FINAL VERSION ACCEPTED FOR PUBLICATION IN THE JOURNAL OF SPORTS SCIENCES

Effects of breaking up sedentary time with 'chair squats' on postprandial metabolism

Nabeha S.A. Hawari[1], John Wilson[1], Jason M.R. Gill[1]

[1]Institute of Cardiovascular and Medical Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom.

Short title: 'Chair squats' and postprandial metabolism

Address for correspondence:
Professor Jason M R Gill
BHF Glasgow Cardiovascular Research Centre
Institute of Cardiovascular and Medical Sciences
College of Medical, Veterinary and Life Sciences
University of Glasgow
Glasgow
G12 8TA
United Kingdom

Telephone: + 44 (0) 141 3302916
Fax: + 44 (0) 141 3302522
Email: jason.gill@glasgow.ac.uk

Keywords: postprandial, sedentary, energy expenditure, substrate utilisation, insulin, glucose, triglyceride

Abstract

Prolonged sitting induces adverse metabolic changes. We aimed to determine whether breaking up prolonged sedentary time with short periods of repeated sit-to-stand transitions ('chair squats') every 20 minutes influences postprandial metabolic responses. Fourteen participants (11 men, 3 women), age 37±16 years, BMI 30.5±3.8 $kg.m^{-2}$ (mean±SD) each participated in two experimental trials in random order, in which they arrived fasted, then consumed a test breakfast (8 $kcal.kg^{-1}$ body weight, 37% energy from fat, 49% carbohydrates, 14% protein) and, 3.5 hours later, an identical test lunch. Expired air and blood samples were taken fasted and for 6.5 hours postprandially. In one trial (SIT) participants sat continuously throughout the observation period; in the 'Chair squat' trial (SIT/STAND), participants performed 'chair squats' (10 x standing and sitting over 30 seconds, every 20 minutes). Compared to SIT, energy expenditure was 409.7±41.6 kJ (16.6±1.7%) higher in SIT/STAND ($p<0.0001$). Postprandial insulin concentrations over the post-breakfast period were 10.9±8.4% lower in SIT/STAND than SIT ($p=0.047$), but did not differ between trials in the post-lunch period. Glucose and triglyceride concentrations did not differ significantly between trials. These data demonstrate that a simple, unobtrusive intervention to break up sedentary time can induce some favourable metabolic changes.

Keywords: sitting; standing; energy expenditure; glucose; insulin; triglyceride

Abstract: 196 words

Main text: 3745 words

Introduction

Sedentary behaviour has been defined as waking activities in a sitting or reclining posture with energy expenditure ≤1.5 METS (where 1 MET is resting energy expenditure) (Tremblay et al., 2017). There is a growing body of epidemiological evidence that high levels of sedentary behaviour are associated with adverse cardio-metabolic biomarker risk profiles and with increased risk of cardiovascular disease, diabetes, metabolic syndrome, obesity and death from any cause (Edwardson et al., 2012; Wilmot et al., 2012; Thorp, Owen, Neuhaus, & Dunstan, 2011; Healy, Matthews, Dunstan, Winkler, & Owen, 2011; Celis-Morales et al., 2012). This association is generally independent of time spent engaged in moderate-to-vigorous physical activity (MVPA) (>3 METS), except when levels of physical activity are very high (Ekelund et al., 2016).

In addition, observational studies suggest that the *pattern* as well as total amount of sedentary behaviour may be important. It has been reported that individuals who regularly break up their periods of sedentary time have a more favourable cardio-metabolic risk profile, particularly with respect to adiposity-related variables, than those who habitually engage in prolonged periods of uninterrupted sedentary time, independent of total time spent sedentary (Healy et al., 2008; Healy et al., 2011; Cooper et al., 2012). While the observational nature of these data mean that a causal relationship cannot be assumed, the findings suggest that interventions which change the pattern, as well as the total amount, of sitting and standing behaviour could potentially elicit cardio-metabolic health benefits. This hypothesis requires testing in experimental studies.

A growing body experimental data have shown that breaking up periods of prolonged sitting with periods of standing increases energy expenditure over the course of the day (Reiff, Marlatt, & Dengel, 2012; Speck & Schmitz, 2011). In some (Thorp et al., 2014; Henson et al., 2016; Buckley, Mellor, Morris, & Joseph, 2014), but not all (Bailey & Locke, 2015), studies favourable changes to postprandial glucose and/or insulin responses have also been observed when sitting is replaced by standing. Building on this work, we recently demonstrated that the pattern of sitting and standing influences postprandial energy expenditure and substrate utilisation independent of overall sitting and standing time. In a proof-of-priniciple study, 10 overweight men underwent three experimental conditions in random order; one where they consumed a test breakfast and test lunch and sat continously for 8 hours; one where they undertook 16 bouts of standing for 15 minutes during the 8-hour postprandial observation period; and one where they undertook 160 bouts of standing for 1.5 minutes during the 8-hour observation period (Hawari et al., 2016). Despite total time spent standing and sitting being identical in the two standing conditions, the increase in metabolic rate (21% vs 11% increase) and fat oxidation (18% vs 7% increase), compared to the sitting condition, was significantly greater in the latter condition with a greater number of sit-to-stand transitions. This provides a potential explanation for the independent effect of frequency of sedentary breaks on indices of adiposity observed in large epidemiological studies and suggests that increasing the number of sit-to-stand transitions undertaken may provide an alternative strategy to increasing the total amount of time spent standing when designing interventions to counteract the adverse metabolic effects of prolonged sitting. To test this hypothesis, the aim of the present study was therefore to determine whether, compared to continuous sitting, breaking up prolonged sedentary time by undertaking ‘chair squats’ – repeated sit-to-stand transitions over a short period (sitting and standing 10 times over 30 seconds, every 20 minutes) – provided measureable metabolic benefits.

Methods

Screening and inclusion criteria

Overweight (BMI > 25 $kg.m^{-2}$) men and women aged 18-65 years, who were non-smokers and not achieving current physical activity guidelines were recruited. Women were only included if they were postmenopausal, as postprandial metabolic responses vary over the course of the menstrual cycle, which could confound outcome measures (Gill, Malkova, & Hardman, 2005)). Other exclusion criteria included: frank diabetes (physician diagnosed or fasting glucose (>7$mmol.l^{-1}$ on screening); uncontrolled hypertension (>160/90 mmHg on anti-hypertensive medication); previous history of established CHD; or current medications known to affect lipid or glucose metabolism. Participants were recruited to the study by local advertising and personal contacts. They were invited for screening where they attended the laboratory in the fasted state and completed a health history questionnaire; had a blood sample taken to determine fasting glucose concentration; had blood pressure measured using an automated monitor (Omron Healthcare, Inc., Illinois, USA); had height, body mass and waist circumference measured using standard International Society for the Advancement of Kinanthropometry protocols (Marfell-Jones, Olds, Stewart, & Carter, 2006); and completed the International Physical Activity Questionnaire to assess habitual physical activity levels.

Participants

Fourteen participants (11 men, 3 women) meeting the inclusion criteria, aged 37 ± 16 years, with body mass index (BMI) 30.5 ± 3.8 $kg.m^{-2}$, and waist circumference 102.3 ± 10.7 cm [mean ± SD] were recruited for this study. The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the College of Medical, Veterinary and Life Sciences Research Ethics Committee at the University of Glasgow. All participants provided written informed consent.

129

130 ***Study design***

131 Participants each completed two experimental trials with an interval of 1-2 weeks. In one
132 trial, participants sat continuously for a 6.5-hour observation period (SIT). In the other
133 participants interrupted their sitting by standing from their chair and sitting down again 10
134 times over a 30-second period ('chair squats'), every 20 minutes throughout the observation
135 period (SIT/STAND). Order of testing was randomised by selection of folded papers
136 indicating trial order, with seven participants undertaking the SIT trial first and the other
137 seven undertaking the SIT/STAND trial first. Participants were asked to weigh and record
138 their food intake and refrain from planned exercise (undertaking only the activities of normal
139 daily living) and alcohol on the two days preceding their first main experimental trial and to
140 replicate this for the two days preceding subsequent trials. The experimental protocol is
141 shown in Figure 1 and described below.

142

143 ****Figure 1 near here****

144

145 ***Experimental protocol***

146 **Uninterrupted sitting trial (SIT):** Participants arrived at the metabolic suite after a 12-hour
147 overnight fast. They sat comfortably for 10 minutes, before a 5-minute expired air sample
148 was collected via a mouthpiece into a Douglas bag to determine oxygen uptake (VO_2) and
149 carbon dioxide production (VCO_2) enabling calculation of metabolic rate and substrate
150 utilisation by indirect calorimetry (Frayn & Macdonald, 1997). A cannula was then inserted
151 into an antecubital vein for repeated blood sampling: this was kept patent by flushing with
152 saline throughout the day. A baseline fasting blood sample was collected into a K_2EDTA tube

and placed immediately on ice. Participants were then given a standardised breakfast comprising a buttered bagel and a meal replacement drink (Complan Foods Ltd, UK) made up with whole milk, which provided 8 kcal energy per kg body mass (37% energy from fat, 49% carbohydrates, 14% protein) and was consumed within 10 minutes. Further blood samples were taken at 30, 60, 120 and 180 minutes after breakfast. Three and a half hours after breakfast, participants consumed a test lunch, which was identical to breakfast, and further blood samples were taken before and 30, 60, 120 and 180 minutes after lunch (i.e. 210, 240, 270, 330 and 390 minutes after breakfast). Expired air samples for the determination of metabolic rate and substrate utilisation were collected throughout the 6.5-hour observation period (see 'Chair Squat' trial below for the frequency of expired air measurements). Participants sat comfortably (reading, watching TV, doing paperwork etc) throughout the observation period and were permitted to drink water throughout the day. They were directly observed by investigators throughout to ensure compliance to the protocol. A comfort break to the toilet (which was ~20 m from the metabolic investigation suite) was permitted during the interval between the 180-minute post-breakfast blood sample and lunch.

'Chair squats' trial (SIT/STAND): This was identical to the SIT trial, except that every 20 minutes during the 6.5-hour observation period, participants were asked to perform 10 'chair squats' involving standing up and sitting back down in their chair, without using their arms to assist them, over a 30 second period. To accurately quantify the effects of the chair squats on metabolic rate and substrate utilization, taking into account effects during the recovery period, separate expired air samples were taken for the minute prior to commencing chair squats; over the 30-second chair squat period; and during the post-chair squat recovery period from 0 to 1 minute; 1 to 2 minutes; 2 to 3 minutes; 3 to 4.5 minutes; and 19 to 20 minutes.

178 The final of these samples immediately preceded the next round of chair squats. These
179 expired air sampling time periods were chosen as our pilot data indicated that energy
180 expenditure returned to resting levels within ~3 to 4 minutes of completion of the chair
181 squats, so this sampling protocol enabled accurate quantification of energy expenditure, while
182 ensuring participant comfort by minimising the time that they spent breathing though a
183 mouthpiece. The expired air sampling periods were identical in the SIT trial.
184
185 ***Calculation of energy expenditure and substrate utilization***
186 Energy expenditure and energy substrate utilisation were calculated using indirect
187 calorimetry (Frayn & Macdonald, 1997). In brief, fat oxidation (in $g.min^{-1}$) was calculated as
188 1.67 VO_2 – 1.67 VCO_2 – 1.92 n; carbohydrate oxidation (in $g.min^{-1}$) was calculated as 4.55
189 VCO_2 – 3.21 VO_2 – 2.87 n. For these calculations, VO_2 and VCO_2 were expressed in $l.min^{-1}$,
190 and urinary nitrogen excretion (n) was assumed to be 0.11 $mg.kg^{-1}.min^{-1}$ throughout each
191 trial, based on data from previous studies in the literature (Flatt, Ravussin, Acheson, &
192 Jequier, 1985; Melanson, Donahoo, Dong, Ida, & Zemel, 2005). Energy expenditure (in
193 $kJ.min^{-1}$) was calculated as fat oxidation x 39.0 + carbohydrate oxidation x 15.5 + protein
194 oxidation x 17.0, where protein oxidation was estimated to be 6.25 n.
195
196 ***Blood processing and analysis***
197 Venous blood samples were collected into K_2 EDTA tubes, placed immediately on ice, and
198 centrifuged to separate plasma within 15 minutes. Plasma glucose concentrations were
199 measured immediately using a benchtop analyser (YSI 2300 STAT Plus™ Glucose and
200 Lactate Analyser, YSI (UK) Ltd.). The remaining of plasma was stored at -80ºC for later
201 analysis. Insulin concentration was determined using a commercially available ELISA
202 (Mercodia AB, Uppsala, Sweden). TG concentrations were determined by commercially

enzymatic colorimetric kit (Randox Laboratories, Crumlin, UK) using an autoanalyser (ILab^{TM} 600, Clinical Chemistry System, Instrumentation Laboratory, USA).

Power calculation

As the most consistent association between frequency of sedentary breaks and health outcomes related to adiposity variables (Healy et al., 2008; Healy et al., 2011; Cooper et al., 2012), we primarily based our sample size on the number of participants needed to detect a difference in overall energy expenditure over the observation period. Our previous data had shown that the within-person SD for difference in resting oxygen uptake was 6.1% (Farah & Gill, 2013). We assumed that the within-person SD for differences in energy expenditure between trials here would be similar. Accordingly, we calculated that ten participants would enable detection of a ~6% difference in energy expenditure between trials with 80% power at $p < 0.05$. In addition, based on our earlier observations that the within-person SD for postprandial glucose, TG and insulin responses were 3.4%, 10.1% and 22.9%, respectively (Gill et al., 2005), our sample would enable detection of respective differences between trials of ~3%, ~10% and ~23%, in glucose, TG and insulin responses.

Statistical analysis

Statistical analyses were performed using Statistica (Version 10, StatSoft, Inc.) and Minitab (Version 14, Mintab Inc.). Data were tested for normality using the Anderson-Darling normality test, and where necessary, data were logarithmically transformed prior to statistical analysis. The area under curve (AUC), calculated using the trapezium rule was used as a summary measure of the postprandial responses for energy expenditure, fat oxidation and carbohydrate oxidation. This provides a measure of total amount of energy expended or

substrate used over the observation period. For glucose, insulin and TG concentrations, the time-averaged AUC (i.e. AUC divided by the duration of the observation period) was used as a summary measure. This provides a measure of the average concentration over the observation period. AUC was calculated separately for the post-breakfast (0 to 180 mins) and post-lunch (210-390 mins) as well as the overall observation period. Comparisons of summary measures between trials were made by paired t-test. Where appropriate (i.e. when differences were observed in baseline values between conditions) statistical analyses of postprandial responses were adjusted for fasting values. Cohen's d effect sizes were calculated to describe the magnitude of differences between trials (>0.8 large, 0.5-0.8 medium, <0.5 small, <0.2 trivial) (Cohen, 1992). Data are presented as mean ± SEM unless otherwise stated, and $p < 0.05$ was considered significant.

Results

Baseline values

Baseline values in the two trials are shown in Table 1. There were no differences in body mass, fat oxidation or carbohydrate oxidation, or plasma glucose, insulin or TG concentrations between experimental conditions in the fasted state, before the interventions were commenced, but baseline energy expenditure was ~7% higher in the SIT/STAND trial than the SIT trial.

****Table 1 near here****

Energy expenditure and substrate utilisation during the interventions

Figure 2 shows energy expenditure and substrate utilisation over the 6.5-hour observation period, with summary data for these responses shown in Table 2. Compared to the SIT trial, total energy expenditure over the 6.5 hours was 409.7 ± 41.6 kJ (16.6 ± 1.7%) higher in the

SIT/STAND trial ($p<0.0001$). This difference remained statistically significant after adjustment for baseline energy expenditure ($p = 0.0007$). Total carbohydrate oxidation was 21.0 ± 4.5 g (33.9 ± 8.2%) higher in the SIT/STAND trial than the SIT trial ($p = 0.0005$); the difference in total fat oxidation between trial over the 6.5-hour observation period was not statistically significant (2.2 ± 1.3 g (9.7 ± 5.3%) higher in SIT/STAND, $p = 0.11$). As we previously observed differences in the effects of standing on postprandial responses in post-breakfast and post-lunch observation periods (Hawari, Al-Shayji, Wilson, & Gill, 2016), we decided to analyse these periods separately, these summary data are presented in Table 3. Energy expenditure over the both post-breakfast period (0-180 mins) (by 219.3 ± 20.9 kJ (19.9 ± 1.6%)) and post-lunch period (210-390 mins) (by 184.6 ± 21 kJ (15.8 ± 2%)) were both significantly higher in the SIT/STAND than the SIT trial ($p<0.0001$ for both). Similarly, carbohydrate oxidation was higher in the SIT/STAND than the SIT trial over both the post-breakfast (by 9.4 ± 2.2 g (44.1 ± 13.6%)) and post-lunch (by 10.6 ± 2.3 g (31 ± 7.1%)) periods (both $p < 0.001$). Fat oxidation was higher in the SIT/STAND trial than the SIT trial over the post-breakfast period (by 1.9 ± 0.7 g (15.9 ± 5.8%), $p = 0.01$), but did not differ significantly between trials over the post-lunch period ($p = 0.48$).

****Figure 2 near here****

****Table 2 near here****

****Table 3 near here****

Blood glucose, insulin and TG responses during the interventions

Blood glucose, insulin and TG responses over the 8-hour observation period are shown in Figure 3, with summary data for these responses shown in Tables 2 and 3. Postprandial insulin concentrations over the post-breakfast period were 10.9 ± 8.4% lower in the

SIT/STAND trial than the SIT trial ($p = 0.047$), but the insulin response in the post-lunch period, or when taken over the overall 6.5-hour observation period did not differ significantly between the two trials. There were no significant differences between the two trials in glucose and TG responses and Cohen's d effect sizes for both responses were trivial.

****Figure 3 near here****

Discussion

The major finding of the this study is that breaking up prolonged sedentary time with repeated 'chair squats' for 30 seconds every 20 minutes significantly increased energy expenditure by 16.6% over a 6.5-hour observation period during which a test breakfast and test lunch were consumed. Over the 3 hours following breakfast, post-prandial fat oxidation was 15.9% higher and postprandial insulin concentrations were 10.9% lower, but these changes did not persist in to the post-lunch period. There were no differences between the two trials in postprandial glucose or insulin responses.

Epidemiological studies have shown that a high level of sedentary behaviour is associated with increased risk of obesity (Thorp et al., 2011). It is conceivable that this may be mediated, at least in part, by the low energy expenditure associated with sitting. A number of experimental studies have shown that replacing sitting with standing increases energy expenditure over the course of the day (Reiff et al., 2012; Speck & Schmitz, 2011). Building on this work, we recently observed that intermittently standing for 1.5 minutes 10 times every 30 minutes led to 9% higher energy expenditure over an 8-hour postprandial period than standing continuously for 15 minutes every 30 minutes over the same time-frame

(Hawari et al., 2016), indicating that the number of transitions between sitting and standing influenced energy expenditure independently of the overall amount of time spent sitting and standing. In that study there were 144 additional sit-to-stand transitions in the intermittent standing condition and 296 kJ additional energy was expended: from this it was possible to calculate that a sit-to-stand transition expended ~2 kJ of energy. The findings from the present study are consistent with this, energy expenditure was 410 kJ higher in the SIT/STAND compared with the SIT condition and 180 additional sit-to-stand transitions were undertaken in the former – equivalent to 2.3 kJ energy expenditure per transition. Thus, the present data provide confirmation that previously observed differences in energy expenditure between continuous and intermittent standing (Hawari et al., 2016) can be fully accounted for by the energy expended in the transition from sitting to standing and taken together these independent observations provide a robust estimation of energy expended in a sit-to-stand transition cycle.

Previous investigations of the effects of breaking up prolonged sitting with standing have had equivocal results in terms of alterations in glucose and insulin metabolic responses with some (Thorp et al., 2014; Henson et al., 2016; Buckley et al., 2014), but not all (Bailey & Locke, 2015; Hawari et al., 2016) studies observing favourable changes when sitting is replaced by standing. In studies which have assessed postprandial TG responses, replacing sitting with standing has generally not resulted in significant changes (Henson et al., 2016; Hawari et al., 2016). In the present study, we observed that breaking up prolonged sitting by with 10 chair-squats every 20 minutes reduced insulin concentrations in the post-breakfast period, although this did not persist into the post-lunch period. This could conceivably be mediated by the skeletal muscle contractions needed to move between sitting and standing stimulating contraction-mediated glucose uptake (Krook, Wallberg-Henriksson, & Zierath, 2004), thereby reducing the requirement for insulin to maintain glucose homeostasis. Indeed, the

repeated sit-to-stand transitions over 30 seconds, in effect represents multiple sets of bodyweight squats over the course of the day. However, the chair-squat intervention did not significantly affect postprandial glucose or TG concentrations. Interestingly, Dempsey and colleagues recently reported that breaking up prolonged sitting with 3 minutes of bodyweight resistance exercises every 30 minutes over a 7-hour postprandial observation period reduced postprandial glucose, insulin and TG concentrations in adults with type 2 diabetes (Dempsey et al., 2016). This more potent intervention effect in Dempsey's study may reflect two things. First, the volume of resistance exercise undertaken in that study (6 vs 1.5 mins per hour) was substantially higher than in the present study. It may well be that a larger volume of sit-to-stand transitions – for example 60 seconds of 'chair squats', rather than 30 seconds, every 20 minutes – may elicit more substantial effects on postprandial insulin, glucose and TG responses. Secondly, the participants in the present study were normoglycaemic, and it may be the case that the stimulus required to positively affect postprandial metabolic responses may be greater in healthy normoglyaemic individuals than those with metabolic dysfunction where there is greater capacity for improvement. For example, lab-based interventions breaking up sitting with standing have been effective at reducing postprandial glucose and insulin concentrations in post-menopausal women with impaired glucose regulation (Henson et al., 2016), but this effect has not been replicated in similar interventions in younger, normoglycaemic individuals (Bailey & Locke, 2015; Hawari et al., 2016; Miyashita et al., 2013). Thus, going forward, studies are needed i) to determine whether the present intervention is effective at reducing postprandial glucose, insulin and TG responses in individuals with impaired glucose regulation and ii) to determine whether the metabolic benefits observed here would be enhanced in normoglycaemic individuals with an increased 'dose' of 'chair squats'.

The intervention undertaken in the present study is simple, requires no equipment and little space and only takes 1.5 minutes per hour. The additional 410 kJ of energy expended over the course of the trial, would equate to 8.2 MJ over 4 weeks if the intervention was carried out on 5 days of the week, which is equivalent to over 1 kg weight loss. This, together with the modest reductions in postprandial insulin concentrations, suggest that pragmatic, low volume, interventions of this nature may have the potential to elicit benefits to metabolic health. Thus, the 'chair squat' approach used in the present study could potentially be developed into an alternative strategy which would be used as an alternative to, or in combination with, other interventions, such as standing desks, to break up periods of prolonged sitting in individuals, such as office workers, to who need to work at a desk throughout the day. This would require substantial further development, and the present findings provide a rationale for undertaking longer-term randomised controlled trials to determine whether interventions of this nature are acceptable to individuals and sustainable in practice and whether they induce long-term benefits to metabolic health.

This study does have some limitations. Firstly, although it had sufficient power to clearly detect an effect of the intervention on energy expenditure, with 14 participants, it may have been underpowered to detect clear effects on the postprandial insulin response in the post-lunch period. Secondly, we did not consider different doses of sit-to-stand transitions to determine the nature of the dose-response relationship. Further research is required to define whether effects can be generalised to other population such as the non-obese and patients with impaired glucose regulation or type 2 diabetes.

In conclusion, this study demonstrated that a simple, unobtrusive intervention of performing 10 'chair squats' over 30 seconds every 20 minutes over a 6.5-hour observation period increased energy expenditure by over 400 kJ, a 16.6% increase over prolonged sitting on

normoglycaemic overweight and obese men and women. The intervention also reduced insulin concentrations in the post-prandial period following breakfast. Further study is needed to determine whether larger doses of 'chair squats' would induce greater metabolic benefits and whether this approach can be translated into an effective longer-term intervention.

Funding details

NSAH was supported by a Scholarship from the Government of Saudi Arabia.

Disclosure statement

The authors have no conflicts of interest relevant to this work.

References

Bailey, D. P. & 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,* 294-298.

Buckley, J. P., Mellor, D. D., Morris, M., & Joseph, F. (2014). Standing-based office work shows encouraging signs of attenuating post-prandial glycaemic excursion. *Occup.Environ.Med., 71,* 109-111.

Celis-Morales, C. A., Perez-Bravo, F., Ibanez, L., Salas, C., Bailey, M. E., & Gill, J. M. (2012). Objective vs. Self-Reported Physical Activity and Sedentary Time: Effects of Measurement Method on Relationships with Risk Biomarkers. *PLoS.ONE., 7,* e36345.

Cohen, J. (1992). Statistical power analysis. *Curr.Dir.Psychol., 1,* 98-101.

Cooper, A. R., Sebire, S., Montgomery, A. A., Peters, T. J., Sharp, D. J., Jackson, N. et al. (2012). Sedentary time, breaks in sedentary time and metabolic variables in people with newly diagnosed type 2 diabetes. *Diabetologia, 55,* 589-599.

Dempsey, P. C., Larsen, R. N., Sethi, P., Sacre, J. W., Straznicky, N. E., Cohen, N. D. et al. (2016). Benefits for Type 2 Diabetes of Interrupting Prolonged Sitting With Brief Bouts of Light Walking or Simple Resistance Activities. *Diabetes Care, 39,* 964-972.

Edwardson, C. L., Gorely, T., Davies, M. J., Gray, L. J., Khunti, K., Wilmot, E. G. et al. (2012). Association of sedentary behaviour with metabolic syndrome: a meta-analysis. *PLoS.ONE., 7,* e34916.

Ekelund, U., Steene-Johannessen, J., Brown, W. J., Fagerland, M. W., Owen, N., Powell, K. E. et al. (2016). Does physical activity attenuate, or even eliminate, the detrimental

409 association of sitting time with mortality? A harmonised meta-analysis of data from more
410 than 1 million men and women. *Lancet, 388,* 1302-1310.

411 Farah, N. M. & Gill, J. M. (2013). Effects of exercise before or after meal ingestion on fat
412 balance and postprandial metabolism in overweight men. *Br.J Nutr., 109,* 2297-2307.

413 Flatt, J. P., Ravussin, E., Acheson, K. J., & Jequier, E. (1985). Effects of dietary fat on
414 postprandial substrate oxidation and on carbohydrate and fat balances. *J Clin Invest, 76,*
415 1019-1024.

416 Frayn, K. N. & Macdonald, I. A. (1997). Assessment of substrate and energy metabolism in
417 vivo. In B.Draznin & R. Rizza (Eds.), *Clinical Research in Diabetes and Obesity Part I:*
418 *Methods, Assessment, and Metabolic Regulation.* (pp. 101-124). Totowa NJ: Humana Press.

419 Gill, J. M., Malkova, D., & Hardman, A. E. (2005). Reproducibility of an oral fat tolerance
420 test is influenced by phase of menstrual cycle. *Horm.Metab Res., 37,* 336-341.

421 Hawari, N. S., Al-Shayji, I., Wilson, J., & Gill, J. M. (2016). Frequency of Breaks in
422 Sedentary Time and Postprandial Metabolic Responses. *Med.Sci.Sports Exerc., 48,* 2495-
423 2502.

424 Healy, G. N., Dunstan, D. W., Salmon, J., Cerin, E., Shaw, J. E., Zimmet, P. Z. et al. (2008).
425 Breaks in sedentary time: beneficial associations with metabolic risk. *Diabetes Care, 31,* 661-
426 666.

427 Healy, G. N., Matthews, C. E., Dunstan, D. W., Winkler, E. A., & Owen, N. (2011).
428 Sedentary time and cardio-metabolic biomarkers in US adults: NHANES 2003-06. *Eur.Heart*
429 *J, 32,* 590-597.

Henson, J., Davies, M. J., Bodicoat, D. H., Edwardson, C. L., Gill, J. M., Stensel, D. J. et al. (2016). Breaking Up Prolonged Sitting With Standing or Walking Attenuates the Postprandial Metabolic Response in Postmenopausal Women: A Randomized Acute Study. *Diabetes Care, 39,* 130-138.

Krook, A., Wallberg-Henriksson, H., & Zierath, J. R. (2004). Sending the signal: molecular mechanisms regulating glucose uptake. *Med.Sci.Sports Exerc., 36,* 1212-1217.

Marfell-Jones, M., Olds, T., Stewart, A., & Carter, L. (2006). *International standards for anthropometric assessment*. Potchefstroom, South Africa: ISAK.

Melanson, E. L., Donahoo, W. T., Dong, F., Ida, T., & Zemel, M. B. (2005). Effect of low- and high-calcium dairy-based diets on macronutrient oxidation in humans. *Obes.Res., 13,* 2102-2112.

Miyashita, M., Park, J. H., Takahashi, M., Suzuki, K., Stensel, D., & Nakamura, Y. (2013). Postprandial lipaemia: effects of sitting, standing and walking in healthy normolipidaemic humans. *Int.J.Sports Med., 34,* 21-27.

Reiff, C., Marlatt, K., & Dengel, D. R. (2012). Difference in caloric expenditure in sitting versus standing desks. *J Phys.Act.Health, 9,* 1009-1011.

Speck, R. M. & Schmitz, K. H. (2011). Energy expenditure comparison: a pilot study of standing instead of sitting at work for obesity prevention. *Prev.Med, 52,* 283-284.

Thorp, A. A., Kingwell, B. A., Sethi, P., Hammond, L., Owen, N., & Dunstan, D. W. (2014). Alternating bouts of sitting and standing attenuate postprandial glucose responses. *Med.Sci.Sports Exerc., 46,* 2053-2061.

Thorp, A. A., Owen, N., Neuhaus, M., & Dunstan, D. W. (2011). Sedentary behaviors and subsequent health outcomes in adults a systematic review of longitudinal studies, 1996-2011. *Am.J.Prev.Med., 41*, 207-215.

Tremblay, M. S., Aubert, S., Barnes, J. D., Saunders, T. J., Carson, V., Latimer-Cheung, A. E. et al. (2017). Sedentary Behavior Research Network (SBRN) - Terminology Consensus Project process and outcome. *Int.J.Behav.Nutr.Phys.Act., 14*, 75.

Wilmot, E. G., Edwardson, C. L., Achana, F. A., Davies, M. J., Gorely, T., Gray, L. J. et al. (2012). Sedentary time in adults and the association with diabetes, cardiovascular disease and death: systematic review and meta-analysis. *Diabetologia, 55*, 2895-2905.

Figure Legends

Figure 1. Study protocol. Participants completed two trials in random order: Uninterrupted sitting (SIT) and sitting broken up with 10 ‘chair squats’ every 20 minutes (SIT/STAND).

Figure 2. Energy expenditure (panel a), fat oxidation (panel b) and carbohydrate oxidation (panel c) over the 6.5-hour observation period. Values are mean ± SEM. Boxes indicate test breakfast and test lunch.

Figure 3. Glucose (panel a), insulin (panel b) and triglyceride (panel c) responses over the 6.5-hour observation period. Values are mean ± SEM. Boxes indicate test breakfast and test lunch.

Table 1. Baseline values in the fasted state in the two experimental conditions.

	SIT	SIT/STAND	*p*
Body mass (kg)	92.5 ± 3.8	92.5 ± 3.8	0.93
Energy expenditure ($kJ.min^{-1}$)	5.44 ± 0.22	5.85 ± 0.26	0.01
Fat oxidation ($g.min^{-1}$)	0.08 ± 0.01	0.09 ± 0.01	0.18
Carbohydrate oxidation ($g.min^{-1}$)	0.07 ± 0.02	0.08 ± 0.02	0.89
Plasma glucose ($mmol.l^{-1}$)	4.9 ± 0.1	5.0 ± 0.1	0.47
Plasma insulin ($mU.l^{-1}$)	12.0 ± 1.1	12.6 ± 1.4	0.52
Plasma TG ($mmol.l^{-1}$)	1.28 ± 0.13	1.23 ± 0.13	0.46

Values are mean ± SEM, n = 14

Table 2. Summary postprandial responses over post-breakfast, post-lunch and overall 6.5-hour postprandial observation period in the two experimental conditions.

	Overall postprandial response (0 to 390 mins)			
	SIT	**SIT/STAND**	***p***	**Cohen's d effect size**
Total Energy Expenditure (kJ)	2503 ± 105	2912 ± 123	<0.0001	2.55
Total Fat Oxidation (g)	26.7± 2.2	28.9 ± 2.4	0.11	0.67
Total CHO Oxidation (g)	67.1 ± 5.5	88.0 ± 7.1	0.0005	1.17
Plasma Glucose ($mmol.l^{-1}$)	5.89 ± 0.24	5.90 ± 0.17	0.94	0.05
Plasma Insulin ($mU.l^{-1}$)	86.1 ± 13.8	75.2 ± 10.1	0.10	0.38
Plasma TG ($mmol.l^{-1}$)	1.71 ± 0.21	1.68 ± 0.21	0.71	0.16

Values are mean ± SEM, n=14. Plasma glucose, insulin and TG concentrations are time-averaged mean postprandial values over the post-breakfast, post-lunch and overall postprandial observation periods.

Table 3. Summary postprandial responses over post-breakfast, post-lunch postprandial observation period in the two experimental conditions.

	Post-Breakfast period (0 to 180 mins)				**Post-Lunch period (210-390 mins)**			
	SIT	**SIT/STAND**	***p***	**Cohen's d effect size**	**SIT**	**SIT/STAND**	***p***	**Cohen's d effect size**
Total Energy Expenditure (kJ)	1106 ± 47	1325 ± 59	< 0.0001	2.81	1202 ± 52	1387 ± 57	<0.0001	2.35
Total Fat Oxidation (g)	13.4 ± 1.1	15.3 ± 1.3	0.01	1.87	11.3 ± 1.0	11.7 ± 1.1	0.48	0.20
Total CHO Oxidation (g)	25.1 ± 2.9	34.5 ± 3.6	0.001	1.15	36.6 ± 2.4	47.2 ± 3.2	0.001	1.22
Plasma Glucose ($mmol.l^{-1}$)	6.18 ± 0.29	6.11 ± 0.21	0.72	0.10	5.82 ± 0.24	5.87 ± 0.2	0.75	0.09
Plasma Insulin ($mU.l^{-1}$)	91.7 ± 14.7	75.5 ± 10.9	0.047	0.58	87.5 ± 14.6	79.8 ± 11.0	0.21	0.35
Plasma TG ($mmol.l^{-1}$)	1.43 ± 0.15	1.38 ± 0.16	0.53	0.17	2.0 ± 0.27	2.0 ± 0.26	0.98	0.01

Values are mean ± SEM, n=14. Plasma glucose, insulin and TG concentrations are time-averaged mean postprandial values over the post-breakfast, post-lunch and overall postprandial observation periods.

Figure 1

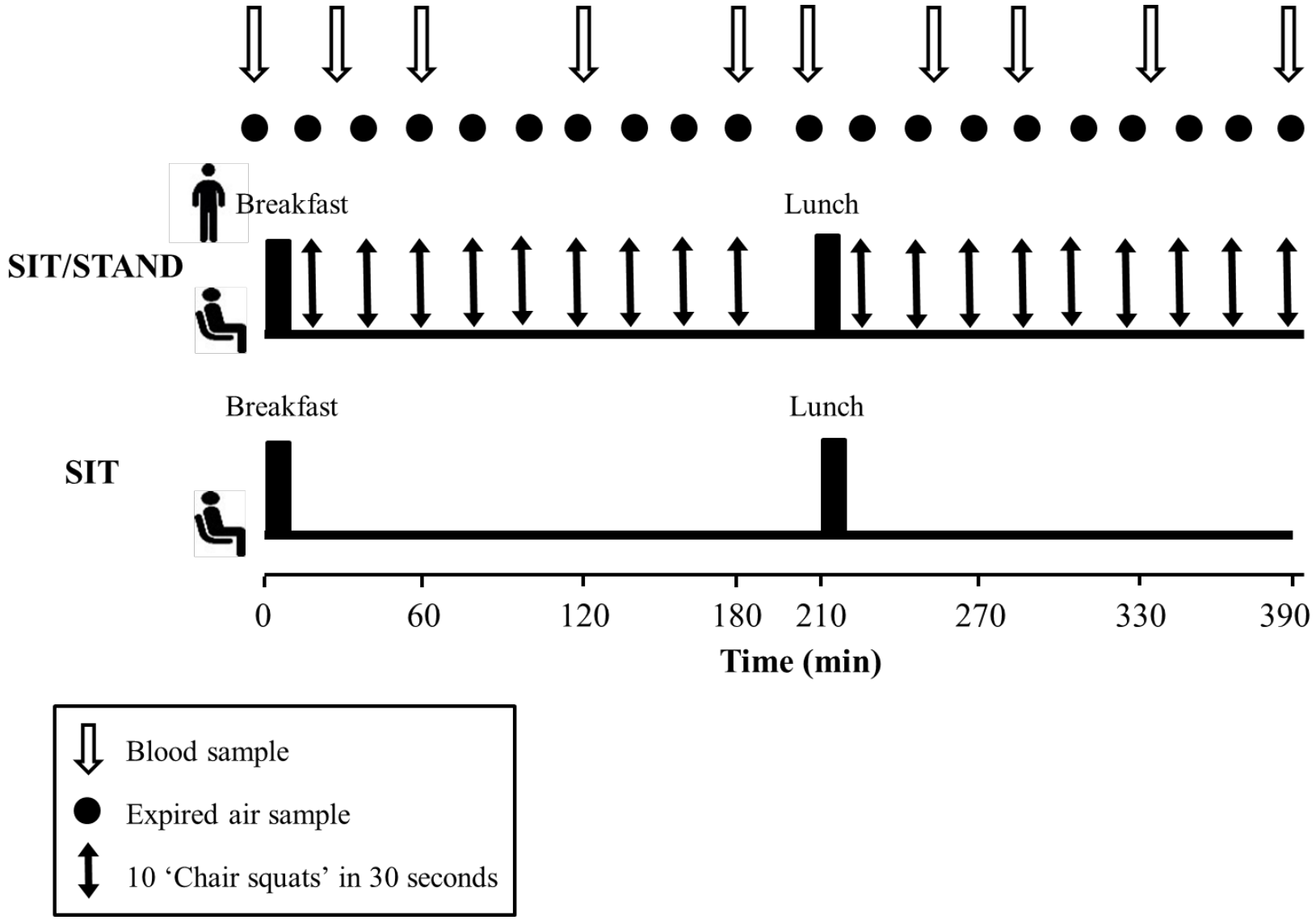

Breakfast
Lunch
SIT/STAND
Breakfast
Lunch
SIT
0
60
120
180
210
270
330
390
Time (min)
Blood sample
Expired air sample
10 'Chair squats' in 30 seconds

Figure 2

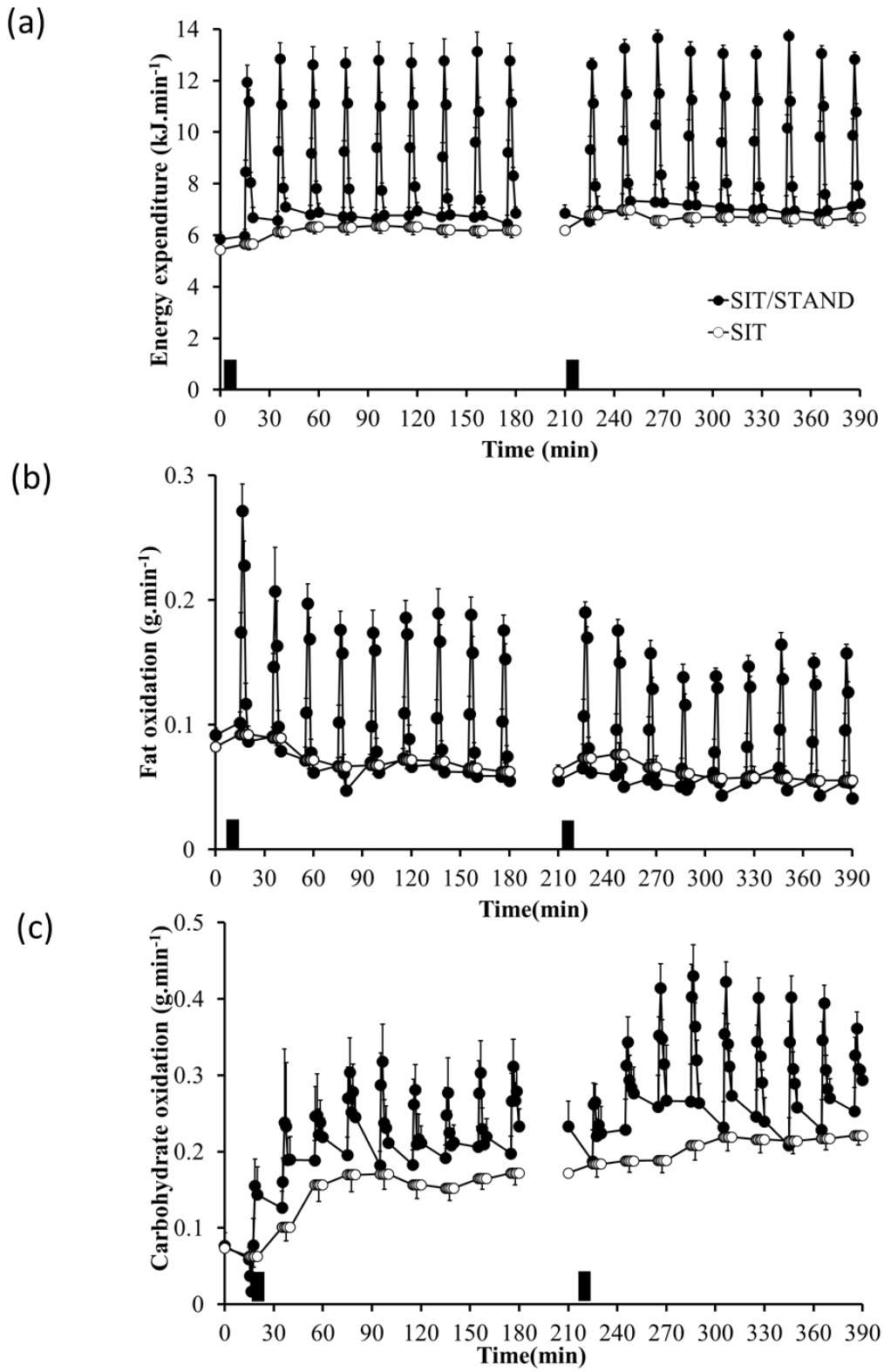
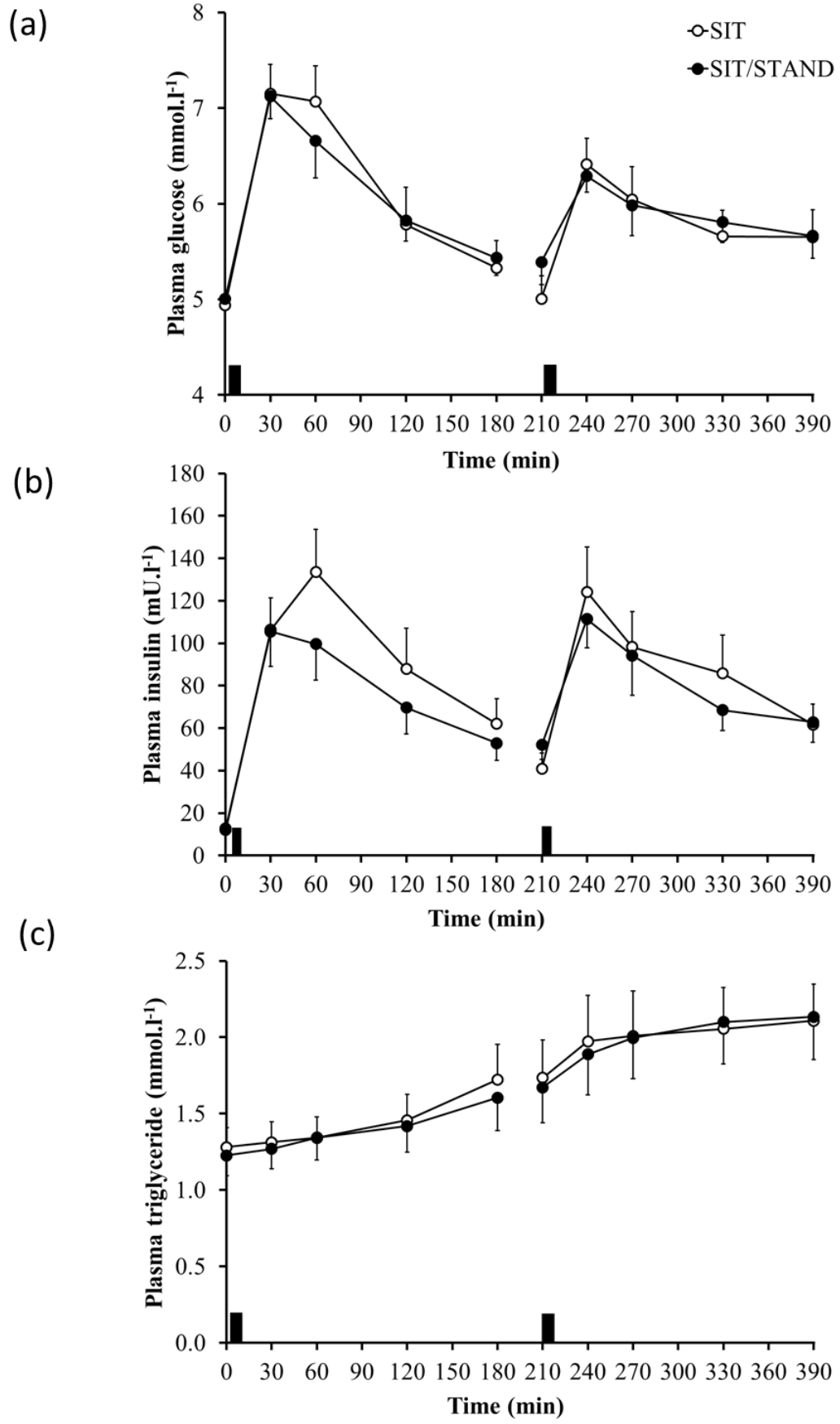

(a)
Energy expenditure (kJ.min-1)
SIT/STAND
SIT
Time (min)
(b)
Fat oxidation (g.min-1)
Time(min)
(c)
Carbohydrate oxidation (g.min-1)
Time(min)

Figure 3


(a)
SIT
SIT/STAND
Plasma glucose (mmol.l-1)
8
7
6
5
4
0 30 60 90 120 150 180 210 240 270 300 330 360 390
Time (min)
(b)
Plasma insulin (mU.l-1)
180
160
140
120
100
80
60
40
20
0
0 30 60 90 120 150 180 210 240 270 300 330 360 390
Time (min)
(c)
Plasma triglyceride (mmol.l-1)
2.5
2.0
1.5
1.0
0.5
0.0
0 30 60 90 120 150 180 210 240 270 300 330 360 390
Time (min)