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Breaking up prolonged sitting time with walking does not affect appetite or gut hormone concentrations but does induce an energy deficit and suppresses postprandial glycaemia in sedentary adults

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Abstract

Background: Breaking up periods of prolonged sitting can negate harmful metabolic effects but the influence on appetite and gut hormones is not understood and is investigated in this study.

Methods: Thirteen sedentary (7 female) participants undertook three, 5 h trials in random order: 1) uninterrupted sitting (SIT), 2) seated with 2 min bouts of light-intensity walking every 20 min (SIT+LA), and 3) seated with 2 min bouts of moderate-intensity walking every 20 min (SIT+MA). A standardised test drink was provided at the start and an *ad libitum* pasta test meal provided at the end of each trial. Subjective appetite ratings and plasma acylated ghrelin, peptide YY, insulin, and glucose were measured at regular intervals. Area under the curve (AUC) was calculated for each variable. **Results:** AUC values for appetite and gut hormone concentrations were unaffected in the activity breaks conditions compared to uninterrupted sitting (linear mixed modelling: $p>0.05$). Glucose AUC was lower in SIT+MA than SIT+LA ($p=0.004$) and SIT ($p=0.055$). There was no difference in absolute *ad libitum* energy intake between conditions ($p>0.05$), however, *relative* energy intake was lower in SIT+LA (39%; $p=0.011$) and SIT+MA (120%; $p<0.001$) than SIT. **Conclusion:** Breaking up prolonged sitting does not alter appetite and gut hormone responses to a meal over a 5 h period. Increased energy expenditure from activity breaks could promote an energy deficit that is not compensated for in a subsequent meal.

Keywords

Sedentary behaviour; activity breaks; prolonged sitting; appetite-regulating hormones; acylated ghrelin; energy intake

Introduction

In recent years it has emerged that engaging in prolonged periods of uninterrupted sitting increases risk of obesity, morbidity, and mortality (Biswas et al. 2015; Hamilton et al. 2007). This behaviour reduces daily energy expenditure but this is not accompanied by compensatory reductions in appetite or energy intake and may thus contribute to weight gain (Stubbs et al. 2004). Energy expended through acute bouts of structured exercise ($36\text{--}81\% \dot{V}O_{2\max}$ for 30-120 min) has a trivial effect on absolute energy intake but does create a large energy deficit in the immediate hours post-exercise (Schubert et al. 2013). This may thus have potential for causing negative energy balances over time.

A shift away from emphasis on continuous exercise bouts is now emerging with research highlighting the importance of regularly interrupting prolonged periods of sitting with brief activity bouts as this improves chronic disease risk independent of time spent in moderate-to-vigorous physical activity (Dempsey et al. 2014). Experimental evidence shows that interrupting sitting with 2 min bouts of light- or moderate-intensity walking every 20 min suppresses postprandial glycaemia and insulinaemia in overweight and non-overweight individuals (Bailey and Locke 2015; Dunstan et al. 2012). Such findings has led to the publication of an Expert Statement providing guidelines on avoiding prolonged periods of sedentary work that recommend initially accumulating at least 2 h of standing and light activity (light walking) per working day (Buckley et al. 2015). To our knowledge, only one study has examined appetite and appetite-regulating hormone responses to breaking up prolonged sitting reporting that hourly 5 min bouts of moderate-intensity cycling led to suppressions in appetite compared to an energy-matched continuous bout of exercise performed in the morning or 12 h of uninterrupted sitting (Holmstrup et al. 2013). This may support the use of activity breaks to interrupt sitting time since this is likely to be a more achievable movement strategy at work and in leisure time. However, these observations require further support and appetite responses to different protocols for breaking up prolonged sitting need to be examined.

Regulation of appetite and food intake is complex and influenced by a range of neuroendocrine and psychological factors (Hussain and Bloom 2013). Several secreted peptide hormones such as acylated ghrelin and peptide YY (PYY) communicate with the central nervous system to control energy intake and respond to changes in energy status and structured exercise (Stensel 2010). On a meal-to-meal basis, episodic peptide hormones secreted from the gut act to control energy intake (Neary and Batterham 2009). Ghrelin is secreted from the stomach and is the only gut hormone known to stimulate appetite (Kojima et al. 1999). The post-translationally modified form of this hormone, acylated ghrelin, is the form of ghrelin solely responsible for appetite stimulation (Delhanty et al. 2012). The other episodic gut hormones act as satiety signals to inhibit food intake and highly prominent of these is PYY, which is secreted chiefly from the distal intestine and colon in direct proportion to the energy content of an ingested meal (le Roux et al. 2006). Single bouts of exercise can have a marked impact on these hormones with changes evident soon after initiation of exercise (Bailey et al. 2015; Broom et al. 2009; Stensel 2010; Ueda et al. 2009b). However, these perturbations are short-lived and customary circulating levels are restored soon after exercise (King et al. 2013; Ueda et al. 2009b). A consistent finding is that a single bout of exercise does not cause compensatory changes in appetite-regulating hormones in the direction that would lead to increased appetite and food intake (Schubert et al. 2014). The response of these hormones to short regular activity bouts has been reported in only one study that did not observe any change in total PYY in individuals performing hourly 5 min moderate-intensity cycling bouts (Holmstrup et al. 2013). Further research investigating a wider array of appetite-regulating hormones is warranted to clarify their response to energy expended through regular activity breaks that are now being widely prescribed.

The primary objective of this study was to investigate the effects of breaking up prolonged sitting with frequent bouts of light- or moderate-intensity walking on subjective appetite, energy intake, and circulating concentrations of acylated ghrelin and total PYY in sedentary, inactive adults.

Secondary objectives were to investigate the effects of this activity regimen on postprandial glucose and insulin concentrations.

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1 **Materials and Methods**

2 *Participants and enrolment process*

3 This randomised three-way crossover study was approved by the University of Bedfordshire Ethics
4 Review Committee and was in accordance with the principles set out in the Declaration of Helsinki.
5 Thirteen sedentary (7 females), inactive but otherwise healthy participants (mean \pm SD; age, 26.6 \pm
6 8.5 years; body fat, 24.4 \pm 8.2%) gave written informed consent to participate in the study following
7 a verbal and written explanation of the nature and risks involved. Exclusion criteria included any
8 known blood borne disease, pregnancy, clinically diagnosed diabetes, taking glucose-lowering
9 and/or lipid-lowering medication, employment in a non-sedentary occupation, currently watching <2
10 h of television/day, regularly engaged in moderate-intensity physical activity (150 min/week) for at
11 least 3 months, and known physical activity contraindications, major illness/injury, or other health
12 issues that may limit the ability to perform the necessary activity bouts.

13 Participants attended a familiarisation session where they had their body fat percentage
14 measured using the Tanita BC-418 Body Composition Analyzer (Tanita Corporation, Tokyo, Japan)
15 and familiarised with use of the Borg Rating of Perceived Exertion (RPE) scale (Borg 1982). In line
16 with previous research (Bailey and Locke 2015; Dunstan et al. 2012), participants then became
17 accustomed to the light-intensity treadmill walking speed (3.2 km/h; Woodway PPS55 Med-i, GmbH,
18 Germany) during which RPE was recorded to ensure walking speed was equivalent to light-intensity
19 activity for each participant (RPE of 6-9). During the moderate-intensity walking familiarisation, the
20 treadmill speed that yielded an RPE rating between 12 and 14 for each participant was recorded and
21 used during that experimental condition (Dunstan et al. 2012).

22

23 *Study protocol*

24 Each trial was separated by ≥ 7 days. In line with previous research, participants were asked to
25 refrain from exercise, alcohol, and caffeine in the 48 h before each main trial (Dunstan et al. 2012).
26 During this 48 h time period, physical activity levels were objectively measured with an Actigraph

27 GT3X accelerometer (Actigraph, Pensacola, FL., USA) worn around the hip during waking hours.
28 Participants were required to have a daily wear time of ≥ 10 h and data were recorded in 5 s epochs
29 with previously validated accelerometer counts applied (Freedson et al. 1998). Physical activity levels
30 did not differ significantly between trials ($p > 0.05$; data not shown).

31 Participants reported to the laboratory in the morning (~08:30) following an overnight fast
32 and were asked to minimise their activity prior to attending e.g. travel in by car. Each participant was
33 required to arrive at the same time of day for each of their trials. Participants weighed (Salter Disc
34 Electronic Kitchen Scale, HoMedics Group Ltd, UK) and recorded food intake for 24 h before the first
35 main trial and were asked to replicate the quantity and timings of eating prior to each subsequent
36 testing day (Bailey et al. 2015; Deighton et al. 2013). After fasting blood collection (-1 h), participants
37 remained seated for 1 h before consuming a standardised test drink (consumed within 5 min). The
38 drink contained 75 g carbohydrate (100% dextrose monohydrate powder; Thornton & Ross Ltd, UK)
39 in 100 mL water combined with 50 g fat (Calogen; Nutricia, UK). The specific nutritional components
40 were energy, 3,211 kJ; fat, 50.0 g; saturated fat, 5.3 g; carbohydrate, 79.3 g; sugars, 4.0 g; protein,
41 nil; fibre, nil; and sodium, 7.0 mg. Following consumption, participants were guided through the 5 h
42 trial and supervised at all times by a member of the research team to ensure full compliance with
43 the protocols. Hourly blood samples were taken prior to the activity bouts (trial conditions 2 and 3
44 below).

45 The trial conditions were based upon previous research that demonstrated suppressed
46 postprandial glucose and insulin concentrations (Bailey and Locke 2015; Dunstan et al. 2012) and
47 were as follows:

- 48 1. *Uninterrupted sitting* (SIT): participants remained seated throughout the experimental
49 period and were instructed to minimise excessive movement.
- 50 2. *Sitting + light-intensity activity breaks* (SIT+LA): participants rose from the seated position
51 every 20 min and completed 2 min bouts of light-intensity walking on a motorised treadmill

52 with a level surface at 3.2 km/h, providing a total of 28 min activity. They then returned to
53 the seated position.

54 3. *Sitting + moderate-intensity activity breaks* (SIT+MA): identical procedure to the sitting +
55 light-intensity activity breaks condition, but participants completed 2 min bouts of
56 moderate-intensity walking on the treadmill at between 5.8 and 7.9 km/h every 20 min,
57 providing a total of 28 min moderate-intensity activity.

58

59 Energy expenditure of the activity bouts was estimated using the ACSM Metabolic Equations for
60 walking (ACSM 2014). Estimated activity energy expenditure during SIT+LA was 458 ± 96 kJ and
61 SIT+MA was 921 ± 226 kJ. Participants watched television or DVDs; read books, magazines, or
62 newspapers; talked; or worked on a laptop computer throughout the trials but were devoid of food
63 cues at all times. Participants were permitted to rise from a seated position to void as necessary.

64 At the end of each trial, an *ad libitum* test meal (instant pasta: 74.5% carbohydrate, 21%
65 protein, and 4.5% fat; Tesco, UK) was provided and participants were instructed to “eat as much as
66 they like until satisfied”. The meal was consumed in isolation so that social influence did not affect
67 food selection and participants were not made aware that their food intake was being measured. In
68 line with previous research (Ueda et al. 2009a; Ueda et al. 2009b), a small bowl was filled with the
69 test pasta (~200 g) and repeatedly filled before the participant had emptied it to ensure blindness to
70 the amount of food eaten. No time limit was set for eating. During all trials, the participants and
71 experimenters were instructed to abstain from talking about the meal. After consumption of the test
72 meal, any remaining food was weighed (Salter Disc Electronic Kitchen Scale) and the amount
73 determined subtracted from the pre-meal value to obtain the total amount of food ingested. Then,
74 absolute energy intakes from the test meal in each session were calculated from the amount of food
75 eaten and nutritional information provided on the food label (5.3 kJ/g).

76

77 *Ratings of perceived appetite*

78 During each trial subjective feelings of hunger (“How hungry do you feel?”), satisfaction (“How
79 satisfied do you feel?”), fullness (“How full do you feel?”), and prospective food consumption (PFC;
80 “How much do you think you can eat?”) were reported on paper using a validated 100 mm Visual
81 Analogue Scale (VAS) (Flint et al. 2000). Appetite perceptions were measured fasted (-1 h) and every
82 30 min during the 5 h trial period. An overall appetite rating was calculated as the mean value of the
83 four appetite perceptions after inverting the values for satisfaction and fullness (Stubbs et al. 2000).

84

85 *Blood sampling*

86 During each main trial, venous blood was collected via a cannula (Vasofix®, B. Braun Medical Ltd,
87 Sheffield, UK), which was inserted into an antecubital vein. A fasting sample was taken upon arrival
88 at the laboratory (-1 h) and then every hour after commencement of each trial while seated.
89 Samples were collected into two 4.9 mL EDTA vacuettes (VACUETTE®, Greiner Bio-One, Austria).
90 One vacuette was immediately centrifuged at 1,500 x g for 10 min at a temperature of 4°C (Heraeus
91 Multifuge X3R, Thermo Scientific, Loughborough, UK). The plasma supernatant was then dispensed
92 into separate 2 mL cryovials, flash frozen in liquid nitrogen, and stored at -80°C until later analysis of
93 insulin and total PYY concentrations. From each sample, 50 µL blood samples were collected into a
94 microvette and analysed immediately to determine glucose concentrations using the YSI 2300 STAT
95 plus glucose and lactate analyser (YSI Inc., Yellow Springs, Ohio, USA). The YSI uses a steady state
96 measurement methodology, where membrane based glucose oxidase catalyses the oxidation of
97 glucose to gluconic acid and hydrogen peroxide. The difference between the sample generated
98 plateau current and the initial baseline current is proportional to the glucose concentration. The YSI
99 was calibrated at the start of every day and every 45 min thereafter.

100 To prevent the degradation of acylated ghrelin, a 50 µL solution containing potassium
101 phosphate buffer, p-hydroxymercuribenzoic acid, and sodium hydroxide was added to one 4.9 mL
102 EDTA vacuette, which was then centrifuged at 1,500 x g for 10 min at 4°C. The plasma supernatant
103 was then dispensed into a storage tube and 100 µL of 1 M hydrochloric acid was added per mL of

104 plasma to preserve acylated ghrelin (Hosoda et al. 2004). Thereafter, samples were spun at 1500 x g
105 for 5 min at 4°C prior to being flash frozen in liquid nitrogen and stored in 2 mL cryovials at -80°C
106 until later analysis.

107

108 *Blood biochemistry*

109 Commercially available enzyme immunoassays were used to determine plasma concentrations of
110 acylated ghrelin (SPI BIO, Montigny le Bretonneux, France), total PYY (Millipore, Watford, UK), and
111 insulin (Merckodia, Uppsala, Sweden). To eliminate interassay variation, samples from each
112 participant were analysed in the same run. The intraassay coefficients of variation were below 15%
113 for each variable, which is comparable to previous work in this field (Bailey et al. 2015; Ueda et al.
114 2009a).

115

116 *Statistical analysis*

117 Analyses were completed using the statistical software package IBM SPSS Statistics version 21.0
118 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 4.03 (GraphPad Software Inc., La Jolla, CA,
119 USA). Prior to any inferential statistical analyses, descriptive statistics tables were generated to
120 check the central tendency (mean, median), and dispersion (standard deviation, minimum,
121 maximum) of the data. Second, quantile-quantile (Q – Q) plots were used to check the normality
122 assumption of the results obtained for each of the conditions across all time points. Standard
123 graphical methods were preferred over null hypothesis significance testing to check statistical
124 assumptions (Grafen and Hails 2002). The two-tailed alpha level for significance testing was set as
125 $p < 0.05$.

126 Linear mixed models were chosen to determine if there were any differences in the
127 dependent variables between the conditions across time. This type of analysis was preferred as it
128 can accurately model different covariate structures for repeated measures data and model between-
129 subject variability (Vandenbogaerde and Hopkins 2010; West et al. 2006). Total area under the curve

130 (tAUC) was calculated for all blood metabolite and appetite variables using the trapezoidal method
131 for the total trial period. Positive incremental area under the curve (iAUC) was also calculated for
132 glucose and insulin based on suggestions that this method for estimating AUC more accurately
133 describes glycaemic responses to food (Le Floch et al. 1990) and to permit comparisons with
134 previous research (Dunstan et al. 2012). Fixed and random factors for the linear mixed model were
135 fit for each AUC variable. The main effect for condition was analysed by plotting the mean values.
136 Although the number of males ($n=7$) and females ($n=6$) studied was low, the main effect of sex and
137 condition x sex interaction effects were tested to generate preliminary data on potential sex
138 differences in the response to breaking up prolonged sitting. Data for condition x sex interaction
139 effects are presented only where significant findings have been observed. No significant main effects
140 for sex were observed for any variable and this data is thus not presented; all $p>0.234$. Step down
141 Hommel (Hommel 1988) adjusted post-hoc pair wise comparisons were calculated if a significant
142 main and/or interaction effect was present. The most appropriate model was chosen using the
143 smallest Hurvich and Tsai's criterion (AICC) in accordance with the principal of parsimony. Normality
144 and homogeneity of variance of the residuals for all variables were checked using Q – Q plots and
145 scatter plots, respectively. Acylated ghrelin and insulin tAUC in addition to absolute energy intake
146 and relative energy intake were non-normally distributed and log transformed prior to analysis. Back
147 transformation of these variables to natural units was applied to allow for meaningful presentation
148 in text and tables. Data are presented as mean \pm SD in the text and mean \pm SE in the figures.

149 Based on previous data (Deighton et al. 2013), a sample size of 12 participants was
150 determined as sufficient to detect a 10% difference in appetite perceptions during a post-exercise
151 period. This was the variable expected to have the smallest worthwhile change with the largest
152 amount of variability. This calculation was performed using G*power with an alpha value of 5% and
153 a power of 80% (Faul et al. 2007).

154

155 **Results**

156 *Appetite perceptions*

157 There were no significant differences in baseline appetite perception between trials (all $p>0.244$).
158 Table 1 shows appetite perception tAUC values for each trial condition. There were no significant
159 differences between trials for hunger, satisfaction, fullness, PFC, and overall appetite tAUC (all
160 $p>0.468$). The analysis of serial measurements confirmed the findings of the AUC analysis by
161 demonstrating no significant differences between trials (all $p>0.586$). There were also no significant
162 condition x time interaction effects observed for any appetite variable (all $p>0.486$). Overall appetite
163 responses over time for each trial are shown in Fig. 1.

164

165 Figure 1 about here

166

167 *Absolute energy intake*

168 Energy intake during the *ad libitum* pasta meal was $1,846 \pm 1,239$ kJ in SIT, $1,670 \pm 1,038$ kJ in
169 SIT+LA, and $1,360 \pm 510$ kJ in SIT+MA. These values did not differ significantly between trials
170 ($p=0.422$).

171

172 *Estimated relative energy intake (REI)*

173 A significant main effect of condition for REI (energy intake during experimental trials minus the net
174 energy expenditure of the activity bouts) was observed ($p<0.001$). As shown in Fig. 2, REI was
175 significantly lower in both the SIT+LA and SIT+MA trials (by 39% $p=0.011$ and 120% $p<0.001$,
176 respectively) than in SIT (SIT = $1,846 \pm 1,239$ kJ; SIT+LA = $1,247 \pm 1,063$ kJ; SIT+MA = 464 ± 632 kJ).
177 REI between SIT+LA and SIT+MA did not differ significantly ($p=0.138$). Closer inspection of the data
178 revealed that 11 out of the 13 participants had a lower REI in SIT+LA than in SIT and all 13
179 participants had a lower REI in SIT+MA than in SIT. There was a significant condition x sex interaction
180 effect ($p=0.047$) with males having a significantly reduced REI in the SIT+MA ($p<0.001$) trial

181 compared with SIT and significantly reduced REI in SIT+MA than SIT+LA ($p=0.029$). The lower REI in
182 SIT+LA than SIT in males was approaching significance ($p=0.112$). Mean REI for males was $2336 \pm$
183 1796 kJ in SIT, 1503 ± 1377 kJ in SIT+LA, and 75 ± 519 kJ in SIT+MA. In females, REI was significantly
184 lower in SIT+MA than SIT ($p=0.001$) and the lower REI in SIT+LA than SIT in females was approaching
185 significance ($p=0.101$). Mean REI for females was 1495 ± 569 kJ in SIT, 1067 ± 846 kJ in SIT+LA, and
186 687 ± 611 kJ in SIT+MA.

187

188 Figure 2 about here

189

190 *Gut hormone concentrations*

191 Gut hormone concentrations did not differ significantly at baseline (both $p>0.384$). Table 2 shows
192 tAUC values for plasma acylated ghrelin and total PYY for each trial condition. Acylated ghrelin and
193 PYY concentrations did not differ significantly between trials (both $p>0.785$). The analysis of serial
194 measurements confirmed the findings of the AUC analysis by demonstrating no significant
195 differences between trials (both $p>0.769$). There were also no significant condition x time interaction
196 effects observed for acylated ghrelin or PYY (both $p>0.258$). Gut hormone concentrations over time
197 for each trial are shown in Fig. 3.

198

199 Figure 3 about here

200

201 *Glucose and insulin concentrations*

202 Plasma glucose and insulin AUC values for each trial can be seen in Table 2. Fasting plasma glucose
203 concentrations differed significantly between trials at baseline with higher concentrations in SIT+MA
204 (4.3 ± 0.4 mmol/L) than SIT (4.0 ± 0.3 mmol/L; $p = 0.017$). Fasting plasma insulin concentrations did
205 not differ at baseline between trials ($p=0.226$). There was a main effect of condition for glucose iAUC
206 ($p=0.004$) with significantly lower concentrations during SIT+MA than SIT+LA ($p=0.004$). Glucose

207 iAUC was also lower in SIT+MA than SIT and this was approaching significance ($p=0.055$). The
208 condition x sex interaction effect for glucose iAUC was approaching significance ($p=0.113$) with post-
209 hoc comparisons revealing lower glucose concentrations in males (1.9 ± 1.6 mmol/L) compared with
210 females during SIT (3.8 ± 1.3 ; $p=0.048$). Furthermore, glucose concentrations were lower in SIT+MA
211 (1.1 ± 1.6 mmol/L) than SIT+LA (3.7 ± 2.2 mmol/L; $p=0.018$) in males and lower in SIT+MA (1.9 ± 1.4
212 mmol/L) than SIT (3.8 ± 1.3 mmol/L; $p=0.009$) in females. Insulin iAUC did not differ significantly
213 between trials ($p=0.308$) and there was also no significant difference in glucose tAUC ($p=0.993$) or
214 insulin tAUC ($p=0.638$) between trials. The analysis of serial measurements demonstrated no
215 significant differences between trials for glucose or insulin (both $p>0.866$). There were also no
216 significant condition x time interaction effects observed for either of these variables (both $p>0.804$).
217 Plasma glucose and insulin concentrations over time for each trial are shown in Fig. 3.

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Discussion

The main finding of this study was that regularly breaking up prolonged sitting with short bouts of light- or moderate-intensity walking does not affect appetite, energy intake or circulating gut hormone concentrations. This leads to an acute energy deficit that is not compensated for in a subsequent meal.

This study observed no change in appetite (hunger, satisfaction, fullness, PFC, and overall appetite) in the activity breaks conditions compared with uninterrupted prolonged sitting. Previous research supports these findings with no alterations in appetite (the desire for food and drink) reported following an acute bout of low- to moderate-intensity exercise ($<60\% \dot{V}O_{2max}$) (Schubert et al. 2013). Transient reductions in appetite, sometimes referred to as “exercise-induced anorexia”, have only been reported to occur following continuous exercise bouts exceeding $60\% \dot{V}O_{2max}$ (Bilski et al. 2009). In a study of similar design to the current research, Holmstrup et al. (2013) observed no difference in 12 h AUC hunger and satiety in male obese participants who completed hourly 5 min walking bouts compared with uninterrupted sitting and continuous exercise performed in the morning. Holmstrup et al. (2013) required participants to consume a 1046 kJ high carbohydrate meal every 2 h and reduced hunger and increased satiety were reported in discrete 2 h time periods later in the day. As the current study examined responses to a single meal it is difficult to make direct comparisons between these studies. Holmstrup et al. (2013) also did not assess subsequent energy intake so it is unknown whether any changes in appetite would result in altered energy intake following regular activity breaks in that study.

In unison with the finding that participants maintained their appetite across all conditions in the current study, absolute energy intake (*ad libitum* consumption of a pasta meal) also did not differ in the activity breaks conditions compared to uninterrupted sitting. The majority of studies that have predominantly utilised *ad libitum* buffet meals to examine post-exercise energy intake have reported that an acute exercise bout does not alter post-exercise energy intake (Martins et al. 2008). However, when completing research of this nature it is important to consider REI (energy

intake minus the net energy expenditure of activity) (Imbeault et al. 1997; King et al. 2010). Estimated REI was lower in both of the activity breaks conditions in the current study compared with uninterrupted sitting for the male and female participants combined. To emphasise the importance of this finding, the majority of participants had a reduced REI in the activity breaks conditions compared with uninterrupted sitting: 11 out of 13 in SIT+LA and 13 out of 13 in SIT+MA. When analysed separately by sex, moderate-intensity activity breaks significantly reduced REI compared with uninterrupted sitting in males and females. However, in males, moderate-intensity activity breaks also lowered REI more than light-intensity activity breaks, but this was not observed in females. This may highlight sex as a potential mediating factor in the response of REI to breaking up prolonged sitting with different intensity activity breaks. However, the number of males and females examined in this study is low and larger sample sizes with greater statistical power are required to further confirm this concept. Nevertheless, the current study's findings suggest that completing 2 min bouts of low- or moderate-intensity walking every 20 min over a 5 h period does not lead to a compensatory increase in energy intake to account for the elevation of energy expenditure induced by the activity bouts. This type of low demanding movement strategy should therefore be further explored to determine its potential role in body weight control.

The current study found no change in circulating acylated ghrelin or total PYY concentrations in response to regular activity breaks compared to uninterrupted sitting. The majority of research examining the acute response of acylated ghrelin and PYY to exercise has focused on moderate-high intensity continuous exercise ($\sim 60\text{-}70\% \dot{V}O_{2\max}$) (Stensel 2010). This work has widely reported acute exercise induced suppression of acylated ghrelin and elevation of PYY concentrations in lean healthy males. These responses are short-lived and generally last for no more than 1 h post-exercise (Stensel 2010). The exercise intensity and duration completed in the current study was lower and shorter than the studies noted above, which may explain the lack of influence on gut hormone concentrations. To the authors' knowledge, this is the first study to examine the response of circulating acylated ghrelin concentrations to regular breaks in sitting time. King et al. (2010)

reported no alteration in acylated ghrelin concentrations over 8 h following a 60 min treadmill walk compared to a resting control trial. The brisk treadmill walking used by King et al. (2010) was ~7 km/h (0% incline), which was a similar pace to the SIT+MA condition in the current study. Energy deficits caused by light- or moderate-intensity walking performed in frequent short bouts or in one continuous bout therefore do not result in compensatory increases in circulating acylated ghrelin.

In comparison to research investigating the response to a single moderate-intensity exercise bout eliciting similar exercise-induced energy expenditure values to the moderate-intensity activity breaks used in the current study (914 ± 226 kJ in SIT+MA), a decrease in acylated ghrelin and increase in circulating total PYY concentrations has been reported (Kawano et al. 2013). However, different modes of exercise (cycling and rope skipping) were used compared to walking in the current study, which may explain disparate findings and it is possible that a continuous bout of exercise is necessary to affect appetite hormones. Holmstrup et al. (2013) reported no difference in total PYY concentrations over 12 h when comparing hourly 5 min moderate-intensity walking bouts to a duration- and intensity-matched continuous exercise bout performed in the morning; total PYY concentrations in each of these conditions also did not differ compared with uninterrupted sitting. These findings suggest that hourly acylated ghrelin and total PYY concentrations are not sensitive to regular activity breaks comprising of low-moderate intensities and are in conjunction with the current findings that appetite and absolute energy intake did not differ in the activity breaks conditions compared to uninterrupted sitting.

The activity bouts in the current study led to an estimated total activity induced energy expenditure of 458 ± 96 kJ in SIT+LA and 914 ± 226 kJ in SIT+MA. Estimated energy expenditure loss per day at work has been 732 kJ (150 kcal) since 1960 and this accounts for a significant portion of the increase in body weight over the past five decades (Church et al. 2011). The reduction in occupational energy expenditure can be explained by increased amounts of seated technical work or desk-based office work with office workers spending up to 75% of their working hours seated (Thorpe et al. 2012). As more than 50% of this time is accumulated in prolonged periods of sustained sitting

(Thorpe et al. 2012), incorporating regular activity breaks into the working day, such as those used in the current study, could be an attractive strategy to increase daily energy expenditure and reduce periods of prolonged sitting. In respect of current experimental and observational evidence, this could potentially aid in body weight control and reduce chronic disease risk in the long term (Buckley et al. 2015; Eriksen et al. 2015), although intervention studies are required to establish this.

In this study, postprandial plasma glucose iAUC was lower in the moderate-intensity activity breaks condition compared with light-intensity activity breaks and uninterrupted sitting. Conversely, postprandial insulin iAUC did not differ between conditions. Acute suppressions in postprandial glucose and insulin concentrations have been reported in a number of studies where sitting time has been regularly interrupted with short activity breaks (Benatti and Ried-Larsen 2015). In overweight and obese older adults, light- and moderate-intensity activity breaks (2 min walking every 20 min) caused similar suppressions in postprandial glucose and insulin iAUC compared to uninterrupted sitting (Dunstan et al. 2012). In our laboratory, we also observed lower postprandial glucose concentrations when sitting time was interrupted with 2 min light-intensity walking breaks every 20 min in non-overweight participants (Bailey and Locke 2015). The reason light-intensity activity breaks did not suppress glucose concentrations in the present study is not clear. The low glucose levels observed in males during the uninterrupted sitting condition will have reduced the potential for the light-intensity activity breaks to suppress glucose. However, glucose iAUC between the uninterrupted sitting and light-intensity activity breaks conditions was similar in females. Further research is thus required to explore potential sex differences in the response of postprandial glucose to light-intensity activity breaks.

The main strength of this study is the crossover design and the measurement of an array of appetite-related variables. This is one of the first studies to examine appetite and gut hormone responses to regular activity used to break up prolonged sitting, which is now being widely prescribed (Buckley et al. 2015). A limitation of this study is that total PYY was measured rather than PYY₃₋₃₆, which is the form of PYY that is more potent in suppressing hunger (Chelikani et al. 2004).

However, total PYY and PYY₃₋₃₆ are highly correlated (Tsilchorozidou et al. 2008) and changes in total PYY are thus likely to reflect changes in PYY₃₋₃₆. Another potential limitation is that fasting plasma glucose concentrations differed at baseline between trials (higher in SIT+MA than SIT). However, this difference was minimal (4.30 ± 0.36 vs. 4.05 ± 0.34 mmol/L for SIT+MA and SIT, respectively) and we calculated positive incremental AUC for glucose and insulin concentrations and this method is free of influence from baseline values (Le Floch et al. 1990). Males and females were investigated in this study and some data suggests there are physiological differences between sexes in appetite, energy intake, and hormonal responses to short-term exercise (Hagobian et al. 2009). However, more recent evidence has reported no apparent sex differences in hormonal and appetite responses to exercise (Alajmi et al. 2015; Hagobian et al. 2013). Preliminary data from this study suggests that there may be sex differences in the REI and postprandial glucose response to breaking up prolonged sitting and additional studies with larger samples are thus required to further explore this concept. Previous studies have documented increased energy intake following cognitive effort with computer-based tasks (Chaput and Tremblay 2007). The participants in the current study were permitted to engage in a variety of seated activities during the trials and cognitive effort was thus not standardised, which may have had an impact on the energy intake responses observed. Lastly, this study compared the effects of breaking up prolonged sitting with regular activity over a period of 5 h following a single meal. Responses to this type of activity engagement following multiple meals across the course of the day thus cannot be inferred, nor can the long-term chronic responses to breaking up prolonged sitting.

In conclusion, appetite and gut hormone responses to a meal are not altered over a 5 h period when prolonged sitting is interrupted with frequent short bouts of light- or moderate-intensity activity. The increased energy expenditure from regular activity breaks could promote an energy deficit that is not compensated for in a subsequent meal. Future research should examine whether breaking up prolonged sitting will lead to repeated energy deficits over the long term and assist with weight management in obesity.

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Conflicts of interest: None

Draft

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Table 1: Total area under the curve values for appetite perceptions

| | Uninterrupted sitting | Sitting + activity breaks | |
|---------------------------|-----------------------|---------------------------|--------------------|
| | | Light-intensity | Moderate-intensity |
| Hunger (mm/6 h) | 349 ± 112 | 357 ± 92 | 353 ± 117 |
| Satisfaction (mm/6 h) | 166 ± 67 | 167 ± 83 | 193 ± 102 |
| Fullness (mm/6 h) | 171 ± 92 | 171 ± 92 | 189 ± 87 |
| PFC (mm/6 h) | 412 ± 74 | 405 ± 88 | 395 ± 89 |
| Overall appetite (mm/6 h) | 430 ± 76 | 431 ± 76 | 417 ± 90 |

Values are means ± SD. No significant between-trial differences were observed ($p > 0.05$). PFC, prospective food consumption.

Table 2: Area under the curve values for gut hormone, glucose, and insulin concentrations

| | Sitting + activity breaks | | |
|-----------------------------------|---------------------------|-----------------|--------------------|
| | Uninterrupted sitting | Light-intensity | Moderate-intensity |
| Acylated ghrelin tAUC (pg/mL/6 h) | 485 ± 302 | 503 ± 305 | 476 ± 296 |
| Peptide YY tAUC (pg/mL/6 h) | 959 ± 222 | 989 ± 257 | 920 ± 213 |
| Insulin tAUC (μU/mL/6 h) | 159 ± 69 | 162 ± 64 | 153 ± 78 |
| Glucose tAUC (mmol/L/6 h) | 23.3 ± 2.7 | 23.4 ± 2.8 | 23.5 ± 2.3 |
| Insulin iAUC (μU/mL/6 h) | 115 ± 69 | 95 ± 63 | 87 ± 84 |
| Glucose iAUC (mmol/L/6 h) | 2.9 ± 1.7 | 3.5 ± 2.2 | 1.5 ± 1.5* |

Values are means ± SD. *significant difference between light-intensity and moderate-intensity activity breaks conditions ($p=0.004$). tAUC, total area under the curve; iAUC, positive incremental area under the curve

Fig. 1. Changes in overall appetite during uninterrupted sitting (SIT), sitting + light-intensity activity breaks (SIT+LA), and sitting + moderate-intensity activity breaks (SIT+MA). Values are means \pm SE; $n = 13$. Solid arrow indicates standardised test drink, broken arrow indicates *ad libitum* pasta meal.

Fig. 2. Relative energy intake in the uninterrupted sitting (SIT), sitting + light-intensity activity breaks (SIT+LA), and sitting + moderate-intensity activity breaks (SIT+MA) conditions. Values are means \pm SE; $n = 13$. *significantly higher than SIT+LA ($p=0.011$) and SIT+MA ($p<0.001$).

Fig. 3. Changes in plasma concentrations of (A) acylated ghrelin, (B) peptide YY, (C) glucose, and (D) insulin during uninterrupted sitting (SIT), sitting + light-intensity activity breaks (SIT+LA), and sitting + moderate-intensity activity breaks (SIT+MA). Values are mean \pm SE; $n = 13$. Some error bars have been omitted for clarity. Solid arrow indicates standardised test drink, broken arrow indicates *ad libitum* pasta meal.

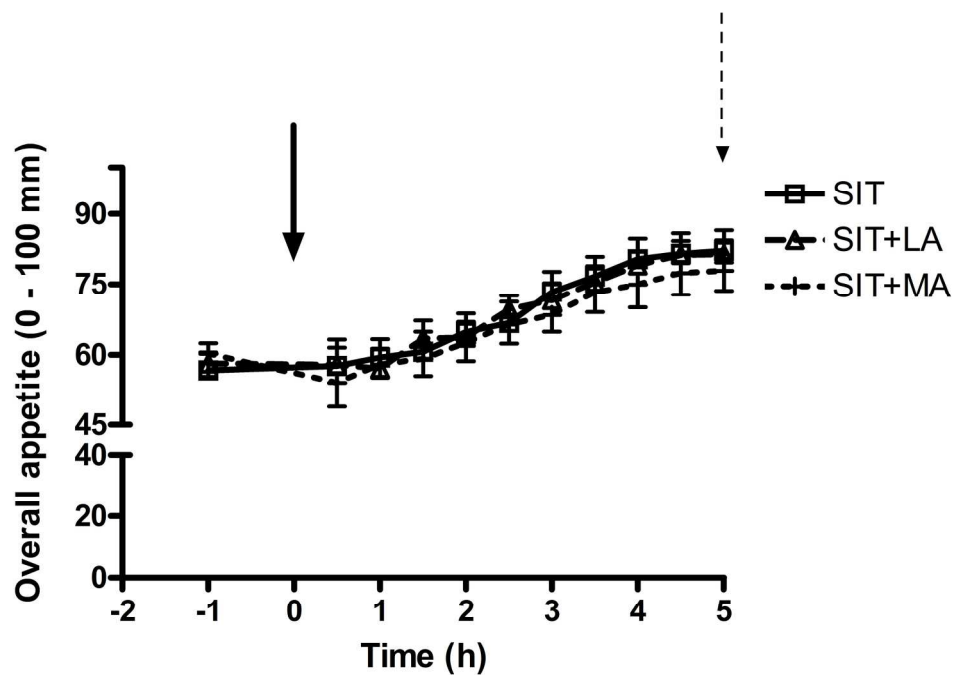


Fig. 1. Changes in overall appetite during uninterrupted sitting (SIT), sitting + light-intensity activity breaks (SIT+LA), and sitting + moderate-intensity activity breaks (SIT+MA). Values are means \pm SE; $n = 13$. Solid arrow indicates standardised test drink, broken arrow indicates ad libitum pasta meal.
91x65mm (600 x 600 DPI)

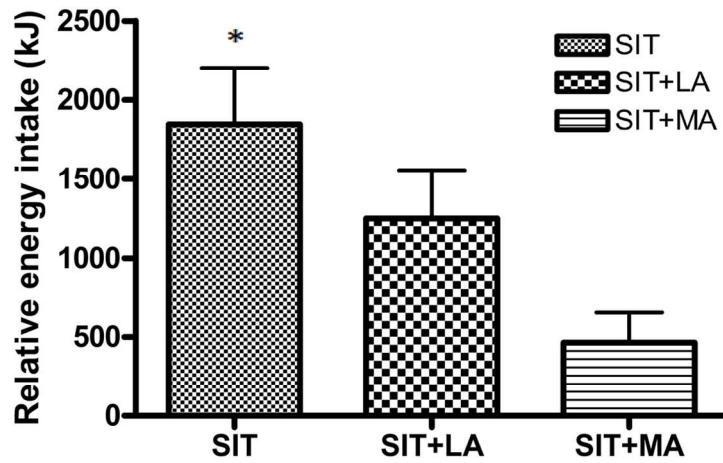


Fig. 2. Relative energy intake in the uninterrupted sitting (SIT), sitting + light-intensity activity breaks (SIT+LA), and sitting + moderate-intensity activity breaks (SIT+MA) conditions. Values are means \pm SE; $n = 13$. *significantly higher than SIT+LA ($p=0.011$) and SIT+MA ($p<0.001$).
67x35mm (600 x 600 DPI)

