Appetite 182 (2023) 106445

Contents lists available at ScienceDirect

Appetite

journal homepage: www.elsevier.com/locate/appet

Breaking up sitting with short frequent or long infrequent physical activity breaks does not lead to compensatory changes in appetite, appetite-regulating hormones or energy intake

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ARTICLE INFO

Handling Editor: S. Higgs

Keywords:
Sedentary behaviour
Physical activity
Activity breaks
Obesity
Appetite hormones
Acylated ghrelin

ABSTRACT

The aim of this study was to determine the appetite-related responses to breaking up prolonged sitting with physical activity bouts differing in frequency and duration among adult females. Fourteen sedentary females aged 34 ± 13 years with a body mass index of 27.1 ± 6.3 kg/m^2 (mean ± SD) took part in a randomised crossover trial with three, 7.5 h conditions: (1) uninterrupted sitting (SIT), (2) sitting with short frequent 2-min moderate-intensity walking breaks every 30 min (SHORT-BREAKS), and (3) sitting with longer duration, less frequent 10-min moderate-intensity walking breaks every 170–180 min (LONG-BREAKS). The intensity and total duration of physical activity was matched between the SHORT-BREAKS and LONG-BREAKS conditions. Linear mixed models were used to compare the outcomes between conditions with significance being accepted as $p \leq 0.05$. There were no significant between-condition differences in hunger, satisfaction, prospective food consumption or overall appetite area under the curve (AUC) (all $p \geq 0.801$). Absolute *ad libitum* energy intake and relative energy intake (REI) did not differ significantly between conditions (all $p \geq 0.420$). Acylated ghrelin and total peptide YY incremental and total AUC did not differ significantly between conditions (all $p \geq 0.388$). Yet, there was a medium effect size for the higher acylated ghrelin incremental AUC in SHORT-BREAKS versus SIT ($d = 0.61$); the reverse was seen for total AUC, which was lower in SHORT-BREAKS versus SIT ($d = 0.69$). These findings suggest that breaking up sitting does not lead to compensatory changes in appetite, appetite hormones or energy intake regardless of physical activity bout duration and frequency among adult females.

1. Introduction

Sedentary behaviour is a risk factor for many adverse health outcomes including obesity, cardiovascular disease, type 2 diabetes and premature mortality (Bailey, Hewson, Champion, & Sayegh, 2019; Wilmot et al., 2012). As sedentary behaviour represents a state of low energy expenditure (i.e., <1.5 METs) (Tremblay et al., 2017), high levels of sedentary time may result in low daily energy expenditure, leading to an energy imbalance whereby energy intake exceeds expenditure. If repeated habitually over a prolonged period, this positive energy balance could result in weight gain (Stubbs et al., 2004). In support of this, a 7-day period of being sedentary was not compensated for by a concomitant reduction in energy intake, with the excess energy being stored as fat (Stubbs et al., 2004). Furthermore, the prevalence of sedentary behaviour is of concern in the context of obesity; for example, it was estimated that occupational-related energy expenditure has fallen by 100 kilocalories per day in U.S. adults over 50 years from 1960, which was considered to be a significant contributor to the population increase in body weight during this period (Church et al., 2011).

Appetite and food intake are regulated by a complex interaction of

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https://doi.org/10.1016/j.appet.2022.106445
Received 25 May 2022; Received in revised form 30 November 2022; Accepted 29 December 2022
Available online 30 December 2022

neuroendocrine and behavioural factors (Hussain & Bloom, 2013). Episodic peptide hormones that are secreted from the gut interact with the brain's hypothalamic appetite centres to modulate energy intake on a meal-to-meal basis (Neary & Batterham, 2009). This includes the acylated form of ghrelin, which is secreted from the stomach and is the only known hormone to stimulate hunger (Delhanty, Neggers, & van der Lely, 2012). Peptide YY (PYY) is secreted from the distal intestine and colon and causes satiation to occur in response to food intake (le Roux et al., 2006). These gut hormones are responsive to acute changes in nutrient intake and physical activity (Hagobian, Sharoff, & Braun, 2008; Suzuki, Jayasena, & Bloom, 2011). A single continuous bout of moderate or vigorous intensity exercise can suppress acylated ghrelin and increase PYY concentrations for the following 2–9 h (Schubert, Sabapathy, Leveritt, & Desbrow, 2014). Yet, exercise-induced changes in gut hormone concentrations do not necessarily translate into reduced feelings of appetite and energy intake (Deighton, Karra, Batterham, & Stensel, 2013). A consistent observation, though, is that exercise does not stimulate compensatory increases in appetite and energy intake, thus leading to an energy deficit that could be relevant for weight management (Schubert, Desbrow, Sabapathy, & Leveritt, 2013).

In contrast to the large body of evidence investigating the effects of single exercise bouts on appetite and energy intake related outcomes, research on the acute effects of short bouts of physical activity used to break up prolonged periods of sitting remains in its infancy. This is a key area of study based on evidence that prolonged sitting in a state of energy balance acutely increases hunger (Granados et al., 2012). Thus, the potential for increased energy expenditure through breaks in prolonged sitting to offset any potential increases in appetite is of high relevance. The available research has shown that an energy deficit induced by 2-min of light or moderate-intensity walking every 20 min over 5 h did not result in compensatory changes in acylated ghrelin, total PYY, subjective appetite or subsequent energy intake in sedentary non-overweight males and females (Bailey et al., 2016). Similar findings were reported in response to 2-min of moderate-intensity walking every 30 min in a sample of men and women who were normal weight and overweight (Mete et al., 2018). Brisk walking for 2-min every 20 min acutely increased glucagon-like peptide-1 (GLP-1) and total PYY in men and women with central overweight/obesity, which supports potential appetite regulating effects of breaking up sitting (Chen et al., 2022). However, the effects on subjective appetite and energy intake were not evaluated in this study (Chen et al., 2022). In males and females with obesity, 5-min moderate-intensity walking bouts performed hourly did not affect total PYY concentrations over a 12-h period (Holmstrup, Fairchild, Keslacy, Weinstock, & Kanaley, 2013). Yet, hunger was significantly reduced in the afternoon compared with prolonged sitting and a single continuous energy-matched exercise bout performed in the morning (Holmstrup et al., 2013). These between-study discrepancies in findings suggest that the effects of breaking up sitting on appetite may differ between individuals based on their weight status. The physical activity characteristics may also be a factor, with longer duration breaks (e.g. 5 min bouts) (Holmstrup et al., 2013) potentially exerting a greater stimulus for appetite suppression than shorter breaks (e.g. 2 min) (Bailey et al., 2016; Mete et al., 2018). The effects of frequency and duration of physical activity breaks on these outcomes thus requires direct comparison.

Appetite and appetite-hormone responses to exercise and energy deficits may be sex-dependent. Females demonstrate fat-preserving mechanisms compared with men, i.e. higher acylated ghrelin after an energy deficit and no inhibition of appetite in a state of energy balance after exercise (Hagobian et al., 2008). However, the majority of research suggests that there may not be sex differences in these outcomes in response to exercise (Dorling et al., 2018). In the context of sedentary behaviour, prolonged sitting in a state of energy balance led to increased acylated ghrelin in men with no accompanying changes in subjective appetite, but attenuated leptin, reduced acylated ghrelin and increased subjective appetite (higher hunger and lower fullness) in women (Granados et al., 2012). Study samples comprising of both men and women may, therefore, lead to larger variations in data that may not reflect the true response in each sex. To date, there is no research that has investigated the effects of breaking up sitting with different frequencies and durations of physical activity on appetite and energy balance in a single-sex sample. This could be important for identifying optimal tailored physical activity and sedentary break patterns as part of weight management strategies. The aim of this study, therefore, was to compare subjective appetite (primary outcome), appetite-regulating hormones and energy intake in response to short frequent physical activity breaks versus longer duration less frequent breaks in sitting, matched for total volume and intensity among adult females exhibiting a range of body mass index values. It was hypothesised that both physical activity break regimes would suppress subjective appetite relative to uninterrupted sitting and would not lead to any compensatory increases in energy intake.

2. Materials and methods

2.1. Study overview

This randomised, crossover trial was approved by the University of Bedfordshire Institute for Sport and Physical Activity Research Ethics Committee (approval number 2015ISPAR012). Written informed consent was provided by each participant prior to participating in the study. Participants first attended a preliminary testing session and then took part in three experimental conditions each lasting 7.5 h. The conditions were separated by a $\geq$7-day washout period and took place during the self-reported follicular phase to minimise variations in appetite regulation that may occur during the menstrual cycle (Hirschberg, 2012). The order of the conditions was counterbalanced using an incomplete Latin square method that was generated by a member of the research team. The participants remained blinded to condition order until they were informed of which condition they were taking part in on the morning of arrival to the first two experimental conditions days. All testing took place at the University of Bedfordshire Sport and Exercise Science Laboratories.

2.2. Participants

Eligible participants for this study were females aged 18–50 years who were sedentary (self-reported sitting $\geq$7 h/day based on data that this was the threshold above which all-cause mortality risk increased (Chau et al., 2013)) and physically inactive (i.e. engaged in less than 150 min/week of moderate-to-vigorous physical activity). Exclusion criteria were pregnancy, diagnosed diabetes, a known blood-borne disease, the use of glucose or lipid medication, major illness or injury, employed in a non-sedentary occupation, contraindications to physical activity, allergies to any of the foods being provided during the study, unstable body mass in the past six months and currently dieting. Participants were also ineligible if they were vegetarian, reported a dislike of >3 of the food items being provided at the *ad libitum* meal or if they were a restrained eater as defined by the Three-Factor Eating Questionnaire (Stunkard & Messick, 1985). Participants were informed of the aims of the study before providing informed consent, but the expected direction of the results based on previous evidence were not stated to minimise the potential influence of pre-conceptions on the study outcomes.

2.3. Sample size

The primary outcome for this study was subjective appetite. Sample size calculations were performed using G*Power (Faul, Erdfelder, Lang, & Buchner, 2007) and were based on previous data using moderate-intensity steady state exercise (Deighton et al., 2013). To detect an effect size of d = 0.35 for differences in hunger between

conditions, it was estimated that 13 participants would be required when allowing for a within-person correlation of 0.5, 80% power, and α = 0.05.

2.4. Preliminary testing session

Participants had their height, weight and body fat % measurements taken during a preliminary testing session. Weight and body fat % (bioelectrical impedance analysis) were measured using the Tanita BC-418 Segmental Body Composition Analyzer (Tanita Corp., Tokyo, Japan). A four-stage submaximal exercise test was then completed on a treadmill (Woodway PPS55Med-I, Woodway GmbH, Weil am Rhein, Germany). The test started at a comfortable walking speed and increased by 1.5 km/h for each of the 4-min stages. A maximal oxygen uptake ($VO_{2\ max}$) test was then performed on the same treadmill after a 30 min rest period. This test started at a comfortable walking speed and increased by 1 km/h every 3-min until volitional exhaustion was reached. Expired air was measured breath-by-breath during both tests using the Cortex Metalyzer 3B online gas analysis system (Cortex Biophysik GmbH, Leipzig, Germany). A valid $VO_{2\ max}$ value was considered to have been attained if there was a plateau in VO_2 despite increasing workload or meeting ≥2 of the following end-point criteria: (1) Rating of Perceived Exertion (RPE) ≥18, (2) respiratory exchange ratio >1.1, and (3) heart rate within 10 bpm of age predicted maximum (Mier, Alexander, & Mageean, 2012). A line of best fit for the relationship between VO_2 and treadmill speed was produced for each participant to identify the speed that elicited 65% of $VO_{2\ max}$ for the experimental conditions.

2.5. Experimental protocol

To standardise physical activity and dietary intake, participants refrained from engaging in exercise and consuming alcohol or caffeine for 48 h before taking part in each condition. Participants were asked to use the same mode of travel (preferably by vehicle to minimise physical activity) to the laboratories on the day of the experimental conditions. The weight and time of all food and liquid intake was recorded in a food diary during the 24 h before the first experimental condition. This intake was then replicated during the 24 h before the remaining two conditions (Bailey et al., 2016). On the day of the experimental conditions, participants attended the laboratories at ~08:30 after an overnight fast; water intake was permitted *ad libitum*. Prior to the commencement of each condition, an Actiheart monitor (CamNtech Ltd., Cambridge, UK) was fitted to the chest of the participant to provide a valid and reliable measure of physical activity energy expenditure (Brage, Brage, Franks, Ekelund, & Wareham, 2005). The Actiheart was calibrated for each participant based on their expired air data from the $VO_{2\ max}$ test. A cannula was then inserted into an antecubital vein and a fasting blood sample was drawn. The 7.5 h experimental condition period (shown in Fig. 1) then commenced. The three conditions were: (1) uninterrupted sitting (SIT) at a desk, (2) sitting with short frequent 2-min moderate-intensity physical activity (walking) breaks every 30 min (SHORT-BREAKS), and (3) sitting with longer duration, less frequent 10-min moderate-intensity physical activity (walking) breaks every 170–180 min (LONG-BREAKS).

Physical activity intensity (65%$VO_{2\ max}$) and total volume (30 min) were matched between the SHORT-BREAKS and LONG-BREAKS conditions. This volume and intensity was selected as an accumulated 30 min of moderate-intensity physical activity on five days per week would meet the guidelines of 150 min/week of moderate-intensity physical activity (Department of Health and Social Care, 2019). This was in addition to previous research demonstrating that moderate-intensity exercise increases PYY, reduces acylated ghrelin and suppresses subjective appetite acutely (Schubert et al., 2014). RPE was measured with the Borg scale (Borg, 1982) in the last 30 s of each physical activity break. Participants were seated when not performing physical activity and used a laptop computer, read, or talked. A researcher supervised the participants to ensure compliance with the protocols. Participants were permitted to walk to the toilets when needed which were located ~30 m from the testing area.

2.5.1. Food and water intake

Standardised breakfast and lunch meals were consumed at 15 min and 180 min, respectively, during each condition. The breakfast meal provided 15% and the lunch meal provided 25% of each participant's daily energy requirement. A validated equation (Mifflin et al., 1990) was used to estimate each participant's energy requirements with a 1.4 physical activity factor applied to represent a sedentary day. Breakfast was cornflakes and whole milk (glycaemic index of 80) and lunch was white bread, roast chicken slices, margarine, chocolate and crisps (glycaemic index of 58). The macronutrient composition was 58% carbohydrate, 28% fat and 14% protein for breakfast and 46% carbohydrate, 40% fat and 14% protein for lunch. An *ad libitum* cold food buffet was provided at 300 min. The items available were white bread, wholemeal bread, margarine, mayonnaise, cheese, ham, crisps, chocolate bars, cereal bars, cookies, apples, oranges, bananas, milk and orange juice. Participants were not made aware of the purpose of the buffet meal and were instructed to "eat as much as they like until comfortably full and satisfied". They had a maximum of 30 min for consumption and were able to request more of the food items provided. All test meals were consumed in an isolated room with no social influences. Food items were displayed in the same way for each condition with the same crockery and cutlery being used. These protocols are similar to previous research

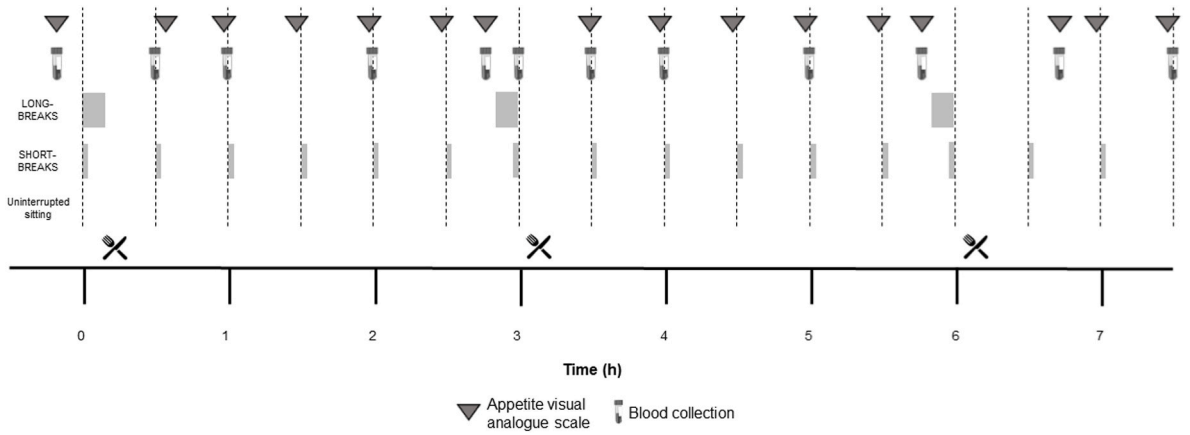

Fig. 1. Schematic of experimental protocol. Open rectangles indicate 10-min physical activity breaks in LONG-BREAKS condition and 2-min physical activity breaks in SHORT-BREAKS condition.

(Hagobian et al., 2013). The consumption time of each meal was recorded during the first condition and participants were asked to replicate this as closely as possible in the subsequent conditions. Water was provided *ad libitum* during the first condition and this volume was replicated by spreading consumption across the day in the subsequent conditions.

2.6. Data collection

2.6.1. Appetite perceptions

A validated 100 mm visual analogue scale (VAS) was used to measure subjective appetite (Flint, Raben, Blundell, & Astrup, 2000) upon arrival to the laboratory (fasted) and then every 30 min during the experimental conditions. The VAS measured perceptions of hunger ("How hungry do you feel?"), satisfaction ("How satisfied do you feel?"), fullness ("How full do you feel?") and prospective food consumption (PFC; "How much do you think you can eat?"). A composite appetite score was calculated at each time point as the mean of the four appetite parameters after inverting satisfaction and fullness values (van Can et al., 2014): $Composite\ appetite = \frac{hunger+(100-satisfaction)+(100-fullness)+PFC}{4}$

2.6.2. Energy intake

Energy intake at the *ad libitum* meal was determined by weighing the leftover food and liquid items individually with digital scales (Salter 1036BKSSDR, HoMedics Group Ltd., Kent, UK) and calculating the weighted difference of the buffet items before and after consumption using energy values from the food packaging.

2.6.3. Blood collection and biochemistry

Blood samples were taken using a cannula and collected into two pre-cooled EDTA-containing vacuettes (Greiner Bio-One, Kremsmünster, Austria) at baseline and 35, 60, 120, 165, 180, 210, 240, 300, 345, 405, 450 min. To preserve acylated ghrelin, a 50-μL solution containing potassium phosphate buffer, p-hydroxymercuribenzoic acid and sodium hydroxide was mixed into one of the vacuettes before they were spun at 1500×*g* for 10 min at 4 °C. The plasma from the treated vacuette was mixed with 100 μL of 1 mol/L hydrochloric acid per mL of plasma and was spun at 1500×*g* for 5 min at 4 °C. The plasma from the untreated vacuette and the plasma treated to preserve acylated ghrelin were stored at −80 °C until being later analysed for concentrations of total PYY (Merck Millipore, Watford, UK) and acylated ghrelin (Bertin Pharma, Montigny le Bretonneux, France) using commercially available enzyme immunoassays. Total PYY was measured to allow comparison with previous studies in this field. Samples from each participant were analysed in the same run to avoid inter-assay variation. The intra-assay coefficient of variation was 9.9% for total PYY and 7.9% for acylated ghrelin.

2.7. Data analysis

Total area under the curve (AUC) was calculated using the trapezoidal rule for each subjective appetite and appetite hormone variable across each 7.5 h experimental condition period. Net incremental area under the curve (iAUC) was additionally calculated for the appetite hormone concentrations by subtracting the area under the baseline (fasting) value from total AUC. Relative energy intake (REI) was calculated by subtracting physical activity energy expenditure from energy intake for each condition. SPSS v26 (SPSS Inc., Armonk, N.Y., USA) was used to conduct statistical analyses. To check normality of the data, standard graphical methods (Q-Q plots) were used as preferred to significance testing (Grafen & Hails, 2002). None of the variables were deemed to be non-normally distributed. The outcomes were compared between conditions using linear mixed models with experimental condition entered as a fixed factor. A condition x time analysis was also conducted using linear mixed models for each outcome with condition and time as fixed factors. Participants were entered as a random factor in all models but were subsequently removed as this parameter was redundant in the covariance structure. Covariates included in the models were age, body fat % and baseline values for the outcome where relevant in each model. Data is presented as mean (95% confidence interval [CI]) unless stated otherwise. The level of significance was set at $p < 0.05$. Cohen's d effect sizes were calculated for the between-condition comparisons (trivial effect $d < 0.2$, small effect $d = 0.2–0.49$; medium effect $d = 0.5–0.79$, large effect $d \geq 0.8$) (Cohen, 1988).

3. Results

Participant recruitment took place between November 2015 and August 2016. Fourteen participants were recruited for the study and completed all three experimental conditions. The descriptive characteristics of the sample can be seen in Table 1.

For the breakfast meal, carbohydrate, fat and protein intake were 43 ± 8 g, 9 ± 2 g and 11 ± 2 g, respectively, with an energy intake of 1274 ± 240 kJ. For the lunch meal, carbohydrate, fat and protein intake were 58 ± 10 g, 22 ± 4 g and 18 ± 3 g, respectively with an energy intake of 2123 ± 221 kJ. Physical activity energy expenditure for the physical activity bouts was similar between the SHORT-BREAKS (587 kJ; 95% CI: 449, 725) and LONG-BREAKS (607 kJ; 473, 740) conditions ($p = 0.992$). The mean RPE of the physical activity bouts was significantly higher in the SHORT-BREAKS (11.7 ± 1.7) than LONG-BREAKS condition (10.4 ± 1.4; $p = 0.001$).

There were no differences in any of the subjective appetite variables between conditions at baseline (all $p \geq 0.513$). The main effect of condition was non-significant for hunger, satisfaction, fullness, PFC and overall appetite total AUC (see Table 2) with trivial to small effect sizes for comparisons between conditions ($d = 0.01$ to 0.29). The condition x time analysis supported the AUC data, with no significant main effect of condition or condition × time interactions for each of the appetite perception variables (all $p \geq 0.185$); overall appetite during each condition is illustrated in Fig. 2.

Baseline acylated ghrelin and total PYY concentrations did not differ between SIT, SHORT-BREAKS and LONG-BREAKS (see Table 2). For postprandial acylated ghrelin and total PYY iAUC and total AUC, there was no main effect of condition. There was a medium effect size for higher acylated ghrelin iAUC in SHORT-BREAKS versus SIT ($d = 0.61$); the reverse was seen with lower acylated ghrelin total AUC in SHORT-BREAKS versus SIT ($d = 0.69$). Effect sizes were small for all other acylated ghrelin and total PYY between-condition comparisons. The condition x time analysis demonstrated that there were no significant main effects of condition or condition × time interactions for acylated ghrelin and PYY concentrations (all $p \geq 0.148$); Fig. 3 shows appetite hormone concentrations over time for each condition.

Carbohydrate, fat, protein and total energy intake at the *ad libitum* meal was not significantly different between conditions (Table 2). There were medium effects sizes for the lower *ad libitum* energy intake in LONG-BREAKS compared with SIT ($d = 0.52$), higher carbohydrate intake in SHORT-BREAKS versus SIT ($d = 0.56$) and lower fat intake in SHORT-BREAKS versus LONG-BREAKS ($d = 0.67$). There were also large

Table 1
Descriptive characteristics of the sample (n = 14).

Characteristic	Mean ± SD/*n* (%)
Age (years)	34 ± 13
Body mass (kg)	76.4 ± 17.4
Body mass index (kg/m^2)	27.1 ± 6.3
Healthy weight (18.5–24.9 kg/m^2)	5 (36)
Overweight (25.0–29.9 kg/m^2)	5 (36)
Obese (≥30 kg/m^2)	4 (28)
Body fat %	32.9 ± 10.9
Maximal oxygen uptake (mL/kg/min)	34.5 ± 6.6

Table 2
Appetite perceptions, appetite hormone concentrations and *ad libitum* energy intake for each condition.

Variable	SIT	SHORT-BREAKS	LONG-BREAKS	Main effect of condition (p)
Appetite perceptions				
Hunger total AUC (mm·7.5 h)	214 (142, 285)	224 (153, 296)	213 (140, 285)	0.895
Satisfaction total AUC (mm·7.5 h)	443 (374, 511)	463 (394, 531)	453 (383, 523)	0.801
Fullness total AUC (mm·7.5 h)	455 (381, 528)	468 (395, 541)	456 (381, 530)	0.888
PFC total AUC (mm·7.5 h)	299 (223, 375)	280 (205, 356)	297 (219, 375)	0.861
Composite appetite total AUC (mm·7.5 h)	279 (212, 346)	268 (201, 335)	275 (207, 343)	0.935
Baseline concentrations				
Acylated ghrelin (pg/mL)	130.8 (60.5, 201.0)	121.8 (51.5, 192.0)	135.9 (65.7, 206.2)	0.314
Total PYY (pg/mL)	116.1 (81.6, 150.7)	120.7 (85.8, 155.6)	100.8 (66.3, 135.3)	0.240
Postprandial concentrations				
Acylated ghrelin iAUC (pg/mL·7.5 h)	−210.0 (−296.6, −123.5)	−150.3 (−237.0, −63.6)	−206.9 (−291.5, −116.2)	0.388
Total PYY iAUC (pg/mL·7.5 h)	189.4 (−13.5, 392.4)	146.2 (−58.7, 351.0)	166.2 (−37.5, 369.8)	0.805
Acylated ghrelin total AUC (pg/mL·7.5 h)	745.6 (536.4, 954.7)	617.0 (407.7, 826.4)	767.5 (543.2, 991.8)	0.566
Total PYY total AUC (pg/mL·7.5 h)	1025.1 (822.2, 1228.0)	981.9 (777.0, 1186.7)	1001.8 (798.2, 1205.5)	0.805
***Ad libitum* buffet intake**				
Total energy intake (kJ)	3442 (2629, 4254)	3187 (2374, 3999)	3014 (2183, 3846)	0.579
Total carbohydrate (g)	85 (69, 100)	94 (78, 109)	78 (63, 94)	0.374
Total fat (g)	25 (19, 30)	23 (17, 28)	19 (14, 25)	0.400
Total protein (g)	34 (23, 45)	34 (23, 45)	33 (22,44)	0.989

Data are mean (95% CI). SIT, prolonged sitting; SHORT-BREAKS, sitting with short frequent physical activity breaks; LONG-BREAKS, sitting with longer duration, less frequent physical activity breaks; AUC, area under the curve.

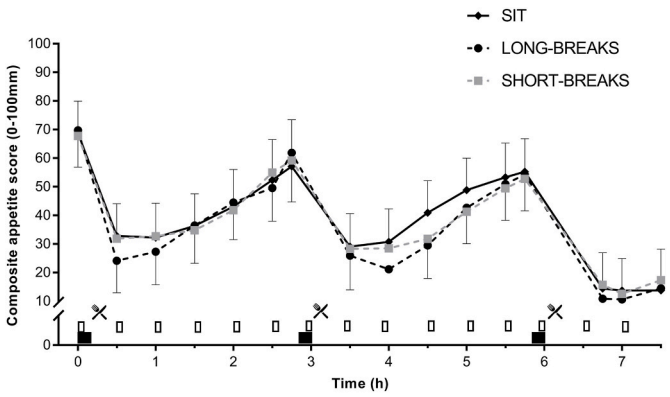

Fig. 2. Composite appetite score during the experimental conditions (SIT, prolonged sitting; SHORT-BREAKS, sitting with short frequent physical activity breaks; LONG-BREAKS, sitting with longer duration, less frequent physical activity breaks). Open rectangles indicate 2-min physical activity breaks; Solid rectangles indicate 10-min physical activity breaks.

effects for lower carbohydrate intake in SHORT-BREAKS versus LONG-BREAKS (d = 1.00) and for lower fat intake in SIT compared with LONG-BREAKS (d = 1.00). Relative energy intake was 755 (571, 938) kJ during SIT, 652 (468, 835) kJ during SHORT-BREAKS and 623 (434, 813) during LONG-BREAKS. Although the main effect of condition was not significant (p = 0.420), there was a medium effect for lower REI in LONG-BREAKS (d = 0.71) and SHORT-BREAKS (d = 0.56) compared with SIT.

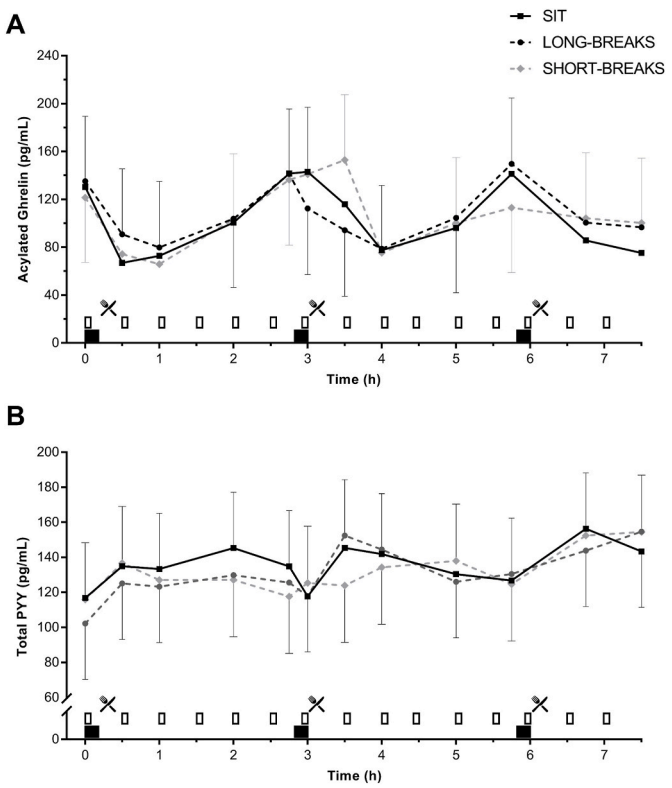

Fig. 3. Acylated ghrelin and peptide YY concentrations during the experimental conditions (SIT, prolonged sitting; SHORT-BREAKS, sitting with short frequent physical activity breaks; LONG-BREAKS, sitting with longer duration, less frequent physical activity breaks). Open rectangles indicate 2-min physical activity breaks; Solid rectangles indicate 10-min physical activity breaks.

4. Discussion

The main findings of this study are that breaking up sitting with volume- and intensity-matched short frequent or longer less frequent bouts of moderate-intensity walking did not lead to any compensatory changes in subjective appetite, appetite hormones or *ad libitum* energy intake in sedentary females. This is synonymous with previous research in males and females without obesity that observed no differences in subjective appetite or appetite hormone concentrations in response to 2-min light or moderate-intensity walking every 20 min, compared with uninterrupted sitting (Bailey et al., 2016). There was also no difference in subjective appetite or *ad libitum* energy intake in response to 2-min moderate-intensity walking breaks every 30 min over two days in males and females who were healthy/normal weight (Mete et al., 2018). In females and males with obesity, 5-min moderate-intensity walking every 60 min reduced hunger compared with prolonged sitting, whereas satiety was only higher when compared with a volume and intensity-matched continuous exercise bout, but not prolonged sitting (Holmstrup et al., 2013). Other than potential hunger suppression in individuals with obesity, there appears to be limited effects of breaking up sitting on subjective appetite, regardless of the frequency or duration of the physical activity breaks. The current study extends previous findings by showing that appetite and energy intake are not altered to compensate for the increased physical activity energy expenditure accumulated in short frequent or longer less frequent breaks among a

sample of adult females.

In contrast to previous research that reported a significantly lower REI in response to breaking up sitting (Bailey et al., 2016), REI in the present study was not significantly different between the uninterrupted sitting and physical activity breaks conditions. There was a medium effect for the 103 kJ and 131 kJ lower REI when sitting was interrupted with both short frequent and longer less frequent physical activity breaks, respectively, compared with uninterrupted sitting. This magnitude of energy deficit is greater than the 62 kJ/day minimum energy gap proposed for preventing weight gain at population level in the long-term (Hill, 2009). The present study was powered to detect differences in the primary outcome (subjective appetite) rather than REI; a larger sample size may have resulted in statistically significant reductions in REI. Intervention studies that are sufficiently powered for energy balance outcomes and conducted over the long-term are needed to establish the efficacy of breaking up prolonged sitting for weight management.

Studies employing a similar volume of moderate-intensity physical activity to the present study (i.e., 30 min), but in one single continuous exercise bout, have reported increases in PYY_{3-36} concentration, decreases in appetite and energy intake, and no change in total ghrelin (Ueda, Yoshikawa, Katsura, Usui, & Fujimoto, 2009; Ueda, Yoshikawa, Katsura, Usui, Nakao, et al., 2009). However, the acylated form of ghrelin that is responsible for exerting appetite stimulating effects was not measured in these previous studies, limiting direct comparisons to the present study.

A previous study in males and females with obesity found no differences in total PYY between breaking up sedentary time with 5-min moderate-intensity walking every 60 min over 12-h, an intensity and energy-matched continuous 1-h exercise bout performed in the morning, or a 12-h period of prolonged sitting (Holmstrup et al., 2013). This agrees with the present study and previous research in which females and males who did not have obesity completed 2 min of light or moderate-intensity walking breaks every 20 min (Bailey et al., 2016). One study reported significant appetite hormone concentration changes (increased GLP-1 and total PYY) in response to breaking up sitting with 2-min moderate-intensity walking breaks every 20 min; this was conducted in a sample of females and males with central obesity (Chen et al., 2022). It may, therefore, be postulated that short frequent physical activity breaks provide a sufficient stimulus for increased appetite hormone concentrations in individuals with obesity. It was not possible to determine the effects of the different physical activity break protocols on appetite hormone responses in participants who were overweight or obese in the present study, or the study by Bailey et al. (2016), as the samples included participants of varying weight status. Based on these findings, it appears that the short frequent or longer duration less frequent physical activity breaks are insufficient to significantly suppress appetite, modify appetite hormones or reduce energy intake in sedentary females with variable body weight status.

Although not statistically significant, a medium effect size was found in the current study for acylated ghrelin total AUC, with lower concentrations in the short frequent breaks condition compared with uninterrupted sitting. Yet, acylated ghrelin iAUC was higher (medium effect) in the short frequent breaks condition compared with uninterrupted sitting. This suggests that breaking up sitting could have potentially meaningful effects on postprandial acylated ghrelin, but this change may not be favourable (i.e. increased concentrations) when responses are evaluated relevant to fasting hormone concentrations. This phenomenon should be investigated in studies that are powered to detect changes in appetite hormones to further elucidate the potential effects of breaking up sitting in the context of controlling for fasting concentrations on these outcomes.

The strengths of this study include the randomised crossover design and the measurement of an array of appetite, appetite hormone and energy intake outcomes in a controlled laboratory environment where the physical activity breaks were matched for total volume and intensity, allowing for a direct determination of the independent effect of bout frequency and duration. The standardisation of dietary intake and exercise prior to the experimental conditions is a further strength. The study was also conducted in females who are less represented in terms of appetite responses to physical activity and breaking up sitting. This study's sample was homogenous with regards to sitting time and physical activity status, meaning that the results can be generalised to populations who are inactive and sit for long periods and may thus have compromised appetite sensitivity (Beaulieu, Hopkins, Blundell, & Finlayson, 2016). However, the findings cannot be generalised to the male population. Also, our sample included a mixture of individuals with healthy weight, overweight and obesity; it is possible that appetite responses to physical activity may depend on weight status. This may have inflated variability in the data, meaning that the findings cannot be applied to a specific weight status. Further limitations include the measurement of total PYY instead of PYY_{3-36} as the latter is a more potent suppressor of appetite (Chelikani, Haver, & Reidelberger, 2004). That said, total PYY and PYY_{3-36} are strongly correlated and are thus likely to reflect changes in one another (Tsilchorozidou, Batterham, & Conway, 2008).

In conclusion, the findings of this study suggest that breaking up sitting with short frequent or longer duration less frequent physical activity breaks do not cause a compensatory change in appetite, appetite hormones or subsequent energy intake in females with variable weight status. There were potentially meaningful beneficial changes in relative energy intake and acylated ghrelin that should be explored further in studies powered to detect changes in these outcomes. The effects of different patterns of physical activity used to break up sitting on appetite and energy intake should be explored in longer-term trials that control for individual weight status to inform health promotion and weight management strategies.

Ethical statement

This randomised crossover trial was approved by the University of Bedfordshire Institute for Sport and Physical Activity Research Ethics Committee (approval number 2015ISPAR012). Written informed consent was provided by each participant prior to participating in the study.

Author contributions

BDM, JKZ-F, DJS and DPB contributed to conceptualisation and methodology. BDM, CJO and DPB contributed to data collection. BDM and DPB conducted data analysis. BDM, JKZ-F and DPB interpreted the data. BDM and DPB drafted the manuscript. All authors critically reviewed and have approved the final article.

Funding

This work was supported by the Royal Society [grant number RG140199].

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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