Metabolic Effects of Three Different Activity Bouts during Sitting in Inactive Adults

SHENG-XIA MA, ZHENG ZHU, LIN ZHANG, XIAO-MEI LIU, YAN-YU LIN, and ZHEN-BO CAO

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ABSTRACT

MA, S.-X., Z. ZHU, L. ZHANG, X.-M. LIU, Y.-Y. LIN, and Z.-B. CAO. Metabolic Effects of Three Different Activity Bouts during Sitting in Inactive Adults. *Med. Sci. Sports Exerc.*, Vol. 52, No. 4, pp. 851–858, 2020. **Purpose:** This study aimed to determine whether interrupting prolonged sitting with three different walking-bout schedules improves glycemic metabolism relative to continuous prolonged sitting in sedentary adults. **Methods:** In a randomized, crossover trial, 16 inactive healthy adults (7 men, 24 ± 3 yr old) completed four 26-h laboratory sessions, including 22.5 h in an energy expenditure (EE)-testing calorimeter chamber. The four 9-h intervention periods were as follows: 9-h uninterrupted sitting (SIT), 30-min sitting/3-min brisk (60% $\dot{V}O_{2max}$) treadmill walk (WALK3), 45-min sitting/5-min (WALK5), or 60-min sitting/8-min (WALK8). Coprimary outcomes included the difference in the mean interstitial glucose concentration, total area under the curve (tAUC), and incremental area under the curve (iAUC) for the entire 26 and 9 h (intervention period) during three activity-bout conditions compared with SIT. **Results:** Compared with SIT, the 26-h mean glucose concentration was attenuated in WALK8 (Δmean −0.22 mmol·L^{-1} [95% confidence interval = −0.43 to −0.001], P = 0.048) without adjustment for EE and attenuated in WALK3 (Δ−0.47 mmol·L^{-1} [−0.75 to −0.10]), WALK5 (Δ−0.47 mmol·L^{-1} [−0.83 to −0.10]), and WALK8 (Δ−0.53 mmol·L^{-1} [−0.92 to −0.13]) after adjustment for EE (all P < 0.01). The 26-h tAUC was reduced in WALK3 (Δ−11.18 mmol·L^{-1} per 26 h [−20.07 to −2.29]), WALK5 (2.12.67 mmol·L^{-1} per 26 h [−22.54 to −2.79]), and WALK8 (Δ−13.85 mmol·L^{-1} per 26 h [−24.60 to −3.10]) (all P < 0.01), as well as the iAUC (all P < 0.05), only after adjustment for EE. The 9-h mean glucose concentration, tAUC, and iAUC decreased in the three activity-break conditions regardless of EE adjustment (all P < 0.05). **Conclusions:** All three walking-bout conditions improved glycemic metabolism compared with SIT, independent of EE, in inactive, healthy adults. **Key Words:** PHYSICAL ACTIVITY, SEDENTARY BEHAVIOR, POSTPRANDIAL PERIOD, BLOOD GLUCOSE, INSULIN RESISTANCE, ENERGY METABOLISM

Evidence shows that sedentary lifestyles are associated with the incidence of type 2 diabetes (T2D) (1–4), metabolic syndrome (4,5), cardiovascular disease and mortality (1–4,6), cancer incidence and mortality (1), and all-cause mortality (1,3,4,6,7), independent of moderate to vigorous physical activity (MVPA) (1,2,4). However, a few studies found that high levels of MVPA (i.e., about 60–75 min·d^{-1}) eliminate the increased risk of all-cause mortality associated with high sitting time in adults (7,8), and the latest American guideline recommends "move more and sit less" throughout the day (8).

In the recent decade, both observational (9–11) and experimental evidence (12–17) suggests that frequent interruptions to prolonged sitting are beneficially associated with metabolic outcomes. Previous experimental studies have used various activity-bout patterns for interrupting prolonged sitting (based on the frequency, duration, and intensity of activity bouts). Experimental protocols have used 100-s to 5-min bouts of light- or moderate-intensity physical activity every 20–30 min (12–24), with the most common pattern being 2 min of walking every 20 min. However, both the practicality and feasibility of these much more frequent activity-break patterns may be questioned in the real world. Further, studies pertaining to lower activity-break frequency (e.g., every 60 min) (25–27) are limited.

In addition, as sedentary behaviors are defined by low levels of energy expenditure (EE, ≤1.5 metabolic equivalents) (28), interrupting sitting with short-bout physical activity may elicit health benefits via increases in EE. Therefore, whether the effects of interrupting sitting time at different frequencies are identical after controlling for EE needs to be investigated.

Thus, the primary aim of this study was to examine the effects of interrupting prolonged sitting with varying intermittent walking bouts of varying frequencies and durations on glycemic metabolism in sedentary adults. Our primary hypothesis was that interrupting prolonged sitting with a 3-min intermittent walk bout performed in every 30-min block (WALK3), a 5-min intermittent walk bout performed in every 45-min block (WALK5), and an 8-min intermittent walk bout

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Submitted for publication January 2019.

Accepted for publication October 2019.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.acsm-msse.org).

0195-9131/20/5204-0851/0

MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/MSS.0000000000002212

performed in every 60-min block (WALK8) will improve glucose response relative to continuous prolonged sitting (SIT) over a 26-h period, after adjusting for EE.

SUBJECTS AND METHODS

Study Overview

This randomized, four-group crossover study was undertaken between October 1, 2017, and August 6, 2018, and was registered in the Chinese Clinical Trial Registry (ChiCTR1800018829). The Ethics Committee of Shanghai University of Sport approved this trial, and all participants provided informed consent.

Participants made 10 separate visits to the laboratory: a detailed screening visit, a preliminary test visit, four continuous glucose monitor-wearing visits (CGM, Dexcom Platimum G4; Dexcom, San Diego, CA), and four experiment-related visits. Participants completed the following experimental conditions in random order: 1) uninterrupted sitting (SIT), 2) 3-min walking breaks with 30-min sitting between each bout (WALK3), 3) 5-min walking breaks with 45 min between each bout (WALK5), and 4) 8-min walking breaks with 60 min sitting between each bout (WALK8). A 5–14 d washout between conditions was used to eliminate potential carryover effects.

Participant Enrollment (Visit 1)

Interested individuals responded to a promotional advertisement (a scannable QR code) posted on the most widely used Chinese multipurpose messaging and social medial app—WeChat (https://weixin.qq.com/) or flyers placed on university campuses in the city of Shanghai. An initial screening was conducted via WeChat where demographic information was obtained. This was followed by a detailed screening of physical and medical conditions per study eligibility criteria at our laboratory. All participants were required to be 20–35 yr old, nonobese ($18 < \text{BMI} < 28\ \text{kg}\cdot\text{m}^{-2}$), inactive, and sedentary (i.e., current sedentary behavior: $\geq 8\ \text{h}\cdot\text{d}^{-1}$ and not meeting the physical activity guideline of $\geq 150\ \text{min}\cdot\text{wk}^{-1}$ of moderate-intensity or $\geq 75\ \text{min}\cdot\text{wk}^{-1}$ of vigorous-intensity exercise for at least 3 months) (29), and free of diabetes and cardiovascular disease. Exclusion criteria were smoking, pregnancy, change of body weight >2 kg in the past 3 months, fasting blood glucose $>6.1\ \text{mmol}\cdot\text{L}^{-1}$, fasting triglyceride concentration $>2.5\ \text{mmol}\cdot\text{L}^{-1}$, fasting total cholesterol concentration $>6\ \text{mmol}\cdot\text{L}^{-1}$, systolic/diastolic blood pressure >140/90 mm Hg, current use of any medication or supplement that affects glucose and lipid metabolism, and known contraindications for physical activity.

After the initial WeChat screening, all potentially eligible participants were invited to an orientation, provided with a study overview, an informed consent, and screened for exclusion criteria. This included a fasting blood test, blood pressure, medical history, body mass, height, and physical activity by the International Physical Activity Questionnaire.

Preliminary Testing (Visit 2)

At least 7 d before participating in their initial intervention session, eligible participants visited our laboratory for a familiarization visit. Participants completed a body composition test via dual-energy x-ray absorptiometry (Lunar Prodigy; GE Healthcare, Chicago, IL) and a maximal aerobic-capacity assessment ($\dot{V}O_{2max}$) using a modified version of the Bruce protocol on a treadmill (Pulsar 4.0; H/p/Cosmos, Nussdorf-Traunstein, Germany), with gas-exchange measurement using a TrueOne 2400 metabolic cart (Model QMC; ParvoMedics, Inc., East Sandy, UT). The speed and the inclination corresponding to 60% $\dot{V}O_{2max}$ were used in the regular activity-break intervention trials.

Participants wore an accelerometer placed on their hip on the right anterior axillary line for seven consecutive days after familiarization (GT3X+; ActiGraph, Pensacola, FL) to measure time spent on sedentary behavior, light physical activity, and MVPA in daily life.

Experimental Regimen (Visit 3–Visit 10)

Restricted period (visits 3, 5, 7, and 9). Participants wore an accelerometer and were asked to refrain from structured physical activities (i.e., no physical activity beyond activities of daily living), caffeine, and alcohol. One day before their first condition, participants recorded their diets and copied the diet before each subsequent condition. Participants wore a CGM and consumed a standardized meal at dinner time the day before each condition day. The CGM sensor was inserted into the subcutaneous fat in the abdominal region 2 cm from the umbilicus. For subsequent conditions, new sensors were inserted within approximately 1 cm of the initial insertion site. All CGM devices were calibrated three times over 26 h according to the manufacturer's instructions with a glucose meter (ACCU-CHEK Performa; Roche Healthcare, Mannheim, Germany). The same CGM and glucose meter were used in all four conditions for each participant to eliminate error caused by variations among devices.

Experimental stage (visits 4, 6, 8, and 10). Participants performed four continuous 26-h experimental conditions in our research facility, including 22.5 h inside and 3.5 h outside the human calorimeter (HC) chamber. Each 26-h period of observation comprised a 30-min steady-state period before entering the chamber, a 9-h intervention period (0830–1730 h), a postintervention period (5.5 h, 1730–2300 h), a sleep period (8 h, 2300–0700 h), and day 2 breakfast period (0700–1000 h outside the chamber). Condition order was randomly assigned using computer-generated random numbers (block randomization and balanced block sizes). Participants were blinded to each of the respective conditions until their arrival at the research facility. Participants performed the same procedure in the four experimental conditions, except the 9-h intervention periods as follows (see Fig. 1):

1. Prolonged sitting (SIT): The control trial was a no-exercise, prolonged sitting trial where subjects were free

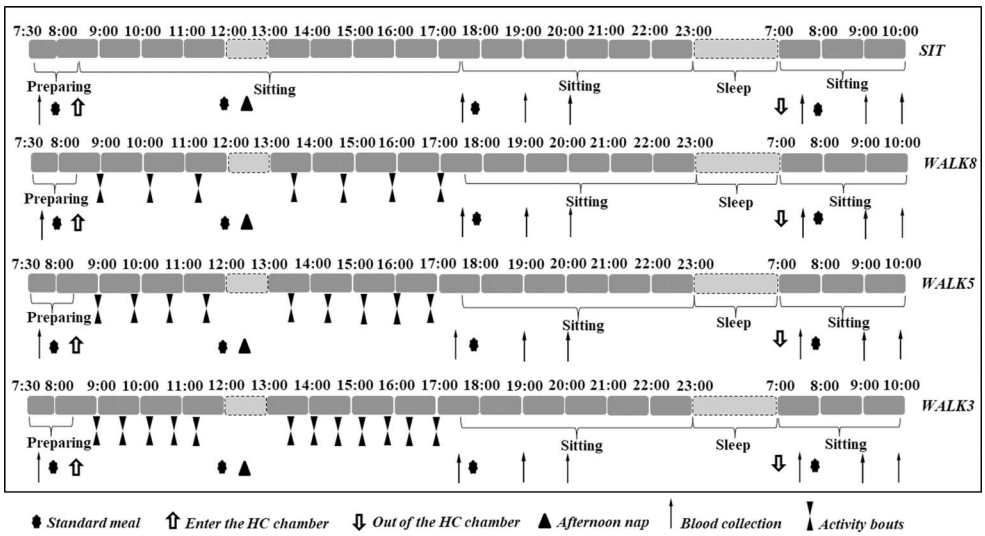

FIGURE 1—Study protocol for the experimental conditions.

to use the Internet, work on their computer, or read during the 9 h spent in the HC chamber, with 80-min break for lunch and afternoon nap (1140–1300 h) and breaks to use the toilet whenever needed similar to a typical workday.

2. A 3-min bout of walking with 30 min sitting between each bout (WALK3): 12, 3-min bouts (accumulating 36 min) of brisk walking on a treadmill (60% $\dot{V}O_{2max}$), with 30 min between each bout, commencing at 0900 h and 1330 h.
3. A 5-min bout of walking with 45 min sitting between each bout (WALK5): 9, 5-min bouts (accumulating 45 min) of brisk walking on a treadmill (60% $\dot{V}O_{2max}$), with 45 min between each bout, commencing time is the same as above.
4. An 8-min bout of walking with 60-min sitting between each bout (WALK8): 7, 8-min bouts (accumulating 56 min) of brisk walking on a treadmill (60%$\dot{V}O_{2max}$), with 60 min between each bout, and the commencing time is the same as above.

Aside from the structured exercise during each of the three conditions involving treadmill walking, participants spent the remainder of the 9 h in the HC chamber sitting, as in the control condition. In the four conditions, after the 9-h intervention period, participants maintained a seated position only while awake during the postintervention and day 2 breakfast periods, except for toilet and wash breaks (2240–2300 h on day 1, 0700–0720 h the next morning). CGM continued throughout the four conditions, and sedentary time and physical activity were also measured using an accelerometer. Participants complied with the protocol under direct supervision from research staff. Female participants completed these visits between days 1 and 14 of their menstrual cycle to minimize variation in metabolic functions, such as insulin sensitivity, that may be caused by the menstrual cycle (30).

Meals

Food intake was standardized starting the night before each condition day. Participants consumed breakfast (0800–0815 h), lunch (1140–1200 h), and dinner (1740–1800 h) on day 1 and breakfast (0745–0800 h) the next morning. All meals, which were standardized among conditions and individualized to meet daily estimated energy requirements (Schofield equation [31], 1.5 physical activity factor), were consumed in our research facility. We ordered all standardized meals from a very popular convenience chain, Family Mart, in Shanghai, and prepared Chinese food was provided to the participants. Breakfast (outside the chamber) mainly consisted of sticky rice, sausages, eggs, and milk. Lunch (in the chamber) mainly consisted of rice, doufu, and chicken. Supper (in the chamber) mainly consisted of noodles and fried meat sauce. Breakfast, lunch, and supper, which provided approximately 25%, 40%, and 35% of daily energy intake, respectively, were entirely the same in all four conditions for each participant. Participants consumed all food under each condition and were instructed to consume the meal at the same time for each experimental condition.

Energy Expenditure (EE)

With the aim of characterizing the interventions, the condition was completed in a chamber (FHC-20S; Fuji Medical Science Co., LTD, Chiba, Japan) from 0830 h on day 1 to 0700 h the next morning for testing EE (EE measurement began at 0900 h, accumulating 22 h). The chamber covers an area of 11.4 m^2; its internal volume is 27.5 m^3 (3.85 m width × 2.85 m depth × 2.50 m height), and it contains a toilet, a wash stand, a bed, a desk with chair, and a treadmill. The temperature and the relative humidity of incoming fresh air were maintained at 25.0°C ± 0.5°C and 50.0% ± 3.0%, respectively. The sample air is dehumidified using a gas-sampling unit (SCC-C: ABB Corp., Kyoto, Japan) and analyzed using a mass spectrometer (Prima PRO; Thermo Fisher Scientific, Cheshire, UK), calibrated biweekly using standard gas. Carbon dioxide production ($\dot{V}CO_2$) and oxygen consumption ($\dot{V}O_2$) were calculated using the formula of Henning et al. (32).

Blood Collection and Biochemical Analysis

A total of seven venous blood samples were collected via venipuncture for each intervention condition. On day 1, a

baseline 10-h fasting blood sample was collected after at least 20 min of quiet resting after arrival in the morning (0730 h), and blood was then sampled at predinner (30 min after the last activity bout), at 1- and 2-h postdinner time, at prebreakfast, and at 1- and 2-h postbreakfast time the next morning. During postprandial intervals before sample times, participants were asked to refrain from drinking water. All samples were collected directly into standard coagulant separation gel tubes and stored at room temperature (22°C to 25°C) for 30 min; they were centrifuged (3000 rpm for 15 min at 4°C), and the serum was stored at −80°C for subsequent analysis. Code-labeled samples were sent to an independent laboratory (ADICON Clinical Laboratories, Sheng, China, INS, accredited by CAP, ISO15189 and CMA) for testing serum glucose (GLU) and insulin (INS) after all samples under four conditions from each participant were collected. All samples from the same participant were assayed in a single run.

Serum glucose concentrations were measured using hexokinase enzymatic method on a Beckman-Coulter AU680 analyzer (Beckman-Coulter Trading, Shanghai, China). Serum insulin concentrations were measured using chemiluminescent microparticle immunoassay methods on an Abbott Architect 12000SR (Abbott Laboratories, Shanghai, China). Kits and calibrators for glucose were sourced from Beckman-Coulter Diagnostics (Pasadena, CA), and the kits and calibrators used for insulin analysis were sourced from Abbott Diagnostics (Chicago, IL). Interassay coefficients of variation were 2.07% and 3.25% for serum glucose and insulin, respectively. Intra-assay coefficients of variation were between 2.86% and 3.62% for serum glucose, and between 3.19% and 4.78% for serum insulin.

Statistical Analysis

Sample size calculations were based on a previous study using similar methodology in overweight/obese adults (33), assuming an 11% reduction in 26-h mean glucose concentrations for the activity-break conditions. Therefore, we estimated that 12 paired observations would be needed to achieve 80% power to detect the expected effect size (Cohen $d = 0.60$) in the primary outcome variables between the interventions (activity breaks vs control), with two-sided alpha = 0.05. To allow for potential withdrawals, 18 participants were randomized. Although the glucose total area under the curve (tAUC) and the incremental area under the curve (iAUC) of 26 and 9 h were not priori powered, these outcomes have been widely studied and reported in the literature (15,16,22,33,34) related to interrupting prolonged sitting. In addition, the treatment had a clinically meaningful effect on change in these outcomes, as indicated by an average effect size (across the three experimental conditions) of >0.80 and power of >95%. On the basis of these observations, we treat these outcomes as part of our primary end points. All other outcomes were considered exploratory. Across all trial conditions and participants, there were no missing outcome values.

The tAUC and iAUC for interstitial glucose, serum glucose, and insulin levels were calculated using concentrations of each condition using analysis software (Prism 6.01; GraphPad, San Diego, CA). The tAUC was calculated between the curve and a baseline concentration of zero, and iAUC was calculated by subtracting baseline concentrations over the corresponding period from tAUC.

CGM data were summarized into a total 26-h condition period (800 h day 1–1000 h next morning) and separated into four segmentation periods according to the partition of each condition (intervention period, postintervention period, sleep period, and day 2 breakfast period). For iAUC calculations, the preprandial values obtained just before each meal were used as a baseline (interstitial glucose baseline: mean of glucose during the 15 min before each period). Homeostatic model assessment (HOMA)—insulin sensitivity (HOMA-IS) was calculated using the equation of HOMA-IS = 10,000/(fasting glucose [mg/dL] × fasting insulin[μU/mL]) (35). For accelerometer data, the cut points of Sasaki et al. (36) and the <200 counts (37) were used to categorize activity intensity and sedentary behavior, respectively.

Descriptive data are presented as mean ± SD, except range for age. All other data were normally distributed and presented as marginal mean ± SD, and nonnormal distributions were square root-transformed to achieve normality. Linear mixed models with random intercepts were used to evaluate the differential effects of the conditions on all outcome variables. A random effect of participants was used to account for individual differences in all models. Model 1 was adjusted for potential confounders explaining residual outcome variance (age, sex, percent body fat, $\dot{V}O_{2max}$, and preprandial value) and period effects (treatment order). Model 2 was additionally adjusted for corresponding EE (e.g., EE of the intervention phase as a covariate for the 9-h mean glucose, iAUC, and 2-h dinner iAUC; total 26-h EE for 26-h mean glucose, iAUC, and 2-h breakfast iAUC the next morning). If there was a main effect of the condition, a Bonferroni-corrected test (corrected P value = observed P value [uncorrected] × 6 [the number of comparisons]) was applied to *post hoc* analysis. All statistical calculations were performed using IBM SPSS Statistics 22.0 (IBM, Armonk, NY). A two-tailed probability-level criterion of 0.05 was adopted.

RESULTS

Participant characteristics. Of 48 participants who signed up for the screening visit, 18 were randomized and 16 completed all conditions and were included in the final analysis. Causes of elimination and dropout are detailed in Supplemental Figure 1, http://links.lww.com/MSS/B833. Participant characteristics are summarized in Table 1. Female participants had higher %fat ($P = 0.006$) but lower high-density lipoprotein cholesterol level, systolic/diastolic blood pressure, and $\dot{V}O_{2max}$ (all $P < 0.05$). Free-living physical activity accelerometer data confirmed that participants were physically inactive (MVPA, 16 ± 6 min·d^{-1}) and sedentary (sitting time, 618 ± 73 min·d^{-1}).

Food intake and EE. The mean energy intake values for breakfast, lunch, and dinner were 504 ± 57, 887 ± 105, and 767 ± 103 kcal, respectively, and the target macronutrient

TABLE 1. Participant characteristics.

Characteristic	All (n = 16)	Male (n = 7)	Female (n = 9)
Age (range, yr)	24 (21–30)	26 (21–30)	23 (21–29)*
Anthropometrics			
Height (cm)	166 ± 7	171 ± 1	162 ± 1*
Weight (kg)	61.7 ± 10.5	69.1 ± 2.1	55.9 ± 0.8*
BMI (kg·m^{-2})	22.2 ± 2.3	23.4 ± 0.5	21.3 ± 0.3
Percent body fat (%)	29.0 (8.8)	22.6 (7.4)	33.9 (6.5)*
Metabolic and cardiovascular risk factors			
Fasting glucose (mmol·L^{-1})	4.76 ± 0.25	4.82 ± 0.30	4.72 ± 0.21
Fasting insulin (pmol·L^{-1})	52.0 ± 16.0	56.4 ± 17.1	46.4 ± 13.6
Fasting triglyceride (mmol·L^{-1})	0.86 ± 0.31	0.80 ± 0.27	0.95 ± 0.36
Total cholesterol (mmol·L^{-1})	4.37 ± 0.70	4.33 ± 0.68	4.42 ± 0.78
HDL cholesterol (mmol·L^{-1})	1.36 ± 0.20	1.44 ± 0.21	1.25 ± 0.12*
LDL cholesterol (mmol·L^{-1})	2.64 ± 0.58	2.61 ± 0.58	2.68 ± 0.63
Systolic blood pressure (mm Hg)	100 ± 7	105 ± 7	96 ± 3*
Diastolic blood pressure (mm Hg)	63 ± 5	66 ± 6	60 ± 3*
Aerobic fitness			
Absolute $\dot{V}O_{2max}$(L·min^{-1})	2.41 ± 0.55	2.94 ± 0.07	1.99 ± 0.03*
Relative $\dot{V}O_{2max}$(mL·kg^{-1}·min^{-1})	38.8 ± 5.1	43.1 ± 0.9	35.6 ± 0.4*
Accelerometer data			
Sedentary time (min·d^{-1})	618 ± 73	653 ± 64	590 ± 71
LPA time (min·d^{-1})	237 ± 44	252 ± 51	226 ± 37
MVPA time (min·d^{-1})	16 ± 6	18 ± 6	15 ± 5

Data are expressed as mean ± SD unless otherwise stated.
P values were generated using independent two-sample t-test.
*P < 0.05 vs male.
LPA, light physical activity.

TABLE 2. Glycemic control over 26 h and during activity-bout period (continuous glucose monitoring data, n = 16).

	SIT	WALK3	WALK5	WALK8	P
Model 1					
26-h glucose (0800 h day 1–1000 h day 2)					
Mean	5.64 ± 0.45	5.44 ± 0.45	5.45 ± 0.44	5.43 ± 0.44*	0.031
tAUC	146.4 ± 11.8	141.7 ± 11.8	141.5 ± 11.7	141.2 ± 11.7	0.091
iAUC	28.96 ± 8.85	25.72 ± 8.82	24.59 ± 8.75	24.97 ± 8.74	0.086
9-h glucose (intervention period 0830–1730 h)					
Mean	6.10 ± 0.50	5.69 ± 0.50*	5.73 ± 0.49*	5.69 ± 0.49*	0.001
tAUC	58.03 ± 4.74	54.11 ± 4.72*	54.54 ± 4.68*	54.17 ± 4.68*	0.001
iAUC	14.45 ± 4.33	10.82 ± 4.31*	11.35 ± 4.28*	11.29 ± 4.27*	0.002
Model 2					
26-h glucose (0800 h day 1–1000 h day 2)					
Mean	5.84 ± 0.54	5.42 ± 0.44*	5.38 ± 0.45*	5.32 ± 0.47*	0.005
tAUC	152.2 ± 14.5	141.0 ± 11.7*	139.5 ± 11.9*	138.3 ± 12.4*	0.007
iAUC	32.46 ± 11.16	25.31 ± 8.74	23.39 ± 8.97*	23.09 ± 9.41*	0.028
9-h glucose (Intervention period 0830–1730 h)					
Mean	6.46 ± 0.74	5.65 ± 0.49*	5.62 ± 0.51*	5.48 ± 0.59*	0.001
tAUC	61.45 ± 7.03	53.75 ± 4.64*	53.52 ± 4.84*	52.13 ± 5.55*	0.001
iAUC	17.84 ± 6.40	10.46 ± 4.27*	10.34 ± 4.44*	9.27 ± 5.08*	0.001

Values are adjusted mean of covariates ± SD. Mean values were compared using linear mixed models for the main effect condition followed by a Bonferroni multiple-comparisons test. Units of mean glucose and tAUC/iAUC are mmol·L^{-1} and mmol·L^{-1}·h, respectively. Model 1 was adjusted for age, sex, percent of body fat, relative $\dot{V}O_{2max}$, treatment order, and corresponding baseline value. Model 2 was additionally adjusted for EE.
*P < 0.05 vs SIT.

profile was about 14% energy from protein, 37% from fat, and 49% from carbohydrate.

Compared with SIT, the 22-h EE increased by mean 208 kcal (95% confidence interval [CI] = 148–269), 254 kcal (194–315), and 289 kcal (229–350) in WALK3, WALK5, and WALK8, respectively (all P < 0.001), and the value for WALK8 was also significantly higher than that for WALK3 (P = 0.002). During the intervention period, the 9-h EE was higher in WALK3 (Δ 217 kcal [34–403]), WALK5 (Δ 263 kcal [78–447]), and WALK8 (Δ 325 kcal [141–510]) than that in SIT (all P < 0.001), and it was also significantly higher in WALK8 than in WALK3 (P < 0.001) and WALK5 (P = 0.05).

Continuous glucose monitoring. An overview of the mean 26-h interstitial glucose profiles for all participants by condition is presented in Figure 2, and numerical values are displayed in Table 2. During the 9-h intervention period, compared with SIT, WALK3, WALK5, and WALK8 reduced iAUC for CGM by, mean difference (95% CI), 3.63 mmol·L^{-1} per 9 h (0.94–6.33; P = 0.003), 3.11 mmol·L^{-1} per 9 h (0.46–5.75; P = 0.013), and 3.17 mmol·L^{-1} per 9 h (0.59–5.74; P = 0.009), respectively. Similar findings were observed for the mean glucose and tAUC for CGM (Table 2). These effects remained after adjustment for EE (Table 2). However, the effects of the activity-bout conditions on 26-h glycemia, as measured by the mean glucose and tAUC for CGM, were only significant when EE was included in the statistical models (Table 2). The effects of the activity-bout conditions on measures of 26-h glycemia (mean glucose, tAUC, and iAUC) were not significant when standard covariates were included in the model (Table 2). This is likely to be driven largely by the intervention period, as no significant treatment effects were observed during the 5-h postcondition and 8-h sleep periods in the two models (Supplemental Table 1, http://links.lww.com/MSS/B834). Besides, no significant treatment effects were observed among the three activity-break conditions in any period. Model parameter estimates revealed that EE was not significantly associated with 26-h mean glucose concentration (unstandardized β = −0.08 mmol·L^{-1}·MJ^{-1}, P = 0.16), 26-h tAUC (β = −0.79 mmol·L^{-1}·h·MJ^{-1}, P = 0.66), and 26-h iAUC (β = −1.03 mmol·L^{-1}·h·MJ^{-1}, P = 0.45).

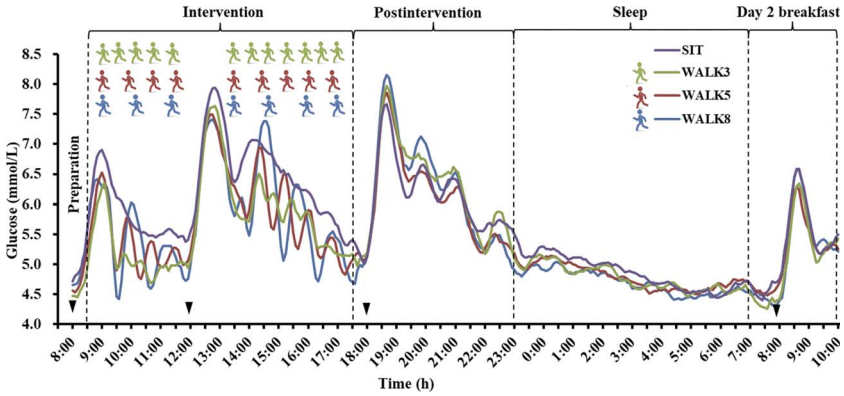

FIGURE 2—Mean 26-h interstitial glucose profiles for SIT, WALK3, WALK5, and WALK8. Note: mean activity bouts; mean meal point in time.

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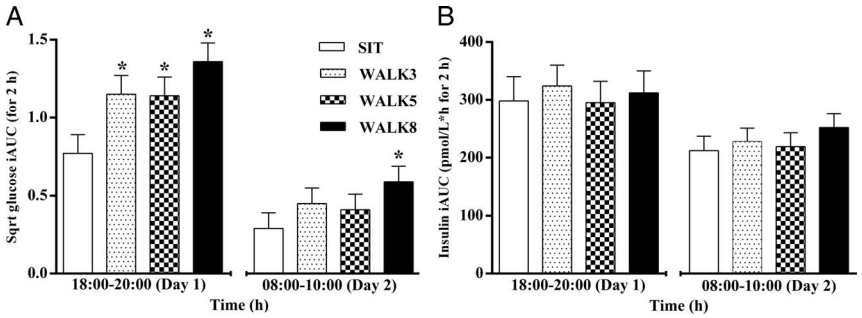

FIGURE 3—The 2-h dinner (day 1) and breakfast (day 2) serum glucose iAUC (A) and insulin iAUC (B) measured during SIT, WALK3, WALK5, and WALK8. Data are presented as adjusted mean of covariates (SEM) and adjusted for age, sex, percent of body fat, relative $\dot{V}O_{2max}$, treatment order, and corresponding baseline value. *$P < 0.05$ vs SIT.

Serum glucose and insulin. Postprandial serum glucose and insulin responses are shown in Figure 3, and corresponding numerical values are displayed in Supplemental Tables 2 and 3, http://links.lww.com/MSS/B835 and http://links.lww.com/MSS/B836. Compared with SIT, WALK3, WALK5, and WALK8 increased iAUC for 2-h postdinner serum glucose by, square root mean difference (95% CI), 0.38 (0.003–0.76; $P = 0.047$), 0.37 (−0.01 to 0.76; $P = 0.06$), and 0.60 (0.19–1.01; $P = 0.001$), respectively. A similar finding was observed for tAUC only in WALK8, and the 2-h postdinner CGM findings (data not shown) were basically the same with these above results. However, the negative finding of the activity-bout conditions on 2-h postbreakfast serum glucose response, as measured by tAUC and iAUC, was only significant in WALK8 the next morning. All these above effects were lost after adjustment for EE (Supplemental Table 3, http://links.lww.com/MSS/B836).

Compared with SIT, no significant treatment effects of activity-bout conditions were observed on the 2-h postprandial serum insulin tAUC and iAUC both for dinner and breakfast (day 2). However, WALK3, WALK5, and WALK8 increased HOMA-IS by, mean difference (95% CI), 4.80 (0.51–9.17), 5.17 (0.25–10.10), and 6.39 (1.29–11.49) (all $P < 0.05$), respectively, relative to SIT the next morning after overnight fasting, and these effects remained after adjustment for EE (Supplemental Table 3, http://links.lww.com/MSS/B836). Moreover, no significant treatment effects on all the above variables were found among three activity-bout conditions.

DISCUSSION

In inactive healthy young people with very prolonged sitting (618 ± 73 min·d^{-1}), we observed that all three regular activity-break modes in prolonged sitting, with workload of 60% $\dot{V}O_{2max}$, attenuated the 26-h mean glucose concentration and tAUC compared with SIT after controlling for EE (all mean change ↓7%–9%) and lowered iAUC in WALK5 and WALK8 (all mean change ↓28%–29%). During the 9-h intervention period, the three activity-break conditions reduced the mean glucose, tAUC, and iAUC regardless of controlling for EE. The magnitude of reduction was nearly equal among the three activity-break conditions. Interrupted prolonged sitting only provided a benefit during the 9 h activity-bout period and had no lasting effect into the evening and sleep periods.

The result was similar to that of previous studies, that is, whether with overweight/obese (13,18,21,24) and/or T2D/impaired fasting glucose/high risk of T2D participants (14,15,23,33,34) or with healthy participants (12,19,20,22,38), activity-break conditions reduced glucose responses compared with prolonged sitting condition. However, the results of the present study is inconsistent with a recent study (39) that all three activity-bout conditions (2 min/20 min, 6 min/60 min, and 12 min/120 min) did not reduce the postprandial plasma glucose response to prolonged sitting in healthy, sedentary, centrally obese men. This discrepancy may predominantly be explained by the intensity of physical activity during intervention, which was much lower in previous studies (30% $\dot{V}O_{2max}$) than in our study. Most of these previous studies focused on the effects during the activity-bout intervention period, and only few studies observed the postintervention effects (21,33,34,39), which demonstrated that interrupted prolonged sitting not only reduced glucose responses over the whole observed period (21,33,34), but also lowered evening assessment (21,33) and sleep period (21,33,34,39) after the activity-bout intervention period. However, inconsistent with previous studies, no significant treatment effect was observed in the evening assessment and sleep period in the present study. This may be explained by differences in the study population (normal weight, healthy vs overweight/obese (21,33,34,39) with T2D or impaired fasting glucose (33,34). Moreover, it should be noted that the period after activity-bout intervention in previous studies was performed under free-living conditions without monitoring.

This study confirmed that the beneficial effect of interrupting prolonged sitting is independent of EE, suggesting that the interruptions in sitting *per se* seem to provide a metabolic benefit. The result was different from a previous study (40) in which there was significant negative correlation between EE of different modes of breaks and glucose responses in a dose-dependent manner. When interpreting this inconsistency, it should be noted that the previous study, which used a small sample of individuals (n = 6–9) from past studies, was a pooled-analysis study. Future studies are needed to better

understand the role of EE, which has been shown to be important in examining the effect of interrupting prolonged sitting on glycemic control.

The novel finding in this study is that the postdinner glucose iAUC was higher in three activity-break conditions immediately after the intervention period and persisted in the postbreakfast measurement of WALK8 the next morning, without controlling for EE. This result was contradictory to previous studies in which the dinner postprandial glucose was lower in the activity-break condition after the intervention phases in overweight/obese adults (21) with T2D (34) and with impaired fasting blood glucose or prehypertension (33). This may be also explained by differences in the study population (healthy vs overweight/obese, with or without T2D). As a previous review suggested that for metabolically healthy individuals, interrupting sitting time may help reduce the amount of insulin required to maintain normal glycemia, and for those with greater insulin resistance, interrupting time spent sitting may elicit greater benefit by improving poorer glucose clearance as well as improving insulin efficiency (41). This is consistent with our study that both the premeal serum insulin levels of dinner and breakfast (day 2) and the insulin sensitivity were improved in the three activity-break conditions. In addition, the increases in postprandial serum glucose response may be explained as a compensatory response for hepatic glucose production during activity-break periods because, from CGM glucose profile (Fig. 2), there were times that descending levels of the blood glucose reached fasting concentrations in the activity conditions, especially in WALK8, which may suggest a possible role of hepatic glucose production.

With regard to the comparison among the three activity-bout conditions, the treatment effects on glucose response were almost identical during the 9-h experimental condition when EE was included or not included in the statistical models. However, the 2-h postprandial glucose response only increased in WALK8 relative to SIT the next morning when standard covariates were included in the model.

There are several strengths to our study. This is the first study to report an interrupted prolonged sitting condition with EE monitoring during the entire condition process. The highly accurate HC chamber allowed precise EE testing in all four conditions. Furthermore, our study was under strict behavioral supervision and incorporated standardized feeding of a typical Chinese diet for 26 continuous hours in the laboratory, and the incorporation of a randomized crossover design strengthened the internal validity and reliability of our data and permitted a smaller sample size. The comprehensive 26-h restrictive lifestyle and dietary control, alongside CGM use extended by several hours to observe treatment effects beyond the activity-break period, increased the experimental rigor of our results.

The present study also has some limitations. First, as an acute intervention study, it failed to extrapolate longer-term exposures to the particular conditions examined, and it is unknown whether our activity-break modes are beneficial over a longer period. Second, in our study, the sample size is too small to allow us to examine meaningful differences between male and female subjects in our outcomes. Third, the tAUC and iAUC results for serum glucose and insulin are limited by the fact that the meal responses were only observed for three time points at 2 h. In addition, the possible role of hepatic glucose production cannot be determined from this study. Finally, our laboratory-based study still lacks external validity to some extent.

In conclusion, this study demonstrates that interrupting prolonged sitting by performing 3-, 5-, and 8-min bouts of moderate-intensity walking with corresponding 30, 45, and 60-min sitting intervals over 9 h improved glucose responses for 26 h, independent of EE, in inactive, sedentary, and healthy adults. However, no meaningful treatment differences were noted among the three different activity-break patterns. Meanwhile, we showed a significant effect of three treatments on glucose response during the activity-bout period, but lack of a lasting effect. Underlying mechanisms and the long-term effects of interrupted prolonged sitting need to be investigated in the future.

The authors gratefully acknowledge the kind assistance from Sheng Zhao and Li-Qiong Ma. Most importantly, they thank the study participants for their time and commitment to the study protocol; this study would not have been possible without them. This study was funded by the Science and Technology Commission of Shanghai Municipality (grant no. 16080503300) and the National Natural Science Foundation of China (grant no. 31571226).

The authors report no conflicts of interest. The results of the present study do not constitute endorsement by the American College of Sports Medicine. These results are presented as clearly as possible, honestly, and without fabrication, falsification, or inappropriate data manipulation.

EPIDEMIOLOGY

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