Adipose tissue responses to breaking sitting in men and women with central adiposity

Running title: Adipose tissue responses to breaking sitting

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Abstract

Purpose: Breaking prolonged sitting reduces postprandial glucose and insulin concentrations and influences skeletal muscle molecular signalling pathways but it is unknown whether breaking sitting also affects adipose tissue. **Methods:** Eleven central overweight participants (7 men and 4 post-menopausal women) aged 50 ± 5 years (means ± SD) completed two mixed-meal feeding trials (PROLONGED SITTING *versus* BREAKING SITTING) in a randomised, counterbalanced design. The BREAKING SITTING intervention comprised walking for 2 min every 20 min over 5.5 h. Blood samples were taken at regular intervals to examine metabolic biomarkers and adipokine concentrations. Adipose tissue samples were taken at baseline and at 5.5 h to examine changes in mRNA expression and secretion of selected adipokines *ex-vivo*. **Results:** Postprandial glycaemia and insulinaemia were attenuated by approximately 50% and 40% in BREAKING SITTING compared to PROLONGED SITTING (iAUC: 359 ± 117 *versus* 697 ± 218 mmol·330 min·L^{-1}, $p = 0.001$ and 202 ± 71 *versus* 346 ± 150 nmol·330 min·L^{-1}, $p = 0.001$, respectively). Despite these pronounced and sustained differences in postprandial glucose and insulin concentrations, adipose tissue mRNA expression for various genes (IL-6, leptin, adiponectin, PDK4, IRS1/2, PI3K and Akt1, etc.) and *ex-vivo* adipose tissue secretion of IL-6, leptin and adiponectin were not different between trials. **Conclusions:** This study demonstrates that breaking sitting with short bouts of physical activity has very pronounced effects on systemic postprandial glucose and insulin concentrations but this does not translate into corresponding effects within adipose tissue.

Key words: Sedentary, physical activity, gene expression, postprandial, insulin signalling

INTRODUCTION

Adipose tissue is a sizeable and complex endocrine organ that plays a role in metabolic control in part through the secretion of adipokines such as adiponectin and Interleukin-6 (IL-6) (1). Adipose dysfunction is characterised by tissue-specific insulin resistance, local inflammation, fibrosis, and the abnormal secretion of adipokines (2). These adipokines regulate various physiological processes and play a crucial role in the pathophysiology of chronic diseases such as cardiovascular diseases and type 2 diabetes (1). Thus, there is a clear need to develop interventions and strategies that successfully target adipose tissue function.

Regular prolonged exercise improves various aspects of adipose tissue function, including measures of inflammation and insulin sensitivity (3). This form of prolonged physical activity also has demonstrably important effects on various health outcomes including risk factors for cardiovascular disease and diabetes (4). Unfortunately, many people struggle to undertake such structured exercise and alternative forms of physical activity could potentially play an important role. In many modern societies, people spend much of their time engaged in sedentary behaviours such as using a computer and watching television. In addition to causing a positive energy balance and weight gain, several studies have shown that sedentary behaviour is associated with increased risk of abnormal glucose metabolism, metabolic syndrome, type 2 diabetes and cardiovascular disease (5). Thus, targeting this sedentary behaviour may be important for health.

In support of this suggestion, short bouts of physical activity lasting just a few minutes spread over the course of a day improve postprandial triglyceridaemia, glycaemia and insulinaemia (6-9). Breaking prolonged sitting with short bouts of physical activity also affects skeletal muscle signalling pathways related to carbohydrate metabolism (8). However, there has been no assessment of whether breaking prolonged sedentary behaviour impacts upon adipose tissue physiology. If this strategy is effective, it would have major implications for the prevention and management of disorders

associated with dysfunctional adipose tissue. Thus, this study aims to examine the acute effect of breaking prolonged sedentary behaviour (sitting) on adipose tissue.

MATERIALS AND METHODS

Experimental design

Eleven participants (7 men and 4 post-menopausal women) aged between 35 and 64 years with increased central adiposity were recruited via local advertisement. An initial screening was followed by two subsequent main trials (PROLONGED SITTING and BREAKING SITTING). Trials were conducted in a randomised, counterbalanced design separated by a 3–4 week wash-out period. Two identical meals based on participants' total body mass were provided on the main trial days (morning and lunchtime). Blood was taken regularly during the course of each trial and adipose tissue biopsies were taken at baseline and at the end of each trial. The study protocol was approved by Bristol Research Ethics Committee (REC reference number: 13/SW/0321) and is registered at ClinicalTrials.gov (ID: NCT02870088). All participants provided written informed consent before taking part.

Power calculations

There are no data regarding the effect of breaking prolonged sitting on adipose tissue. Adipose tissue is highly responsive to insulin (10) and we have therefore determined sample size based on differences in insulin responses to a standard meal. A previous similar study reported breaking prolonged sitting to reduce postprandial insulinaemia from 3337 IU·9 h·L^{-1} to 2470 IU·9 h·L^{-1} (9). Based on an estimated SD of 600 IU·9 h·L^{-1}, we would require 9 participants to show a difference in iAUC for insulin with 95% power and 5% alpha using a 2-tailed paired *t-test*. Eleven participants were recruited to allow for drop out.

Inclusion and exclusion criteria

Participants were required to be central overweight with at least a waist circumference of 80 cm for women and 94 cm for men (11) and weight stable (no self-reported change in weight ± 3%) (12) for at least 3 months prior to taking part in this study. Participants completed a health questionnaire to exclude any existing cardiovascular and metabolic diseases and a Physical Activity Readiness Questionnaire (PAR-Q) to ensure that participants were able to walk on the treadmill safely. Participants were asked to walk on a treadmill for 2 min to ensure that this would be tolerated. Smokers, pre-menopausal women and volunteers who used any medications which could influence metabolic and inflammatory responses were excluded. A summary of participants' physical characteristics is shown in Table 1.

[INSERT TABLE 1 ABOUT HERE]

Pre-trial assessments

Physical activity assessment

As part of the pre-trial assessments, participants wore a combined heart rate/accelerometer monitor for 7 days to assess habitual physical activity energy expenditure (Actiheart, Cambridge Neurotechnology Ltd., Cambridge, UK). This was attached to the left of the chest via 2 adhesive ECG pads for 24 h per day except for during showering/bathing/swimming (13).

Body composition analysis

Body mass was assessed using digital scales post-void (TANITA corp., Tokyo, Japan). Waist and hip circumferences were assessed based on World Health Organisation guidelines (11). Body composition was determined by using Dual Energy X-ray Absorptiometry (DEXA; Discovery, Hologic, Bedford, UK). Abdominal subcutaneous and visceral adipose tissue mass was estimated from a central region between L1-L4 (14).

120

121 **Trial days**

122 In the 72 h prior to each main trial, participants were asked to refrain from performing any vigorous-
123 intensity exercise. In the 48 h prior to each main trial, consuming alcohol/caffeine was not allowed
124 and a weighed food and fluid record was completed. Participants were asked to replicate this diet
125 prior to their second main trial. In addition, in the 48 h prior to each main trial, participants were
126 asked to wear a pedometer (Yamax, Japan) to restrict their step counts to under 4,000 steps per day
127 in order to mimic a sedentary lifestyle (15) and eliminate any acute effects from recent physical
128 activity.

129

130 A 12 h fast was performed before arriving at the laboratory on main trial days between 08:00–09:00
131 am. After anthropometric measurements, participants rested on a bed for 15 min, followed by two 5
132 min expired gas sample collections using Douglas bags (Hans Rudolph, MO, USA) to determine
133 resting metabolic rate (RMR) (16) from substrate oxidation as described (17).

134

135 After RMR assessment, a 20-gauge cannula (BD, Venflon^{TM} Pro) was inserted into an antecubital
136 forearm vein and a 10-ml baseline venous blood sample was taken and allocated into tubes with either
137 EDTA or serum separation beads (Sarstedt Ltd, Leicester, UK). Plasma samples were centrifuged
138 immediately at 3,465 *g* at 4°C for 10 min. Serum samples were left for 45 min to clot before
139 centrifugation. Subcutaneous adipose tissue samples (~1 g) were subsequently taken under local
140 anaesthetic (1 % lidocaine) from the area around the waist approximately 5 cm lateral to the umbilicus
141 with a 14 G needle using an aspiration technique (18) followed by adipose tissue cleaning and
142 processing as previously described (19).

143

144 Two identical meals (breakfast and lunch) were provided and consumed within a 15-min period during
145 each main trial. The first meal (breakfast) was consumed after taking the baseline adipose and blood

146 sample. The second meal (lunch) was consumed 3 h after the first. The test meal was prescribed
147 according to total body mass and provided 0.35 g fat, 1.17 g carbohydrate, 0.29 g protein and 37 kJ
148 energy per kilogram body mass (6). The percentage of energy from macronutrients was 35 % fat, 52
149 % carbohydrate and 13 % protein (6). The meal comprised white bread (Hovis; soft white bread,
150 medium sliced), sliced cheese (Sainsbury; cheese slices, basic), butter (Unilever; I can't believe its
151 not butter), mayonnaise (Hellmann; light mayonnaise), lettuce (Sainsbury; Iceberg lettuce), tomato
152 (Sainsbury; tomatoes, basics), ham (Sainsbury; British honey roast), whole milk (Sainsbury; British),
153 cocoa powder (Nesquik; cocoa powder), and yoghurt (Müller; fruit corner strawberry).
154
155 **Prolonged sitting and breaking prolonged sitting**
156 The study protocol is shown in Figure 1. In the PROLONGED SITTING trial, participants sat on a
157 chair for the whole period. In the BREAKING SITTING trial, after consuming the first meal,
158 participants walked 2 min on a treadmill at 6.4 $km \cdot h^{-1}$ speed (7) every 20 min for the following 180
159 min. After finishing the second meal, participants continued 2 min of breaking prolonged sitting
160 physical activity at the same speed every 20 min for the following 120 min. For the remainder of the
161 time participants sat on a chair. Thus, in total, participants performed 15 two min bouts of walking
162 throughout the trial (i.e., 30 min of physical activity over 300 min).
163
164 Ratings of perceived exertion (RPE) and heart rate were collected in the last 30 seconds of each 2-
165 min bout of walking during the breaking sitting trial. Two, 1-min expired air samples were collected
166 during the last minute of walking during the breaking sitting trial to estimate energy expenditure and
167 substrate utilization. In addition, expired air samples were taken using Douglas bags (Hans Rudolph,
168 MO, USA) during two 5-min periods of sitting to calculate total energy expenditure under resting
169 conditions. While sitting, participants were only allowed to read, use a laptop or watch television but
170 were otherwise asked to keep as still as possible throughout (including specific instructions to avoid
171 fidgeting). In the first trial, participants were allowed to consume water *ad libitum* and the volume

ingested was replicated for the second trial. In each main trial, baseline blood samples were collected before the first meal and hourly for the following 5 h. Additional blood samples were collected every 15 min for the first hour after each meal. A total of 14 blood samples were collected for each trial (Figure 1).

[INSERT FIGURE 1 ABOUT HERE]

Adipose tissue culture and gene expression

After cleaning and mincing the adipose tissue biopsy sample, a 200 mg portion of adipose tissue was homogenised in 5 mL TRIzol (Invitrogen, Paisley, UK) in a 15 mL centrifuge RNase/DNase-free sterile tube (Invitrogen, Paisley, UK) and stored at −80°C for gene expression analysis. The remaining adipose tissue was directly placed in sterile culture plates in duplicate (Nunc, Roskilde, Denmark) with endothelial cell basal media (ECBM) (Promocell, Germany) containing 0.1 % fatty acid-free bovine serum albumin 100 $U \cdot mL^{-1}$ penicillin and 0.1 $mg \cdot mL^{-1}$ streptomycin (Sigma-Aldrich, Gillingham, UK). Adipose tissue was incubated with a final ratio of 100 mg tissue per 1 mL ECBM media for 3 h (19). A 37°C, 5 % CO_2 and 95 ± 5 % relative humidity incubator was used (MCO-18A1C CO_2 incubator; Sanyo, Osaka, Japan). After the 3-h incubation, media was transferred to sterile eppendorfs and stored at −80°C. Adipokine secretion from cultured adipose explants was normalised to explant adipose mass and then L1-L4 fat mass as described previously (20).

Real-time PCR

An RNeasy Mini Kit (Qiagen, Crawley, UK) was used to extract RNA from TRIzol-digested adipose tissue as described (18). The amount of RNA was quantified using a Qubit 2.0 fluorimeter (Life Technologies, Paisley, UK). RNA was reversed transcribed (1 μg) to cDNA using a High Capacity Reverse Transcription Kit (Applied Biosystems, Warrington, UK). Real-time PCR was performed using a StepOne (Applied Biosystems, Warrington, UK). Predesigned primers and probes were

obtained from Applied Biosystems for the measurement of expression of interleukin 6 (IL-6) (Hs00985639_m1), adiponectin (Hs00605917_m1), leptin (Hs00174877_m1), interleukin 18 (IL-18) (Hs00155517_m1), tumour necrosis factor alpha (TNF-α) (Hs99999043_m1), monocyte chemoattractant protein-1 (MCP-1) (Hs00234140_m1), 5' AMP-activated protein kinase (AMPK) (Hs01562315_m1 and Hs00178903_m1 combined), glucose transporter type 4 (GLUT4) (Hs00168966_m1), hormone-sensitive lipase (HSL) (Hs00193510_m1), insulin receptor substrate 1 (IRS1) (Hs00178563_m1), insulin receptor substrate 2 (IRS2) (Hs00275843_s1), sterol regulatory element binding protein 1c (SREBP-1c) (Hs01088691_m1), pyruvate dehydrogenase kinase isozyme (PDK4) (Hs00176875_m1), peroxisome proliferator-activated receptor γ (PPARγ) (Hs01115513_m1), peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1α) (Hs01016719_m1), RAC-alpha serine/threonine-protein kinase (Akt1) (Hs00178289_m1), adipose triglyceride lipase (ATGL) (Hs00386101_m1), fatty acid translocase (FAT)/CD36 (Hs00169627_m1), forkhead box protein O1 (FOXO1) (Hs01054576_m1), hexokinase 2 (HK2) (Hs00606086_m1), phosphoinositide 3-kinase 85α (PI3K-85α) (Hs00933163_m1), fatty acid synthase (FAS) (Hs00188012_m1), peptidylpropyl isomerase A (PPIA) was used as an endogenous control (21). The comparative Ct method was used to process data where ΔCt = Ct target gene − Ct PPIA. Ct target genes were normalised to an internal calibrator (lowest ΔCt for each target gene) and baseline.

Biochemical analyses

Concentrations of plasma glucose, plasma triglycerides and serum non-esterified fatty acids were analysed using commercially available assay kits and analyser (Daytona Rx; Randox, Crumlin, UK). Serum insulin (Mercodia, Uppsala, Sweden), and both serum and adipose explant secretion of IL-6, leptin, and adiponectin (R&D systems) were measured using Enzyme-linked immunosorbent assay (ELISA).

Statistical analysis

Descriptive data are presented in text and tables as means ± standard deviation (SD); variance bars on figures are presented as means and standard error of the mean (SEM). Time series data were examined using a two-way ANOVA (trial × time) with repeated measures using SPSS version 22 (IBM, Armonk, NY, USA). Green-house–Geisser corrections were applied to intra-individual contrasts where $\varepsilon < 0.75$; however, for less severe asphericity the Huynh–Feldt correction was selected (22) . Incremental area under curve (iAUC) was calculated using the trapezoid method (23) and the differences in summative scores between trials were analysed using paired *t-tests*. Data for iAUC represent the period from the consumption of the first meal to the conclusion of the second meal (330 min). Analysis of iAUCs and gene expression data were conducted using logged transformed data as previously described (18). Statistical significance was set at $p \leq 0.05$.

RESULTS

Blood glucose, insulin, triglyceride and NEFA concentrations in prolonged and breaking sitting trials

Trial × Time interaction effects were found for blood glucose and insulin between PROLONGED and BREAKING SITTING trials (all $p \leq 0.05$, Figure 2A and C). The glucose and insulin iAUC were attenuated significantly by BREAKING SITTING compared to PROLONGED SITTING (359 ± 117 *versus* 697 ± 218 mmol·330 min·L^{-1}, $p = 0.001$ and 202 ± 71 *versus* 346 ± 150 nmol·330 min·L^{-1}, $p = 0.001$, respectively, Figure 2B and D). There was no interaction effect for blood triglyceride and NEFA (Figure 2E and G). Triglyceride iAUC was significantly higher in BREAKING SITTING compared to PROLONGED SITTING trial (283 ± 36 vs. 232 ± 32 mmol·330 min·L^{-1}, $p = 0.002$, respectively, Figure 2F).

[INSERT FIGURE 2 ABOUT HERE]

Adipose tissue mRNA gene expression

There was no interaction effect (trial × time) in adipose tissue mRNA gene expression between BREAKING SITTING and PROLONGED SITTING trials for any of the genes analysed (Figure 3). However, there were time effects for IL-6, MCP1, PDK4, IRS1 and 2, PI3K, HSL and SREBP1c (all $p \leq 0.05$, Figure 3).

[INSERT FIGURE 3 ABOUT HERE]

Serum adipokine concentrations and adipose tissue adipokine secretion *ex vivo*

There was no difference between PROLONGED SITTING and BREAKING SITTING in serum IL-6, leptin and adiponectin (Figure 4A, C and E) or the secretion *ex vivo* of IL-6, leptin and adiponectin (Figure 4B, D and F). There was a time effect for serum IL-6 and leptin ($p \leq 0.05$) (Figure 4).

[INSERT FIGURE 4 ABOUT HERE]

Pre-trial physical activity and resting metabolic rate measures

The average of 48 h step counts prior to PROLONGED and BREAKING SITTING trials was not different (3,868 ± 1,304 *versus* 3,669 ± 1,090 steps·day^{-1}, respectively) and there was no difference in pre-fed RMR on the trial day between trials (1,882 ± 303 *versus* 1,833 ± 338 kcal·day^{-1}, respectively).

Physiological response during trials

During the 15 bouts of two min walking, the average heart rate was 135 ± 12 beats· min^{-1} with an RPE (6–20 scale) of 10 ± 2. The difference in energy expenditure between trials was 216 kcal over the 5.5 h period (Table 2).

[INSERT TABLE 2 ABOUT HERE]

DISCUSSION

The present study investigated whether breaking sitting with regular short bouts of walking influences adipose tissue gene expression and secretion of selected proteins from adipose tissue *ex vivo*. We found that regularly participating in short bouts of walking reduced postprandial glycaemic and insulinaemic responses by 48% and 42%, respectively. However, despite the profound difference in postprandial insulin and glucose concentrations, changes in subcutaneous adipose gene expression were similar in both trials. There was also no difference in adipose tissue explant secretion and circulating concentrations of IL-6, adiponectin or leptin.

The effect of breaking sitting on postprandial glycaemia and insulinaemia

The extent of postprandial glucose and insulin excursions are associated with cardiovascular disease (24, 25). Therefore, strategies to attenuate postprandial glucose and insulin responses would be extremely useful. Breaking prolonged sitting has been shown to reduce postprandial glucose and insulin levels in most (7, 9, 26) but not all studies (27). In the present study, we found that participating in regular short bouts of walking attenuated postprandial glucose and insulin concentrations in sedentary middle-aged central overweight men and women (glucose and insulin iAUC's were attenuated by 48% and 42%, respectively). The additional energy cost of walking during the BREAKING SITTING trial was supported by carbohydrate oxidation and thus we propose that oxidation of carbohydrate may be partly responsible for the relatively lower blood glucose observed in this trial. Thus, breaking prolonged sitting is very effective at reducing postprandial glycaemia and insulinaemia.

Adipose tissue responses to breaking prolonged sitting

In skeletal muscle, breaking sitting with either light or moderate-intensity walking increases the expression of genes involved in glucose metabolism, including PDK4 (8). In the present study, we did not find any differences between PROLONGED SITTING and BREAKING SITTING trials in

the expression of these genes in adipose even though our walking speed, frequency and duration was very similar to that previous study (8). Thus, it appears that acutely breaking prolonged sitting with short bouts of walking reduces postprandial glycaemia/insulinaemia and leads to corresponding changes in key pathways in skeletal muscle; whereas a similar effect on postprandial glycaemia/insulinaemia is not associated with corresponding changes within adipose tissue. These divergent effects are likely to reflect the different roles of these tissues. Skeletal muscle plays a major role in the disposal of exogenously ingested glucose (especially during physical activity), whereas adipose tissue plays a relatively minor role in the acute regulation of blood glucose concentrations (28). Instead, during the postprandial period, adipose tissue plays a major role in responding to feeding and storing ingested lipids (29). Given the effect of glucose and insulin infusion on adipose tissue gene expression (10, 30), it is possible that the magnitude of postprandial glycaemia and insulinaemia in the present study was a more-than-adequate stimulus for adipose tissue, even during the BREAKING SITTING trial. We have recently shown that feeding exerts a potent effect on adipose tissue and can blunt the normal responses to prolonged structured exercise (20). Thus, we propose that the effect of feeding is likely to overcome the impact of physical activity on adipose tissue – whether this is during repeated short bouts of physical activity to break prolonged sitting or during more prolonged structured exercise.

There was an apparent increase in triglyceride iAUC with BREAKING SITTING trial and this may (ostensibly) appear to be a negative response. However, this is likely explained by an enhanced lipid mobilisation in the BREAKING SITTING trial combined with technical considerations related to the assay used for the assessment of plasma triglyceride. Our method for the assessment of plasma triglyceride does not discriminate between free glycerol, bound glycerol in chylomicrons and very-low-density lipoprotein; and thus it is likely that the greater triglyceride iAUC in the BREAKING SITTING trial reflects an increase in circulating free glycerol due to adipose tissue lipolysis.

Temporal changes in adipose tissue gene expression after feeding

Even though we did not observe any differences in gene expression between BREAKING and PROLONGED SITTING trials, there were temporal changes in both trials that could reflect the effect of feeding *per se*. The expression of adipose SREBP1c was increased in both trials and this response is probably indicative of lipid synthesis after feeding (31). Moreover, there was a decrease in HSL and PDK4 in both trials, which may reflect a reduction in lipid mobilisation and oxidation. In addition, insulin receptor, IRS1 and IRS2 gene expression were consistently up-regulated and down-regulated respectively at the end of both trials. IRS-1 plays a central role as an insulin signalling receptor (32) and, similar to the present study, adipose IRS-2 mRNA expression was down-regulated in lean, overweight and obese populations after feeding (33). Taken together, the changes in SREBP1c, HSL, PDK4 and IRS-1 and IRS-2 in both trials probably reflects the normal physiological and metabolic response to feeding.

We also observed an increase in adipose IL-6 and MCP-1 in both trials. Other studies have shown that adipose IL-6 and MCP-1 gene expression were up-regulated after meal consumption in lean, overweight and obese men (33), in people with metabolic syndrome (34), in people with type 2 diabetes (35) and in healthy lean populations (36). A meal-induced up-regulation of adipose IL-6 and MCP-1 gene expression might support the notion that feeding induces acute transient adipose tissue inflammation. However, we cannot discount the possibility that these changes could also reflect an adipose circadian rhythm (37).

Experimental Design Considerations

In the present study, only two adipose biopsies were taken and therefore we do not have a complete time-course of whether the temporal changes observed in both trials was due to meal consumption and/or an entrained biological rhythm. Repeated adipose biopsies might help resolve this issue but this could be problematic due to potential confounding from local inflammation (36). We should also

emphasise that each condition (trial arm) was conducted over the course of a single day. Given the large reduction in serum insulin concentrations in the BREAKING SITTING trial and the powerful effect of insulin on adipose tissue (10, 38), it is possible that if this intervention was repeated for multiple days and/or months then this difference in postprandial insulin would lead to chronic changes in adipose in the longer term. We also do not know how the present results would compare to the same duration of exercise but performed in a different way (e.g., a single bout of 30 min or three bouts of 10 min). In the present study, we provided two mixed meals with a total ~1,725 kcal, which is more than 2-fold the energy intake in the single meal provided by Latouche et al (8). Given the extended timescale ~1,725 kcal is not an unreasonable amount of energy to consume (39), but it is possible that the physiological pressure of processing and storing this amount of energy might outweigh the impact of small bouts of physical activity in adipose tissue. Had we provided smaller meals, it is possible that physical activity could have had a moderating effect on adipose tissue-related outcomes.

Conclusions

Whilst breaking prolonged sitting is an effective strategy for acutely managing postprandial glycaemia and insulinaemia, this does not elicit corresponding temporal changes in adipose tissue.

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Conflict of Interest

The authors declare no competing interests. The results of the present study do not constitute endorsement by the American College of Sports Medicine. The results of this study are presented clearly, honestly, and without fabrications, falsification, or inappropriate data manipulation.

Author contributions

Yung-Chih Chen was responsible for study design and conduct, data collection, data analysis, data interpretation, statistical analysis and manuscript revision; James Betts was responsible for study design and manuscript revision; Jean-Philippe Walhin assisted with adipose tissue biopsies and manuscript revision. Dylan Thompson was responsible for funding, study design, data interpretation and manuscript revision.

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514

515 **Figure Legends**

516

517 **Figure 1. Experimental protocol in PROLONGED SITTING and BREAKING SITTING trials.**
518 In the PROLONGED SITTING trial, participants sat on a chair throughout. In the BREAKING
519 SITTING trial, participants walked on a treadmill at 6.4 $km \cdot h^{-1}$ for 2 min every 20 min.

520

521 **Figure 2: Blood glucose (A), insulin (C), triglyceride (E) and NEFA (G) concentrations in**
522 **PROLONGED SITTING and BREAKING SITTING trials. iAUC for glucose (B), insulin (D)**
523 **and triglyceride (F).** iAUC is not shown for NEFA because values in both trials are mostly below
524 baseline. The sample size is n = 10 due to difficulty in cannulating one female participant. Values are
525 means ± SEM. # denotes significant interaction effect between PROLONGED SITTING and
526 BREAKING SITTING trials. * denotes significantly different between prolonged sitting *versus*
527 breaking sitting trials ($p \leq 0.05$). The shaded box denotes meal time. Dashed lines with grey colour
528 denotes female.

529

530 **Figure 3. Fold changes in relative adipose tissue gene expression under PROLONGED**
531 **SITTING and BREAKING SITTING trials** (all n = 9, due to lack of sufficient tissue samples for
532 two participants). The dashed line indicates baseline. Data normalised to PPIA, internal calibrator
533 and baseline. Samples below the detectable limit (Ct > 35) were excluded from the analysis. † denotes
534 significant time effect and * denotes significant trial effect ($p \leq 0.05$). Values are means ± SEM.

535

536 **Figure 4: Circulating serum IL-6 (A) leptin (C) and adiponectin (D) concentrations in**
537 **PROLONGED SITTING and BREAKING SITTING trials** (sample size is n = 10 due to difficulty
538 in cannulating one participant). **Adipose explant protein secretion of IL-6 (B), leptin (D) and**
539 **adiponectin (F) at baseline (AM) and at end of the trial (PM)** (all n = 9, due to lack of sufficient
540 tissue samples for one male and one female participant). † denotes a significant time effect ($p \leq$

0.05).Values are means ± SEM. The shaded box denotes meal time. Dashed lines with grey colour denotes female.

544 **Table 1. Participants physical characteristics (n = 11)**

Characteristics	Mean ± SD
Age (years)	50 ± 5
Body mass (kg)	97.6 ± 20.8
Height (m)	1.73 ± 0.08
Waist circumference (cm)	109 ± 14
Hip circumference (cm)	112 ± 12
Body mass index ($kg \cdot m^{-2}$)	32.5 ± 6.7
Fat mass (%)	35 ± 6
Fat mass (kg)	33.8 ± 11.0
Fat in L1-L4 region (kg; DEXA)	4.7 ± 1.9
Physical activity level (PAL)	1.48 ± 0.16
Systolic blood pressure (mmHg)	137 ± 12
Diastolic blood pressure (mmHg)	89 ± 7

545 Fat mass in L1-L4 region was assessed as described previously (14).

546

547 **Table 2.** Energy expenditure and substrate oxidation during each 330 min trial (n = 11)

	PROLONGED SITTING	BREAKING SITTING
Energy expenditure (kcal) *	577 ± 129	793 ± 149
Carbohydrate oxidation (g) *	112 ± 45	168 ± 48
Lipid oxidation (g)	17 ± 13	18 ± 10

548 Values are means ± SD. * denotes significantly different between PROLONGED SITTING *versus*
549 BREAKING SITTING trials ($p \leq 0.05$).

550

551

Figure 1

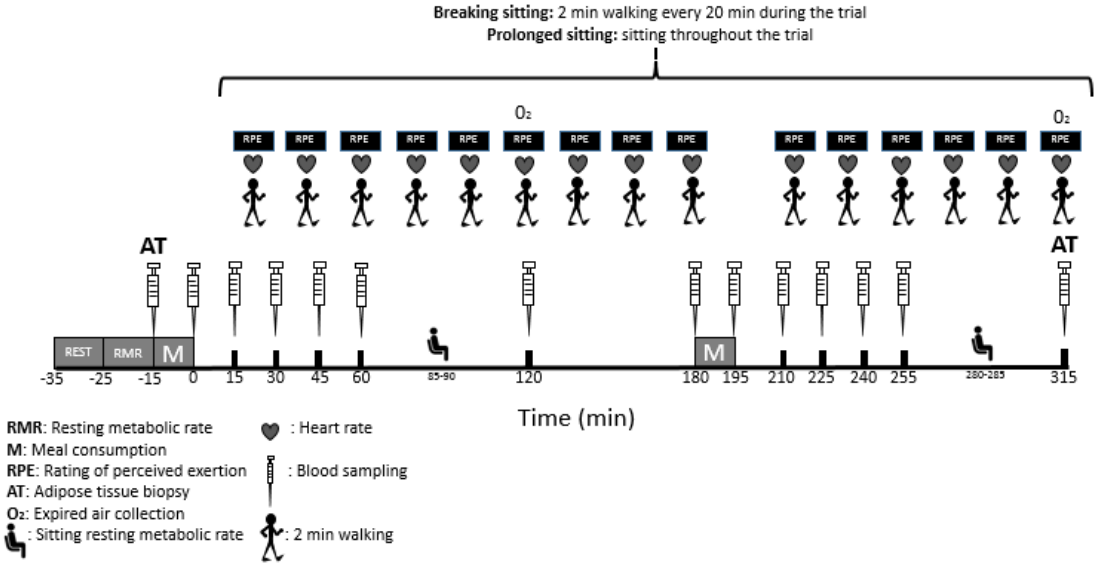

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Figure 2

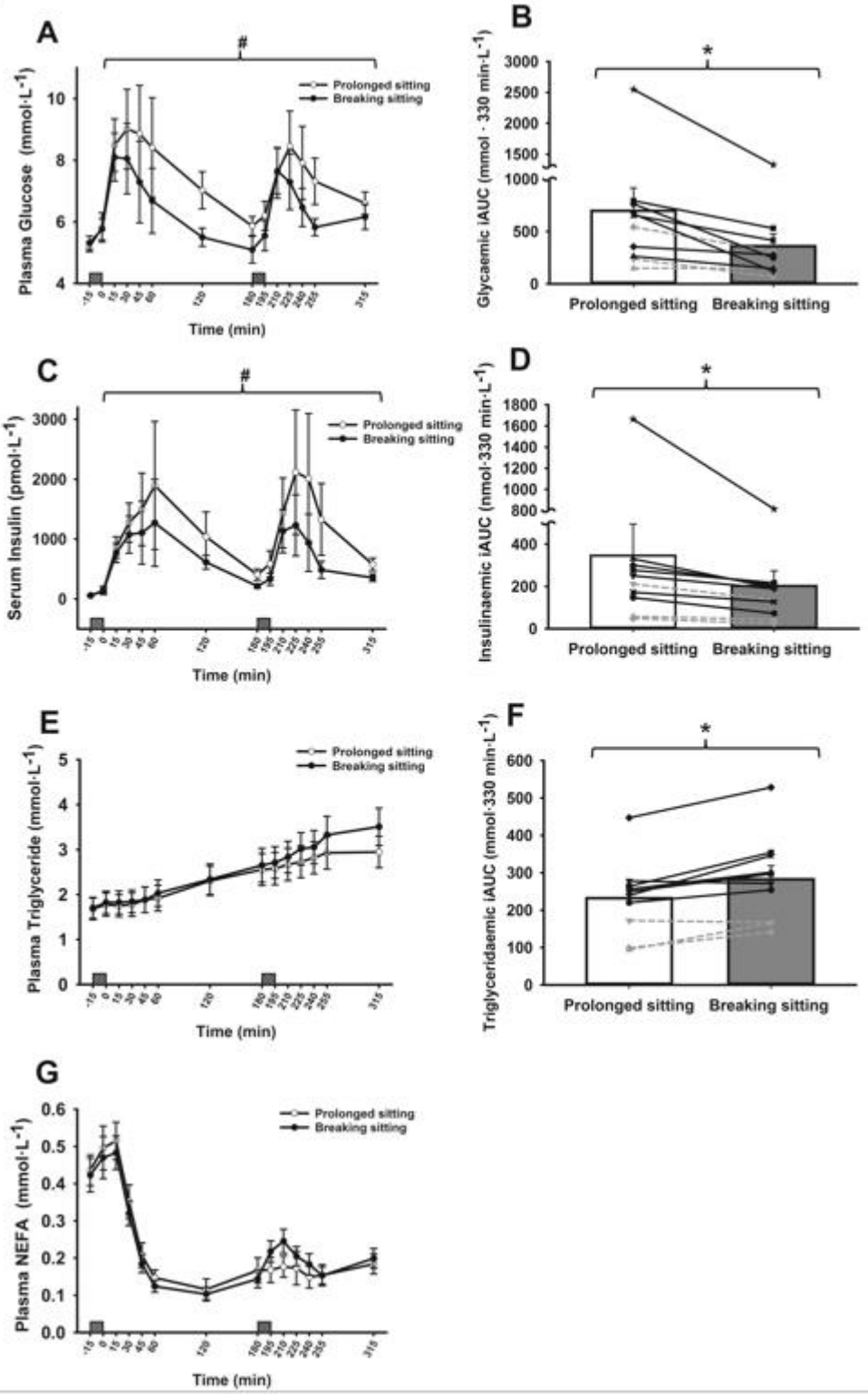

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Figure 3

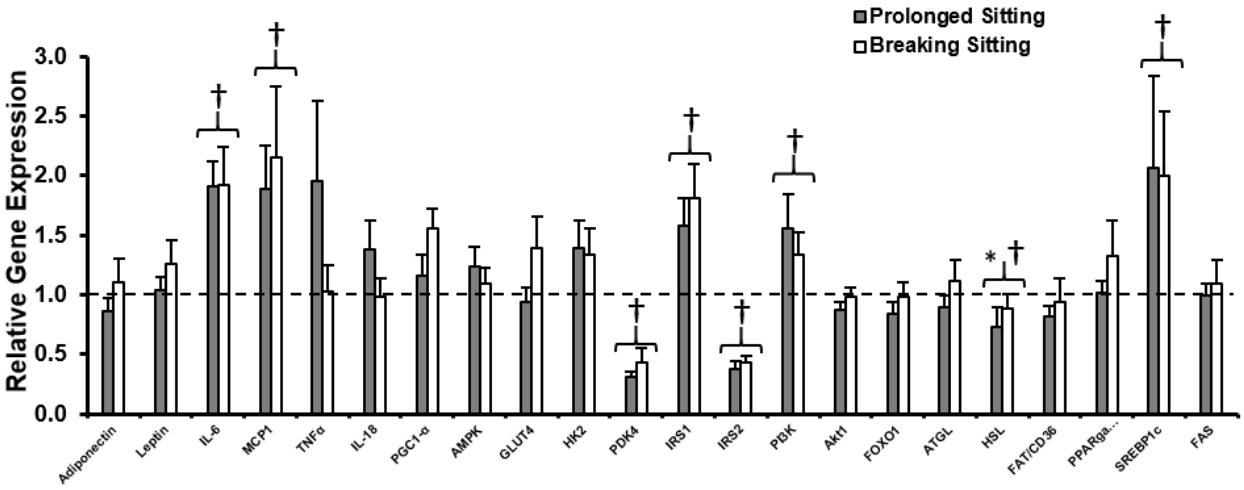

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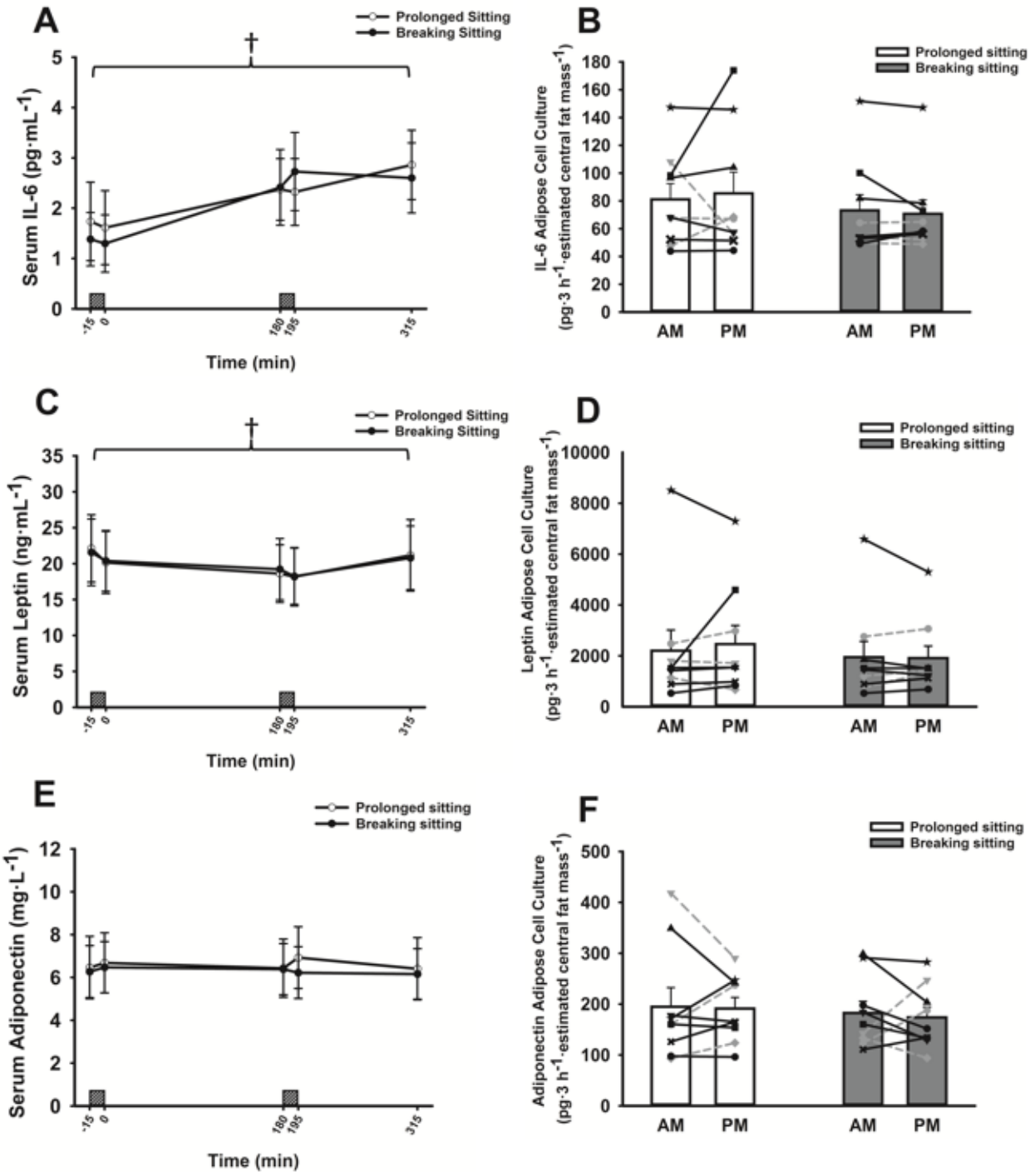
Figure 4


A
Prolonged Sitting
Breaking Sitting
†
Serum IL-6 (pg·mL-1)
Time (min)
B
Prolonged sitting
Breaking sitting
IL-6 Adipose Cell Culture (pg·3 h-1·estimated central fat mass-1)
AM
PM
C
Serum Leptin (ng·mL-1)
D
Leptin Adipose Cell Culture (pg·3 h-1·estimated central fat mass-1)
E
Serum Adiponectin (mg·L-1)
F
Adiponectin Adipose Cell Culture (pg·3 h-1·estimated central fat mass-1)

558