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RESEARCH ARTICLE

Interrupting prolonged sitting with repeated chair stands or short walks reduces postprandial insulinemia in healthy adults

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Abstract

We determined if interrupting prolonged sitting with practical "activity snacks" could reduce postprandial glycemia and insulinemia in healthy adults. Fourteen participants (7 males, 7 females; 24 ± 5 yr; 25 ± 5 kg/m^2; 40 ± 8 mL/kg/min; 7,033 ± 2,288 steps/day) completed three 7.5-h trials in a randomized order consisting of uninterrupted sitting (SIT), sitting with intermittent (every 30 min) walking (WALK; 2 min at 3.1 mph), or sitting with intermittent squats (SQUAT; 15 chair stands with calf raise). Mixed-macronutrient liquid meals provided 20% ("breakfast") and 30% ("lunch") of daily energy needs to mimic Western meal patterns. Blood samples were obtained for analysis of postprandial plasma glucose and insulin concentrations, and skeletal muscle biopsy samples were collected to measure markers of contraction- and insulin-mediated glucose uptake signaling. Postprandial glucose and insulin did not differ across conditions following breakfast. After lunch, peak insulin concentration was lower in SQUAT (52 ± 27, $P < 0.01$) and WALK (62 ± 35, $P < 0.05$) compared with SIT (79 ± 43 μIU/mL). The insulin incremental area under the curve (iAUC) 1 h following lunch was 37 and 29% lower in SQUAT ($P < 0.01$) and WALK ($P < 0.05$) compared with SIT, respectively; however, 3-h insulin iAUC was reduced in SQUAT only (24% vs. SIT, $P < 0.05$). The 3-h insulin:glucose iAUC was reduced following lunch in both SQUAT (30%) and WALK (23%) compared with SIT ($P < 0.05$). Phosphorylation of AKT^{Thr308}, AKT^{Ser473}, and AS160^{Ser318} was not different between conditions ($P > 0.05$). Interrupting prolonged sitting with short walks or repeated chair stands reduces postprandial insulinemia in healthy adults. Our results may have implications for mitigating cardiometabolic disease risk in adults who engage in periods of prolonged sitting.

NEW & NOTEWORTHY Breaking up prolonged sitting with intermittent walking breaks can improve glycemic control. Here, we demonstrated that interrupting prolonged sitting every 30 min with 1 min of repeated chair stands was as effective as 2-min treadmill walks for lowering postprandial insulinemia in healthy adults. Markers of contraction- and insulin-mediated muscle glucose uptake were unchanged. Repeated chair stands as a form of body-weight resistance activity may represent a cost- and space-efficient activity break for mitigating cardiometabolic-disease risk.

glucose; insulin; physical activity; sedentary behavior; skeletal muscle

INTRODUCTION

Prolonged periods of sedentary behavior in a sitting or reclined posture are highly prevalent in modern society (1, 2). Defined as any waking behavior characterized as 1.5 metabolic equivalents (METs) or less (3), sedentary behavior is characteristic of many modern occupations, methods of transportation, and leisure-time activities. Accumulating evidence suggests that a high volume of uninterrupted sedentary time is an independent risk factor for cardiometabolic diseases, including type 2 diabetes and cardiovascular disease (4, 5), as well as all-cause mortality (6, 7). While performing volumes of physical activity above that of current public health recommendations (i.e., ~60–75 min/day) may attenuate some risks associated with high daily sitting time (8), most adults fail to meet, let alone exceed, physical activity recommendations (9, 10). Considering most adults spend the majority of waking hours in a sedentary state (1, 2), innovative strategies that reduce and/or interrupt prolonged sitting are needed to mitigate the independent consequences of a sedentary lifestyle and improve public health.

Encouraging reports suggest that interrupting sitting with short bouts of light-intensity physical activity can reduce cardiometabolic disease risk factors associated with prolonged sitting. For example, breaking up sitting with ~2–3 min of walking or cycling every ~20–30 min has been shown to lower elevations in glucose and insulin concentrations following meals (11–14), major precipitating factors for the development of cardiometabolic diseases (15–18). Increases in skeletal muscle glucose uptake are thought to contribute to activity-break-induced improvements in glycemic control (19); however, limited studies have explored skeletal muscle mechanisms.

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Importantly, reductions in postprandial glycemia and insulinemia have been observed not only in inactive (20, 21) and overweight or obese (13) but also healthy adults (12, 14, 22), highlighting the wide utility of activity breaks for the mitigation of sedentary behavior-induced cardiometabolic disease in a range of individuals. However, implementing such strategies outside of the laboratory may be constrained by the cost of specialized equipment (e.g., treadmill or bicycle), conflicting workplace requirements/norms (e.g., the need to stay at one's desk), and/or limited physical space beyond one's sedentary area (e.g., an office cubicle or constrained to one's home) (23, 24). Thus identifying cost-effective and practical interventions that do not require extra equipment or space may help reduce real and/or perceived barriers to adopting this efficacious disease risk-reduction behavior.

While sometimes underappreciated, resistance exercise can also improve glycemic control in healthy individuals (25, 26) and is associated with reduced risk of type 2 diabetes (27) and all-cause mortality (28). Thus body-weight resistance exercise may represent an accessible and cost-effective activity break for improving postprandial glycemic control and mitigating sitting-induced cardiometabolic-disease risk. While various iterations of predominantly walking breaks have been shown to improve postprandial glycemic control during prolonged sitting, there are limited reports investigating the efficacy of intermittent body-weight resistance exercise. Improvements in glycemic control with 3-min resistance exercise breaks every 30 min has recently been demonstrated in adults with obesity (29) and type 2 diabetes (30); however, the efficacy in inactive but otherwise healthy adults remains to be explored. Considering that prolonged periods of sitting among seemingly healthy adults can also compromise metabolic health (31) and increase cardiometabolic disease risk (4, 5), investigating the efficacy of accessible and cost-effective activity breaks in this population is needed.

The purpose of the present study was to determine the effect of interrupting prolonged sitting every 30 min with body-weight resistance exercise ("repeated chair stands with calf raise") or light-intensity walking on postprandial glycemia and insulinemia in healthy adults. We also obtained skeletal muscle biopsy samples at the end of each trial to explore potential mechanisms. The body-weight resistance exercise protocol selected did not require specialized equipment nor space beyond one's immediate sedentary area and could be performed "on the spot" with the use of a chair. We hypothesized that postprandial glycemia and insulinemia would be similarly reduced when prolonged sitting was interrupted with short breaks of body-weight resistance exercise or treadmill walking.

MATERIALS AND METHODS

Participants and Ethics Approval

Fourteen inactive but otherwise healthy adults between the ages of 18 and 35 yr were recruited from the Greater Toronto Area via poster advertisement and online trial registration (NCT03896828). Participants were deemed recreationally inactive based on performing <150 min of moderate-intensity physical activity per week, completing <3 exercise sessions per week, and having a peak oxygen uptake ($\dot{V}O_2$peak) categorized as "good" or below based on ACSM age- and sex-normative values (32). Inclusion criteria also included a self-reported sitting time of >7 h per day. Female participants were eumenorrheic and not on hormonal contraceptive medication. Exclusion criteria included an inability to perform physical activity as determined by the Physical Activity Readiness Questionnaire (PAR-Q), regular tobacco or drug use, and/or a diagnosed medical condition under the care of a physician. Sample size was determined using the previously reported effect size ($d = 0.89$) for the change in our primary outcome, postprandial insulin incremental area under the curve (iAUC) (13), with light-intensity walking breaks in overweight adults. To detect a significant effect ($P < 0.05$) of activity breaks compared with prolonged sitting with 80% power, $n = 12$ was required. To account for a potentially lower effect size with the healthier population investigated in the present study, $n = 14$ were recruited. Muscle signaling was a secondary outcome not included in sample size calculations. The experimental protocol was approved by the University of Toronto Health Sciences Research Ethics Board, and all procedures were conducted in accordance with the Declaration of Helsinki. All participants were informed of the study purpose, experimental procedures, and potential risks before obtaining written informed consent.

Baseline Testing

Following a 10-h overnight fast, body mass and composition were assessed with air displacement plethysmography (BodPod; Cosmed USA, Inc., Concord, CA). Participants were then provided a 10% carbohydrate beverage (1.2 g/kg) to consume ad libitum before performing an incremental exercise test on a treadmill (LifeFitness 9500HR, Mettawa, IL) for determination of $\dot{V}O_{2peak}$. Participants performed a 2-min warm-up at a self-selected pace between 5.5 and 6.5 mph at 0%, after which the incline increased by 2% every 2 min until volitional exhaustion as previously described (33). Heart rate was measured continuously using a chest-worn strap (Polar A3, Lake Success, NY) and respiratory gases (iWorx GA-300, Dover, NH) were obtained for determination of $\dot{V}O_{2peak}$. A respiratory exchange ratio above 1.1 was achieved for all tests, and $\dot{V}O_{2peak}$ was defined as the highest average oxygen consumption over 30 s. Following a brief rest, participants were familiarized with the experimental trial exercise breaks before leaving the laboratory with an accelerometer (Actigraph; GT3X-BT, Pensacola, FL) to wear for 3 days for determination of habitual daily step count.

Experimental Trial Overview

Using a repeated-measures crossover design, participants underwent three trials at least 1 wk apart. Females were tested in the midfollicular phase of the menstrual cycle (*days 4* to *10*), which was determined from participant-reported onset of menses, based on previously reported fluctuations in insulin sensitivity across the menstrual cycle (34). In a counterbalanced randomized order, participants completed three 7.5-h interventions on separate days: *1*) uninterrupted sitting (SIT); *2*) sitting with 2-min walks at 3.1 mph every 30 min (WALK); and *3*) sitting with 15 "body-weight squats" (1 min) every 30 min (SQUAT). During SIT, participants remained seated at a desk for 7.5 h, with the only exception being visits to a nearby restroom (~50-m walk away) as

required. In WALK, participants interrupted sitting every 30 min with 2-min walks at 3.1 mph on treadmill positioned beside the desk. This is similar to previous research implementing walking breaks (13, 14) and also reflects average human walking speed (35). In SQUAT, participants interrupted sitting every 30 min with 1 min of repeated squats, performed at a pace of 15 squats per min (or 1 "squat" every 4 s) using an online metronome. The squats performed were a "chair stand with calf raise," which was selected to reduce the risk of injury, standardize squat depth, and recruit similar muscle groups as walking. The SQUAT activity break was also selected based on pilot data (n = 10 adults) demonstrating similar exercise-induced energy expenditure as the 2-min WALK activity break (data not shown as we also measure energy expenditure in the current investigation; P = 0.41). Throughout all trials, participants were permitted to read or work on a laptop to pass the time.

Pretrial Dietary and Physical Activity Controls

Participants were provided with standardized diets consisting of prepackaged foods the day before experimental trials. Resting energy expenditure obtained from the BodPod, which uses the Nelson prediction equation, was multiplied by an activity factor of 1.4 for determination of total energy intake. The diet provided 2,027 ± 410 kcal/day (55 ± 1% carbohydrate, 29 ± 3% fat, and 16 ± 2% protein), and participant consumption was verified with a checklist. Participants were also asked to refrain from structured physical activity for 4 days before experimental trials and to not exceed 7,500 steps/day the day before the experimental trials (monitored via accelerometry). On the morning of experimental trials, participants were asked to continue wearing the accelerometer and arrive to the laboratory by vehicle or public transit.

Sample Collection Protocol

Following a 10-h overnight fast, participants arrived to the laboratory between 0630 and 0730 h. An indwelling catheter was inserted into a forearm vein, and a single fasting blood sample was obtained. Thereafter, the 7.5-h trial began, and blood samples were collected every 30 min, which were immediately followed by the activity breaks in WALK and SQUAT. At the end of each trial, a skeletal muscle biopsy sample was obtained ~30–45 min after the final activity break using procedures we have previously described (36). Briefly, muscle samples were obtained from the vastus lateralis under local anesthesia (1% lidocaine) using a Bergstrom needle adapted with suction, immediately snap frozen in liquid nitrogen, and stored at −80°C for future analysis. On the second trial, a resting biopsy was taken at the same time as the pretrial fasting blood sample to normalize the signaling of the posttrial biopsies (see below). All blood samples were placed into EDTA-coated vacutainers, and catheters were flushed with ~1 mL of 0.9% saline to prevent coagulation between blood draws. Blood samples were immediately centrifuged at 2,500 *g* for 15 min. In the rare instance the centrifuge was not available, blood samples sat on ice for a maximum of 30 min before centrifugation. Centrifuged samples were placed at 4°C until plasma was aliquoted and stored at −80°C until further analyses.

Energy expenditure of the activity breaks was estimated using indirect calorimetry during the 5fifth activity break in SQUAT and WALK. Oxygen consumption ($\dot{V}O_2$; L consumed) was measured during 5 min of seated baseline, the activity break, and seated recovery for a total of 10 min. Activity-break induced $\dot{V}O_2$ was determined by subtracting resting $\dot{V}O_2$ (estimated from the final 3 min of seated baseline) from total $\dot{V}O_2$ during the activity break and seated recovery. Energy expenditure of the activity breaks was estimated using the assumption that 1 liter of oxygen consumed is equivalent to 5 kcal of energy expended. Step count was measured via accelerometry on trial days until participants left the laboratory (~1530 h).

Experimental Trial Dietary Intake

Participants consumed mixed-macronutrient liquid meals that mimicked the energy distribution and macronutrient composition of meals in Western society (37). After a fasting blood sample (and resting muscle biopsy in *trial 2*) was obtained, breakfast and lunch were provided at 0 and 3 h and consisted of 20 and 30% of daily energy intake, respectively. Participants consumed the liquid meals within 5 min, and the macronutrient composition was based on a diet consuming 55% carbohydrate and 1.2 g protein/kg/day, with the remaining energy requirements met with dietary fat. Thus breakfast and lunch consisted of 406 ± 87 and 610 ± 130 kcal, respectively (55% carbohydrate, 17 ± 1% protein and 28 ± 1% fat). Total carbohydrate content at breakfast and lunch was 56 ± 12 and 84 ± 18, respectively. Carbohydrate and fat content were provided with commercially available carbohydrate powder (Tang; Kraft, Don Mills, Canada and Polycal), protein-free powder (PFD-1; Mead Johnson, Evansville, IN), and grapeseed oil; protein was provided as crystalline amino acids (Ajinomoto North America, Inc., Raleigh, NC) modeled on the basis of egg protein. Activity breaks were performed immediately following blood sample collection, with the exception of the two mealtime points where the order of events was blood sample, and meal consumption (consumed within 5 min), activity break.

Analytical Procedures

Plasma glucose.

Plasma glucose was analyzed photometrically using the glucose oxidase reaction in conjunction with an auxiliary (peroxidase) reaction (Infinity, ThermoScientific, Canada). Briefly, 10 μL of sample and 200 μL of assay reagent were added to a 96-well plate. Following a 30-s shake, plates were incubated at 37°C for 10 min and read at 540 nm. To minimize interassay variability, all samples from a given participant (13 samples/trial; 39 samples/participant) were run in duplicate on the same 96-well plate. Investigators prepared glucose standards (0, 2.5, 5.0, 7.5, and 10 mmol/L) using laboratory grade glucose anhydrous [D-(+)-glucose, Millipore Sigma, Canada], and a glucose control (5.56 mmol/L; glucose standard, Millipore Sigma, Canada) was added to each plate to verify accuracy of the standard curve. Glucose concentrations were visibly inspected, and in the event the coefficient of variation (CV) between duplicates was >6.0%, the sample was rerun in duplicate on a separate plate to determine which repeat from the original plate was correct. Across all

samples analyzed, the intra-assay CV between sample duplicates was 1.8 ± 1.6%.

Plasma insulin.

Plasma insulin was measured using an enzyme-linked immunosorbent assay (ELISA; ALPCO Immunoassays). Samples were run in duplicate following manufacturer instructions using the standards, controls, and reagents provided within the ELISA kit. To minimize interassay variability, all samples from a given participant (13 samples/trial; 39 samples/participant) were run in duplicate on the same insulin assay. Insulin concentrations were visibly inspected, and in the event the CV between duplicates was >10% (n = 23 samples of 540), an attempt was made to identify the most likely value by using the preceding and succeeding time points. If this was not possible, an average of the two duplicates was used. Across all samples analyzed, the intra-assay CV between sample duplicates was 4.0 ± 5.2%, which is consistent with the advertised intra-assay precision by the manufacturer (3.2–10.3%).

Muscle homogenization and immunoblotting.

Skeletal muscle samples were homogenized in radioimmunoprecipitation assay buffer (65 mM Tris-base, 150 mM NaCl, 1%NP-40, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate), supplemented with protease and phosphatase inhibitors (Roche, Mannheim, Germany), using a handheld motorized tissue homogenizer. Myofibrillar and cytoplasmic fractions were separated by centrifugation at 700 *g* for 5 min at 4°C, with the cytoplasmic fraction (supernatant) used for immunoblotting analysis. Protein concentrations of cytoplasmic fractions were determined by a commercially available bicinchoninic acid assay (Fisher Scientific, Toronto).

Immunoblotting procedures were conducted as described previously (36). Primary antibodies utilized were as follows: Akt^{Thr308} (no. 2965), Akt^{Ser473} (no. 4060), $AS160^{Ser318}$ (no. 8619), and ACC^{Ser79} (no. 3661) and were all purchased from Cell Signaling Technology (Danvers, MA) and used at a dilution of 1:1,000 in 5% BSA in TBS Tween. Bands were quantified using Protein Simple AlphaView SA software and normalized to Ponceau S and a gel control (identical generic sample run on every gel) to account for differences in total protein loaded and gel-to-gel variability (38).

Calculations and Data Analysis

A surrogate index of insulin resistance was calculated from average fasting plasma insulin and glucose concentrations across trials using the homeostatic model assessment (HOMA2) online calculator (https://www.dtu.ox.ac.uk/homacalculator/) version 2.2.3. The insulin:glucose ratio at all blood sampling time points was calculated as a measure of the insulin response to postprandial glycemia. Plasma glucose, insulin, and the insulin:glucose ratio in response to breakfast and lunch were plotted across time for all subjects and the positive incremental area under the curve (iAUC) over 1 and 3 h postprandial was computed using an online program (Prism 8, GraphPad Software, San Diego, CA). Positive iAUC was selected as it represents glycemic responses to food more accurately than total AUC (39) and also allowed for direct comparison with the majority of previous research in this field. Peak glucose and insulin in response to breakfast and lunch were determined. Phosphorylation of proteins in muscle samples obtained at the end of each trial are reported as fold change from the baseline muscle sample obtained on the morning of the second trial.

Statistical Analyses

Statistical analyses were performed on Prism 8 (GraphPad Software, San Diego, CA). Normality of the data was confirmed using the Kolmogorov-Smirnov test and Q-Q plots. A one-way repeated measures ANOVA was used to test for differences in step counts, glucose, insulin, and immunoblotting outcome variables across trials (SIT, SQUAT, and WALK). In the event of significance with the ANOVA, a Holm-Sidak post hoc test was performed to isolate differences between the activity breaks and SIT. A paired Student's t test was used to compare activity-induced $\dot{V}O2$ in SQUAT and WALK. Blood analysis is from n = 14; however, muscle samples were only collected from a subset of participants and thus muscle outcomes reflect n = 12 (7 males and 5 females). The level of significance for all analyses was $P \leq 0.05$. Results are presented as means ± SD unless otherwise stated.

RESULTS

Participant Characteristics

Fourteen males and females (n = 7 each) completed the experimental protocol (Table 1). Females were tested on day 7 ± 2 of the menstrual cycle (range: 4 to 10 days), and average cycle length throughout the study was 28 ± 4 days (range: 22 to 35 days). There were no differences between trials in the preceding day step count (5,717 ± 2612 steps/day, P = 0.93), or fasting plasma glucose (5.3 ± 0.3 mmol/L, P = 0.89) and insulin (8.2 ± 2.9 μIU/mL, P = 0.96) concentrations on the morning of experimental trials.

Activity Break Characteristics

Activity breaks in SQUAT and WALK were completed with 100% compliance. Activity breaks elicited similar increases in $\dot{V}O2$ in SQUAT (1.15 ± 0.40 liters) and WALK (1.22 ± 0.58 liters; P = 0.46), resulting in comparable estimates of energy expenditure for single (~6 kcal) and cumulative (~78 kcal) activity breaks throughout the day. Trial day step count was elevated in WALK (5,639 ± 1,338, $P < 0.001$) but not SQUAT (2,765 ± 904, P = 0.07), compared with SIT (1,971 ± 863 steps).

Postprandial Glycemic Control

Plasma glucose, insulin, and the insulin:glucose ratio throughout the trials are depicted in Fig. 1. Postprandial peak glucose and glucose iAUC following breakfast and lunch were similar across trials (Table 2). Postprandial insulin concentrations did not differ in response to breakfast; however, following lunch the peak insulin concentration was lower in SQUAT (51.6 ± 26.7, P = 0.0008) and WALK (62.2 ± 34.9, P = 0.0195) compared with SIT (78.9 ± 43.0 μIU/mL; Table 2). The insulin iAUC 1 h following lunch was 37 and 29% lower in SQUAT (P = 0.0037) and WALK (P = 0.0101) compared with SIT, respectively (Table 2); however, the 3-h insulin iAUC was reduced in SQUAT only (24% vs. SIT, P = 0.0112; WALK vs. SIT, P = 0.1063; Fig. 2). The insulin:glucose iAUC was also reduced

Table 1. *Participant characteristics*

	Males	Females	All
Sample size (*n*)	7	7	14
Age, y	24 ± 6	24 ± 4	24 ± 5
Body mass, kg	82.8 ± 21.7	59.8 ± 4.5*	71.3 ± 19.2
BMI, kg·m^{-2}	26.6 ± 6.3	22.3 ± 1.8	24.5 ± 5.3
Body fat, %	23.6 ± 8.3	27.3 ± 4.9	25.5 ± 7.3
Resting energy expenditure, kcal·day^{-1}	1,684 ± 558	1,234 ± 130*	1,459 ± 304
Daily step count, steps·day^{-1}	6,688 ± 1,806	7,380 ± 2,491	7,033 ± 2,288
$\dot{V}O_2$peak, ml·kg^{-1}·min^{-1}	41.8 ± 8.8	37.8 ± 4.1	40 ± 8
FPG, mmol·L^{-1}	5.4 ± 0.3	5.1 ± 0.3	5.3 ± 0.3
FPI, μIU·mL^{-1}	8.9 ± 3.1	7.5 ± 2.4	8.2 ± 2.9
HOMA2-IR	1.1 ± 0.7	1.0 ± 0.3	1.1 ± 0.4

Values are means ± SD BMI, body mass index; $\dot{V}_{O2peak}$, peak oxygen uptake; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HOMA2-IR, homeostatic model assessment 2 of insulin resistance. *$P < 0.05$, different from males.

following lunch in SQUAT and WALK compared with SIT (1-h iAUC: SQUAT vs. SIT $p = 0.024$, WALK vs. SIT $P = 0.087$; 3-h iAUC: SQUAT vs. SIT 0.016, WALK vs. SIT $P = 0.039$; Table 2 and Fig. 2). Glucose and insulin responses over the combined 6-h period were not different between trials (Supplemental Table S1, available at https://doi.org/10.5683/SP2/T3F7NI).

Skeletal Muscle Outcomes

AS160^{Ser318} phosphorylation was similar across all trials (SIT: 1.13 ± 0.31-fold change from baseline, SQUAT: 1.03 ± 0.35, WALK: 1.04 ± 0.37, $P = 0.61$; Fig. 3). Furthermore, AKT phosphorylation at Serine 473 (SIT: 1.49 ± 0.83, SQUAT: 1.61 ± 0.68, WALK: 1.36 ± 1.08; Fig. 3) and Threonine 308 (SIT: 1.28 ± 0.34, SQUAT: 1.21 ± 0.35, WALK: 1.16 ± 0.51; Fig. 3) was similar across trials ($p = 0.51$ and 0.57, respectively). Finally, ACC^{Ser79} was probed during immunoblotting as a marker of AMPK activity and contraction-related signaling; however, no discernable bands were detected (data not shown).

DISCUSSION

Prolonged periods of sedentary behavior have been linked with elevations in postprandial glycemia and insulinemia (12, 14, 21), major precipitating factors in the development of metabolic disease (15–18). The major novel finding from the present study was that interrupting prolonged sitting every 30 min, with either 1 min of repeated chair stands or 2 min of treadmill walks, lowered postprandial insulinemia following lunch in healthy men and women. To our knowledge, this is the first investigation to directly compare the efficacy of body-weight resistance exercise breaks to that of treadmill walking breaks for mitigating sitting-induced cardiometabolic disease risk factors in adults without type 2 diabetes.

In healthy adults with normal glucose tolerance, postprandial hyperinsulinemia is suggestive of peripheral insulin resistance (15) and is often recognized as a first step in the etiology of type 2 diabetes (18). Thus interventions that reduce postprandial insulinemia have been suggested to be an efficacious strategy to delay and/or prevent the development of lifestyle-induced metabolic diseases. Accumulating evidence suggests that interrupting sitting with light- or moderate-intensity walking breaks can attenuate postprandial insulin excursions (13, 14, 20, 21); however, the efficacy of other types of exercise that do not require equipment nor space beyond one's immediate sedentary area remain to be investigated. Our study demonstrates that frequently interrupting sitting with repeated chair stands is an effective alternative to walking breaks for reducing postprandial insulinemia in healthy adults. To the best of our knowledge, we are the first to demonstrate that a form of body-weight resistance activity breaks is as effective as volume-matched treadmill walking for reducing postprandial insulinemia in healthy adults. Previously, a 3-min routine of squats, lunges, and calf raises performed every 30 min lowered blood glucose excursions in adults with type 2 diabetes (40) and reduced insulinemia in adults with obesity (41). Our findings extend the beneficial effects of body-weight resistance activity breaks to healthy adults and also demonstrate the efficacy of a more time-efficient protocol requiring only 1 min of activity every 30 min. Considering previously reported financial and/or spatial constraints associated with implementation of walking breaks outside of the laboratory (23, 24),

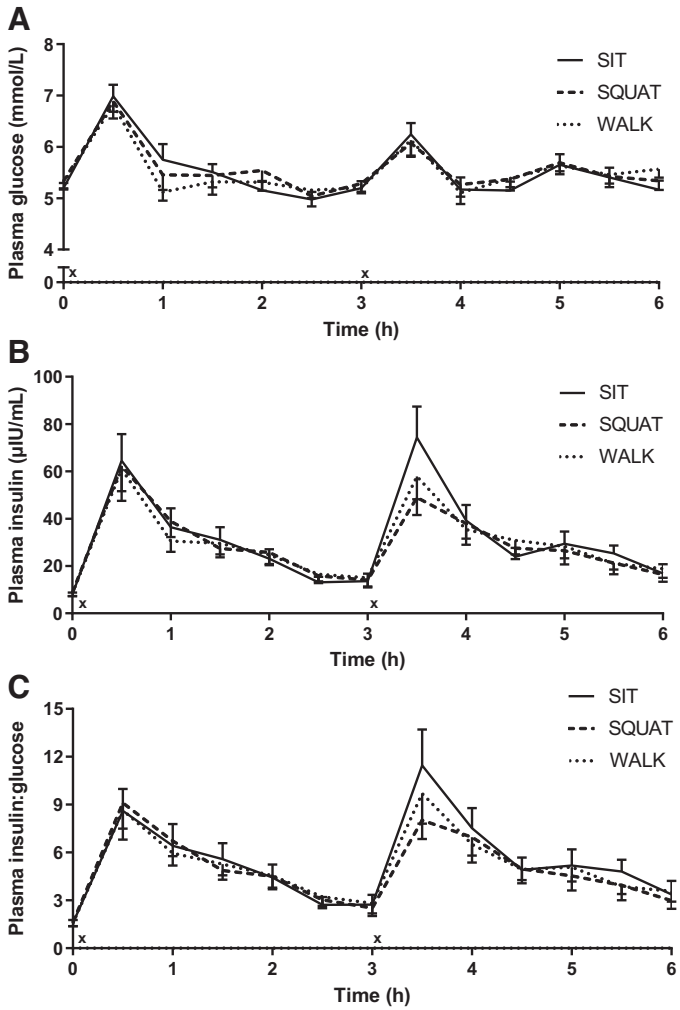

Figure 1. Mean (±SE) plasma glucose, insulin, and insulin:glucose measured over 6-h trials in SIT, SQUAT, and WALK. X denotes meal provision, immediately following blood sample.

Table 2. *Postprandial glucose and insulin responses during prolonged sitting (SIT) or sitting interrupted with activity breaks (SQUAT and WALK)*

	Breakfast			Lunch		
	SIT	SQUAT	WALK	SIT	SQUAT	WALK
Glucose iAUC, mmol·L^{-1}·3 h	100 ± 79	92 ± 77	72 ± 47	89 ± 93	72 ± 74	87 ± 83
Glucose iAUC, mmol·L^{-1}·1 h	58 ± 41	51 ± 31	46 ± 26	37 ± 35	29 ± 33	37 ± 38
Insulin iAUC, μIU·mL^{-1}·3 h	3,972 ± 2500	3,871 ± 2596	3,717 ± 2580	3,954 ± 2261	2,992 ± 1736*	3,421 ± 2314
Insulin iAUC, μIU·L^{-1}·1 h	2,138 ± 1383	2,012 ± 1388	1,908 ± 1519	2,232 ± 1540	1,412 ± 902*	1,575 ± 1145*
Insulin:glucose iAUC, AU·3 h	652 ± 378	617 ± 368	614 ± 407	700 ± 413	489 ± 311*	540 ± 417*
Insulin:glucose iAUC, AU·1 h	266 ± 157	295 ± 201	283 ± 221	334 ± 222	232 ± 156*	267 ± 229
Peak glucose, mmol·L^{-1}	6.9 ± 0.8	6.9 ± 0.7	6.8 ± 1.0	6.4 ± 0.7	6.5 ± 0.7	6.6 ± 0.8
Peak insulin, μIU·mL^{-1}	62 ± 33	60 ± 33	55 ± 39	79 ± 43	52 ± 27*	62 ± 35*

Values are means ± SD. iAUC, insulin incremental area under the curve. AU, arbitrary units. *$P < 0.05$, significantly different vs. SIT.

repeated chair stands may represent a cost-effective and practical activity break for mitigating sitting-induced cardiometabolic-disease risk.

The activity breaks in the present study lowered postprandial insulinemia in response to the second but not first meal of the day, suggesting that a minimum volume of activity may need to be accrued before reductions in postprandial insulinemia are observed. Others have reported reduced insulin excursions in response to the first meal of the day with short walking breaks (13, 20, 21); however, these protocols involved faster walking speeds (e.g., up to 5.2 mph) (20) and intensities requiring a jog-like pace (e.g., 65% $\dot{V}_{O2peak}$) (21). The activity breaks in the present study were more modest and based on the metabolic cost of WALK (2 min at 3.1 mph), which was selected to reflect average human walking speed (35). It is possible that the lower volume and/or intensity of activity breaks in the present study contributed to the delayed improvement in postprandial insulinemia throughout the day compared with previous reports. However, whether the energy expenditure of activity breaks modulates the reduction in insulinemia associated with interrupting sitting remains controversial (29, 31, 42, 43). Simply interrupting sitting may be more important, in which case the greater frequency of activity breaks in other studies (e.g., every 20 min) (13, 21) may also explain the discrepancy. From a practical perspective, high-volume and high-frequency activity breaks may not be possible at home or in the workplace (23). Thus identifying minimum thresholds could be important for elucidating efficacious and feasible approaches for mitigating sitting-induced elevations in postprandial insulinemia, for which our study may provide insight. It is also worth mentioning that the reduction in insulinemia with the activity breaks occurred in response to the meal that elicited the highest insulin excursion. While the greater insulin concentration following the second meal may be due to its higher energy and carbohydrate content relative to the first meal, it may also be a reflection of known diurnal fluctuations in insulin secretion (44). Future research should consider the interaction between circadian rhythms and the efficacy of activity breaks to improve postprandial glycemic control.

We did not see a reduction in postprandial glycemia throughout the day in WALK or SQUAT, which contrasts with some (12–14, 22) but not all (20, 21, 29, 45–48) studies investigating the impact of activity breaks on glycemia. With respect to body-weight resistance activity breaks, previous work has demonstrated no change to postprandial glycemia relative to a prolonged sitting condition in middle-aged (~35–55 yr) adults (45, 46). Differences in study design, the activity-break protocol implemented, measurement techniques, and study populations make comparisons among studies inherently challenging. However, accumulating evidence suggests reductions in glycemia with activity breaks are more likely to occur in those with lower baseline fitness (49), higher degree of insulin resistance (30), and/or elevated body mass index (BMI) (50). Indeed, improvements in glycemia are more consistent and of greater magnitude in those with some degree metabolic dysfunction, including adults

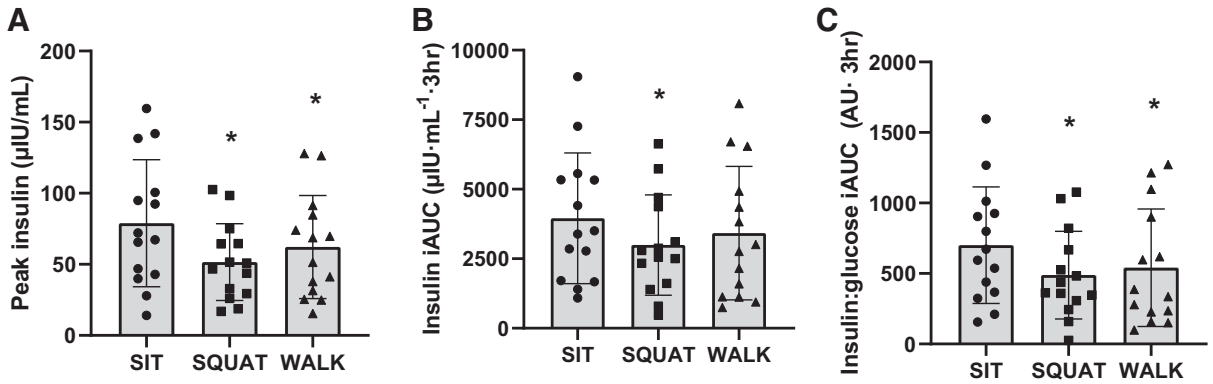

Figure 2. Postprandial insulin responses to lunch. Peak plasma insulin (*A*) insulin incremental area under the curve (iAUC) (*B*) and insulin:glucose iAUC (*C*) during 3 h postprandial.*Significantly different vs. SIT ($P < 0.05$) determined by post hoc analysis following a significant ($P < 0.05$) ANOVA.

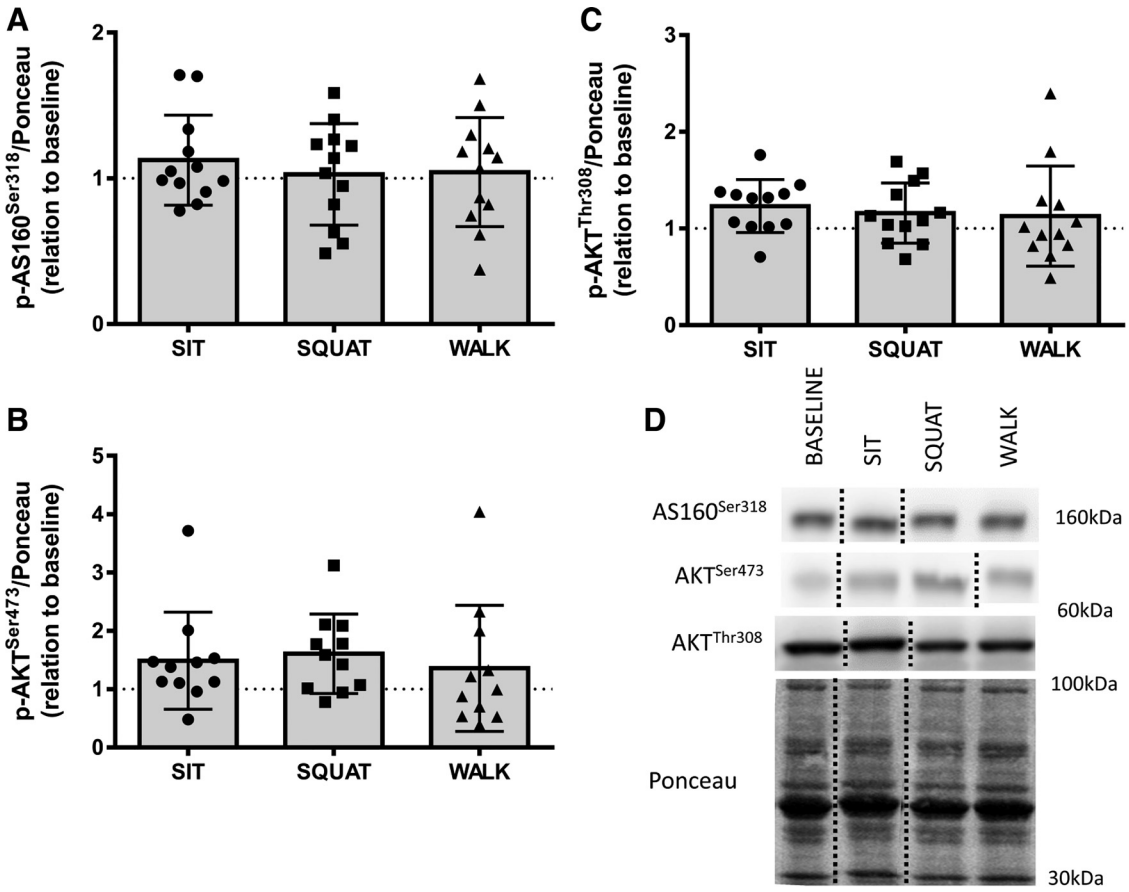

Figure 3. Insulin-related intramuscular signaling. AS160^{Ser318} (*A*), AKT^{Ser473} (*B*), and AKT^{Thr308} (*C*) phosphorylation following each experimental trial day. Representative images of immunoblots for each target and loading control (Ponceau) are displayed in *D*. Dashed vertical lines denote where the order of bands was adjusted so that all representative blots could be displayed in a consistent format.

with obesity (13, 51) and type 2 diabetes (30, 40). The participants in the present study were relatively fit based on $\dot{V}_{O2peak}$ and normoglycemic based on fasting plasma glucose and insulin concentrations. The lack of improvement to glycemia may therefore be unsurprising, and our findings corroborate the notion that activity breaks in normoglycemic adults are more likely to reduce the amount of insulin required to maintain euglycemia (19). It is also possible that the modest energy and carbohydrate content of the mixed-macronutrient meals provided in the present study did not stimulate a large enough glucose excursion for attenuation by the activity breaks. The size and composition of meals were designed to reflect dietary patterns in Western society, whereby a substantial portion of energy intake (~50%) is reserved for evening consumption (37). It is possible that consuming larger and/or more carbohydrate dense test meals throughout the intervention, similar to other studies (22, 51), would yield different results.

The reduced insulin:glucose ratio following lunch in SQUAT and WALK is suggestive of an "insulin sparing" effect in the maintenance of postprandial glycemia. While this is likely to reflect a coordinated response from a number of tissues including the pancreas and liver, mechanisms in skeletal muscle may be primarily involved. Activity breaks are thought to stimulate skeletal muscle blood flow and glucose uptake via insulin-independent mechanisms (19), which may have reduced the amount of insulin required to maintain glycemia during the postprandial period. We selected activity breaks requiring large muscle groups of the lower body that were similar to others reporting upregulated contraction-mediated glucose uptake pathways in muscle samples obtained at the end of the day (~45 min following the final activity break) (52, 53). However, we were not able to detect an increase in the phosphorylation of ACC in muscle samples obtained at a similar time point, which may be a result of the higher fitness and younger age of our participants than earlier reports (52). The reduced insulin:glucose ratio following lunch may also suggest that activity breaks enhanced insulin-mediated glucose uptake during the postprandial period. However, proteins in the insulin-signaling pathway were unchanged in response to a single day of activity breaks in our study and others (52), although this may be a result of the suboptimal end-of-day timing of muscle biopsies (4.5 h following meal provision). Future studies that obtain muscle samples in closer proximity to the activity breaks and/or meal ingestion are needed to understand the role of contraction- and insulin-mediated muscle glucose uptake on activity break-induced improvements in whole-body glycemia and insulinemia.

Our study has a number of strengths, among which includes an equal representation of male and female participants and careful standardization of menstrual cycle phase

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on trial days. Given the potential influence of menstrual cycle phase on insulin sensitivity (34), we performed calendar-based tracking to ensure metabolic trials were restricted to the midfollicular phase of the menstrual cycle (*days 7 ± 2*). However, our study was not powered to assess sex-based differences, which should be considered a limitation in light of recent evidence reporting superior improvements in blood pressure (54) and glycemic control (55) in response to activity breaks in women. It is also worth noting that step counts in SIT reached ~2,000 steps by the end of the trial (~1530 h), which may seem high for a prolonged sitting condition. However, we allowed participants to commute to the laboratory in the morning and walk to a nearby restroom throughout the trial as needed, which we believe improves the ecological validity of our findings. Also, it appears the accelerometer inaccurately recorded chair stands during trials as "steps," as reflected by the additional ~700 steps/trial in SQUAT compared with SIT. Upon further investigation, we determined that ~40 "steps" were recorded during the 1-min activity break (which would equate to ~600 "steps" throughout the full trial), likely explaining the (nonsignificant) increase in steps in SQUAT compared with SIT. Lastly, it is also important to recognize that while the strict experimental controls and crossover nature of our study enhanced internal validity of our findings, the implications of single day, laboratory-controlled trials for mitigating disease risk should be interpreted with caution. As such, our findings should not be interpreted to suggest that activity breaks represent a replacement for accumulating daily moderate-vigorous intensity physical activity. Research is needed that evaluates the effectiveness of both walking and body-weight resistance activity breaks in real-world settings (e.g., at home and in the workplace), over longer periods (e.g., multiple days or weeks) and in healthy and at-risk populations (e.g., Metabolic Syndrome, older adults, etc.).

To conclude, our study demonstrates that interrupting sitting with brief bouts of repeated chair stands is an effective alternative to treadmill walking for mitigating sitting-induced elevations in postprandial insulinemia in healthy adults. Repeated chair stands require no equipment or space beyond one's sedentary area and may represent a practical strategy for mitigating cardiometabolic disease risk associated with prolonged sitting. Long-term studies evaluating the efficacy and feasibility of body-weight resistance breaks at home and in the workplace are needed as are studies exploring mechanisms underlying the whole-body improvements in glycemic control, both in healthy and at-risk populations.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.B.G. and D.R.M. conceived and designed research; J.B.G., S.E., E.W., N.H., J.M.M., and D.R.M. performed experiments; J.B.G., S.E. and N.H. analyzed data; J.B.G., S.E. and N.H. interpreted results of experiments; J.B.G. prepared figures; J.B.G. drafted manuscript; J.B.G., S.E., E.W., N.H., J.M.M., D.A.K., and D.R.M. edited and revised manuscript; J.B.G., S.E., E.W., N.H., J.M.M., D.A.K., and D.R.M. approved final version of manuscript.

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