FINAL VERSION ACCEPTED FOR PUBLICATION IN MEDICINE AND SCIENCE IN SPORTS AND EXERCISE

Frequency of breaks in sedentary time and postprandial metabolic responses

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Short title: Sedentary breaks and postprandial metabolism

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Abstract

Purpose: To compare the metabolic effects of breaking up sedentary time with prolonged periods of standing versus multiple shorter standing bouts with the same total duration to determine whether, in principle, altering the frequency of 'standing breaks' in sedentary time, influences metabolic responses over the course of the day.

Methods: Ten normoglycaemic overweight/obese men (age 33±13 years; BMI 28.3±3.0 $kg.m^2$; mean±SD) each participated in three experimental trials in random order, in which they arrived fasted, then consumed a test breakfast (8 $kcal.kg^{-1}$ body weight, with 37% energy from fat, 49% from carbohydrates, 14% from protein) and, 4 hours later, an identical test lunch. Expired air and blood samples were taken fasted and for eight hours postprandially. In one trial (SIT) participants sat continuously throughout the observation period; in the prolonged standing trial (PRO-Stand), participants stood still for 15 minutes every 30 minutes; and in the intermittent standing trial (INT-Stand), they stood for 1.5 minutes, 10 times every 30 minutes.

Results: Compared to SIT energy expenditure was 320±62 kJ (10.7±2.0%) higher in PRO-Stand and 617±76 kJ (20.4±2.3%) higher in INT-Stand: energy expenditure in INT-Stand was 296±78 kJ (9.0±2.3%) higher than PRO-Stand (mean±SEM; all $p<0.001$). However, there were no significant differences between trials in postprandial glucose, insulin or triglyceride responses.

Conclusions: These data demonstrate an independent effect of frequency of sedentary breaks on energy expenditure which provides an explanation for the association between frequency of sedentary breaks and adiposity observed in epidemiological data. However, it may be necessary to break up sitting with activities of greater intensity than quiet standing to positively influence glucose, insulin and triglyceride metabolism in relatively young, normoglycaemic overweight/obese men.

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

Introduction

There is a large body of observational data showing strong associations between time spent engaged in sedentary behaviour – defined as non-sleeping activities in a sitting or reclining posture with energy expenditure ≤1.5 METS (where 1 MET is resting energy expenditure) (25) – and a number of adverse health outcomes, including mortality, cardiovascular disease, type 2 diabetes and obesity (5, 12, 29, 30). These relationships are often independent of time spent engaged in moderate-to-vigorous physical activity (>3 METS) (5, 12, 29, 30). In addition, recent observational data in almost 700 adults from the AusDiab study, using a postural sensor to objectively monitor time spent sitting, standing and stepping, suggested that isotemporally replacing sitting with standing was associated with favourable changes to glucose and lipid metabolism (13). There is also observational evidence to suggest that individuals who break up sedentary time more frequently have a more favourable cardio-metabolic risk profile – particularly with respect to adiposity variables – than those who habitually engage in prolonged periods of uninterrupted sedentary time, independent of total time spent sedentary (3, 11, 12). However, the mechanisms by which more frequent breaks in sedentary time may impart these benefits, independent of total sedentary time, are unclear. A number of short-term intervention studies have shown that interrupting sedentary periods with multiple short (≤3 min) bouts of light or moderate activity throughout the day can reduce postprandial glucose, insulin and triglyceride (TG) responses, and blood pressure, on the same or following day (4, 18, 22, 23). Other studies have shown that interrupting prolonged sitting with periods of static standing ranging from five minutes every 30 minutes (14) to 30 minutes every hour (28), can reduce postprandial glucose concentrations. However, in all of these studies sedentary time was replaced by standing or walking leading to a reduction in total time spent sedentary, so the effects of altering the frequency of breaks in sedentary time, independent of changing total time sedentary, on these metabolic responses

are not known. It is also not known whether altering the frequency of breaks in sedentary time influences metabolic rate and substrate utilisation, which may provide an explanation for the association between frequency of sedentary breaks and adiposity observed in the epidemiological data (3, 11, 12). The aim of this study was therefore to compare the metabolic effects of breaking up sedentary time with prolonged periods of standing *versus* multiple shorter standing bouts with the same total duration to determine whether – in principle – altering the frequency of breaks in sedentary time, influences metabolic responses over the course of the day.

Methods

Participants

Ten men, aged 33 ± 13 years, with body mass index (BMI) 28.3 ± 2.8 $kg.m^{-2}$, waist circumference 100.2 ± 9.5 cm [mean ± SD], and low levels of habitual physical activity (less than 2 hours per week of moderate-to-vigorous physical activity as assessed by the International Physical Activity Questionnaire), were recruited for this study though personal contacts and local advertising. All participants had BMI >25 $kg.m^{-2}$, were non-smokers, had no known history of CVD or diabetes (and fasting glucose <6.0 $mmol.l^{-1}$ on screening), and were not taking any medications known to affect lipid or glucose metabolism. 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.

Study design

Participants each completed three 8-hour experimental trials; uninterrupted sitting (SIT), prolonged standing (PRO-Stand), and intermittent standing (INT-Stand) in a randomised

order, with an interval of 1-2 weeks between trials. Participants were asked to weigh and record their food intake and refrain from planned exercise (undertaking only the activities of normal daily living) and alcohol on the two days preceding their first main experimental trial and to replicate this for the two days preceding subsequent trials. Physical activity and sedentary behaviour during these days was assessed using ActivPAL accelerometers (PAL Technologies Ltd., Glasgow, UK). The experimental protocol is shown in Figure 1 and described below.

Experimental protocol

Uninterrupted sitting trial (SIT): Participants arrived at the metabolic suite after a 12-hour overnight fast. They sat comfortably for 10 minutes, before two sequential 5-minute expired air samples were collected via a mouthpiece into a Douglas bag to calculate metabolic rate and substrate utilisation using indirect calorimetry (9). The second of these samples was used as the baseline value. A cannula was then inserted into an antecubital vein for repeated blood sampling, with was kept patent by flushing with saline throughout the day. A baseline fasting blood sample was drawn in 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, 180 and 240 minutes after breakfast. Four hours after breakfast, participants consumed a test lunch, which was identical to breakfast, and further blood samples were taken 30, 60, 120, 180 and 240 minutes after lunch (i.e. 270, 300, 360, 420 and 480 minutes after breakfast). Expired air samples for the determination of metabolic rate and substrate utilisation were collected at 15-minute intervals throughout the 8-hour observation period. Participants sat comfortably (reading,

watching TV, doing paperwork etc) throughout the observation period and were permitted to drink water throughout the day. Comfort breaks to the toilet (which was ~20m from the metabolic investigation suite) were permitted.

Prolonged standing trial (PRO-Stand): This was identical to the SIT trial, except that in each 30-minute period throughout the day, participants were asked to sit for 15 minutes and stand stationary for 15 minutes, so that in total they stood for 4 hours and sat for 4 hours, with 16 sit-to-stand and 16 stand-to-sit transitions over the 8-hour observation period. All blood samples were taken during 15-minute sitting periods.

Intermittent standing trial (INT-Stand): This was identical to the SIT trial, except that in each 30-minute period, participants sat for 5 minutes; then undertook 10 cycles of standing for 90 seconds followed by sitting for 30 seconds (20 minutes in total); then sat for 5 minutes. Thus they stood for 15 minutes and sat for 15 minutes every 30 minutes, but the standing occurred in 10 x 90-second blocks, rather than a single 15-minute block. Thus, over the 8-hour observation period they stood for 4 hours and sat for 4 hours, with 160 sit-to-stand and 160 stand-to-sit transitions. All blood samples were taken during the 10 minutes of continuous sitting in each 30-minute period.

Calculation of energy expenditure and substrate utilization

Energy expenditure and energy substrate utilisation were calculated using indirect calorimetry (9). For these calculations urinary nitrogen excretion was assumed to be 0.11 $mg.kg^{-1}.min^{-1}$ throughout each trial, based on data from previous studies in the literature (8, 21).

Blood processing and analysis

Venous blood samples were collected into K_2 EDTA tubes, placed immediately on ice, and centrifuged to separate plasma within 15 minutes. Plasma glucose concentrations were measured immediately using a benchtop analyser (YSI 2300 STAT Plus™ Glucose and Lactate Analyser, YSI (UK) Ltd.). The remaining of plasma was stored at -80ºC for later analysis. Insulin concentration was determined using a commercially available ELISA (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 (3, 11, 12), 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% (6). 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 (10), 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. Comparisons between trials were made using repeated measures ANOVA, with post-hoc Fisher LSD tests used to identify where any differences lay. 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) (2). Data are presented as mean ± SEM unless otherwise stated, and $p < 0.05$ was considered significant.

Results

Baseline values

There were no differences in body mass, rates of energy expenditure, fat oxidation or carbohydrate oxidation, or plasma glucose, insulin or TG concentrations between the three experimental conditions in the fasted state, before the interventions were commenced (Table 1), indicating that control of lifestyle in the days preceding the trials was sufficient to ensure that the baseline metabolic state in all trials was the same.

Energy expenditure and substrate utilisation during the interventions

Energy expenditure and substrate utilisation over the 8-hour observation period are shown in Figure 2, with summary data for these responses shown in Table 2. Compared to the SIT trial total energy expenditure over the 8 hours was 320 ± 62 kJ (10.7 ± 2.0%) higher in the PRO-Stand trial and 617 ± 76 kJ (20.4 ± 2.3%) higher in the INT-Stand trial: energy expenditure in the INT-Stand trial was 296 ± 78 kJ (9.0 ± 2.3%) higher than the PRO-Stand trial (all $p<0.001$). The Cohen's d effect sizes for all of these differences were large. Total fat

oxidation over the observation period was 7.1 ± 1.9 g (20.2 ± 6.7%) greater in the INT-Stand trial than the SIT trial ($p<0.01$), with a large effect size, but the 2.5 ± 2.2 g (7.6 ± 5.4%) difference in fat oxidation between the PRO-Stand and SIT trials was not statistically significant and the effect size was small. Total fat oxidation was 4.6 ± 2.6 g (13.7 ± 7.6%) greater in the INT-Stand trial than the PRO-Stand trial ($p=0.06$), with a large effect size. Compared to the SIT trial, total carbohydrate oxidation was 14.4 ± 5.2 g (30.8 ± 12.6%) higher in the PRO-Stand trial ($p<0.05$) and 22.0 ± 6.0 g (44.0 ± 12.8%) higher in the INT-Stand trial ($p<0.01$). The difference in carbohydrate oxidation between the INT-Stand and PRO-Stand trials (7.6 ± 7.8 g; 15 ± 12.4%) was not statistically significant and had a small effect size. In *post-hoc* observations, it became apparent that the pattern of substrate utilization between trials differed between the post-breakfast (0-240 minute) and post-lunch (240-480 minute) postprandial observation periods. We therefore decided to analyse these periods separately. In the post-breakfast period 19.6 ± 1.5 g, 20.1 ± 1.5 g, and 25.0 ± 1.8 g of fat were oxidised in the SIT, PRO-Stand and INT-Stand trials, respectively. Fat oxidation over this period was significantly higher in the INT-Stand trial than the other two trials ($p < 0.001$ for both), but did not differ significantly between the SIT and PRO-Stand trials ($p = 0.68$). In contrast, fat oxidation over the post-lunch period did not differ significantly between any of the trials (SIT: 18.9 ± 1.4 g; PRO-Stand: 20.8 ± 1.8 g; INT-Stand: 20.5 ± 1.4 g). In the post-breakfast period, carbohydrate oxidation was significantly higher than SIT (31.2 ± 3.2 g) in the PRO-Stand stand trial (41.0 ± 3.3 g) ($p = 0.007$) and tended to be higher than SIT in the INT-Stand trial (38.0 ± 2.7 g) ($p = 0.055$), but did not differ significantly between the PRO-Stand and INT-Stand trials ($p = 0.36$). Carbohydrate oxidation was significantly higher in the INT-Stand trial (48.1 ± 3.6 g) than both the SIT trial (32.8 ± 3.1 g) ($p = 0.002$) and the PRO-Stand trial (37.4 ± 3.4 g) ($p = 0.02$) but did not differ significantly between the SIT and PRO-Stand trials ($p = 0.30$). Thus, the increment in energy expenditure in the INT-Stand trial over

the PRO-Stand trial was largely mediated by an increase in fat oxidation in the post-breakfast period and an increase in carbohydrate oxidation in the post-lunch period.

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 Table 2. There were no significant differences between the three trials in glucose, insulin and TG responses. The effect sizes for the differences between trials in the insulin and TG responses were trivial to small. Although not statistically significant, a medium effect size was observed when comparing the glucose response in the PRO-Stand trial with the SIT trial ($p = 0.16$) and the INT-Stand trial ($p = 0.48$).

Discussion

The main finding of the this study is that increasing the frequency of breaks in sedentary time, while keeping total sedentary time constant, increased energy expenditure and fat oxidation over an 8-hour postprandial observation period. This is the first time that an independent effect of the number of sedentary breaks on day-long metabolic responses has been demonstrated and these findings provide an explanation for the association between frequency of sedentary breaks and adiposity observed in the epidemiological data (3, 11, 12).

A number of studies have reported that energy expenditure during quiet standing is 2-33% higher than observed during sitting (16, 19, 24, 27). The present findings are consistent with this. In the PRO-Stand condition – where participants alternated 15 minutes of sitting with 15 minutes of standing throughout the observation period – energy expenditure was 10.7%

higher than the SIT condition, an absolute increase in expenditure of 320 kJ over 8 hours. In the INT-Stand condition – where participants undertook 10 1.5-minute bout of standing in every half-hour – there was a further increase in energy expenditure of 9.0% (296 kJ), despite participants sitting and standing for the same total duration in both trials. To put these figures into context, if participants replicated the protocol in the trial for 4 weeks, energy expenditure in the PRO-Stand and INT-Stand conditions would be 9.0 MJ and 17.3 MJ higher than the SIT condition. Assuming no change in energy intake, this would equate to ~1.2 kg weight loss, relative to SIT, in the PRO-Stand condition and a ~2.2 kg weight loss in the INT-Stand condition. Interestingly a large proportion of the increase in energy expenditure from increasing the frequency of sedentary breaks was in fat oxidation. Participant oxidised 7.1 g more fat and 7.7 g more carbohydrate in the INT-Stand compared with the PRO-stand trials, which equates to 277 kJ increased fat and 131 kJ increased carbohydrate oxidation in terms of energy. This disproportionate increase in fat oxidation with increasing sit-to-stand transitions may have implications for the long-term regulation of body weight as high levels of fat oxidation have been shown to be protective against long-term weight gain, independent of metabolic rate (20, 26, 31).

The increased energy expenditure in the INT-Stand compared with the PRO-Stand condition, was likely mediated by the increased concentric and eccentric muscular activity associated with the larger number of sit-to-stand transitions. A recent study by Judice and colleagues (16) attempted to quantify the energy expended in sit-to-stand transitions per se by comparing the energy expended over 10 minutes when participants stood and sat down immediately once per minute for10 minutes with 10 minutes of sitting, reporting the energy cost of a single sit-to-stand transition was ~0.02 kJ per kg body mass. In the present study, participants stood for 4 hours and sat for 4 hours, with 16 sit-to-stand (and 16 stand-to-sit) transitions in

the PRO-Stand condition and stood and sat for the same duration but with 160 sit-to-stand (and 160 stand-to-sit) transitions in the INT-Stand condition. Thus, the 296 kJ difference in energy expenditure represents the energy expended in 144 sit-to-stand/stand-to-sit transitions, i.e. ~2 kJ per transition or ~0.02 kJ per kg, in line with Judice et al's calculations. Thus the present findings suggest that the 'snapshot' calculation of the energy expended during short-duration sit-to-stand transitions in the fasted state, can be extended over the course of a day under 'real-life' postprandial conditions.

We found no significant effects of either prolonged or intermittent standing breaks on postprandial incremental glucose, insulin or TG responses. The effect sizes for the difference in incremental insulin and TG responses between trials were trivial to small. Thus the lack of a statistically significant effect of prolonged or intermittent standing on these responses appears to reflect the absence of a physiologically important influence of the standing interventions on these outcomes, rather than a lack of statistical power to detect a clinically relevant effect. The postprandial glucose response was ~3% lower in the PRO-Stand trial, but ~1% *higher* in the INT-Stand, than the SIT trial. Neither of these differences were statistically significant, but there was a medium effect size for the difference between the PRO-Stand and SIT conditions, suggesting that this difference could conceivably be physiologically relevant, but that the study did not have sufficient statistical power to detect it. However, while we cannot definitively exclude a potential glucose-lowering effect of PRO-Stand – albeit a relatively modest one – it is intriguing that a similar pattern was not observed for INT-Stand, where the glucose response was not lower than the SIT condition. This could conceivably be a consequence of the concentric and eccentric muscular activity associated with the repeated sit-to-stand and stand-to-sit transitions in INT-Stand condition, which are essentially equivalent to performing 160 bodyweight squats over the observation

period. Thus the INT-Stand condition could be considered analogous to a session of resistance exercise spread over a number of hours. While resistance exercise training programmes have been shown to improve insulin sensitivity and reduce glucose concentrations over the long-term, particularly in people with type 2 diabetes (15), there is evidence of a transient increase in plasma glucose concentrations in response to resistance exercise (7, 17). Thus it is conceivable that an acute muscle contraction-mediated glucose-raising effect could have offset any potential glucose-lowering effect of standing *per se* in the INT-Stand condition. Further work is therefore needed to confirm whether this hypothesis is correct and, importantly, to determine whether over the longer-term, adaptations in skeletal muscle in response to such repeated contractions could elicit favourable effects of high frequency breaks in sedentary behaviour on glucose metabolism.

A number of previous reports have demonstrated that breaking up continuous sitting time with ≤3-minute intervals of light or moderate intensity physical activity every 20-30 minutes can reduce postprandial glucose, insulin and TG concentrations (4, 18, 22, 23). Studies evaluating the effects of breaking up sitting with static standing on these postprandial blood responses have had more mixed results. Henson and colleagues (14) recently reported that in postmenopausal women (mean age 66 years) with impaired glucose regulation, breaking up sitting time with 5 minutes of quiet standing every 30 minutes over a 7.5-hour postprandial observation period reduced the glucose and insulin incremental AUCs by 34% and 20%, respectively, with no significant effect on the postprandial TG response. In an intervention by Thorp and colleagues (28), in which overweight/obese middle-aged participants (mean age 48 years) performed normal work tasks over an 8-hour workday either seated or alternating 30 minutes of sitting and 30 minutes of standing using a sit-to-stand workstation, the incremental glucose response was 11% lower in the sit-to-stand condition, but there was no

significant effect of the intervention on insulin or TG responses. In contrast, Bailey and Locke (1) recently reported that in young (mean age 24 years) non-obese adults, breaking up prolonged sitting with 2 minutes of standing every 20 minutes had no effect on postprandial glucose or TG responses over a 5-hour period, but breaking up sitting with 2 minute breaks of light ambulation (3.2 km/h walking) every 20 minutes reduced glucose (but not TG) responses by ~16%. In the present study we found no significant effects of either prolonged or intermittent standing breaks on postprandial incremental glucose, insulin or TG responses in our group of relatively young (mean age 33 years), overweight/obese, normoglycaemic men, although we could not definitely exclude a modest potential glucose-lowering effect in the PRO-Stand condition. Thus, no intervention study has observed a statistically significant acute effect of standing on postprandial insulin or TG concentrations in normoglycemic adults – in contrast to the findings of studies where sitting was broken up by light to moderate physical activity (4, 18, 22, 23) – suggesting that a greater stimulus than standing is needed to positively alter these responses in young to middle-aged adults without pre-existing dysglycaemia. Observational data from AusDiab study of middle-aged and older adults (mean age 57.9 years) reported that reallocation of 2 hours of sitting with 2 hours of standing per day was associated with ~2% lower fasting glucose and ~11% lower fasting TG concentration (13). While the causality and direction of these associations cannot be confirmed from such a cross-sectional analysis, these data do raise the possibility that metabolic benefits of standing may be more clearly observed in interventions undertaken in an older population. Further study is therefore needed to determine i) whether interventions to replace sitting with standing improve postprandial glucose, insulin and TG metabolism in older individuals, and ii) whether interventions to increasing the frequency of interruptions to sitting might enhance the previously reported benefits of standing breaks on postprandial glucose, insulin and TG metabolism in those with glucose dysregulation (14).

In conclusion, this study was designed to determine whether, in principle, the number of transitions between sitting and standing could influence postprandial metabolic responses independent of total time spent sitting and standing. Our data clearly indicate that the frequency of interruptions to sedentary time has a marked independent influence on metabolic rate, which is likely due to the increased energy expended due to muscular contractions in the sit-to-stand and stand-to-sit transitions. Each additional sit-to-stand transition cycle expended ~2 kJ energy, which can help explain the epidemiological observation between sedentary breaks and adiposity (3, 11, 12). While our INT-Stand protocol, with 20 sit-to-stand transition cycles per hour is clearly impractical to implement in 'real world' settings, these findings can help inform the design of practical interventions to reduce sedentary behaviour. For example, performing 4 sit-to-stand transition cycles per hour (i.e. standing then sitting once every 15 minutes) over the course of the waking day would lead to ~100-120 kJ of additional daily energy expenditure over and above the increment in metabolic rate elicited by standing *per se*. We found no evidence that standing, either in prolonged bouts or intermittent bouts could influence postprandial insulin or TG responses in these normoglycaemic participants (although we cannot definitively exclude a potential modest glucose lowering effect of prolonged standing from the present data) suggesting that it may be necessary to break up sitting with activities of greater intensity than quiet standing to positively influence postprandial metabolism in relatively young, normoglycaemic overweight/obese men.

Acknowledgments

NSAH was supported by a Scholarship from the Government of Saudi Arabia. We would like to thank Ms Hannah Martin, Ms Sandra Perry, Ms Claire Robertson and Mr Kyriakos Tsitoglou for assistance with data collection.

Conflict of Interest

The authors have no relevant conflicts of interest. The results of the present study do not constitute endorsement by ACSM.

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Figure Legends

Figure 1. Study protocol. Participants completed three trials in random order: Uninterrupted sitting (SIT), Prolonged standing (PRO-Stand), and Intermittent standing (INT-Stand). The grey boxes represent each 30-minute intervention period throughout the day, with the protocol undertaken during each 30-minute period expanded below.

Figure 2. Energy expenditure (panel a), fat oxidation (panel b) and carbohydrate oxidation (panel c) over the 8-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 8-hour observation period. Values are mean ± SEM. Boxes indicate test breakfast and test lunch.

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

	SIT	PRO-Stand	INT-Stand
Body mass (kg)	89.9 ± 3.4	89.8 ± 3.4	89.7 ± 3.3
Energy expenditure ($kJ.min^{-1}$)	5.61 ± 0.18	5.39 ± 0.16	5.41 ± 0.19
Fat oxidation ($g.min^{-1}$)	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01
Carbohydrate oxidation ($g.min^{-1}$)	0.10 ± 0.02	0.11 ± 0.01	0.08 ± 0.01
Plasma glucose ($mmol.l^{-1}$)	5.2 ± 0.2	5.2 ± 0.1	5.4 ± 0.2
Plasma insulin ($mU.l^{-1}$)	7.4 ± 0.9	8.1 ± 1.4	8.8 ± 1.7
Plasma TG[a] ($mmol.l^{-1}$)	1.18 ± 0.24	1.24 ± 0.14	1.16 ± 0.21

Values are mean ± SEM, n = 10. [a]statistics performed on log-transformed data. There were no significant differences in any variable between trials

Table 2. Summary postprandial responses over the 8-hour postprandial observation period in the three experimental conditions.

	SIT	PRO-Stand	INT-Stand	SIT *vs* PRO-Stand	SIT *vs* INT-Stand	PRO-Stand *vs* INT-Stand
	Mean ± SEM			*Effect size*		
Total energy expenditure (kJ)	2980 ± 78	3301 ± 112	3597 ± 139	1.64***	2.56***	1.19***
Total fat oxidation (g)	38.4 ± 2.7	40.9 ± 2.9	45.5 ± 3.0	0.36	1.19**	0.54[#]
Total carbohydrate oxidation (g)	64.1 ± 5.9	78.4 ± 5.6	86.1 ± 5.5	0.87*	1.17**	0.31
Plasma glucose AUC ($mmol.l^{-1}.h$)	47.3 ± 1.4	46.0 ± 1.5	47.9 ± 1.9	0.78	-0.19	-0.61
Plasma insulin AUC ($mU.l^{-1}.h$)	355 ± 47	335 ± 60	329 ± 45	0.23	0.24	0.06
Plasma TG AUC[a] ($mmol.l^{-1}.h$)	14.3 ± 2.4	15.0 ± 1.6	13.5 ± 1.9	-0.17	0.23	0.38

Values are mean ± SEM, n = 10. [a]statistics performed on log-transformed data. [#]p=0.06, *p < 0.05, **p<0.01, ***p<0.001.

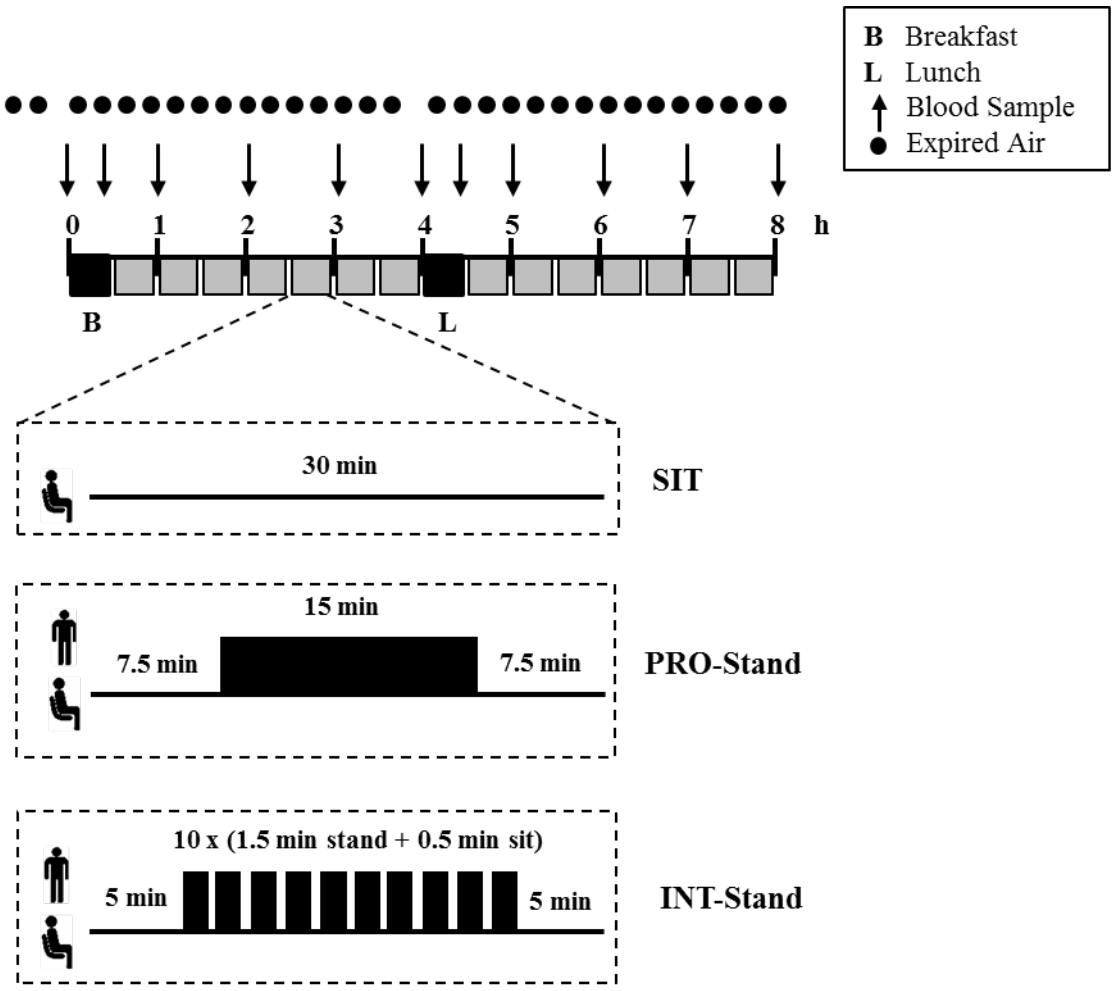

B Breakfast
L Lunch
Blood Sample
Expired Air
0
1
2
3
4
5
6
7
8
h
B
L
30 min
SIT
15 min
7.5 min
7.5 min
PRO-Stand
10 x (1.5 min stand + 0.5 min sit)
5 min
5 min
INT-Stand

Figure 1

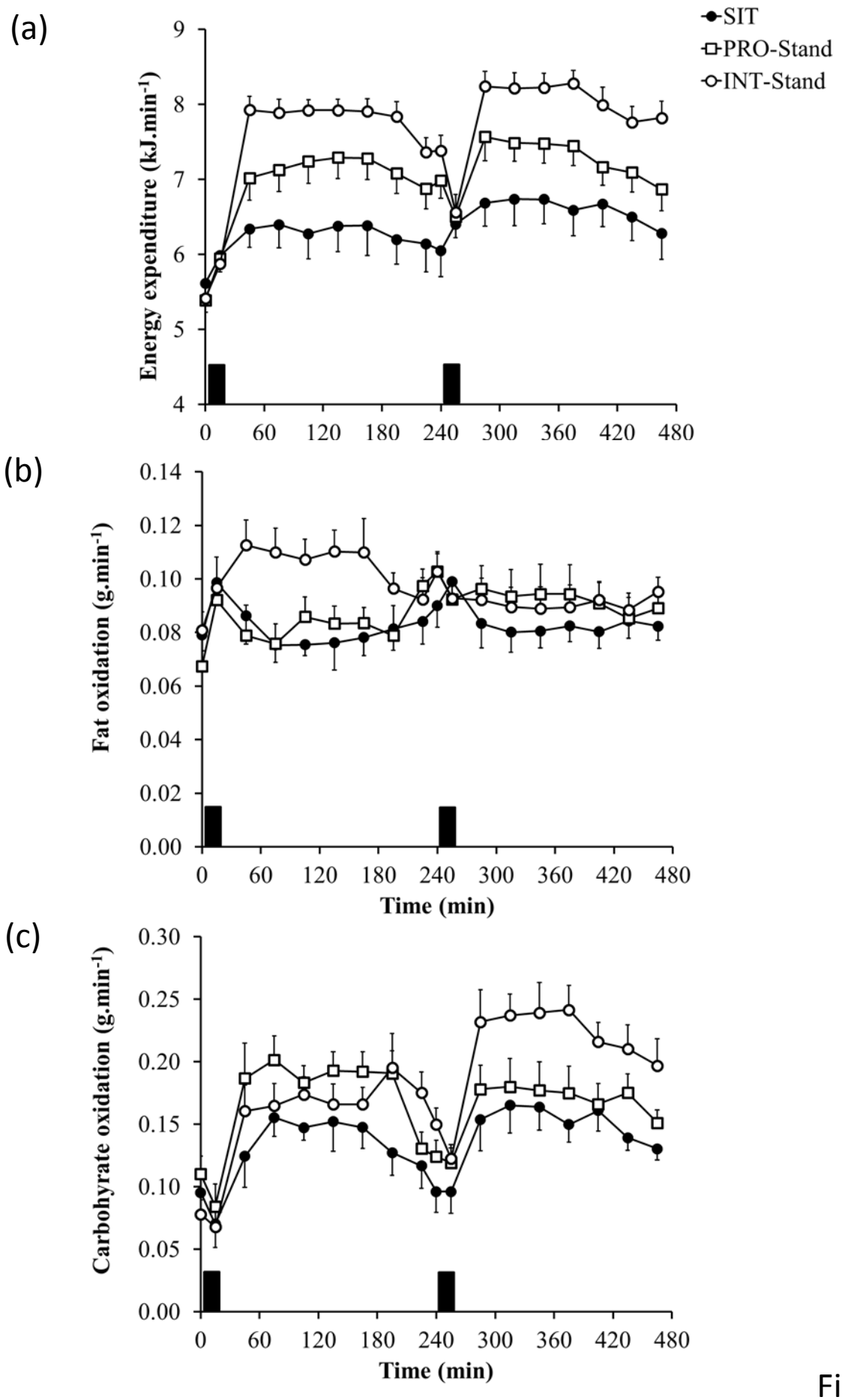
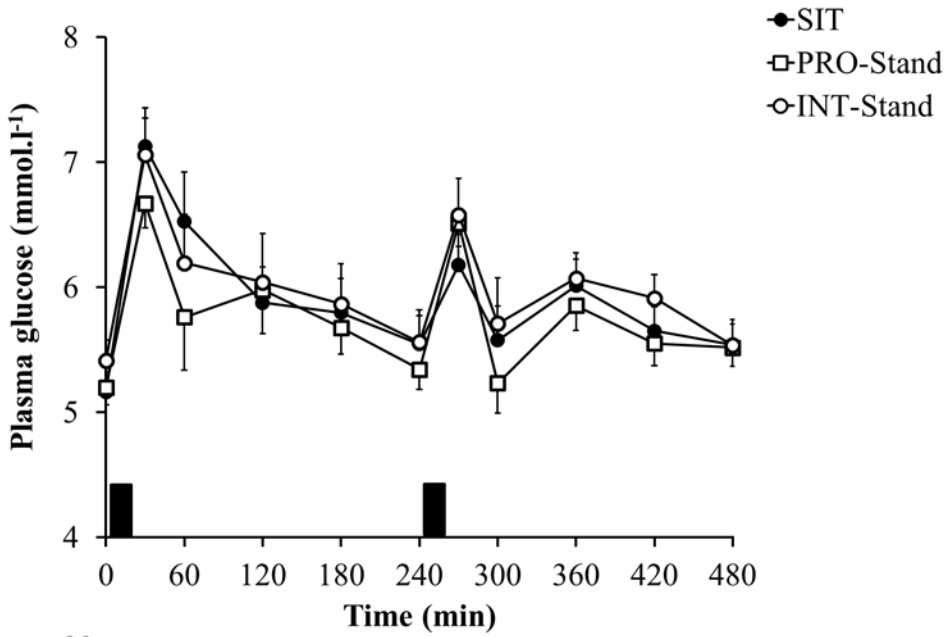
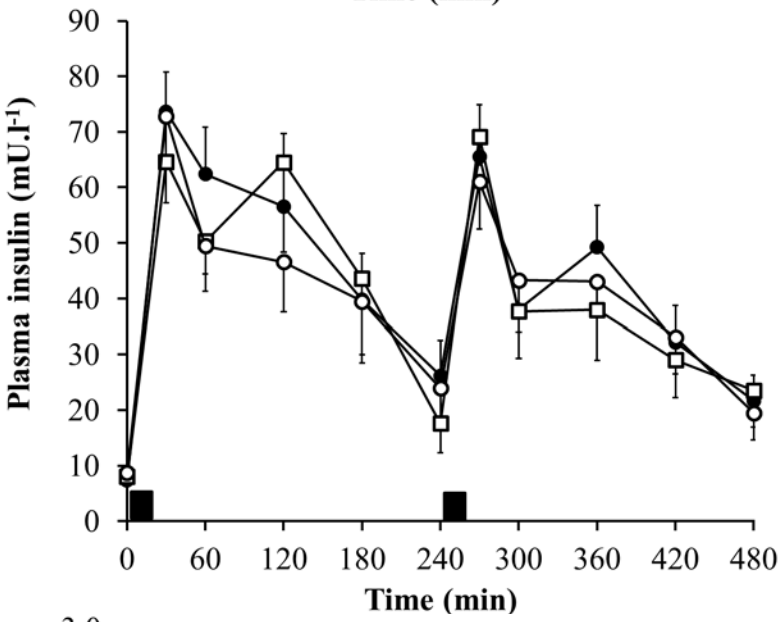

(a)
SIT
PRO-Stand
INT-Stand
Energy expenditure (kJ.min-1)
9
8
7
6
5
4
0
60
120
180
240
300
360
420
480
(b)
Fat oxidation (g.min-1)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Time (min)
(c)
Carbohyrate oxidation (g.min-1)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Time (min)

Figure 2

(a)


SIT
PRO-Stand
INT-Stand
Plasma glucose (mmol.l-1)
8
7
6
5
4
0
60
120
180
240
300
360
420
480
Time (min)

(b)


Plasma insulin (mU.l-1)
90
80
70
60
50
40
30
20
10
0
0
60
120
180
240
300
360
420
480
Time (min)

(c)

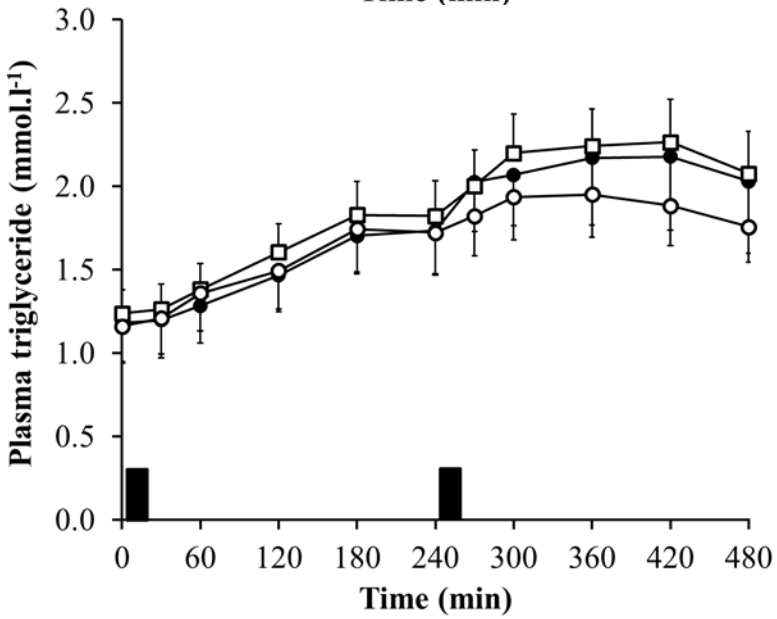

Plasma triglyceride (mmol.l-1)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
60
120
180
240
300
360
420
480
Time (min)

Figure 3