Beneficial postprandial lipaemic effects of interrupting sedentary time with high-intensity physical activity versus a continuous moderate-intensity physical activity bout: a randomised crossover trial

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Abstract

Objectives: To compare the postprandial cardiometabolic response to prolonged sedentary time, continuous moderate-intensity physical activity (PA) followed by prolonged sedentary time, and interrupting prolonged sedentary time with hourly high-intensity PA breaks. *Design*: Three-condition randomised crossover trial. *Methods*: Fourteen sedentary and inactive adults aged 29 ± 9 years took part in three, 8-h conditions: 1) prolonged sitting (SIT), 2) a continuous 30-min moderate-intensity PA bout followed by prolonged sitting (CONT-SIT), and 3) sitting interrupted hourly with 2 min 32 s high-intensity PA bouts (SIT-ACT). The treadmill PA in conditions 2 and 3 were matched for energy expenditure. Two standardised test meals were consumed during each condition. Incremental area under the curve (iAUC) for each 8-h condition was calculated for glucose, insulin, triglyceride, and high-density lipoprotein cholesterol (HDL-C) concentrations. Statistical analyses were completed using linear mixed models. *Results*: Compared with SIT, SIT-ACT lowered triglyceride iAUC by 2.23 mmol/L·8 h (95% CI -4.33, -0.13) and raised HDL-C iAUC by 0.99 mmol/L·8 h (0.05, 1.93) (all p≤0.038). There was no significant difference in triglyceride or HDL-C iAUC between CONT-SIT and SIT or SIT-ACT (p≥0.211). There were no significant differences between conditions for glucose or insulin iAUC (p≥0.504). *Conclusions*: This study suggests that interrupting prolonged sitting with hourly high-intensity PA breaks acutely improves postprandial triglyceride and HDL-C concentrations compared with prolonged sitting, whereas a continuous moderate-intensity PA bout does not.

Key words

Sedentary behaviour; Postprandial metabolism; Prolonged sitting; Cardiometabolic disease; Physical activity

Abbreviations

CI – Confidence interval

CVD - cardiovascular disease

HDL-C – High-density lipoprotein cholesterol

iAUC – Incremental area under the curve

MVPA – moderate-to-vigorous physical activity

PA – Physical activity

RPE – Rating of perceived exertion

Introduction

Cardiometabolic diseases, such as type 2 diabetes and cardiovascular disease (CVD), are highly prevalent in modern society.[1] These diseases are associated with poor regulation of postprandial triglyceride, glucose and insulin, which can cause oxidative stress, inflammation, and endothelial dysfunction, subsequently increasing cardiometabolic disease risk.[2] Thus, interventions to reduce fluctuations in these cardiometabolic risk markers are important, particularly as the waking day is predominantly spent in a postprandial state.

Although engaging in ≥60 minutes per day of moderate intensity physical activity (PA) may offset the increased mortality risk associated with high sitting time,[3] higher total sedentary time and lower number of interruptions in sedentary time are often associated with increased cardiometabolic disease risk independent of moderate-to-vigorous PA (MVPA).[4,5] Acute experimental studies report beneficial postprandial triglyceride, glucose and insulin responses to interrupting prolonged sitting every 20-30 min with light or moderate-intensity PA.[6-8] When sitting is interrupted less frequently (i.e., hourly) with moderate-intensity cycling, postprandial glucose, triglyceride, and high-density lipoprotein cholesterol (HDL-C) concentrations were not affected compared with uninterrupted sitting.[9,10] However, continuous moderate-intensity PA may not increase skeletal muscle GLUT4 expression and lipoprotein lipase activity to the same extent as high-intensity PA.[11,12] Thus, although interrupting sitting with moderate-intensity PA every 30-min reduces postprandial glucose,[8] higher-intensity PA may be necessary if sitting is interrupted hourly. Additionally, the limited active muscle mass with hourly PA interruptions may have been insufficient for stimulating whole-body glucose and lipid metabolism.[9,10]

Interrupting sitting may have similar or more pronounced cardiometabolic effects than continuous moderate-intensity PA in various populations.[7,8] Reductions in postprandial glucose and insulin concentrations were observed when sitting was interrupted with moderate-intensity walking compared with a single, continuous energy and intensity-matched PA bout

performed in the morning in healthy, normal weight adults.[8] Interrupting sitting with moderate-intensity walking also attenuated postprandial triglycerides to a similar extent as a continuous energy and intensity-matched PA bout performed in the morning in postmenopausal women.[7] The postprandial cardiometabolic response to interrupting sitting with high-intensity PA compared with an energy-matched continuous moderate-intensity PA bout has not been studied in any population. Therefore, this study compared the postprandial cardiometabolic response to prolonged sitting, continuous moderate-intensity PA followed by prolonged sitting, and interrupting sitting with hourly high-intensity PA.

Methods

This was a three-condition randomised crossover trial approved by the University of Bedfordshire Institute for Sport and Physical Activity Research Ethics Committee (approval number 2015ISPAR004). All testing took place at the University of Bedfordshire Sport and Exercise Science Laboratories. Fourteen (seven female) sedentary and inactive adults (self-reported sitting ≥7 h/day and MVPA <150 min/week) aged 18-55 years gave informed consent to participate. Exclusion criteria were any known blood borne disease, pregnancy, diabetes, taking glucose-lowering and/or lipid-lowering medication, known PA contraindications, major illness/injury, allergies to the test meals being provided, or other health issues that may limit the ability to perform the necessary activity bouts.

Sample size estimations were based on postprandial glucose and triglyceride incremental area under the curve (iAUC). Based on an effect size of F=0.61 for change in postprandial glucose,[13] 10% within-group error variance, a within-person correlation of 0.6, 90% power, and α=0.05, it was estimated that nine participants would be required for this study. Using these same parameters, but with an effect size of F=0.45 for postprandial triglycerides,[14] it was estimated that 12 participants would be required. To allow for dropout, 14 participants were recruited.

115 Stature was measured using a stadiometer (Holtain Ltd., Crymych, Wales). Body mass and
116 body fat were measured using the Tanita BC-418 Segmental Body Composition Analyzer
117 (Tanita Corp., Tokyo, Japan). Participants were then familiarised with the Borg Rating of
118 Perceived Exertion (RPE) scale[15] and completed a maximal oxygen uptake ($\dot{V}O_{2max}$) test on a
119 motorised treadmill (Woodway PPS55Med-I, GmbH, Germany). Expired air was measured
120 continuously using an online gas analysis (Cortex Metalyzer 3B, GmbH, Germany).
121 Participants began the test with a 3-min stage at a speed they could comfortably maintain for
122 30-min. This was then increased by 1 km/h every 3-min until volitional exhaustion. $\dot{V}O_{2max}$ was
123 taken as the highest $\dot{V}O_2$ value averaged over a 10 s period and was accepted as having been
124 achieved when meeting ≥2 of the following end-point criteria: 1) heart rate within 10 bpm of
125 age predicted maximum (220 – age), 2) respiratory exchange ratio >1.15, 3) plateau of $\dot{V}O_2$
126 despite increasing workload, and 4) RPE ≥18. Subsequently, the $\dot{V}O_2$ ($mL \cdot kg^{-1} \cdot min^{-1}$)
127 representing 60% and 85% $\dot{V}O_2$ Reserve ($\dot{V}O_2R$) were determined and the treadmill speeds
128 corresponding to these intensities were estimated and used for the experimental conditions.
129
130 After preliminary measures, participants completed three, 8-h experimental conditions
131 separated by washout periods of 6-35 days and conducted in an incomplete counterbalanced
132 order pre-determined using the Latin square method (see Figure 1). The washout period was
133 longer for females to ensure the experimental conditions were completed during the follicular
134 phase only to minimise the effects of the menstrual cycle on cardiometabolic outcomes.[16] The
135 conditions were as follows:
136
137 1) Prolonged sitting (SIT): participants remained seated throughout the experimental period.
138 2) Continuous moderate-intensity PA followed by prolonged sitting (CONT-SIT): after 30-min
139 rest, participants completed a 30-min continuous moderate-intensity PA bout (60% $\dot{V}O_2R$)
140 followed by prolonged sitting for the remainder of the condition.

3) Sitting interrupted with high-intensity PA breaks (SIT-ACT): after 30-min rest, participants completed 2 min 32 s bouts of high-intensity PA (85% $\dot{V}O_2R$) at 60-min intervals. This resulted in eight bouts equalling a total of 20 min 16 s PA.

To match PA energy expenditure between CONT-SIT and SIT-ACT, it was estimated that 20 min 16 s of PA at 85% $\dot{V}O_2R$ would elicit similar energy expenditure to 30-min of PA at 60% $\dot{V}O_2R$.[17] The 20 min 16 s of PA during SIT-ACT was spread equally across the eight hourly bouts, resulting in a bout duration of 2 min 32 s. This approach ensured that the PA bout duration and proximity of PA bouts to the blood samples were standardized between participants. All PA was performed on a motorised treadmill.

Participants were asked to refrain from exercise, alcohol, and caffeine in the 48-h before each experimental condition, which was confirmed verbally upon arrival. Participants weighed and recorded all food and liquid intake in a food diary for 24-h before the first experimental condition. The quantity and timings were then replicated prior to each subsequent experimental condition. All participants attended the laboratory at ~0800 h after an overnight fast and were instructed to minimise PA during their commute. Upon arrival, participants were fitted with an Actiheart monitor (CamNtech Ltd., Cambridge, UK) to provide a valid and reliable estimate of PA energy expenditure throughout each experimental condition.[18] The monitor was individually calibrated using expired air data from the $\dot{V}O_{2max}$ test. Subsequently, participants had a cannula (Vasofix, B. Braun Medical Ltd, Sheffield, UK) inserted into an antecubital vein and a fasting blood sample was collected. A standardised breakfast meal was then consumed and the 8-h experimental condition commenced following the last mouthful. A second standardised meal was consumed at 4-h. Participants were permitted to void when needed during each condition. The toilets were located ~30 m from the laboratory. Participants were supervised throughout the conditions to ensure adherence to the protocols and were permitted to watch DVDs, read, talk, or work on a laptop when seated. During sitting periods, participants were asked to minimise excessive movement.

The standardised breakfast and lunch meals provided 15% and 30%, respectively, of individual daily energy requirements estimated using the Mifflin equation[19] with a PA factor of 1.4 applied to represent a sedentary day. The Mifflin equation[19] provides a more accurate estimation of resting energy expenditure than other commonly used prediction equations.[20] Both meals consisted of cornflakes and whole milk, contained 55% carbohydrate, 30% fat, and 15% protein and had a high glycaemic index (79). The mean carbohydrate, fat, and protein content was 47±8 g, 11±2 g, and 13±2 g for breakfast and 71±12 g, 17.2±3 g, and 19±3 g for lunch. Participants were asked to consume each meal within 15-min. During the first experimental condition, consumption times were recorded and participants were asked to replicate this as closely as possible in each subsequent condition. Water was provided *ad libitum* during the first condition and the volume consumed was replicated during subsequent conditions.

Venous blood samples were collected via cannulation in the fasted state and then hourly into two 4.9 mL EDTA-containing vacuettes (Vacuette, Greiner Bio-One, Austria). Whole blood was pipetted in volumes of 50 μl from one vacuette into a microvette and analysed immediately in duplicate to determine blood glucose concentrations using the YSI 2300 STAT plus glucose and lactate analyzer (YSI Inc., Yellow Springs, OH, USA). Further volumes of 30 μl of whole blood were aliquoted onto two separate Reflotron test strips (Roche Diagnostics, Burgess Hill, UK) for the determination of triglyceride and HDL-C concentrations using the Reflotron Plus system (Roche Diagnostics, Burgess Hill, UK). The vacuettes were then spun using a refrigerated centrifuge (Heraeus, Heraeus Multifuge X3R, Thermo Scientific) at 1500 x g for 10-min at 4^{o}C. The plasma was stored at -80^{o}C for later batch analysis of insulin using an enzyme-linked immunosorbent assay kit (Mercodia, Uppsala, Sweden). The intra-assay and inter-assay CVs were 9.4% and 12.2%, respectively.

The primary outcomes were net iAUC for the cardiometabolic variables as this method is most appropriate for describing postprandial glycaemic and lipaemic responses.[21,22] Total AUC (tAUC) was calculated using the trapezoidal method to permit comparisons with previous research.[7,13,14] The net iAUC was calculated by subtracting the area under the baseline concentration from the tAUC.

Statistical analyses were completed using SPSS version 22.0 (SPSS INC., Armonk, N.Y., USA). Normality assumptions were assessed using Quantile-Quantile plots. Linear mixed models were used to determine any differences in the dependent variables between conditions. Condition and covariates (age, gender, body fat%, and fasting outcome values) were fixed factors and participants were random factors within all models analysing cardiometabolic outcomes. No covariates were entered into the models when comparing PA energy expenditure between conditions as age, gender, and body mass index are used in the algorithms to estimate PA energy expenditure. Sidak correction for multiple comparisons was used for post-hoc analysis when a significant main effect was present. Cohens' d effect sizes were calculated to describe the magnitude of differences between conditions; 0.2, 0.5 and 0.8 indicated a small, medium and large effect. Data are presented as mean (95% confidence interval [CI]) unless stated otherwise. Statistical significance was accepted as $p \leq 0.05$.

Results

Table 1 shows the descriptive characteristics of the participants. Total PA energy expenditure did not differ significantly (p=0.236) between CONT-SIT (661 kJ; 476, 828) and SIT-ACT (732 kJ; 539, 891). Table 2 shows fasting concentrations, iAUC and tAUC for each cardiometabolic risk marker and Figure 2 (supplementary material) shows cardiometabolic risk marker responses over time for each condition. Fasting concentrations did not differ significantly between conditions (Table 2).

There was a significant main effect of condition for triglyceride iAUC (Table 2). Triglyceride iAUC was reduced by 2.23 mmol/L·8 h in SIT-ACT compared with SIT (p=0.035) with a medium effect size for this difference (d=0.62). No significant difference was observed between SIT and CONT-SIT (p=0.361; d=0.35) or between CONT-SIT and SIT-ACT (p=0.580; d=0.27) with small effect sizes. There was a trend for triglyceride tAUC being lower in SIT-ACT than SIT (p=0.073). There was a significant main effect of condition for HDL-C iAUC with concentrations being 0.99 mmol/L·8 h higher in SIT-ACT than SIT with a medium effect size (p=0.037; d=0.64). No significant differences were observed between SIT and CONT-SIT (p=0.813; d=0.20) or between CONT-SIT and SIT-ACT (p=0.211; d=0.44) with small effect sizes (Table 2). There was no main effect of condition for HDL-C tAUC. The main effect of condition for glucose and insulin iAUC and tAUC was not significant.

Discussion

The novel findings of this study were that interrupting sitting with short, hourly bouts of high-intensity PA improved postprandial triglyceride and HDL-C concentrations compared with uninterrupted sitting in sedentary adults, whereas a continuous moderate-intensity PA bout followed by prolonged sitting did not.

The reduction in postprandial triglyceride concentrations in response to interrupting sitting with high-intensity PA compared with prolonged sitting reported here is congruent with previous research where postmenopausal women engaged in moderate-intensity walking for 1 min 30 s every 15-min[7] and obese men engaged in moderate-intensity cycling for 3-min every 30-min.[14] However, other studies where sitting was interrupted with moderate-intensity walking for 1 min 40 s every 30-min in healthy, normal weight adults[8] and moderate-intensity cycling for 8-min every hour in young healthy adults[9] did not attenuate postprandial triglycerides, possibly because the combination of frequency, duration and intensity of interruptions in sitting were not sufficient. Alternatively, metabolically healthy participants may not respond to interruptions in sitting[8,9] compared with postmenopausal women,[7] obese men,[14] and our

sedentary and inactive sample who had a relatively higher body fat%. Furthermore, attenuation of postprandial triglyceride concentrations in response to interrupting sitting with moderate-intensity PA may be delayed due to the activity of lipoprotein lipase typically peaking 8-22 h after a single bout of moderate-intensity PA.[23] Given the findings of the present study, it is possible that interrupting sitting regularly may acutely attenuate the decrease in lipoprotein lipase activity that occurs rapidly in response to physical inactivity in animal models.[24] Thus, further research examining lipoprotein lipase in response to interrupting sitting in humans would be valuable.

The increase in postprandial HDL-C net iAUC in response to interrupting sitting may be due to triglyceride-rich lipoproteins undergoing hydrolysis and losing surface phospholipids that are acquired by HDL-C.[25] This increase was not shown in previous studies that measured HDL-C only at the end of the experimental periods,[13,26] indicating that regular measurements across the postprandial period are required to detect significant or potentially meaningful effects. In contrast to our findings, a decrease in HDL-C and increase in triglycerides has been reported in response to interrupting sitting every 40-min with 6-min high-intensity (70% $\dot{V}O_{2max}$) cycling in young females.[26] Thus, treadmill PA, which is weight-bearing and incorporates upper and lower body muscle contractions, as used in the present study, may be necessary to benefit lipid metabolism.[26] However, HDL-C tAUC did not differ between conditions in the present study. Thus, net iAUC, where only the data above baseline are included and the data that drop below baseline are subtracted from the response, may be more sensitive for detecting between-condition effects. Although iAUC is most appropriate for describing postprandial lipaemic responses,[22] tAUC may have physiological relevance and should be examined in future research.

The present study did not observe a beneficial change in postprandial lipids in response to a single continuous bout of moderate-intensity PA with a duration and intensity that aligned to current PA guidelines.[27] Similar to the present study, postprandial triglyceride concentrations

were not attenuated in response to a continuous 30-min moderate-intensity walking bout compared with prolonged sitting.[8] However, other research has reported beneficial triglyceride responses to both continuous 30-min moderate-intensity PA bouts and interrupting sitting with ten, 3-min bouts of moderate-intensity PA every 30-min.[14,28] Importantly, the postprandial test meal challenge in these two studies[14,28] was performed 17-h following PA engagement, which may have permitted a longer duration for lipid metabolism in the presence of elevated lipoprotein lipase activity.[23] Collectively, these findings suggest that reductions in postprandial lipaemia do not occur during a 7-8 h sitting period after engagement in a continuous 30-min moderate-intensity PA bout.

Interrupting sitting with hourly high-intensity PA did not affect postprandial glucose or insulin, which is in agreement with previous research where sitting was interrupted hourly with 8-min moderate-intensity cycling.[9] However, the majority of research has reported beneficial glucose and insulin responses to interrupting sitting with light or moderate-intensity PA for 2-5 min every 20-30 min.[8,13] Thus, the hourly interruptions in sitting time in the present study and previous research[9] may not have been frequent enough to upregulate the physiological mechanisms responsible for glucose disposal, such as translocation of the intracellular glucose transporter protein GLUT-4 and permeability of muscles cells to glucose.[29]

When considering clinical relevance, postprandial dyslipidaemia is associated with oxidative stress that triggers a plethora of atherogenic changes and increases CVD risk.[2] Thus, engaging in short high-intensity PA bouts may provide a time efficient, effective strategy to reduce CVD risk and may be particularly appealing for individuals who find it difficult to engage in continuous moderate-intensity PA. However, our sample were in good general health; thus, the findings may not be generalised to clinical populations at high risk of CVD. Further limitations of the present study are that a single high-intensity PA bout was not included for comparison to isolate the effects of PA frequency from intensity on cardiometabolic health. However, continuous high-intensity PA may not be feasible in sedentary populations due to

lower cardiorespiratory fitness and enjoyment.[30] Although fasting cardiometabolic outcomes did not differ between conditions, the control of menstrual cycle phase over three conditions could have meant that metabolic changes occurred in female participants over the course of the study (~3 months). It may have also been more appropriate to objectively confirm adherence to diet and PA controls before each experimental condition and to have collected blood immediately before the PA breaks to minimise any residual effects from the previous break. Lastly, this study was conducted in a controlled laboratory environment. Future research should evaluate the effects of interrupting sitting in real-life settings, such as at home or the workplace, where high-intensity PA may be less feasible than lower intensities, although this potential disadvantage may be offset by the lower frequency of PA breaks required.

Conclusion

In conclusion, interrupting sitting with hourly high-intensity PA acutely improves postprandial triglyceride and HDL-C concentrations when compared with prolonged sitting, whereas an energy-matched continuous moderate-intensity PA bout followed by prolonged sitting does not. Interrupting sitting with high-intensity PA may, therefore, be a potential strategy to reduce cardiometabolic disease risk in sedentary and inactive adults.

Practical implications

- Interrupting sitting with short high-intensity physical activity breaks every hour acutely improves cardiometabolic disease risk markers.
- The long term cardiometabolic health benefits associated with engaging in 30-minutes of moderate-intensity physical activity per day may not be seen acutely.
- Avoiding prolonged periods of sitting is recommended to reduce cardiometabolic disease risk.

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420 **Table 1** Descriptive participant characteristics (n=14)

Characteristics	Mean ± SD
Age (years)	29 ± 9
Height (cm)	172.8 ± 5.9
Weight (kg)	78.5 ± 20.4
Body mass index (kg/m^2)	26.1 ± 5.8
Body fat (%)	26.1 ± 7.5
Maximum oxygen uptake ($mL \cdot kg^{-1} \cdot min^{-1}$)	38.6 ± 4.2

421

422 **Table 2** Cardiometabolic risk marker values for each condition

Variable	SIT	CONT-SIT	SIT-ACT
Fasting triglycerides (mmol/mL)	1.24 (0.92, 1.56)	1.48 (1.16, 1.81)	1.29 (0.95, 1.62)
Fasting HDL-C (mmol/mL)	1.10 (0.93, 1.27)	0.88 (0.71, 1.05)	0.99 (0.81, 1.16)
Fasting blood glucose (mmol/L)	4.32 (4.09, 4.55)	4.46 (4.23, 4.68)	4.33 (4.10, 4.55)
Fasting plasma insulin (μU/mL)	8.02 (4.91, 11.1)	7.19 (4.08, 10.3)	10.2 (7.13, 13.4)
Triglyceride iAUC (mmol/L·8 h)*	1.36 (-0.46, 3.17)	0.11 (-1.72, 1.94)	-0.88 (-2.73, 0.97)
Mean difference[a] compared with SIT	-	-1.24 (-3.34, 0.86)	**-2.23 (-4.33, -0.13)**
Mean difference[a] compared with CONT-SIT	-	-	-0.99 (-3.18, 1.20)
Triglyceride total AUC (mmol/L·8 h)	11.95 (10.47, 13.42)	10.56 (9.07, 12.05)	9.60 (8.08, 11.13)
Mean difference[a] compared with SIT	-	-1.38 (-3.88, 1.12)	-2.34 (-4.85, 0.17)
Mean difference[a] compared with CONT-SIT	-	-	-0.96 (-3.50, 1.59)
HDL-C iAUC (mmol/mL·8 h)*	-0.13 (-0.91, 0.66)	0.18 (-0.61, 0.96)	0.86 (0.08, 1.65)
Mean difference[a] compared with SIT	-	0.30 (-0.68, 1.28)	**0.99 (0.05, 1.93)**
Mean difference[a] compared with CONT-SIT	-	-	0.69 (-0.27, 1.64)
HDL-C total AUC (mmol/mL·8 h)	7.98 (6.99, 8.97)	7.67 (6.68, 8.65)	8.38 (7.39, 9.37)
Mean difference[a] compared with SIT	-	-0.31 (-1.27, 0.65)	0.40 (-0.52, 1.32)
Mean difference[a] compared with CONT-SIT	-	-	0.71 (-0.21, 1.63)
Blood glucose iAUC (mmol/L·8 h)	0.36 (-1.18, 1.90)	-0.27 (-1.93, 1.38)	0.82 (-0.82, 2.46)
Mean difference[a] compared with SIT	-	-0.64 (-3.30, 2.03)	0.46 (-2.17, 3.08)
Mean difference[a] compared with CONT-SIT	-	-	1.09 (-1.74, 3.92)
Blood glucose total AUC (mmol/L·8 h)	35.40 (33.89, 36.91)	34.86 (33.30, 36.41)	35.65 (34.14, 37.15)

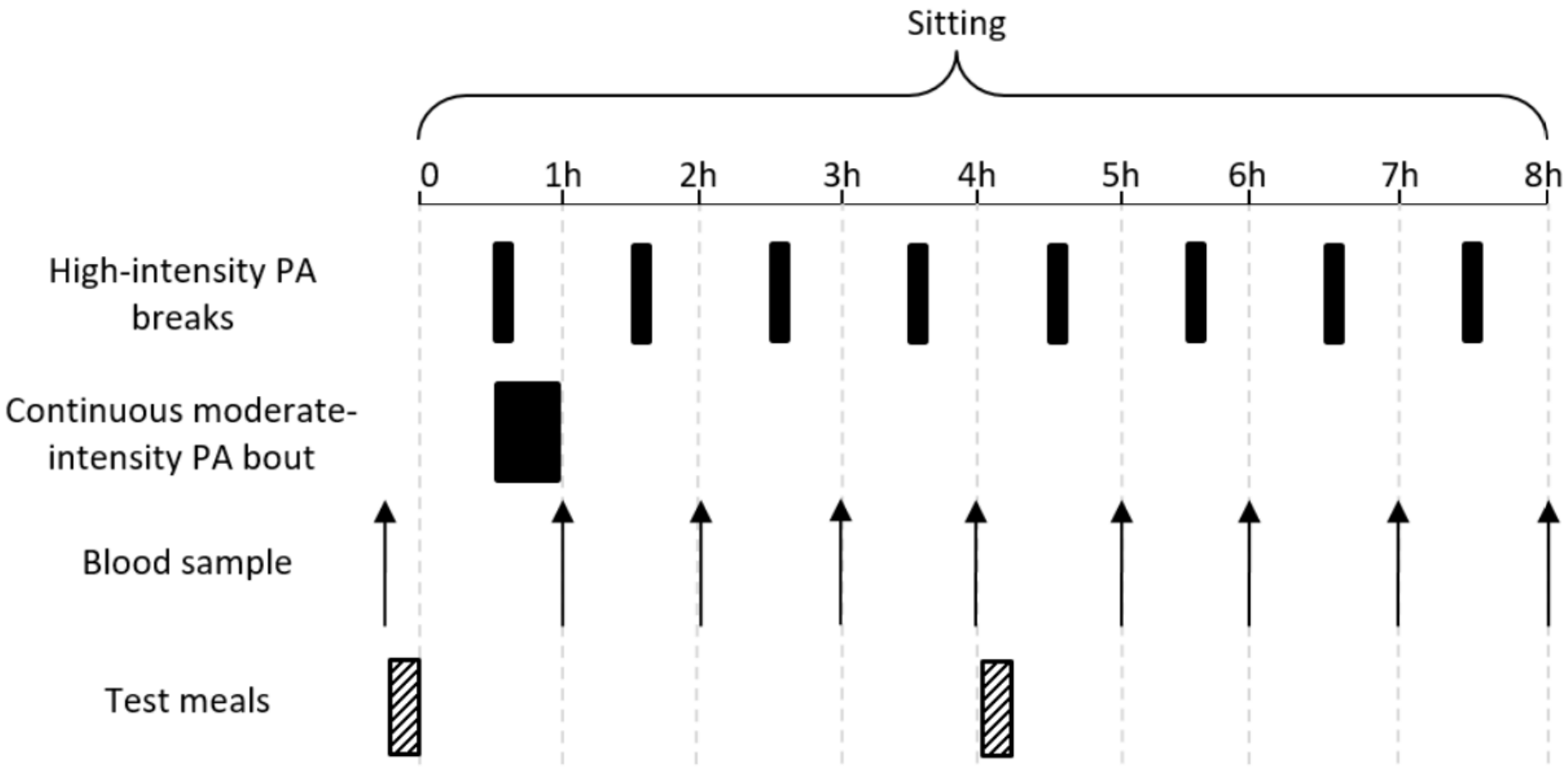
Mean difference[a] compared with SIT	-	-0.54 (-2.68, 1.59)	0.25 (-1.78, 2.27)
Mean difference[a] compared with CONT-SIT	-	-	0.79 (-1.34, 2.92)
Plasma insulin iAUC (μU/mL·8 h)	183.1 (138.7, 227.5)	177.9 (133.3, 222.5)	159.7 (114.8, 204.6)
Mean difference[a] compared with SIT	-	-5.2 (-45.4, 35.0)	-23.4 (-76.9, 30.2)
Mean difference[a] compared with CONT-SIT	-	-	-18.2 (-61.5, 25.1)
Plasma insulin total AUC (μU/mL·8 h)	201.5 (159.5, 243.4)	198.7 (156.5, 240.9)	178.1 (135.5, 220.8)
Mean difference[a] compared with SIT	-	-2.7 (-49.5, 44.1)	-23.3 (-72.1, 25.4)
Mean difference[a] compared with CONT-SIT	-	-	-20.6 (-70.6, 29.3)

Data are mean (95% CI). SIT, prolonged sitting; CONT-SIT, continuous moderate-intensity physical activity followed by prolonged sitting; SIT-ACT, sitting interrupted with high-intensity physical activity; HDL-C, high-density lipoprotein cholesterol; iAUC, incremental area under the curve.

[a]Estimated from pairwise comparisons of marginal means adjusted for age, gender, body fat% and fasting values for each biochemical measure.

*****Denotes a significant main effect of condition ($p \leq 0.05$)

Bold text indicates a significant pairwise comparison ($p \leq 0.05$)



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434 **Figure 1.** Schematic of experimental protocol. PA, physical activity.

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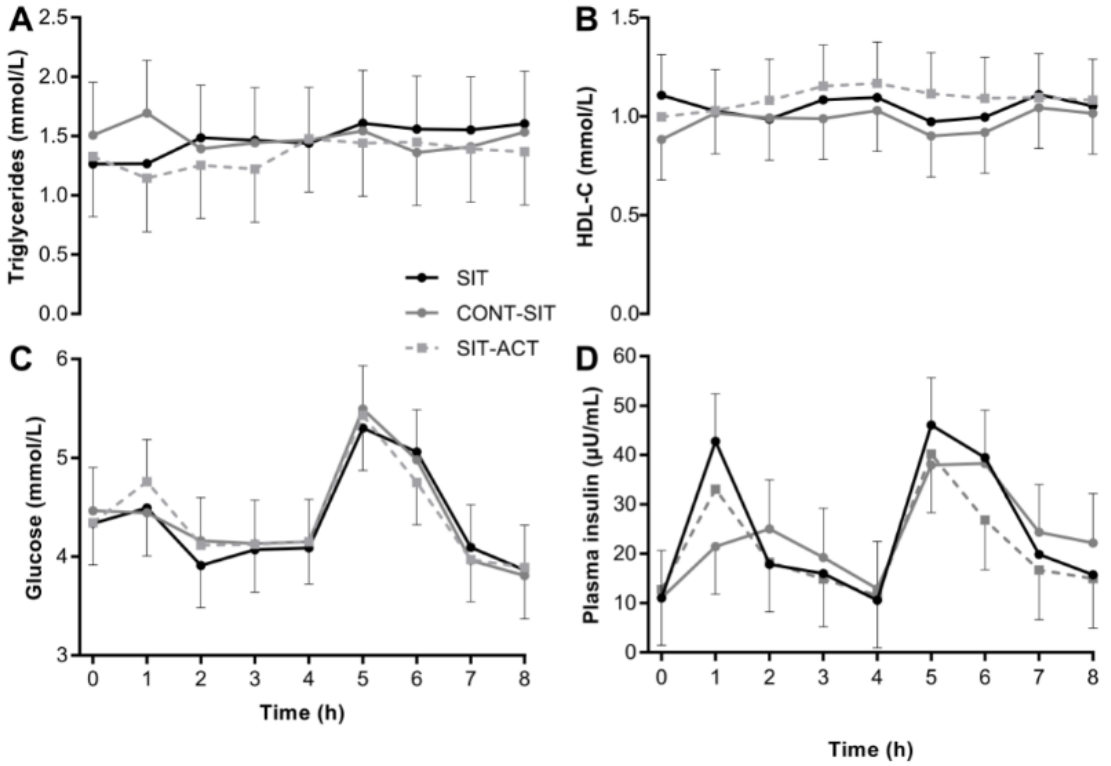

436

437 **Figure 2 (supplementary).** Changes in triglycerides (A), high-density lipoprotein cholesterol
438 (HDL-C) (B), glucose (C), and insulin concentrations during the prolonged sitting (SIT)**,**
439 continuous moderate-intensity physical activity followed by prolonged sitting (CONT-SIT),
440 and sitting interrupted with high-intensity physical activity (SIT-ACT) conditions. Data are
441 mean and 95% confidence interval. Some error bars have been omitted for clarity.

442