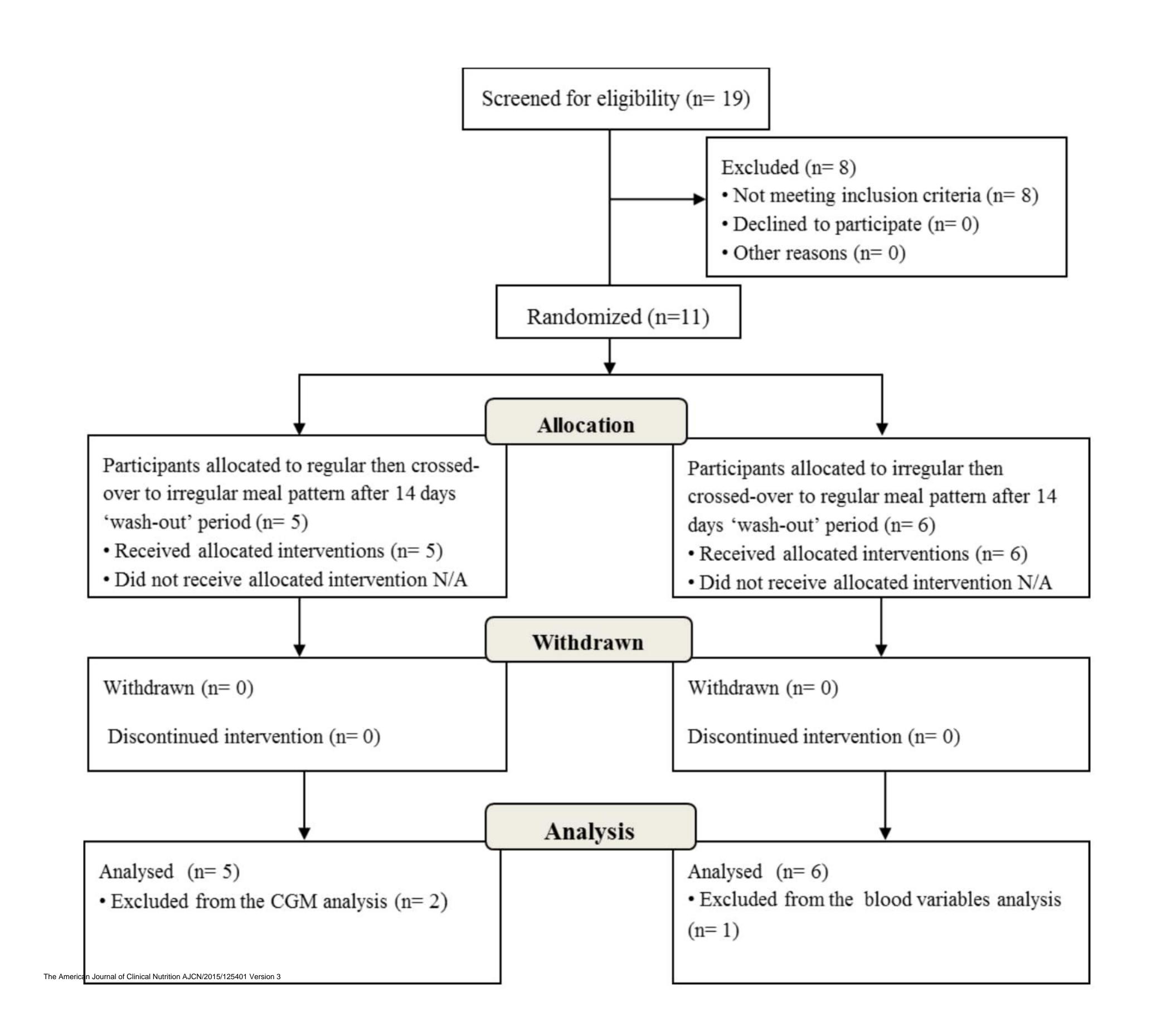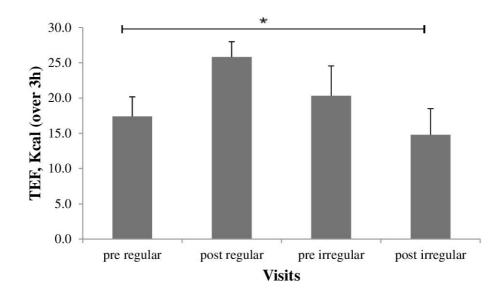
FIGURE 1. Study participant flow diagram.

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

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

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

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

FIGURE 4. Mean (± SEM) iAUC plasma GLP-1, PYY and ghrelin concentrations in ten healthy women in the visits pre and post regular and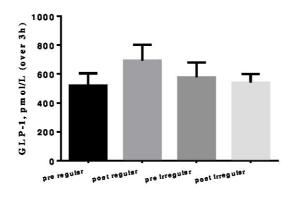 irregular meal pattern, measured by the trapezoidal method. A significant main effect of visit was observed for iAUC plasma PYY (Two-way ANOVA, $p < 0.05$).
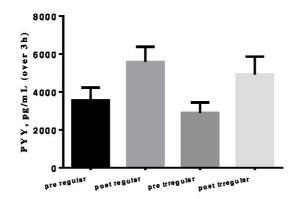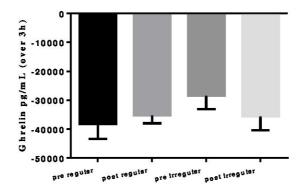
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                    ┌─────────────────────────────────────┐
                    │ Screened for eligibility (n= 19)     │
                    └─────────────────────────────────────┘
                                    │
                                    │          ┌──────────────────────────────────────┐
                                    ├─────────▶│ Excluded (n= 8)                      │
                                    │          │ • Not meeting inclusion criteria (n= 8) │
                                    │          │ • Declined to participate (n= 0)     │
                                    │          │ • Other reasons (n= 0)               │
                                    │          └──────────────────────────────────────┘
                                    ▼
                    ┌─────────────────────────────────────┐
                    │ Randomized (n=11)                    │
                    └─────────────────────────────────────┘
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Allocation

| Participants allocated to regular then crossed-over to irregular meal pattern after 14 days 'wash-out' period (n= 5) • Received allocated interventions (n= 5) • Did not receive allocated intervention N/A | Participants allocated to irregular then crossed-over to regular meal pattern after 14 days 'wash-out' period (n= 6) • Received allocated interventions (n= 6) • Did not receive allocated intervention N/A |

Withdrawn

| Withdrawn (n= 0) Discontinued intervention (n= 0) | Withdrawn (n= 0) Discontinued intervention (n= 0) |

Analysis

| Analysed (n= 5) • Excluded from the CGM analysis (n= 2) | Analysed (n= 6) • Excluded from the blood variables analysis (n= 1) |