Effects of frequency and duration of interrupting sitting on cardiometabolic risk markers

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Abstract

Interrupting prolonged sitting with multiple bouts of moderate-intensity physical activity (PA) can improve postprandial cardiometabolic risk markers. This study examined the effect of high and low frequency PA bouts (matched for total PA duration and energy expenditure) on postprandial cardiometabolic responses when compared with prolonged sitting. In this three-condition randomised crossover trial, fourteen sedentary, inactive females (33.8±13.4 years, BMI 27.1±6.3 kg/m^2) completed three, 7.5 h conditions: 1) prolonged sitting (SIT), 2) high-frequency PA breaks (HIGH-FREQ) consisting of 15 x 2 min bouts of moderate-intensity treadmill PA every 30 min, and 3) low-frequency PA breaks (LOW-FREQ) consisting of 3 x 10 min bouts of moderate-intensity treadmill PA every 180 min. The PA bouts were performed at 65% of peak oxygen uptake. Net incremental area under the curve (iAUC) for each 7.5 h condition was calculated for glucose, insulin and triacylglycerol (TAG) concentrations. Insulin iAUC was significantly ($p<0.026$) lower during HIGH-FREQ (mean [95%CI]; 82.86 [55.02, 110.70] μU/mL·7.5h) than LOW-FREQ (116.61 [88.50, 144.73]) and SIT (119.98 [92.42, 147.53]). Glucose and TAG iAUC did not differ between conditions. Engaging in higher-frequency PA breaks may be effective in attenuating postprandial insulin responses compared with lower-frequency PA breaks and prolonged sitting.

Introduction

Increased postprandial levels of glucose, insulin, and triacylglycerol (TAG) promote oxidative stress, inflammation, and endothelial dysfunction that can increase the risk of cardiometabolic disease [1,2]. Acute responses and chronic adaptations to engaging in physical activity (PA) can attenuate elevations in these cardiometabolic risk markers [3-5]. Accordingly, current UK PA guidelines recommend that adults engage in ≥150 min/week of moderate-to-vigorous physical activity (MVPA) accumulated in bouts of ≥10 min to benefit their health [6].

Several studies have reported that accumulating moderate-intensity PA in regular short bouts of ≤3 min in duration is effective for attenuating postprandial glucose, insulin and TAG responses over a single day [7-10]. Furthermore, the cardiometabolic benefits of short frequent bouts of PA may be equally or more effective than a single continuous bout of PA of the same intensity and volume (typically 30 min in duration) [9-11]. This may be because interrupting muscular inactivity that occurs during prolonged sitting suppresses postprandial glucose, insulin, and TAG levels via different mechanistic pathways than continuous PA [12]. However, the effects of regular short bouts of PA compared with less frequent bouts of ≥10 min that are recommended in government guidelines [6] are unknown.

The aim of this study was to compare the postprandial cardiometabolic effects of frequent short bouts of PA used to interrupt sitting time to an equal volume of PA accumulated in less frequent 10 min bouts in sedentary females.

Materials & Methods

This randomised crossover study was approved by the University of Bedfordshire Institute for Sport and Physical Activity Research Ethics Committee and adhered to published ethical standards [13]. All testing took place at the University of Bedfordshire Sport and Exercise

Science Laboratories. After a preliminary visit, participants completed three experimental conditions in an incomplete counterbalanced order pre-determined using the Latin square method. Participants were blinded to the first two experimental conditions that they were taking part in until they arrived in the morning to complete those respective conditions.

Participants

Fourteen sedentary (defined as self-reported sedentary time ≥7 h/day because volumes above this threshold are associated with increased cardiometabolic disease risk [14]) and inactive (self-reported MVPA <150 min/week or <75 min vigorous PA [6]) females aged 20-55 years provided informed consent to participate in the study. Participants were recruited between November 2015 and August 2016. Exclusion criteria included working in a non-sedentary occupation, any known blood borne disease, pregnancy, diabetes, taking glucose-lowering and/or lipid-lowering medication, known PA contraindications, major illness/injury, or allergies to the test meals being provided.

Preliminary visit

Participants attended a preliminary testing session to have stature (Holtain Ltd., Crymych, Wales), body mass, and body composition (Tanita BC-418 Segmental Body Composition Analyzer, Tanita Corp., Tokyo, Japan) measured. To ascertain a treadmill speed for the experimental conditions, participants completed a 4 x 4 min submaximal and a maximal oxygen uptake test on a motorised treadmill (Woodway PPS55Med-I, GmbH, Germany). Breath-by-breath expired air samples were collected throughout both tests using an online gas analysis system (Cortex Metalyzer 3B, GmbH, Germany). Participants started both tests at a walking speed that they felt they could comfortably maintain for 30 min. The submaximal test speed was increased by 1.5 km/h per stage. After a ~30 min supervised rest, the maximal oxygen uptake test was completed, where the speed was increased by 1 km/h every three min until volitional exhaustion. Maximal oxygen uptake (VO_{2max}) was taken as the

highest VO_2 value over a 10 s period and was accepted as valid if a plateau in VO_2 (≤2.1 $mL \cdot kg^{-1} \cdot min^{-1}$) occurred despite increasing workload [15]. Peak oxygen uptake was taken if a plateau was not attained but ≥2 of the following end-point criteria were satisfied: 1) heart rate within 10 bpm of age predicted maximum, 2) respiratory exchange ratio >1.1, and 3) RPE ≥18 [16]. The relationship between treadmill speed and % VO_{2peak} was used to estimate the treadmill speed that elicited 65% VO_{2peak} for use during the experimental conditions.

Experimental protocol

Due to hormonal alterations in glucose metabolism during the female menstrual cycle [17], experimental conditions were completed during the follicular phase only (days 1-10). There was a minimum of a 7 day washout period between each condition to minimise carryover effects. Prior to each experimental condition, participants refrained from exercise, alcohol, and caffeine for 48 h. Participants recorded the weight and timings of all food and liquid intake in a food diary for 24 h before the first experimental condition and were asked to replicate the quantity and timings of consumption prior to each subsequent condition [7]. Participants arrived at the laboratories at ~08:30 in a fasted state following a vehicular commute. Fasting blood samples were collected following insertion of a cannula into an antecubital vein. Following this, participants undertook one of three, 7.5 h experimental conditions (see Figure 1):

1) SIT: Uninterrupted sitting at a desk.

2) HIGH-FREQ: Sitting interrupted with frequent, 2-min moderate-intensity treadmill PA breaks every 30 min.

3) LOW-FREQ: Sitting interrupted with less frequent, 10-min moderate-intensity treadmill PA breaks at 0 min, 170 min, and 350 min.

To match the PA conditions for PA intensity and energy expenditure, all PA was performed at a treadmill speed that corresponded to 65% VO_{2max} and the total duration of PA was 30 min in both conditions. The moderate-intensity PA was a walking pace for some participants and a jogging pace for others. Rating of Perceived Exertion (RPE) was obtained during the last 30 s of each treadmill bout using the Borg scale [18]. During experimental conditions, participants were permitted to work on a laptop computer, read, or talk during sitting periods and were supervised by a researcher to ensure adherence to the protocols. Participants were permitted to void when necessary with the toilets being located ~30 m from the laboratory.

Two standardised test meals were provided during each condition: one at 15 min and one at 180 min. These were individualised to provide 15% and 25%, respectively, of estimated daily energy requirements for each participant. Energy requirements were estimated using the Mifflin equation with a PA factor of 1.4 applied to represent a sedentary day [19]. The first meal provided 58% carbohydrate (43.1 ± 8.1 g), 28% fat (9.4 ± 1.8 g) and 14% protein (10.7 ± 2.0 g) with a mean energy intake of 1274.4 ± 240.2 kJ). The meal consisted of cornflakes and whole milk (glycaemic index: 80; based on the individual food items' glycaemic index values reported in international tables [20] and taking into account the weighted contribution of carbohydrate from each food item [21]). The second meal provided 46% carbohydrate (57.6 ± 10.0 g), 40% fat (22.3 ± 3.9 g) and 14% protein (17.8 ± 3.1 g) with a mean energy intake of 2123.4 ± 220.9 kJ. This meal consisted of white bread, roast chicken, margarine, crisps and chocolate (glycaemic index: 58). At 6 h, participants were given 30 min *ad libitum* access to a cold food buffet. The items available were white bread, wholemeal bread, margarine, mayonnaise, cheese, ham, crisps, chocolate bars, cereal bars, cookies, apples, oranges, bananas, milk and orange juice. All items were provided in standardised quantities and the total volume provided was expected to exceed the amount that would be consumed. Participants were provided with water *ad libitum* during the first experimental condition; this

same volume was provided spread across the day in the subsequent experimental conditions.

Blood samples were collected into two 4.9 mL EDTA-containing vacuettes (Vacuette, Greiner Bio-One, Austria) at the time points shown in Figure 1. From one vacuette, 50 μl of whole blood was immediately pipetted into a microvette (Microvette CB300 EDTA, Sarstedt Ltd, Leicester, UK) from which glucose concentrations were measured at 0, 35, 60, 90, 120, 150, 165, 180, 210, 240, 270, 300, 330, 345, 405, 420 and 450 min using the YSI 2300 STAT plus glucose and lactate analyzer (YSI Inc., Yellow Springs, OH, USA). The remaining blood was centrifuged (Heraeus, Heraeus Multifuge X3R, Thermo Scientific) at 1500 x g for 10 min at 4^{o}C. The plasma supernatant was stored at -80^{o}C for later batch analysis of insulin and TAG at 0, 35, 60, 120, 165, 180, 210, 240, 300, 345, 405 and 450 min. Plasma insulin concentrations were measured using a commercially available enzyme-linked immunosorbent assay kit (Mercodia, Uppsala, Sweden). Plasma TAG concentrations were determined via spectrophotometry using the lipase hydrolysis method (GOP-PAP; Randox Laboratories Ltd, Crumlin, UK).

Calculations and statistical analysis

Postprandial glucose, insulin and TAG outcomes were calculated for each 7.5 h experimental condition using the trapezoidal method. Total area under the curve (TAUC) was calculated; the area under the baseline value was subtracted to calculate net incremental area under the curve (iAUC). Statistical analyses were completed using SPSS version 22.0 (SPSS Inc., Armonk, N.Y., USA). Normality was assessed using standard graphical procedures [22]. Insulin iAUC was non-normally distributed and was log transformed prior to analysis. The data were back-transformed to natural units to provide meaningful interpretation of the results. Linear mixed models with fixed ('condition') and random ('participant') effects were fitted using the correlation structure yielding the lowest

Hurvich and Tsai's information criterion (AICC) [23]. All models were adjusted for potential covariates (age, body fat% and baseline outcome values). Post hoc analyses were adjusted using the Sidak correction for multiple comparisons. Cohens' d effect sizes were calculated to describe the magnitude of significant differences between conditions with 0.2, 0.5 and 0.8 indicating a small, medium and large effect, respectively [24]. Data are presented as mean (95% confidence interval [CI]) unless stated otherwise. Significance was accepted as $p \leq 0.05$.

Results

14 participants were recruited who each completed the full trial. The characteristics of the participants are shown in Table 1. Nine of the participants attained a VO_2 plateau and the remaining 5 participants met the secondary criteria for VO_{2peak}.

Baseline cardiometabolic risk marker concentrations did not differ significantly between conditions (Table 2). The mean RPE was significantly higher during the LOW-FREQ PA bouts (11.7 ± 1.7) than the HIGH-FREQ PA bouts (10.4 ± 1.4; p=0.001). Total energy and macronutrient intake during the buffet meal was similar between conditions (Table 2). There was a significant main effect of condition for insulin iAUC with concentrations being significantly lower by 31% and 29% in HIGH-FREQ compared with SIT (p=0.026; d=1.34) and LOW-FREQ (p=0.017; d=1.21), respectively. The effect sizes for these differences were large. Insulin TAUC was also significantly lower in HIGH-FREQ than SIT (p=0.020; d=0.56) and LOW-FREQ (p=0.050; d=0.52), with medium effect sizes. There were no significant main effects of condition for glucose or TAG outcomes. Cardiometabolic responses over time for each condition are shown in Figure 2 for descriptive purposes.

Discussion

The main finding of this study was that postprandial insulin concentrations in sedentary, inactive females were attenuated in response to 2-min, frequent moderate-intensity PA breaks, but were not suppressed by engaging in the same duration of PA accumulated in less frequent, 10-min breaks when compared with uninterrupted sitting. Previous research examining the effects of PA breaks on postprandial insulin have yielded mixed findings. There are several acute experimental trials reporting that PA breaks every 20-30 min for durations between 1 min 40 s and 5 min suppressed postprandial insulin by 21-26% in healthy and dysglycaemic adults [10,25-29], whilst other trials incorporating similar designs have reported no suppression of insulin [7,8]. Although study designs are similar, it is possible that the shorter observation periods and provision of only one meal during the experimental conditions could have reduced the potential to detect differences in insulin in some studies [7,8,25]. Indeed, the study by Henson et al. [27] provided two meals across an experimental period the same duration as the present study (7.5 h) and they observed a suppression in postprandial insulin. Alternatively, previous studies reporting no differences [7,8] were not powered to detect changes in insulin and the sample sizes may thus have been insufficient. However, a similar number of participants was used in the present study where significant differences were detected. It is possible that the participants in previous studies [7,8] may have been at the healthy end of the metabolic risk spectrum [30] and able to dispose of more glucose under the presence of similar insulin concentrations in response to PA breaks compared with participants in the current study and the studies by Henson et al. [27] and Chrismas et al. [29]. Further adequately powered studies are required to establish the effects of PA breaks across the metabolic risk spectrum.

This study is the first, to our knowledge, to directly examine the acute effects of accumulating volume and intensity-matched PA in different frequencies on postprandial metabolism. Despite the current UK PA guidelines recommending that MVPA is accumulated in bouts of ≥10 min to benefit health [6], we observed that engaging in three,

10-min PA bouts separated by ~3 h did not suppress postprandial insulin compared to uninterrupted sitting. Conversely, engaging in 2-min PA breaks every 30 min suppressed insulin by ~30% compared with uninterrupted sitting and engaging in 10-min PA bouts spread across the day. Previous research has shown that insulin was suppressed by 18% in response to 1 min 40 s walking breaks every 30 min compared with a single continuous intensity-matched 30 min walking bout [10]. Conversely, another study observed no difference in postprandial insulin between a single continuous 60-min bout of moderate-intensity PA compared with 5-min intensity-matched PA breaks spread over 10 h [31], possibly because the frequency of PA breaks was insufficient. The more frequent PA breaks in the present study and the study by Peddie et al. [10] may have upregulated or maintained insulin sensitivity related pathways, whereas 10-min PA bouts performed approximately every 3 h were not sufficient. This suggests that interrupting sitting with shorter, more frequent PA breaks may be more beneficial than engaging in longer, less frequent PA breaks. Additionally, participants reported lower levels of perceived exertion whilst engaging in the more frequent breaks. This type of PA regime may thus be more achievable for inactive and sedentary adults in addition to offering greater cardiometabolic benefits.

Despite several reports of attenuated postprandial glucose concentrations in response to short, frequent moderate-intensity PA breaks in healthy adults [7,8,32], adults who are overweight/obese [10,33,34] and individuals with type 2 diabetes [35,36], postprandial glucose was unaffected by the PA breaks in the present study. The sample in the present study could be considered metabolically health due to their normal fasting glucose levels. It has been suggested that individuals who are metabolically health may reduce the amount of insulin required to maintain normal glucose homeostasis in response to interrupting sitting [30]. This would thus explain the lack of change in postprandial glucose observed here. Additionally, the lack of change may have been due to the relatively low elevation in blood glucose after the test meals, which could have limited the potential of the PA breaks to

attenuate glucose. We chose to provide participants with a mixed meal to reflect habitual dietary intakes. Although the predicted glycaemic index of the breakfast was high, the lunch had a moderate glycaemic index and potentially did not stimulate a large enough glucose response that could have potentially been attenuated by PA breaks. Suppressions in postprandial glucose in response to PA breaks may therefore only be observed after consumption of higher glycaemic index meals, as postulated previously [8].

The evidence that regular PA breaks can attenuate postprandial TAG is mixed, with some studies observing beneficial changes [11,37,38] and others not [10,39,40]. Due to the heterogenous nature of the PA breaks (intensity, frequency and duration) and sample characteristics across studies, the reasons for these differences are not clear [4]. That said, lipoprotein lipase (LPL) activity typically peaks >8-22 h after moderate-intensity PA [41] and research that measured the postprandial TAG response to a high-fat test meal the day after engaging in regular PA breaks has typically reported beneficial responses [9,42-44]. Thus, the lack of time-lag between the PA and postprandial measurements and the provision of test meals, where 28-40% of the energy was derived from fat, may explain our null findings. It is also possible that the moderate-intensity PA in the current study was not of a sufficient intensity to elevate LPL activity during the 7.5 h experimental period [38]. As physical activity energy expenditure directly affects postprandial lipaemic responses more strongly than PA intensity or duration [45], the 30 min PA completed during the PA conditions may have also been insufficient. Nonetheless, this volume of walking has been effective in previous studies [37] when the postprandial challenge took place the following day, which may suggest PA timing is key.

A strength of the present study is that it was conducted in females who have been less represented in PA and postprandial research. However, this also poses a limitation as it is not known whether the findings would be generalisable to males. A greater attenuation in

postprandial glucose responses to light-intensity PA breaks has been previously seen in females versus males [35]. However, another study found that sex did not affect acute responses to interrupting sitting [46]. Further research investigating potential sex differences is thus required. The experimental protocols in this study were completed in a controlled laboratory environment. Future research should investigate the efficacy of engaging in frequent PA breaks in free-living settings, such as in the workplace and at home.

In conclusion, this study observed a significant attenuation in postprandial insulin concentrations in response to interrupting sitting with frequent 2-min moderate-intensity PA breaks compared with less frequent 10-min PA bouts of the same intensity and total duration and uninterrupted sitting. Frequent PA breaks may, therefore, be a beneficial strategy to reduce cardiometabolic disease risk in sedentary females.

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Table 1. Participant characteristics (n=14)

Characteristic	Mean ± SD
Age (years)	33.8±13.4
Height (cm)	168.1±5.3
Weight (kg)	76.4±17.4
Body mass index (kg/m^2)	27.1±6.3
Body fat (%)	32.9±10.9
Peak oxygen uptake (mL·kg^{-1}·min^{-1})	34.5±6.6

Variable	Prolonged sitting	HIGH-FREQ	LOW-FREQ	Main effect of condition
Baseline concentrations				
Blood glucose (mmol/L)	4.43 (4.28, 4.59)	4.41 (4.25, 4.57)	4.34 (4.18, 4.50)	0.572
Plasma insulin (µU/mL)	7.72 (4.98, 10.46)	10.36 (7.62, 13.10)	6.50 (3.65, 9.35)	0.092
Plasma triglycerides (mmol/L)	0.79 (0.61, 0.96)	0.77 (0.60, 0.95)	0.74 (0.56, 0.92)	0.787
Postprandial concentrations				
Blood glucose iAUC (mmol/L·7.5 h)	0.87 (-0.72, 2.45)	2.42 (0.80, 4.04)	1.94 (0.31, 3.57)	0.145
Plasma insulin iAUC (µU/mL·7.5 h)	119.98 (92.42, 147.53)	82.86 (55.02, 110.70)	116.61 (88.50, 144.73)	**0.013**
Plasma triglycerides iAUC (mmol/L·7.5 h)	0.43 (-0.37, 1.33)	0.79 (-0.05, 1.64)	0.90 (0.03, 1.77)	0.542
Blood glucose TAUC (mmol/L·7.5 h)	33.79 (32.20, 35.38)	35.35 (33.73, 36.98)	34.87 (33.24, 36.50)	0.146
Plasma insulin TAUC (µU/mL·7.5 h)	179.46 (149.52, 209.41)	147.00 (116.69, 177.31)	177.10 (146.61, 207.59)	**0.015**
Plasma triglycerides TAUC (mmol/L·7.5 h)	6.15 (5.32, 6.97)	6.47 (5.65, 7.29)	6.53 (5.67, 7.40)	0.602
Buffet intake				
Total energy intake (kJ)	3442 (2629, 4254)	3187 (2374, 3999)	3014 (2183, 3846)	0.579
Total carbohydrate (g)	84.8 (69.2, 100.4)	93.7 (78.1, 109.3)	78.3 (62.7, 93.9)	0.374
Total fat (g)	24.6 (18.9, 30.1)	22.8 (17.3, 28.4)	19.4 (13.8, 24.9)	0.400

Total protein (g)	33.6 (22.5, 44.6)	34.1 (22.9, 45.1)	32.9 (21.9,44.0)	0.989

Table 2. Biochemical and nutritional intake values for each condition

Bolded text denotes a significant main effect. HIGH-FREQ, sitting interrupted with 2 min PA breaks every 30 min; LOW-FREQ, sitting interrupted with 10 min PA breaks every 180 min; iAUC, incremental area under the curve; PA, physical activity.

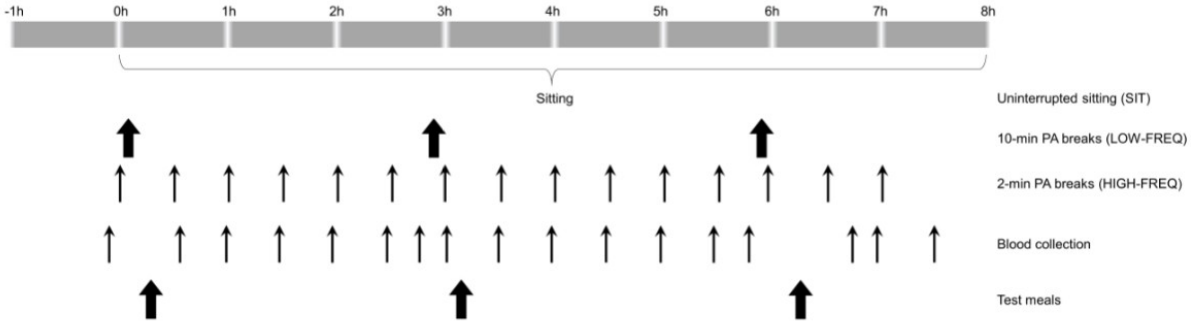

Figure 1. Schematic of experimental condition protocols. PA, physical activity; HIGH-FREQ, sitting interrupted with 2 min PA breaks every 30 min; LOW-FREQ, sitting interrupted with 10 min PA breaks every 180 min.

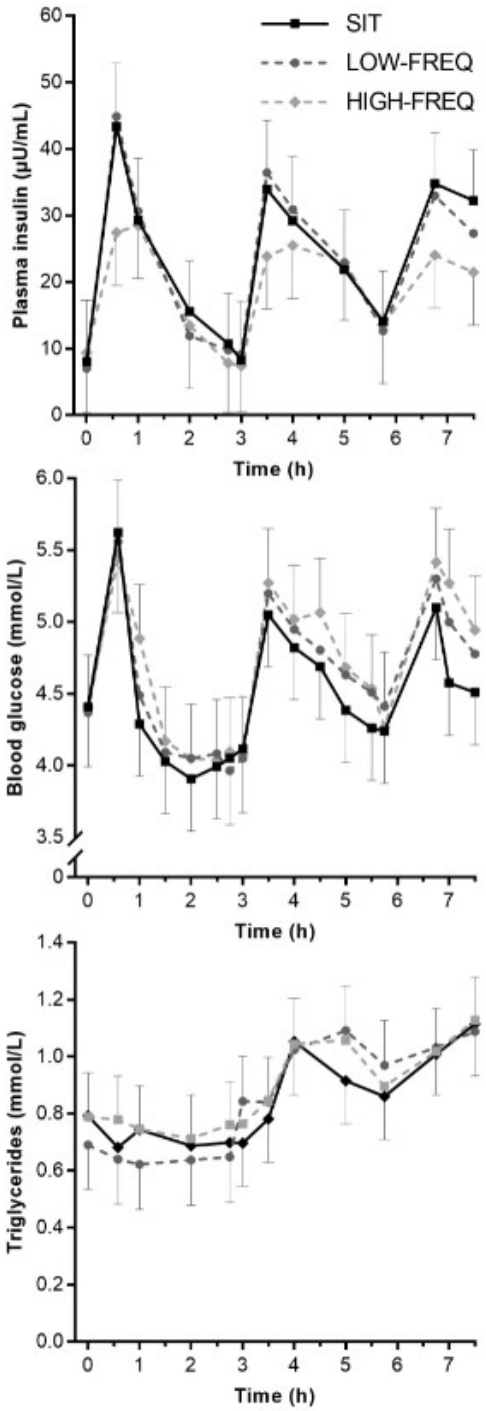

Figure 2. Changes in insulin, glucose, and triacylglycerol concentrations during prolonged sitting (SIT)**,** sitting interrupted with 2 min PA breaks every 30 min (HIGH-FREQ), and sitting interrupted with 10 min PA breaks every 180 min (LOW-FREQ). Data are mean and 95% confidence intervals. Some error bars have been omitted for clarity.