

Volume 41

An NRC Research
Press Journal

Une revue de
NRC Research
Press

2016

www.nrcresearchpress.com

Applied Physiology, Nutrition, and Metabolism

Physiologie appliquée, nutrition et métabolisme

The Canadian Society for Exercise Physiology and the Canadian Nutrition Society have chosen *Applied Physiology, Nutrition, and Metabolism* as their principal medium for the publication of research papers



La Société canadienne de physiologie de l'exercice et la Société canadienne de nutrition ont choisi *Physiologie appliquée, nutrition et métabolisme* comme leur principal organe de publication d'articles de recherche



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

Daniel P. Bailey, David R. Broom, Bryna C.R. Christmas, Lee Taylor, Edward Flynn, and John Hough

Abstract: 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. Thirteen sedentary (7 female) participants undertook three 5-h trials in random order: (i) uninterrupted sitting (SIT), (ii) seated with 2-min bouts of light-intensity walking every 20 min (SIT + LA), and (iii) seated with 2-min bouts of moderate-intensity walking every 20 min (SIT + MA). A standardised test drink was provided at the start of each trial 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. AUC values for appetite and gut hormone concentrations were unaffected in the activity breaks conditions compared with uninterrupted sitting (linear mixed modelling: $p > 0.05$). Glucose AUC was lower in SIT + MA than in 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 in SIT. In 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.

Key words: sedentary behaviour, activity breaks, prolonged sitting, appetite-regulating hormones, acylated ghrelin, energy intake.

Résumé : L'interruption périodique de la position assise prolongée peut contrer les effets néfastes sur le métabolisme, mais on ne connaît pas les effets sur les hormones de l'appétit et de l'intestin, d'où le but de cette étude. Treize personnes sédentaires dont 7 femmes participent selon un ordre aléatoire à 3 essais d'une durée de 5 h chacun : (i) assis sans interruption (« SIT »), (ii) assis + 2 min de marche de faible intensité toutes les 20 min (« SIT + LA ») et (3) assis + 2 min de marche d'intensité modérée toutes les 20 min (« SIT + MA »). Au début de chaque essai, les participants consomment une boisson-test et, à la fin de chaque essai, ils consomment un repas de pâtes ad libitum. À intervalles réguliers, on évalue la sensation de faim et les concentrations plasmatiques de ghréline acylée, de peptide YY, d'insuline et de glucose. On mesure la surface sous la courbe (« AUC ») de chaque variable. Les valeurs de l'AUC de l'appétit et de la concentration des hormones intestinales ne varient pas lors des pauses actives comparativement à la condition assise sans interruption (modèle linéaire mixte, $p > 0,05$). L'AUC du glucose est plus petite dans la condition SIT + MA comparativement aux conditions SIT + LA ($p = 0,004$) et SIT ($p = 0,055$). D'une condition à l'autre, on ne note pas de différences de quantité absolue d'énergie consommée ad libitum ($p > 0,05$), mais on note une plus faible quantité relative d'énergie consommée dans les conditions SIT + LA (39 %; $p = 0,011$) et SIT + MA (120 %; $p < 0,001$) comparativement à la condition SIT. En conclusion, l'interruption par intervalles de la condition assise prolongée ne modifie pas en 5 heures l'appétit et la réponse des hormones intestinales à un mets. L'augmentation de la dépense d'énergie durant les pauses actives pourrait favoriser un déficit énergétique qui n'est pas compensé lors d'un repas subséquent. [Traduit par la Rédaction]

Mots-clés : comportement sédentaire, pauses actives, position assise prolongée, hormones régulant l'appétit, ghréline acylée, apport énergétique.

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%–81% maximal oxygen uptake ($\dot{V}O_{2max}$) for 30–120 min) has a trivial effect on absolute energy intake but does create a large energy deficit in the immediate hours postexercise (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 reg-

Received 3 September 2015. Accepted 9 December 2015.

D.P. Bailey, B.C.R. Christmas,* L. Taylor,† E. Flynn,‡ and J. Hough. Institute for Sport and Physical Activity Research, Department of Sport Science and Physical Activity, University of Bedfordshire, Polhill Avenue, Bedford, MK41 9EA, UK.

D.R. Broom. Academy of Sport and Physical Activity, Sheffield Hallam University, Sheffield, S10 2BP, UK.

Corresponding author: Daniel Bailey (email: daniel.bailey@beds.ac.uk).

*Present address: Sport Science Program, College of Arts and Sciences, Qatar University, Doha, Qatar.

†Present address: ASPETAR, Qatar Orthopedic and Sports Medicine Hospital, Athlete Health and Performance Research Centre, Aspire Zone, Doha, Qatar.

‡Present address: Elucigene Diagnostics, Citylabs, Nelson Street, Manchester, M13 9NQ, UK.

ularly 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 nonoverweight individuals (Bailey and Locke 2015; Dunstan et al. 2012). Such findings has led to the publication of an Expert Statement, which provides guidelines on avoiding prolonged periods of sedentary work and recommends initially accumulating at least 2 h of standing and light activity (light walking) per working day (Buckley et al. 2015). To our knowledge, only 1 study has examined appetite and appetite-regulating hormone responses to breaking up prolonged sitting, reporting that hourly 5-min bouts of moderate-intensity walking led to suppressions in appetite compared with 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 1 study, which did not observe any change in total PYY in individuals performing hourly 5-min moderate-intensity walking 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.

Materials and methods

Participants and enrolment process

This randomised 3-way crossover study was approved by the University of Bedfordshire's Ethics Review Committee and was in accordance with the principles set out in the Declaration of Hel-

sinki. Thirteen sedentary (7 females), inactive but otherwise healthy participants (mean \pm SD; age, 26.6 \pm 8.5 years; body fat, 24.4% \pm 8.2%) gave written informed consent to participate in the study following a verbal and written explanation of the nature and risks involved. Exclusion criteria included any known blood borne disease, pregnancy, clinically diagnosed diabetes, taking glucose-lowering and/or lipid-lowering medication, employment in a non-sedentary occupation, currently watching <2 h of television/day, regularly engaged in moderate-intensity physical activity (150 min/week) for at least 3 months, and known physical activity contraindications, major illness/injury, or other health issues that may limit the ability to perform the necessary activity bouts.

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

Study protocol

Each trial was separated by ≥ 7 days. In line with previous research, participants were asked to refrain from exercise, alcohol, and caffeine in the 48 h before each main trial (Dunstan et al. 2012). During this 48-h time-period, physical activity levels were objectively measured with an Actigraph GT3X accelerometer (Actigraph, Pensacola, Fla., USA) worn around the hip during waking hours. Participants were required to have a daily wear time of ≥ 10 h and data were recorded in 5-s epochs with previously validated accelerometer counts applied (Freedson et al. 1998). Physical activity levels did not differ significantly between trials ($p > 0.05$; data not shown).

Participants reported to the laboratory in the morning (~0830 hours) following an overnight fast and were asked to minimise their activity prior to attending, e.g., travel by car. Each participant was required to arrive at the same time of day for each of their trials. Participants weighed (Salter Disc Electronic Kitchen Scale, Ho-Medics Group Ltd, UK) and recorded food intake for 24 h before the first main trial and were asked to replicate the quantity and timings of eating prior to each subsequent testing day (Bailey et al. 2015; Deighton et al. 2013). After fasting blood collection (~1 h), participants remained seated for 1 h before consuming a standardised test drink (consumed within 5 min). The drink contained 75 g carbohydrate (100% dextrose monohydrate powder; Thornton & Ross Ltd, UK) in 100 mL water combined with 50 g fat (Calogen; Nutricia, UK). The specific nutritional components were energy, 3211 kJ; fat, 50.0 g; saturated fat, 5.3 g; carbohydrate, 79.3 g; sugars, 4.0 g; protein, nil; fibre, nil; and sodium, 7.0 mg. Following consumption, participants were guided through the 5-h trial and supervised at all times by a member of the research team to ensure full compliance with the protocols. Hourly blood samples were taken prior to the activity bouts (trial conditions 2 and 3 below).

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

1. *Uninterrupted sitting* (SIT): participants remained seated throughout the experimental period and were instructed to minimise excessive movement.
2. *Sitting + light-intensity activity breaks* (SIT + LA): participants rose from the seated position every 20 min and completed 2-min

bouts of light-intensity walking on a motorised treadmill with a level surface at 3.2 km/h, providing a total of 28 min of activity. They then returned to the seated position.

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

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

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

Ratings of perceived appetite

During each trial subjective feelings 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?") were reported on paper using a validated 100-mm Visual Analogue Scale (Flint et al. 2000). Appetite perceptions were measured fasted (-1 h) and every 30 min during the 5-h trial period. An overall appetite rating was calculated as the mean value of the 4 appetite perceptions after inverting the values for satisfaction and fullness (Stubbs et al. 2000).

Blood sampling

During each main trial, venous blood was collected via a cannula (Vasofix, B. Braun Medical Ltd, Sheffield, UK), which was inserted into an antecubital vein. A fasting sample was taken upon arrival at the laboratory (-1 h) and then every hour after commencement of each trial while seated. Samples were collected into two 4.9-mL EDTA vacuettes (VACUETTE, Greiner Bio-One, Austria). One vacuette was immediately centrifuged at 1500g for 10 min at a temperature of 4 °C (Heraeus Multifuge X3R, Thermo Scientific, Loughborough, UK). The plasma supernatant was then dispensed into separate 2-mL cryovials, flash frozen in liquid nitrogen, and stored at -80 °C until later analysis of insulin and total PYY concentrations. From each sample, 50- μ L blood samples were collected into a microvette and analysed immediately to determine glucose concentrations using the YSI 2300 STAT plus glucose and lactate analyser (YSI Inc., Yellow Springs, Ohio, USA). The YSI uses a steady-state measurement methodology, where membrane-based glucose oxidase catalyses the oxidation of glucose to gluconic acid and hydrogen peroxide. The difference between the sample-generated plateau current and the initial baseline current

is proportional to the glucose concentration. The YSI was calibrated at the start of every day and every 45 min thereafter.

To prevent the degradation of acylated ghrelin, a 50- μ L solution containing potassium phosphate buffer, *p*-hydroxymercuribenzoic acid, and sodium hydroxide was added to one 4.9-mL EDTA vacuette, which was then centrifuged at 1500g for 10 min at 4 °C. The plasma supernatant was then dispensed into a storage tube and 100 μ L of 1 mol/L hydrochloric acid was added per millilitre of plasma to preserve acylated ghrelin (Hosoda et al. 2004). Thereafter, samples were spun at 1500g for 5 min at 4 °C prior to being flash frozen in liquid nitrogen and stored in 2-mL cryovials at -80 °C until later analysis.

Blood biochemistry

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

Statistical analysis

Analyses were completed using the statistical software package IBM SPSS Statistics version 21.0 (SPSS Inc., Armonk, N.Y., USA) and GraphPad Prism version 4.03 (GraphPad Software Inc., La Jolla, Calif., USA). Prior to any inferential statistical analyses, descriptive statistics tables were generated to check the central tendency (mean, median), and dispersion (standard deviation, minimum, maximum) of the data. Second, quantile-quantile (Q-Q) plots were used to check the normality assumption of the results obtained for each of the conditions across all time points. Standard graphical methods were preferred over null hypothesis significance testing to check statistical assumptions (Grafen and Hails 2002). The 2-tailed α level for significance testing was set as $p < 0.05$.

Linear mixed models were chosen to determine if there were any differences in the dependent variables between the conditions across time. This type of analysis was preferred as it can accurately model different covariate structures for repeated-measures data and model between-subject variability (Vandenbogaerde and Hopkins 2010; West et al. 2006). Total area under the curve (tAUC) was calculated for all blood metabolite and appetite variables using the trapezoidal method for the total trial period. Positive incremental AUC (iAUC) was also calculated for glucose and insulin based on suggestions that this method for estimating AUC more accurately describes glycaemic responses to food (Le Floch et al. 1990) and to permit comparisons with previous research (Dunstan et al. 2012). Fixed and random factors for the linear mixed model were fit for each AUC variable. The main effect for condition was analysed by plotting the mean values. Although the number of males ($n = 6$) and females ($n = 7$) studied was low, the main effect of sex and condition \times sex interaction effects were tested to generate preliminary data on potential sex differences in the response to breaking up prolonged sitting. Data for condition \times sex interaction effects are presented only where significant findings have been observed. No significant main effects for sex were observed for any variable and this data are thus not presented; all $p > 0.234$. Step-down Hommel (1988) adjusted post-hoc pair-wise comparisons were calculated if a significant main and/or interaction effect was present. The most appropriate model was chosen using the smallest Hurvich and Tsai's criterion (AICC) in accordance with the principal of parsimony. Normality and homogeneity of variance of the residuals for all variables were checked using Q-Q plots and scatter plots, respectively. Acylated ghrelin and insulin tAUC in addition to absolute energy intake and relative energy intake were non-normally distributed and log-transformed prior to analysis. Back transformation of these variables to natural

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

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

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

units was applied to allow for meaningful presentation in text and tables. Data are presented as means ± SD in the text and means ± SE in the figures.

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

Results

Appetite perceptions

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

Absolute energy intake

Energy intake during the ad libitum pasta meal was 1846 ± 1239 kJ in SIT, 1670 ± 1038 kJ in SIT + LA, and 1360 ± 510 kJ in SIT + MA. These values did not differ significantly between trials ($p = 0.422$).

Estimated relative energy intake (REI)

A significant main effect of condition for REI (energy intake during experimental trials minus the net energy expenditure of the activity bouts) was observed ($p < 0.001$). As shown in Fig. 2, REI was significantly lower in both the SIT + LA and SIT + MA trials (by 39% $p = 0.011$ and 120% $p < 0.001$, respectively) than in SIT (SIT = 1846 ± 1239 kJ; SIT + LA = 1247 ± 1063 kJ; SIT + MA = 464 ± 632 kJ). REI between SIT + LA and SIT + MA did not differ significantly ($p = 0.138$). Closer inspection of the data revealed that 11 out of the 13 participants had a lower REI in SIT + LA than in SIT and all 13 participants had a lower REI in SIT + MA than in SIT. There was a significant condition × sex interaction effect ($p = 0.047$) with males having a significantly reduced REI in the SIT + MA ($p < 0.001$) trial compared with SIT and significantly reduced REI in SIT + MA than SIT + LA ($p = 0.029$). The lower REI in SIT + LA than SIT in males was approaching significance ($p = 0.112$). Mean REI for males was 2336 ± 1796 kJ in SIT, 1503 ± 1377 kJ in SIT + LA, and 75 ± 519 kJ in SIT + MA. In females, REI was significantly lower in SIT + MA than SIT ($p = 0.001$) and the lower REI in SIT + LA than SIT in females was approaching significance ($p = 0.101$). Mean REI for females was 1495 ± 569 kJ in SIT, 1067 ± 846 kJ in SIT + LA, and 687 ± 611 kJ in SIT + MA.

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 ± SE; $n = 13$. Solid arrow indicates standardised test drink; broken arrow indicates ad libitum pasta meal.

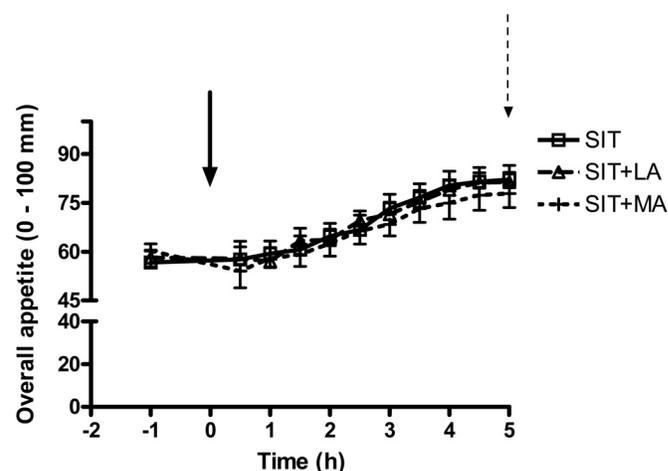
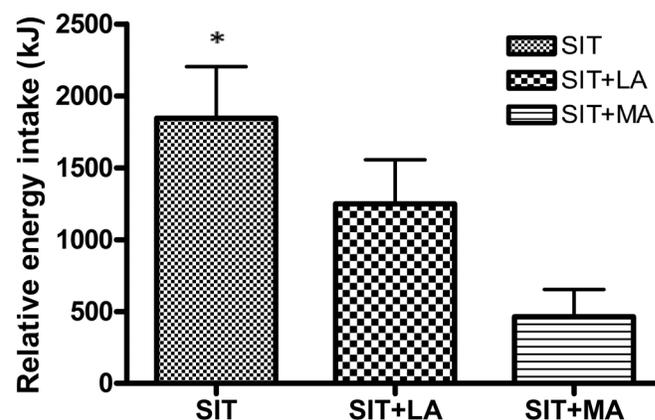


Fig. 2. Relative energy intake in the uninterrupted sitting (SIT), sitting + light-intensity activity breaks (SIT + LA), and sitting + moderate-intensity activity breaks (SIT + MA) conditions. Values are means ± SE; $n = 13$. *, Significantly higher than SIT + LA ($p = 0.011$) and SIT + MA ($p < 0.001$).



Gut hormone concentrations

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

Glucose and insulin concentrations

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

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

	Uninterrupted sitting	Sitting+activity breaks	
		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*

Note: Values are means ± SD. iAUC, positive incremental area under the curve; tAUC, total area under the curve.

*Significant difference between light-intensity and moderate-intensity activity breaks conditions ($p = 0.004$).

lower in SIT + MA than SIT and this was approaching significance ($p = 0.055$). The condition × sex interaction effect for glucose iAUC was approaching significance ($p = 0.113$) with post hoc comparisons revealing lower glucose iAUC in males (1.9 ± 1.6 mmol·L/6 h) compared with females during SIT (3.8 ± 1.3 mmol·L/6 h; $p = 0.048$). Furthermore, glucose iAUC was lower in SIT + MA (1.1 ± 1.6 mmol·L/6 h) than SIT + LA (3.7 ± 2.2 mmol·L/6 h; $p = 0.018$) in males and lower in SIT + MA (1.9 ± 1.4 mmol·L/6 h) than SIT (3.8 ± 1.3 mmol·L/6 h; $p = 0.009$) in females. Insulin iAUC did not differ significantly between trials ($p = 0.308$) and there was also no significant difference in glucose tAUC ($p = 0.993$) or insulin tAUC ($p = 0.638$) between trials. The analysis of serial measurements demonstrated no significant differences between trials for glucose or insulin (both $p > 0.866$). There were also no significant condition × time interaction effects observed for either of these variables (both $p > 0.804$). Plasma glucose and insulin concentrations over time for each trial are shown in Fig. 3.

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.

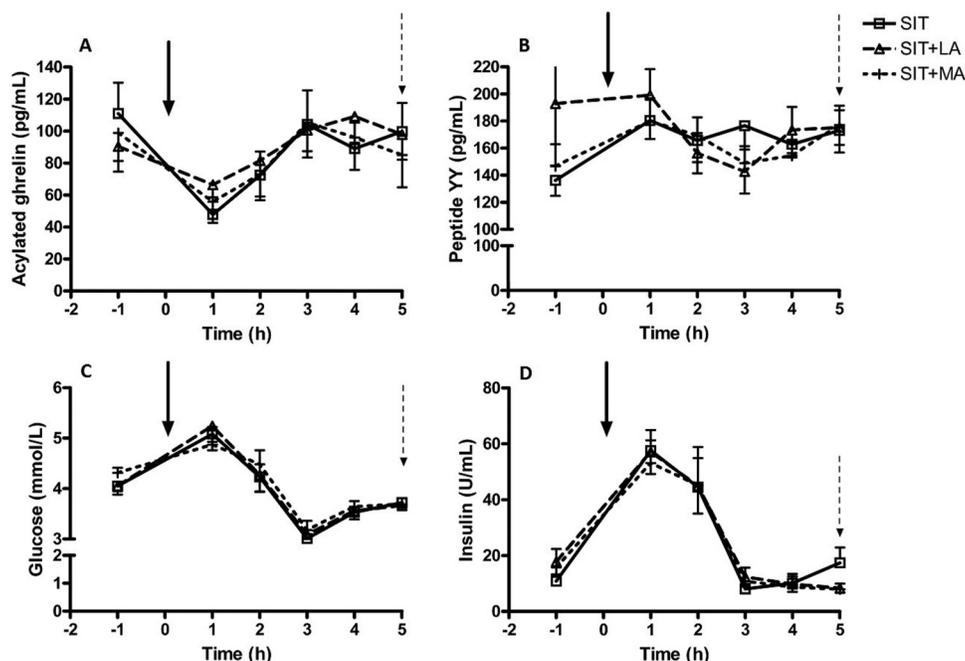
In unison with the finding that participants maintained their appetite across all conditions in the current study, absolute energy intake (ad libitum consumption of a pasta meal) also did not

differ in the activity breaks conditions compared with uninterrupted sitting. The majority of studies that have predominantly utilised ad libitum buffet meals to examine postexercise energy intake have reported that an acute exercise bout does not alter postexercise 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 with 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 ($\sim 60\%–70\% \dot{V}O_{2max}$) (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 postexercise (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 with 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 1 continuous bout therefore do not result in compensatory increases in circulating acylated ghrelin.

In comparison with 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 with 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

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) conditions. Values are means \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.



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 with 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 5 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 (Thorp et al. 2012). As more than 50% of this time is accumulated in prolonged periods of sustained sitting (Thorp 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 with 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

nonoverweight 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 PYY_{3–36}, which is the form of PYY that is more potent in suppressing hunger (Chelikani et al. 2004). However, total PYY and PYY_{3–36} are highly correlated (Tsilchorozidou et al. 2008) and changes in total PYY are thus likely to reflect changes in PYY_{3–36}. 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 iAUC 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. Finally, 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.

Conflict of interest statement

The authors declare no conflicts of interest.

Acknowledgements

This study was funded by the University of Bedfordshire Research Investment Programme.

References

- ACSM. 2014. ACSM's Guidelines for Exercise Testing and Prescription. 7th ed. Lippincott Williams & Wilkins, Pa., USA.
- Alajmi, N., Deighton, K., King, J., Reischak-Oliveira, A., Wasse, L., Jones, J., et al. 2015. Appetite and energy intake responses to acute energy deficits in females versus males. *Med. Sci. Sports Exerc.* [Epub ahead of print.] doi:10.1249/MSS.0000000000000793.
- Bailey, D.P., and Locke, C.D. 2015. Breaking up prolonged sitting with light-intensity walking improves postprandial glycemia, but breaking up sitting with standing does not. *J. Sci. Med. Sport.* 18(3): 294–298. doi:10.1016/j.jsams.2014.03.008. PMID:24704421.
- Bailey, D.P., Smith, L.R., Christmas, B.C., Taylor, L., Stensel, D.J., Deighton, K., et al. 2015. Appetite and gut hormone responses to moderate-intensity continuous exercise versus high-intensity interval exercise, in normoxic and hypoxic conditions. *Appetite*, 89: 237–245. doi:10.1016/j.appet.2015.02.019. PMID:25700630.
- Benatti, F., and Ried-Larsen, M. 2015. The effects of breaking up prolonged sitting time: a review of experimental studies. *Med. Sci. Sports Exerc.* 47(10): 2053–2061. doi:10.1249/MSS.0000000000000654. PMID:26378942.
- Bilski, J., Teległów, A., Zahradnik-Bilska, J., Dembiński, A., and Warzecha, Z. 2009. Effects of exercise on appetite and food intake regulation. *Medicina Sportiva*, 13(2): 82–94. doi:10.2478/v10036-009-0014-5.
- Biswas, A., Oh, P.I., Faulkner, G.E., Bajaj, R.R., Silver, M.A., Mitchell, M.S., et al. 2015. Sedentary time and its association with risk for disease incidence, mortality, and hospitalization in adults: a systematic review and meta-analysis. *Ann. Intern. Med.* 162(2): 123–132. doi:10.7326/M14-1651. PMID:25599350.
- Borg, G.A. 1982. Psychophysical bases of perceived exertion. *Med. Sci. Sports Exerc.* 14(5): 377–381. PMID:7154893.
- Broom, D.R., Batterham, R.L., King, J.A., and Stensel, D.J. 2009. Influence of resistance and aerobic exercise on hunger, circulating levels of acylated ghrelin, and peptide YY in healthy males. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296(1): R29–R35. PMID:18987287.
- Buckley, J.P., Hedge, A., Yates, T., Copeland, R.J., Loosemore, M., Hamer, M., et al. 2015. The sedentary office: a growing case for change towards better health and productivity. Expert statement commissioned by Public Health England and the Active Working Community Interest Company. *Br. J. Sports Med.* 49(21): 1357–1362. doi:10.1136/bjsports-2015-094618. PMID:26034192.
- Chaput, J.P., and Tremblay, A. 2007. Acute effects of knowledge-based work on feeding behavior and energy intake. *Physiol. Behav.* 90(1): 66–72. doi:10.1016/j.physbeh.2006.08.030. PMID:17023010.
- Chelikani, P.K., Haver, A.C., and Reidelberger, R.D. 2004. Comparison of the inhibitory effects of PYY(3-36) and PYY(1-36) on gastric emptying in rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 287(5): R1064–R1070. doi:10.1152/ajpregu.00376.2004. PMID:15242829.
- Church, T.S., Thomas, D.M., Tudor-Locke, C., Katzmarzyk, P.T., Earnest, C.P., Rodarte, R.Q., et al. 2011. Trends over 5 decades in U.S. occupation-related physical activity and their associations with obesity. *PLoS One*, 6(5): e19657. doi:10.1371/journal.pone.0019657. PMID:21647427.
- Deighton, K., Barry, R., Connon, C.E., and Stensel, D.J. 2013. Appetite, gut hormone and energy intake responses to low volume sprint interval and traditional endurance exercise. *Eur. J. Appl. Physiol.* 113(5): 1147–1156. doi:10.1007/s00421-012-2535-1. PMID:23111564.
- Delhanty, P.J., Negggers, S.J., and van der Lely, A.J. 2012. Mechanisms in endocrinology: Ghrelin: the differences between acyl- and des-acyl ghrelin. *Eur. J. Endocrinol.* 167(5): 601–608. doi:10.1530/EJE-12-0456. PMID:22898499.
- Dempsey, P.C., Owen, N., Biddle, S.J., and Dunstan, D.W. 2014. Managing sedentary behavior to reduce the risk of diabetes and cardiovascular disease. *Curr. Diab. Rep.* 14(9): 522. doi:10.1007/s11892-014-0522-0. PMID:25052856.
- Dunstan, D.W., Kingwell, B.A., Larsen, R., Healy, G.N., Cerin, E., Hamilton, M.T., et al. 2012. Breaking up prolonged sitting reduces postprandial glucose and insulin responses. *Diabetes Care*, 35(5): 976–983. doi:10.2337/dc11-1931. PMID:22374636.
- Eriksen, D., Rosthøj, S., Burr, H., and Holtermann, A. 2015. Sedentary work-associations between five-year changes in occupational sitting time and body mass index. *Prev. Med.* 73: 1–5. doi:10.1016/j.ypmed.2014.12.038. PMID:25575798.
- Faul, F., Erdfelder, E., Lang, A.G., and Buchner, A. 2007. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods*, 39(2): 175–191. doi:10.3758/BF03193146. PMID:17695343.
- Flint, A., Raben, A., Blundell, J.E., and Astrup, A. 2000. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int. J. Obes. Relat. Metab. Disord.* 24(1): 38–48. doi:10.1038/sj.ijo.0801083. PMID:10702749.
- Freedson, P.S., Melanson, E., and Sirard, J. 1998. Calibration of the Computer Science and Applications, Inc. accelerometer. *Med. Sci. Sports Exerc.* 30(5): 777–781. doi:10.1097/00005768-199805000-00021. PMID:9588623.
- Grafen, G., and Hails, R. 2002. Modern statistics for the life sciences. Oxford University Press, New York, N.Y., USA.
- Hagobian, T.A., Sharoff, C.G., Stephens, B.R., Wade, G.N., Silva, J.E., Chipkin, S.R., et al. 2009. Effects of exercise on energy-regulating hormones and appetite in men and women. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296(2): R233–R242. PMID:19073905.
- Hagobian, T.A., Yamashiro, M., Hinkel-Lipsker, J., Stredler, K., Evero, N., and Hackney, T. 2013. Effects of acute exercise on appetite hormones and ad libitum energy intake in men and women. *Appl. Physiol. Nutr. Metab.* 38(1): 66–72. doi:10.1139/apnm-2012-0104. PMID:23368830.
- Hamilton, M.T., Hamilton, D.G., and Zderic, T.W. 2007. Role of low energy expenditure and sitting in obesity, metabolic syndrome, type 2 diabetes, and cardiovascular disease. *Diabetes*, 56(11): 2655–2667. doi:10.2337/db07-0882. PMID:17827399.
- Holmstrup, M.E., Fairchild, T.J., Keslacy, S., Weinstock, R.S., and Kanaley, J.A. 2013. Satiety, but not total PYY, is increased with continuous and intermittent exercise. *Obesity (Silver Spring)*, 21(10): 2014–2020. doi:10.1002/oby.20335. PMID:23418154.
- Hommel, G. 1988. A stagewise rejective multiple test procedure based on a modified Bonferroni test. *Biometrika*, 75(2): 383–386. doi:10.1093/biomet/75.2.383.
- Hosoda, H., Doi, K., Nagaya, N., Okumura, H., Nakagawa, E., Enomoto, M., et al. 2004. Optimum collection and storage conditions for ghrelin measurements: octanoyl modification of ghrelin is rapidly hydrolyzed to desacyl ghrelin in blood samples. *Clin. Chem.* 50(6): 1077–1080. doi:10.1373/clinchem.2003.025841. PMID:15161728.
- Hussain, S.S., and Bloom, S.R. 2013. The regulation of food intake by the gut-brain axis: implications for obesity. *Int. J. Obes. (Lond.)*, 37(5): 625–633. doi:10.1038/ijo.2012.93. PMID:22710925.
- Imbeault, P., Saint-Pierre, S., Alméras, N., and Tremblay, A. 1997. Acute effects of exercise on energy intake and feeding behaviour. *Br. J. Nutr.* 77(4): 511–521. doi:10.1079/BJN19970053. PMID:9155502.
- Kawano, H., Mineta, M., Asaka, M., Miyashita, M., Numao, S., Gando, Y., et al. 2013. Effects of different modes of exercise on appetite and appetite-regulating hormones. *Appetite*, 66: 26–33. doi:10.1016/j.appet.2013.01.017. PMID:23402716.
- King, J.A., Wasse, L.K., Broom, D.R., and Stensel, D.J. 2010. Influence of brisk walking on appetite, energy intake, and plasma acylated ghrelin. *Med. Sci. Sports Exerc.* 42(3): 485–492. doi:10.1249/MSS.0b013e3181ba10c4. PMID:19952806.
- King, J.A., Wasse, L.K., Stensel, D.J., and Nimmo, M.A. 2013. Exercise and ghrelin. A narrative overview of research. *Appetite*, 68: 83–91. doi:10.1016/j.appet.2013.04.018. PMID:23624293.
- Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., and Kangawa, K. 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature*, 402(6762): 656–660. doi:10.1038/45230. PMID:10604470.
- Le Floch, J.P., Escuyer, P., Baudin, E., Baudon, D., and Perlemuter, L. 1990. Blood glucose area under the curve: Methodological aspects. *Diabetes Care*, 13(2): 172–175. doi:10.2337/diacare.13.2.172. PMID:2351014.
- le Roux, C.W., Batterham, R.L., Aylwin, S.J., Patterson, M., Borg, C.M., Wynne, K.J., et al. 2006. Attenuated peptide YY release in obese subjects is associated with reduced satiety. *Endocrinology*, 147(1): 3–8. doi:10.1210/en.2005-0972. PMID:16166213.
- Martins, C., Robertson, M.D., and Morgan, L.M. 2008. Effects of exercise and restrained eating behaviour on appetite control. *Proc. Nutr. Soc.* 67(1): 28–41. doi:10.1017/S0029665108005995. PMID:18234129.
- Neary, M.T., and Batterham, R.L. 2009. Gut hormones: implications for the treatment of obesity. *Pharmacol. Ther.* 124(1): 44–56. doi:10.1016/j.pharmthera.2009.06.005. PMID:19560488.
- Schubert, M.M., Desbrow, B., Sabapathy, S., and Leveritt, M. 2013. Acute exercise

- and subsequent energy intake. A meta-analysis. *Appetite*, **63**: 92–104. doi:10.1016/j.appet.2012.12.010. PMID:23274127.
- Schubert, M.M., Sabapathy, S., Leveritt, M., and Desbrow, B. 2014. Acute exercise and hormones related to appetite regulation: a meta-analysis. *Sports Med.* **44**(3): 387–403. doi:10.1007/s40279-013-0120-3. PMID:24174308.
- Stensel, D. 2010. Exercise, appetite and appetite-regulating hormones: implications for food intake and weight control. *Ann. Nutr. Metab.* **57**(Suppl 2): 36–42. doi:10.1159/000322702. PMID:21346335.
- Stubbs, R.J., Hughes, D.A., Johnstone, A.M., Rowley, E., Reid, C., Elia, M., et al. 2000. The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. *Br. J. Nutr.* **84**(4): 405–415. doi:10.1017/S0007114500001719. PMID:11103211.
- Stubbs, R.J., Hughes, D.A., Johnstone, A.M., Horgan, G.W., King, N., and Blundell, J.E. 2004. A decrease in physical activity affects appetite, energy, and nutrient balance in lean men feeding ad libitum. *Am. J. Clin. Nutr.* **79**(1): 62–69. PMID:14684398.
- Thorp, A.A., Healy, G.N., Winkler, E., Clark, B.K., Gardiner, P.A., Owen, N., and Dunstan, D.W. 2012. Prolonged sedentary time and physical activity in workplace and non-work contexts: a cross-sectional study of office, customer service and call centre employees. *Int. J. Behav. Nutr. Phys. Act.* **9**: 128. doi:10.1186/1479-5868-9-128. PMID:23101767.
- Tsilchorozidou, T., Batterham, R.L., and Conway, G.S. 2008. Metformin increases fasting plasma peptide tyrosine tyrosine (PYY) in women with polycystic ovarian syndrome (PCOS). *Clin. Endocrinol. (Oxf.)*, **69**(6): 936–942. doi:10.1111/j.1365-2265.2008.03285.x. PMID:18435831.
- Ueda, S.Y., Yoshikawa, T., Katsura, Y., Usui, T., and Fujimoto, S. 2009a. Comparable effects of moderate intensity exercise on changes in anorectic gut hormone levels and energy intake to high intensity exercise. *J. Endocrinol.* **203**(3): 357–364. doi:10.1677/JOE-09-0190. PMID:19737911.
- Ueda, S.Y., Yoshikawa, T., Katsura, Y., Usui, T., Nakao, H., and Fujimoto, S. 2009b. Changes in gut hormone levels and negative energy balance during aerobic exercise in obese young males. *J. Endocrinol.* **201**(1): 151–159. doi:10.1677/JOE-08-0500. PMID:19158129.
- Vandenbogaerde, T.J., and Hopkins, W.G. 2010. Monitoring acute effects on athletic performance with mixed linear modeling. *Med. Sci. Sports Exerc.* **42**(7): 1339–1344. doi:10.1249/MSS.0b013e3181cf7f3f. PMID:20068494.
- West, B.T., Welch, K.B., and Galecki, A.T. 2006. *Linear Mixed Models: A Practical Guide Using Statistical Software*. Chapman & Hall/CRC Press, Taylor and Francis Group, London, UK.