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Effect of breakfast omission and consumption on energy intake and physical activity in adolescent girls: a randomised controlled trial

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#### 1 Abstract

It is not known if breakfast consumption is an effective intervention for altering daily energy 2 balance in adolescents when compared with breakfast omission. This study examined the acute 3 effect of breakfast consumption and omission on free-living energy intake (EI) and physical activity 4 (PA) in adolescent girls. Using an acute randomised crossover design, forty girls (age  $13.3 \pm 0.8$  y, 5 body mass index  $21.5 \pm 5.0 \text{ kg} \cdot \text{m}^{-2}$ ) completed two, 3-day conditions in a randomised, counter-6 7 balanced order: no breakfast (NB) and standardised (~1962 kJ) breakfast (SB). Dietary intakes were assessed using food diaries combined with digital photographic records and PA was measured via 8 9 accelerometry throughout each condition. Statistical analyses were completed using repeated measures analysis of variance. Post-breakfast EI was 483 ± 1309 kJ/d higher in NB vs. SB 10 (P=0.025), but total daily EI was 1479  $\pm$  1311 kJ/d higher in SB vs. NB (P<0.0005). Daily 11 carbohydrate, fibre and protein intakes were higher in SB vs. NB (P<0.0005), whereas daily fat 12 13 intake was not different (P=0.405). Effect sizes met the minimum important difference of  $\geq 0.20$  for all significant effects. Breakfast manipulation did not affect post-breakfast macronutrient intakes 14 15  $(P \ge 0.451)$  or time spent sedentary or in PA  $(P \ge 0.657)$ . In this sample of adolescent girls, breakfast omission increased post-breakfast free-living EI, but total daily EI was greater when a standardised 16 breakfast was consumed. We found no evidence that breakfast consumption induces compensatory 17 changes in PA. Further experimental research is required to determine the effects of extended 18 periods of breakfast manipulation in young people. 19

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### 21 Introduction

There is a common belief that breakfast is the 'most important meal of the day'<sup>(1)</sup>. However, around 22 one third of young people, including children (pre-pubertal and typically <11 in girls and <13 years 23 in boys) and adolescents (between puberty and adulthood)<sup>(2)</sup>, in many countries skip breakfast 24 regularly<sup>(3,4)</sup>. Cross-sectional reports that show infrequent breakfast consumption to be associated 25 with overweight and obesity have led to premature assumptions that breakfast can be used as an 26 intervention for weight loss<sup>(5)</sup>. Indeed, the lower adiposity status in children who frequently 27 consume breakfast was not observed uniformly across 12 countries<sup>(4)</sup>. Thus, the strength, direction, 28 29 and causal nature of associations between breakfast frequency and adiposity are questionable.

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31 The mechanistic basis for a causal link between breakfast frequency and adiposity may be examined by assessing energy intake (EI) and expenditure. Indeed, a sustained positive energy balance where 32 EI exceeds energy expenditure causes weight gain<sup>(6)</sup>. Despite their higher adiposity, young people 33 who skip breakfast have  $lower^{(7,8)}$  or  $similar^{(9)}$  daily EIs when compared to breakfast consumers. 34 Interventions show that one day of breakfast omission did not increase subsequent EI to compensate 35 for the energy deficit created by breakfast omission in children aged 8 to 10 years<sup>(10)</sup> and in 36 adolescents aged 13 to 17 years<sup>(11)</sup>. Increased lunchtime EI has, however, been reported in men in 37 response to one day of breakfast omission<sup>(12)</sup>. Under free-living conditions, adults generally show 38 higher daily EIs when breakfast is consumed<sup>(13,14,15)</sup>, even when reductions in EI at lunch<sup>(15)</sup> or 39 between 12:00 and 18:00 h<sup>(13)</sup> are observed. However, daily EI was similar in obese adults assigned 40 to daily breakfast omission or consumption for six weeks<sup>(16)</sup>. In overweight and obese "breakfast-41 skipping" females aged 15 to 20 years, daily EI was increased with normal-protein breakfast 42 consumption, but a high-protein breakfast reduced evening snacking and did not increase daily 43 EI<sup>(17)</sup>. In a similar mixed-sex sample, 12 weeks of high-protein breakfast consumption reduced free-44 living daily EI, whereas breakfast skipping and normal-protein breakfast consumption did not<sup>(18)</sup>. 45 However, these studies were based on young people accustomed to breakfast omission and only 46 determined the impact of breakfast addition, not removal<sup>(17,18)</sup>. 47

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When determining free-living EI via self-report in adolescents, compliance and underreporting are major challenges<sup>(19)</sup>. Adolescents report a preference for methods using technology, such as a disposable camera<sup>(19)</sup>, which eliminate the need for participants to estimate portion size and are less burdensome than weighed food diaries<sup>(20,21)</sup>. Digital photography methods have been validated against weighed food diaries and 24 hour recall in adults<sup>(21)</sup> and are reliable and valid when measuring children's food intake in cafeteria settings<sup>(22)</sup>. However, understanding the individual variation in free-living EI assessed using digital photography requires investigation to determine
 clinically meaningful intervention effects<sup>(23)</sup>.

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In addition to EI, physical activity (PA) is a key determinant of energy balance, weight gain and 58 health<sup>(6,24)</sup>. Cross-sectional studies using objective measures of PA (e.g., accelerometry) have 59 reported more frequent breakfast consumption to be associated with higher PA in girls but not 60 boys<sup>(25)</sup>, or in boys but not girls<sup>(26)</sup>, or on weekends but not weekdays<sup>(27)</sup>. In lean<sup>(14)</sup> and obese<sup>(16)</sup> 61 adults assigned to six weeks of daily breakfast consumption or omission, higher PA energy 62 expenditure in the morning was shown in the breakfast consumption groups, and this resulted in 63 increased total daily PA energy expenditure in the lean adults<sup>(14)</sup>. In support, an acute within-64 participant crossover study showed increased morning PA energy expenditure assessed via 65 accelerometry when breakfast was consumed compared with when it was omitted in women<sup>(15)</sup>. 66 However, another study using pedometers and heart rate monitors showed no effect<sup>(28)</sup>. 67 Furthermore, it is not known whether consuming breakfast can increase PA in young people. 68

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The adolescent period is a crucial time to promote dietary and PA behaviours for health, particularly in girls<sup>(29)</sup>. Furthermore, breakfast skipping is highly prevalent in this population<sup>(30)</sup>. Thus, the current study used a randomised, cross-over design to compare the effect of three consecutive weekdays of breakfast omission with standardised breakfast consumption on free-living EI and PA in girls aged 11 to 15 years. In a sub-sample, we examined the natural variability in free-living daily EI assessed using digital photography to determine the interindividual intervention response.

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## 77 Methods

# 78 **Participants**

In this dual centre project, 49 girls aged 11 to 15 years were recruited from schools in the two locations in England. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the respective University Research Ethics Committees. Written informed parental consent and child assent were obtained for all participants. Girls were excluded from the study if they had health related issues identified from a health screen questionnaire (e.g., allergies to the breakfast meals, fitted with a pacemaker) or were unable to walk or wear a PA monitor on their wrist.

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## 87 **Preliminary measurements**

88 Stature was measured to the nearest 0.01 m using a portable Leicester height measure (SECA

89 Corporation, Hamburg, Germany). Body mass was measured and percent body fat estimated to the

nearest 0.1 kg and 0.1% respectively using a Tanita Body Composition Analyser (BC-418 MA, 90 Tanita Corporation, Tokyo, Japan); subsequently, body mass index (BMI) was calculated as body 91 mass divided by stature squared (kg·m<sup>-2</sup>). Using age and sex-specific BMI centiles<sup>(31)</sup>, the girls were 92 then classified as non-overweight (2<sup>nd</sup> to 85th centile) or overweight (85th to 95th centile). Waist 93 circumference was measured to the nearest millimetre on exhalation at the midpoint between the 94 last rib and top of the iliac crest using a non-elastic tape measure<sup>(32)</sup>. To describe the pubertal status 95 of the study sample, the girls were asked to provide a validated<sup>(33,34)</sup> self-assessment of their 96 physical maturation using secondary sexual characteristics with the assistance of a primary home-97 based carer<sup>(35)</sup>. Habitual breakfast frequency was assessed by asking participants the following 98 question: "How often do you usually have breakfast?" Participants were asked to indicate their 99 100 response separately for weekdays and for weekend days. Response categories were 'never' to 'five days' for the week, and 'never' to 'two days' for the weekend. To provide an indication of the 101 102 composition and energy content of the participants' habitual breakfasts, they recorded their breakfast intakes across three days (Tuesday, Wednesday and Thursday, i.e., the weekdays selected 103 104 for the intervention described below) using digital photography and a written food diary. Breakfast EI and macronutrient intakes were calculated using Dietplan 6.7 (Forestfield Software, Horsham, 105 106 UK).

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# 108 Experimental design

Using a within-measures cross-over design, participants completed two, 3-day conditions in a counter-balanced order: no breakfast (NB) and standardised breakfast (SB). The conditions were conducted across the same three weekdays (i.e., Tuesday, Wednesday and Thursday) with either a 4 or an 11 day washout between conditions. For the duration of each 3-day condition, participants were asked to record their diet and wear a wrist-worn accelerometer. The order of the conditions for each participant was produced using a computer-based random number generator by the principal investigator (JZF). All data was collected between December 2013 and July 2014.

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117 On each morning of each 3-day condition, participants arrived at school in the fasted state (no food or drink consumed except water from 21:00 the previous day) and were asked not to eat breakfast 118 (NB) or to consume the SB provided within 30 min (between 08:15-8:45). For NB, participants 119 were provided with 375 mL of water. The participant's first opportunity to consume food or drink 120 during the post-breakfast period was 10:30 (i.e., during school break time); thus, NB involved 121 abstaining from energy-containing food and beverages between 21:00 the previous day and 10:30 122 123 the following morning. The participants were reminded on each day of the experimental conditions to refrain from snacking until 10:30 and reported that they complied with these instructions. The SB 124

consisted of 56.3 g wheat biscuits (Weetabix, Kettering, UK), 188 mL semi-skimmed milk (Tesco 125 Stores Ltd, Chestnut, UK) and 375 mL orange juice (Tesco Stores Ltd, Chestnut, UK). The 126 breakfast was low glycaemic index (GI), with a calculated GI of 54<sup>(36,37)</sup>. We chose a ready-to-eat 127 cereal because this type of breakfast is associated with reduced obesity risk when compared with 128 'other breakfasts'<sup>(7)</sup>. Recommendations suggest breakfast should be 20% of daily EI<sup>(38)</sup> and reviews 129 define breakfast typically as containing 20% to 35% of total daily energy needs<sup>(39)</sup>. Therefore, the 130 SB contained 1994 kJ (500 kcal), equating to ~22-26% of daily energy requirements for 11-15 year 131 old girls, which take into account total daily energy expenditure plus the deposited energy costs for 132 growth (1935 to 2293 kcal/d)<sup>(40)</sup>. The SB was consumed at school rather than home to monitor 133 compliance to the breakfast intervention and record any leftovers. If the SB was not consumed 134 135 completely, it had to represent at least 20% of recommended daily EI for the participant to be included in the final sample<sup>(38)</sup>. 136

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# 138 **Dietary assessment**

Participants recorded their daily diet using a digital camera (Vivitar, ViviCam 46, China) and food 139 diary during each condition. A similar method has been validated previously in adults<sup>(21)</sup> and 140 children<sup>(22)</sup>. The participants were asked to photograph all foods and beverages consumed and use 141 the photographs as a recall method when completing their food diaries each evening. The food 142 diaries included a record of the day, time, type, brand name, preparation method, estimated portion 143 size and any leftovers of all food and drink consumed. Before completing the main conditions, the 144 girls received a tutorial and written instructions on using the digital camera and food diary. On the 145 morning after each day of dietary recording, the research team checked the participants' food 146 diaries for completeness and cross-referenced the food diaries with the corresponding photographs. 147 For missing photographs, portion size was estimated by the participants using the Young Person's 148 Food Atlas<sup>(41,42)</sup> with assistance from the research team; further details were added to the diary 149 when appropriate. The mass of all foods and beverages consumed were estimated by comparing the 150 digital photographs, taken by the participants, to the Young Person's Food Atlas<sup>(41,42)</sup>; this method 151 that has shown good agreement with weighed food diaries in children aged  $\geq 11$  years<sup>(43)</sup>. Food 152 diaries were analysed using Dietplan 6.7 (Forestfield Software, Horsham, UK) to estimate EI and 153 154 macronutrient intakes, which were blocked into three time periods to separate breakfast, lunch and the evening meal and align with the school timetable: (i) 06:00 to 09:00 (including breakfast and 155 early morning snacks), (ii) 10:30 to 14:00 (including school break time snacks and lunch), and (iii) 156 14:00 until 21:00 (including dinner and evening snacks). Percentage breakfast EI compensation was 157 158 calculated for SB relative to NB (i.e., the difference in post-breakfast EI between SB and NB divided by SB breakfast EI multiplied by 100); values of 100% indicated complete compensation 159

for breakfast EI. Portions of fruit and vegetable consumed were quantified using the National
Health Service (NHS) guidelines for 5 portions/d<sup>(44)</sup>. High-fat and sugary snacks were defined as
sweet baked products, cookies, ice cream, cakes, desserts, jams, sugar, sweets, nuts, potato crisps,
cheese products, popcorn and soft drinks<sup>(8)</sup>.

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# 165 Variability of daily EI

To determine the natural variability of daily EI, the primary outcome variable, a sub-sample of 10 166 girls completed two, 3-day free-living diet (FD) conditions on two consecutive weeks at least four 167 weeks before commencing the main study. On each day, the girls were free to eat and drink as they 168 pleased and were instructed to record their dietary intakes using the digital photography and food 169 170 diary method described above (see 'Dietary assessment'). Thus, the data provided an indication of the variability in habitual dietary intakes between two 3-day periods rather than being an assessment 171 172 of measurement reliability. The 95% Limits of Agreement (LoA) for daily EI were calculated by determining a 95% limit above and below the mean difference for FD trial 1 and FD trial 2 173 (systematic error  $\pm$  (1.96  $\times$  random error)), as outlined by Bland and Altman<sup>(45)</sup>. Student's paired t-174 tests were used to identify systematic change in the mean from trial one to two; whereas Pearson's 175 176 product moment correlations between the paired residuals and the mean (proportional error check) and the absolute residuals and the mean (heteroscedasticity check) were examined to ensure the 177 95% LoA were representative of the whole sample. It has been estimated that excessive weight gain 178 could be prevented in children and adolescents by reducing positive energy balance by 628 kJ/d 179  $(150 \text{ kcal/d})^{(46)}$ . Therefore, we deemed that LoA of  $\leq \pm 628 \text{ kJ/d}$  (150 kcal/d) would be an 180 acceptable test-retest error for daily FD EI. 181

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The data on variability of EI was also used as a control arm to quantify the true interindividual 183 differences in the EI response to the breakfast intervention<sup>(23)</sup>. To determine a 'true' effect, 184 Atkinson and Batterham<sup>(23)</sup> suggest comparing the standard deviation (SD) of changes in the 185 intervention arm (i.e., the effect SD) with the SD of changes from the control arm (i.e., the control 186 SD). The SD of the true individual response is:  $\sqrt{(SD_e^2 - SD_c^2)}$ , where SD<sub>e</sub> is the effect SD and SD<sub>c</sub> 187 is the control SD<sup>(23)</sup>. The magnitude of the SD of true individual responses is appraised in terms of 188 clinical importance. As with our LoA analysis, we deemed that a difference of  $\geq \pm 628$  kJ/d (150 189 kcal/d) would be clinically important when comparing the control and intervention SDs<sup>(46)</sup>. 190

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## 192 Physical activity assessment

Wrist-worn accelerometers that have been validated in 8 to 14 year olds (GENEActiv, ActivInsights
 Ltd., Colworth, UK) were used to assess PA for the duration of each 3-day condition<sup>(47)</sup>. The

accelerometers were set to record at 85.7 Hz using a 1-second epoch. The girls were asked to wear the accelerometers on their non-dominant wrist for three days at all times, removing only for bathing and water-based activities. To estimate daily time spent sedentary and in light PA (LPA) and moderate-to-vigorous PA (MVPA), GENEActiv cut-points specific to 8 to 14 year olds were applied and expressed as percentage of total daily wear time<sup>(47)</sup>. The minimal amount of accelerometer data that was considered acceptable was 10 h/d of wear time on all three days of both breakfast conditions<sup>(48)</sup>.

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## 203 **Statistical analyses**

Statistical analyses were completed using IBM SPSS statistics software for Windows version 21 204 205 (IBM Corporation, New York, USA). Total daily EI, macronutient intakes and PA were compared between the two conditions using student's paired t-tests. For post-breakfast EI and macronutrient 206 207 intakes, condition by time of day (2 x 2) repeated measures analysis of variance (ANOVA) were used to examine differences between the conditions across the two time periods (i.e., 10:30 to 14:00 208 209 and 14:00 to 21:00). Weekday habitual breakfast frequency and BMI were considered as covariates, but were not used because the data did not satisfy the assumptions for covariate analysis (i.e., they 210 were not significantly associated with the dependent variables across all conditions). Homogeneity 211 of covariances were examined by Mauchly's test of sphericity, and a Greenhouse-Geisser 212 correction was applied to the degrees of freedom if the sphericity assumption was violated. Cohen's 213 d effect sizes (d) were calculated to gauge the magnitude of differences between conditions for all 214 significant effects. In the absence of published anchors, a d≥0.20 was considered the minimum 215 important difference in all outcome measures, 0.50 to <0.80 moderate and  $\ge 0.80$  large<sup>(49)</sup>. Values 216 are presented as means  $\pm$  SDs unless stated otherwise. 217

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### 219 Justification of sample size

The calculated sample size was based on total daily EI (the primary outcome variable) and LPA (% 220 wear time), as this PA intensity has been shown to be sensitive to breakfast manipulation in 221 adults<sup>(14)</sup>. A worthwhile difference in EI or energy expenditure between the two conditions was 222 defined as 628 kJ/d (150 kcal/d)<sup>(46)</sup>. Our variability study showed that the SD of the EI difference 223 between two, 3-day FD conditions was 1147 kJ/d (274 kcal/d). A 628 kJ/d increase in energy 224 expenditure requires ~45 minutes of LPA at 14.0 kJ/min in adolescent girls<sup>(47,50)</sup>. This would equate 225 to 5% of weekday waking hours and an SD of 6%<sup>(48,51)</sup>. Using these figures, the number of 226 participants estimated to detect a significant change at 90% power with a two-sided significance 227 228 level of 0.05 was 35 for daily EI and 30 for LPA. To be included in the final sample, participants had to meet the following criteria: 1) attended the breakfast intervention club on all days; 2) abstain 229

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# 234 **Results**

# 235 Participant characteristics

The final sample for dietary analysis included 40 participants (nine were excluded: three broke the 236 fast before 10:30 during NB, one did not consume an adequate amount of the SB and five did not 237 record their dietary intakes as specified). Three participants that were included in the final sample 238 did not eat all of the SB, but consumed enough so that SB energy intake was at least 20% of 239 recommended daily EI<sup>(38)</sup>. Table 1 shows the anthropometric characteristics and habitual breakfast 240 frequencies of the final sample. The nine girls who were excluded from the final analyses did not 241 242 have significantly different physical characteristics or breakfast frequencies compared with the 40 who were included ( $P \ge 0.10$ ). 243

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#### **INSERT TABLE 1**

247 Variability of daily energy intake

At the group level (n=10), EI was similar between FD trial 1 and FD trial 2;  $5063 \pm 1332$  vs.  $5244 \pm$ 248 1293 kJ/d (1211  $\pm$  319 vs. 1255  $\pm$  309 kcal/d; t=-0.500; P=0.629). The systematic bias  $\pm$  random 249 error were  $181 \pm 1147$  kJ/d ( $43 \pm 274$  kcal/d). This resulted in 95% LoA of -2067 to 2428 kJ/d, (-250 494 to 580 kcal/d) (see Online Supplementary Document S1). Significant proportional bias was not 251 evident (r=-0.038; P=0.917) and random errors were homoscedastic (r=-0.040, P=0.912). The LoA 252 based on all 10 girls exceeded our *a priori* acceptable test-retest variability of  $\pm$  628 kJ/d (150 253 kcal/d). However, seven of the 10 participants had paired EI values across the repeat measurements 254 that were within  $\pm$  628 kJ/d (150 kcal/d), suggesting the natural variation in FD may be small 255 enough to detect subtle changes in EI that could prevent excessive weight gain<sup>(46)</sup>. In the remaining 256 three girls, one had a particularly large difference of 3141 kJ/d between the repeat measurements. 257 When excluding this participant (n=9), the systematic error  $\pm$  random error was reduced by -148  $\pm$ 258 512 kJ/d, and the 95% LoA were tightened to -1151 to 855 kJ/d, but still exceed the  $\pm$  628 kJ/d cut-259 off. 260

#### 261 Breakfast energy and macronutrient intake

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Accounting for leftovers, breakfast energy and macronutrient intakes for SB were: EI 1962  $\pm$  121 kJ (469  $\pm$  29 kcal), 88.3  $\pm$  6.0 g CHO, 16.4  $\pm$  1.3 g protein, 5.7  $\pm$  0.7 g fat and 5.8  $\pm$  0.6 g fibre. For comparison, the girls consumed 766  $\pm$  439 kJ (183  $\pm$  105 kcal), 29.4  $\pm$  16.1 g CHO, 5.4  $\pm$  3.7 g protein, 5.4  $\pm$  4.5 g fat and 1.2  $\pm$  1.0 g fibre for breakfast habitually. The energy, CHO, protein and fibre intake of the SB were higher than the girls habitual breakfasts (*P*<0.005; d=3.69 to 5.32), whereas fat intakes were similar (*P*=0.672; d=0.09).

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# 269 Daily and post-breakfast energy intake

Fig. 1 shows daily and time-specific EIs for each breakfast condition. Daily EI was higher in SB than NB (P<0.0005). For daily EI, we quantified the true interindividual differences in the intervention responses using the control SD from the variability data described above and the SDs of the residuals from the breakfast conditions<sup>(23)</sup>. The SD (95% confidence interval) of the true individual response was 636 (229 to 1042) kJ/d (152 [55 to 249] kcal/d) for SB vs. NB. Using our cut-off of  $\geq$ 628 kJ/d (150 kcal/d), the large differences in the control and intervention SDs may be clinically important.

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Post-breakfast (i.e., 10:30 to 21:00) EI was  $483 \pm 1309$  kJ/d higher in NB compared with SB (*P*=0.025; d=0.37), independent of the time of day (*P*=0.993) (Fig. 1). The higher post-breakfast EI in NB accounted for  $24 \pm 66\%$  of the standardised breakfast EI.

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#### **INSERT FIGURE 1**

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## 284 Daily and post-breakfast macronutrient intakes

Table 2 shows daily macronutrient intakes for each breakfast condition. Daily CHO, fibre and protein intakes were higher in SB compared with NB (d $\geq$ 0.81 for all comparisons), whereas daily fat intake was not. The effect of condition was not significant for CHO, fat, protein and fibre intakes for the post-breakfast period ( $P \geq 0.451$ ; d $\leq 0.14$  for all comparisons) and there was no interaction with time of day ( $P \geq 0.329$ ). The time of day main effect showed that protein and fibre intakes were higher in the 14:00-21:00 period than the 10:30-14:00 period ( $P \leq 0.026$ ; d $\geq 0.50$ ), but this difference only approached significance for CHO with a small effect (P=0.054; d=0.40).

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## **INSERT TABLE 2**

#### 294 Daily fruit & vegetable and high-fat & sugary snack consumption

The breakfast condition main effect for daily portions of fruit and vegetables consumed was not significant (1.1 ± 1.1 for NB vs. 1.1 ± 0.8 for SB; *P*=0.801). Although the mean number of high-fat and sugary snacks consumed per day tended to be higher for NB (3.0 ± 1.5) compared with SB (2.6 ± 1.3) (*P*=0.097), the effect was only small (d=0.26).

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# 300 Daily physical activity

A total of 35 girls had valid accelerometer data and were included in PA analyses. Wear time was 14.2  $\pm$  1.2 h/d for SB and 14.1  $\pm$  1.3 h/d for NB (*P*=0.488). Daily time spent sedentary or in LPA or MVPA (% wear time) was not different between conditions (Table 3).

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#### **INSERT TABLE 3**

#### 306

# 307 **Discussion**

Using an experimental crossover design, this study showed that total daily EI was higher when adolescent girls consumed a ~1962 kJ standardised breakfast (SB) when compared with no breakfast (NB) over three consecutive weekdays. Although NB increased post-breakfast EI, the degree of EI compensation was small and only accounted for ~24% of the SB. In addition, breakfast manipulation did not affect time spent sedentary or in PA.

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Our study supports previous research showing that breakfast consumption results in higher daily EIs 314 in young  $people^{(10,17)}$  and  $adults^{(12,13,14,15,52)}$  when compared with breakfast omission. In addition to 315 being statistically significant, random within-subject variation and measurement error did not 316 explain the higher daily EI with breakfast consumption, which exceeded the natural variability in EI 317 by more than 150 kcal/d and may thus have clinical importance for weight gain<sup>(23,46)</sup>. The 483 kJ/d 318 (115 kcal/d) increase in post-breakfast EI (i.e., between 10:30 and 21:00) when the girls omitted 319 breakfast amounted to only a quarter of the SB. This incomplete EI compensation may be due to the 320 321 large size of the SB, which contained 2.6 times more energy than the participants' habitual breakfasts. As CHO-based breakfasts containing ~1.3 times less energy than our SB also did not 322 result in EI compensation in young people<sup>(10,17)</sup>, examining the effects of smaller breakfasts similar 323 in energy content to the habitual breakfasts of adolescent girls (~766 kJ in our sample) may enhance 324 325 the ecological validity of the findings and likelihood EI compensation being complete. Although the increased EI with breakfast omission was distributed evenly across the day in our study, reports in 326 adults indicate that these effects are specific to certain time periods<sup>(12,13,15)</sup>. It has also been shown 327 that such effects may depend on the sex and breakfast habits of the sample<sup>(28)</sup>, whereas habitual 328

breakfast frequency was not related to the outcome variables in our sample of adolescent girls. Nevertheless, the 95% confidence interval for the SD of the individual response (229 to 1042 kJ/d) indicates large interindividual variability in compensatory EI responses to breakfast omission. Thus, individual characteristics that may explain this variability, such as eating and PA habits, body composition, age and socioeconomic status warrant clarification.

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Unlike most previous experimental studies in adults that involved unstandardised breakfast 335 manipulation<sup>(13,14,16,28)</sup>, we provided a standardised, wholegrain ready-to-eat cereal-based breakfast. 336 This type of breakfast was chosen because it has strong association with lowered obesity risk in 337 adolescents<sup>(7)</sup> and it is a convenient breakfast choice that requires minimal preparation time, which 338 could be appealing for adolescents who skip breakfast due to a 'lack of time'<sup>(53)</sup>. The small, but 339 statistically significant increase in post-breakfast EI (i.e., between 10:30 and 21:00) with breakfast 340 omission reported here is in contrast with research in "breakfast-skipping" adolescents showing that 341 normal-protein breakfasts do not reduce subsequent EI<sup>(11,17,18)</sup>. Although breakfast GI was not 342 reported in these studies<sup>(11,17,18)</sup>, it is possible that the low GI breakfast in our study promoted a 343 slower release of glucose into the blood, which can reduce hunger and lunchtime EI<sup>(54)</sup> and prolong 344 satiety<sup>(55)</sup> in young people and may explain our discrepant findings<sup>(11,17,18)</sup>. However, the link 345 between GI and EI is controversial<sup>(56,57)</sup>, with the satiating effect of low GI foods possibly being due 346 to their higher fibre content<sup>(56)</sup>. Nevertheless, the 5.8 g of fibre in our SB was within the range of the 347 breakfasts in previous studies (2.0 to 6.1 g)<sup>(11,17)</sup>. Thus, differences in fibre content of the CHO-348 based breakfasts may not explain discrepancies between our results and previous work. Increased EI 349 in response to breakfast omission may be due to a host of metabolic and behavioural responses 350 induced by the appetite regulatory system, including reduced pre-dinnertime neural activation in 351 brain regions controlling food motivation/reward in late adolescent girls<sup>(17)</sup>. Such mechanisms 352 require further research in young people. 353

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Whilst weighed food records are often considered the criterion reference measure of free-living EI 355 in adults, self-reported EI can be underestimated through poor compliance, participant selection 356 bias, recording bias and changes to diet to facilitate recording<sup>(58)</sup>. In adolescents, the reliability and 357 validity of weighed food diaries is less certain and the participant burden is particularly high<sup>(59,60)</sup>. 358 In an attempt to increase compliance to recording dietary intakes in our study, we used a food diary 359 accompanied by photographic evidence rather than a weighed food diary<sup>(19)</sup>. Although previous 360 research has indicated that the addition of photographs to a traditional diet diary can enhance the 361 validity and reliability of dietary recording<sup>(61)</sup>, our comparison of two 3-day records showed 95% 362 LoA for EI of -494 to +580 kcal/d. This high variability of free-living EI, potentially resulting from 363

environmental, biological and methodological factors<sup>(62)</sup>, may limit the potential to detect clinically meaningful differences of 150 kcal/d<sup>(46)</sup>. Previous literature on free-living EI variability using 3-day diet records has used varied statistical approaches and produced mixed findings<sup>(63,64)</sup>. Interestingly, our LoA are narrower than studies reporting 'acceptable' agreement with a 3-day diet record in adults<sup>(65)</sup> and a food menu in free-living young people<sup>(66)</sup>. Thus, the use of a clinically relevant anchor may have affected the interpretation of the LoA in these studies.

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Consistent with cross-sectional reports<sup>(8,9,30)</sup>, the higher daily EI with SB consumption was due to 371 higher intakes of CHO, protein and fibre, whereas breakfast did not affect daily fat intakes. These 372 differences in daily macronutrient intakes were a direct effect of the breakfast meal rather than post-373 374 breakfast intakes. Nevertheless, it is likely that the tendency for higher high-fat and sugary snack consumption contributed to the increased post-breakfast EI when breakfast was omitted. This 375 376 finding also suggests that the nature of the cross sectional association between infrequent breakfast consumption and higher unhealthy snack consumption<sup>(8,9)</sup> may be causal. Overall, the girls 377 consumed around one portion of fruit and vegetables a day, a concerning number considering that a 378 minimum of five portions per day is recommended<sup>(44)</sup>. Although breakfast consumption has been 379 associated with higher fruit and vegetable consumption<sup>(67,68)</sup>, the present study suggests that any 380 such relations are not causal, at least in our sample over a 3-day intervention. As food groups other 381 than fruit and vegetables contribute to micronutrient intakes, whether breakfast manipulation affects 382 micronutrient intakes warrants examination using assessment periods of more than three days<sup>(69)</sup>. 383

384

The small post-breakfast EI compensation in the present study suggests that a higher PA energy 385 expenditure may be more important in contributing to the healthy weight status in frequent 386 breakfast consumers  $^{(1,3,4,5,7)}$ . However, our finding that breakfast did not affect sedentary time or 387 MVPA supports cross-sectional findings in girls aged 9-10 years when using accelerometry to 388 quantify PA on weekdays and weekends<sup>(26)</sup> and experimental research in adults showing no effect 389 of breakfast manipulation on PA assessed via pedometers and heart rate monitors during a working 390 week<sup>(28)</sup>. Although breakfast manipulation did not affect LPA in the girls in the present study, 391 energy expenditure from LPA assessed over seven days was higher in lean adults who consumed 392 breakfast daily compared with those who omitted breakfast daily<sup>(14)</sup>. In the obese cohort of this six 393 week intervention, total PA energy expenditure in the morning was higher in the breakfast group 394 compared with the breakfast omission group<sup>(16)</sup>. An acute randomised crossover trial using 395 accelerometry also showed that consuming breakfast increased PA energy expenditure when 396 compared with breakfast omission in women classified as habitual breakfast eaters<sup>(15)</sup>. The 397 adolescent girls in our study may have responded differently to the adults in previous studies<sup>(14,15,16)</sup> 398

because the provision of breakfast at school meant that they had limited opportunity to engage in
free-living PA directly after consuming breakfast. Thus, providing breakfast at home and including
weekend days may increase the scope for detecting effects on PA.

402

The present study has several limitations. First, breakfast manipulation over three weekdays does 403 not allow us to apply the findings to weekends, where diet and PA patterns are different (27,51,70), or 404 to determine the effects of longer intervention periods. Second, similar to previous studies (17,18), we 405 provided a fixed absolute breakfast portion. However, providing breakfast relative to daily energy 406 requirements may be recommended to reduce between-participant variability in the response to 407 breakfast manipulation. Similarly, methods that provide less variable measures EI would help 408 support our findings, although this is challenging in free-living conditions<sup>(58,59,60)</sup>. In addition, 409 standardising pre-intervention diet and the duration of the washout period between participants 410 would help to minimise the influence of these factors on the study outcomes. Third, future studies 411 employing more sensitive measures to quantify free-living PA or energy expenditure (e.g. combined 412 413 heart rate-accelerometry or doubly labelled water) over longer measurement periods (e.g. seven days) would be valuable in extending the findings reported here. In doing so, the possibility that 414 breakfast consumption may affect PA through an interaction with wake time and sleeping patterns 415 requires consideration. Differences in wake time are unlikely to have confounded the comparison 416 between SB and NB in our study, as the provision of breakfast at school rather than at home meant 417 that the participants were not required to wake up any earlier to consume the SB. Nevertheless, the 418 independent effects of breakfast frequency, timing and composition warrant study. Finally, the 419 generalisability of our findings to adolescent boys and to younger children requires investigation. 420

421

In conclusion, adolescent girls showed a small increase in post-breakfast EI of 483 kJ/d (115 kcal/d) that was not sufficient to compensate completely for three consecutive weekdays of breakfast omission when compared with standardised breakfast consumption (~1962 kJ/d). Thus, total daily EI remained greater when a standardised breakfast was consumed. We also report no evidence of breakfast affecting time spent sedentary or in PA. These findings require examination using extended periods of breakfast manipulation and more sensitive devices to quantify PA energy expenditure in young people.

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434	Figure legends
435	Fig. 1. Energy intake during different times of the day for the no breakfast (NB) and the
436	standardised breakfast (SB) conditions. Error bars represent standard deviation. *Significant main
437	effect of condition for total daily energy intake using paired t-tests and for total energy intake
438	between 10:30-21:00 using a condition by time of day ANOVA ( $P \le 0.025$ ). <sup>†</sup> Significant main effect
439	of time of day using ANOVA ( $P=0.003$ ). n=40.
440	
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443	assistance with data collection for the study.
444	
445	Conflict of interest
446	None.
447	
448	Authorship
449	JKZF and KT designed the study (project conception, development of overall research plan, and
450	study oversight). All authors contributed to the data collection (hands-on conduct of the
451	experiments and data collection). JKZF, TP and KT analysed the data and/or performed statistical
452	analyses. JKZF and KT wrote the paper. JKZF had primary responsibility for final content. All
453	authors have read and approved the final manuscript.
4 - 4	
454	
455	Supplementary material
456	S1. Bland-Altman plot of energy intake (kJ/d averaged across 3 days) for trials 1 and 2. Residual
457	for energy intake is the energy intake in trial 1 minus energy intake in trial 2. The solid line indicates
458	the systematic error. The dashed lines indicate the upper and lower 95% limits of agreement. n=10.
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# Tables

 Table 1. Participant characteristics

	Average	Variability
Age $(y)^1$	13.3	0.8
Stature (m) <sup>1</sup>	1.60	0.08
Body mass (kg) <sup>1</sup>	55.2	15.4
Body fat % <sup>1</sup>	30.7	10.1
Waist circumference (cm) <sup>1</sup>	71.1	12.8
Body mass index $(kg \cdot m^{-2})^1$	21.5	5.0
Breast development <sup>2</sup>	4	1
Pubic hair <sup>2</sup>	4	1
Weekday habitual breakfast frequency (d/week) <sup>1</sup>	3.7	1.6
Weekend habitual breakfast frequency $(d/week)^1$	1.7	0.5

<sup>1</sup>Values are mean and standard deviation. <sup>2</sup>Values are median and interquartile range.

	SB		NB		_ P	d	
-	Mean	SD	Mean	SD	_ 1	u	
Energy, kJ/d	6728	1234	5249	1419	< 0.0005	1.11	
Carbohydrate, g/d	243.1	50.6	158.4	50.0	< 0.0005	1.68	
Fat, g/d	51.3	18.3	48.3	17.2	0.405	0.17	
Protein, g/d	61.4	18.6	47.6	15.4	< 0.0005	0.81	
Fibre, g/d	13.1	3.1	7.5	2.8	< 0.0005	1.91	

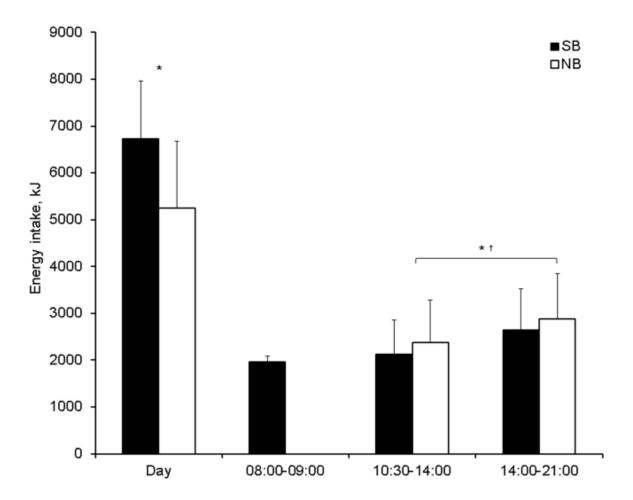
Table 2. Daily energy and macronutrient intakes during three days of no breakfast (NB) or standardised breakfast consumption (SB) using a randomised crossover design (n=40).

<sup>1</sup>SD, standard deviation; d, Cohen's d effect size. <sup>2</sup>Paired t-tests and Cohen's d effect sizes were used to compare the SB and NB conditions.

	SB NB			Р	d	
	Mean	SD	Mean	SD	Γ	u
Sedentary, %WT	70.1	4.9	69.7	4.6	0.769	0.04
LPA, %WT	23.1	2.8	23.2	2.8	0.657	0.06
MVPA, %WT	6.8	2.8	7.1	2.5	0.936	0.01

Table 3. Daily time spent sedentary and in physical activity during three days of no breakfast (NB) or standardised breakfast consumption (SB) using a randomised crossover design (n=35).

<sup>1</sup>SD, standard deviation; d, Cohen's d effect size; %WT, percentage of total wear time; LPA, light physical activity; MVPA, moderate to vigorous physical activity. <sup>2</sup>Paired t-tests and Cohen's d effect sizes were used to compare the SB and NB conditions.



**Fig. 1.** Energy intake during different times of the day for the no breakfast (NB) and the standardised breakfast (SB) conditions. Error bars represent standard deviation. \*Significant main effect of condition for total daily energy intake using paired t-tests and for total energy intake between 10:30-21:00 using a condition by time of day ANOVA ( $P \le 0.025$ ). <sup>†</sup>Significant main effect of time of day using ANOVA (P = 0.003). n=40.