

RESEARCH ARTICLE

Effect of frequent interruptions of sedentary time on nutrient metabolism in sedentary overweight male and female adults

 Nathan P. De Jong,¹ Corey A. Rynders,² David A. Goldstrohm,³ Zhaoxing Pan,⁴ Andrew H. Lange,¹ Carlos Mendez,¹ Edward L. Melanson,^{2,5,6} Daniel H. Bessesen,^{1,7*} and Audrey Bergouignan^{1,8,9*}

¹Division of Endocrinology, Metabolism, and Diabetes and Anschutz Health and Wellness Center, University of Colorado, School of Medicine, Aurora, Colorado; ²Division of Geriatric Medicine, University of Colorado, School of Medicine, Aurora, Colorado; ³Department of Pediatrics, University of Colorado School of Medicine, Perinatal Research Center, Aurora, Colorado; ⁴Department of Biostatistics and Informatics, Anschutz Medical Campus, University of Colorado, Aurora, Colorado; ⁵Division of Endocrinology, Metabolism, and Diabetes, Department of Medicine, Anschutz Medical Campus, University of Colorado, Aurora, Colorado; ⁶Eastern Colorado VA Geriatric Research, Education, and Clinical Center, Denver, Colorado; ⁷Denver Health Medical Center, Denver, Colorado; ⁸Institut Pluridisciplinaire Hubert Curien, Université de Strasbourg, Centre National de la Recherche Scientifique (CNRS), Strasbourg, France; and ⁹UMR 7178, CNRS, Strasbourg, France

Submitted 16 July 2018; accepted in final form 4 January 2019

De Jong NP, Rynders CA, Goldstrohm DA, Pan Z, Lange AH, Mendez C, Melanson EL, Bessesen DH, Bergouignan A. Effect of frequent interruptions of sedentary time on nutrient metabolism in sedentary overweight male and female adults. *J Appl Physiol* 126: 984–992, 2019. First published January 10, 2019; doi:10.1152/jappphysiol.00632.2018.—This study compared 24-h nutrient oxidation responses between a sedentary condition (SED) and a condition in which short 5-min bouts of moderate-intensity physical activity were performed hourly for nine consecutive hours over 4 days (MICRO). To determine whether any shifts in fuel use were due solely to increases in energy expenditure, we also studied a condition consisting of a single isoenergetic 45-min bout of moderate-intensity exercise (ONE). Twenty sedentary overweight or obese adults (10 men/10 women; 32.4 ± 6.3 yr; BMI, 30.6 ± 2.9 kg/m²) completed all three conditions (MICRO, SED, and ONE) in a randomized order. Each condition consisted of a 3-day free-living run-in followed by a 24-h stay in a whole-room calorimeter to measure total energy expenditure (TEE) and substrate utilization. Dietary fat oxidation was also assessed during the chamber stay by administering a [¹⁻¹³C] oleic acid tracer at breakfast. Energy intake was matched across conditions. Both MICRO and ONE increased TEE relative to SED, resulting in a negative energy balance. HOMA-IR improved in both activity conditions. MICRO increased 24-h carbohydrate oxidation compared with both ONE and SED ($P < 0.01$ for both). ONE was associated with higher 24-h total fat oxidation compared with SED, and higher 24-h dietary fat oxidation compared with both SED and MICRO. Differences in substrate oxidation remained significant after adjusting for energy balance. In overweight and obese men and women, breaking up sitting time increased reliance upon carbohydrate as fuel over 24 h, while a single energy-matched continuous bout of exercise preferentially relies upon fat over 24 h.

NEW & NOTEWORTHY Insulin sensitivity, as assessed by HOMA-IR, was improved after 4 days of physical activity, independent of frequency and duration of activity bouts. Temporal patterns of activity across the day differentially affect substrate oxidation. Fre-

quent interruptions of sedentary time with short bouts of walking primarily increase 24-h carbohydrate oxidation, whereas an energy-matched single continuous bout of moderate intensity walking primarily increased 24-h fat oxidation.

carbohydrate oxidation; dietary fatty acid oxidation; microbouts of activity; physical inactivity; whole-room calorimetry

INTRODUCTION

Sedentary behavior is associated with several adverse health outcomes, including obesity, cardiometabolic diseases, diabetes, certain types of cancer, and premature mortality (23, 26). These associations have been observed across sex, age, ethnicity, and even among individuals who meet the current intensity-based physical activity guidelines (i.e., 150-min/week of moderate intensity or 75 min/wk of vigorous exercise) (26). Isotemporal substitution modeling suggests that replacing 1 h of sedentary time with either light or moderate-to-vigorous intensity physical activity (MVPA) in inactive adults was associated with lower mortality and risk factors of metabolic disease, with MVPA associated with the most potent health-enhancing, time-dependent behavior (7, 24). Independent of potential confounders and time spent in other activities, reallocation of 30-min/day of sedentary time with an equal amount of MVPA is associated with lower blood triglycerides, glucose and insulin, and higher insulin sensitivity (7). Additionally, observational studies suggest that interrupting sedentary time with frequent bouts of physical activity is associated with lower plasma glucose and insulin, waist circumference, inflammatory marker C-reactive protein, even in individuals who regularly exercise (15, 17). Adults whose sedentary time was mostly uninterrupted had less healthy cardiometabolic profiles, based on increased blood glucose and triglycerides, compared with those who had more frequent breaks in sedentary time (15–17), even when controlling for total sedentary time, MVPA, age, sex, and ethnicity (15, 17).

* D. H. Bessesen and A. Bergouignan contributed equally to this article.

Address for reprint requests and other correspondence: Audrey Bergouignan, Anschutz Health and Wellness Center, University of Colorado Anschutz Medical Campus, 12348 E. Montview Blvd., Aurora 80045 CO (e-mail: Audrey.bergouignan@ucdenver.edu).

A number of acute and short-term studies (≤ 3 days) have shown that frequent interruptions of prolonged sedentary activities with short bouts of walking decrease postprandial plasma glucose and insulin concentrations compared with a prolonged sedentary condition (2, 5, 9–11, 18, 21, 27, 34). For example, Dunstan et al. (10) compared plasma glucose and insulin responses to uninterrupted sitting and 2-min bouts of activity every 20 min for 5 h in overweight adults. Sitting was either interrupted by light (3.2 km/h) or moderate-intensity (5.8–6.4 km/h) treadmill walking. Relative to uninterrupted sitting, glucose and insulin incremental area under the curve (iAUC) in response to standardized meal were both significantly reduced after the activity-break conditions. A possible hypothesis for the reduction in plasma glucose concentrations is that carbohydrate oxidation is increased to support the increased energy expenditure. To test this hypothesis, we used room calorimetry and stable isotope tracers to compare the short-term effects (4 day) of activity microbouts (5 min of moderate-intensity physical activity performed hourly for nine consecutive hours) to a sedentary condition on 24-h nutrient oxidation in physically inactive adults with overweight and obesity. Because a shift in carbohydrate oxidation with activity microbouts might be solely due to increased energy expenditure, we also studied an energy-matched single bout of moderate-intensity physical activity performed in the morning. Only a few studies have controlled for energy expenditure

by comparing the effect of frequent interruptions of sedentary behavior with bouts of physical activity to an isocaloric continuous bout of physical activity, which remains the most common form of recommended physical activity (5, 12, 18, 27, 34). A finding that short, frequent bouts of activity promote carbohydrate oxidation in sedentary persons may inform an approach to personalize exercise prescription for individuals who have difficulty complying with traditional physical activity recommendations and reducing the exposure to increased plasma glucose observed in highly sedentary populations.

METHODS

Participants

Eligible participants were aged 19–45 yr, with a body mass index (BMI) between 27 and 33 kg/m², weight stable for >3 mo, had fasting plasma insulin concentrations below 25 μ IU/ml, and self-reported >6 h/day of sitting. Women were premenopausal but could use oral contraceptives. Exclusion criteria included clinically diagnosed diabetes, taking glucose- and/or lipid-lowering medication, dyslipidemia, smoking, or being physically active (>150 min/wk moderate-intensity exercise). Participants were recruited between October 2014 and October 2016 from newspapers advertisements, public announcement, and flyers in the Denver and Aurora areas, CO [see Consolidated Standards of Reporting Trials (CONSORT) diagram Fig. 1].

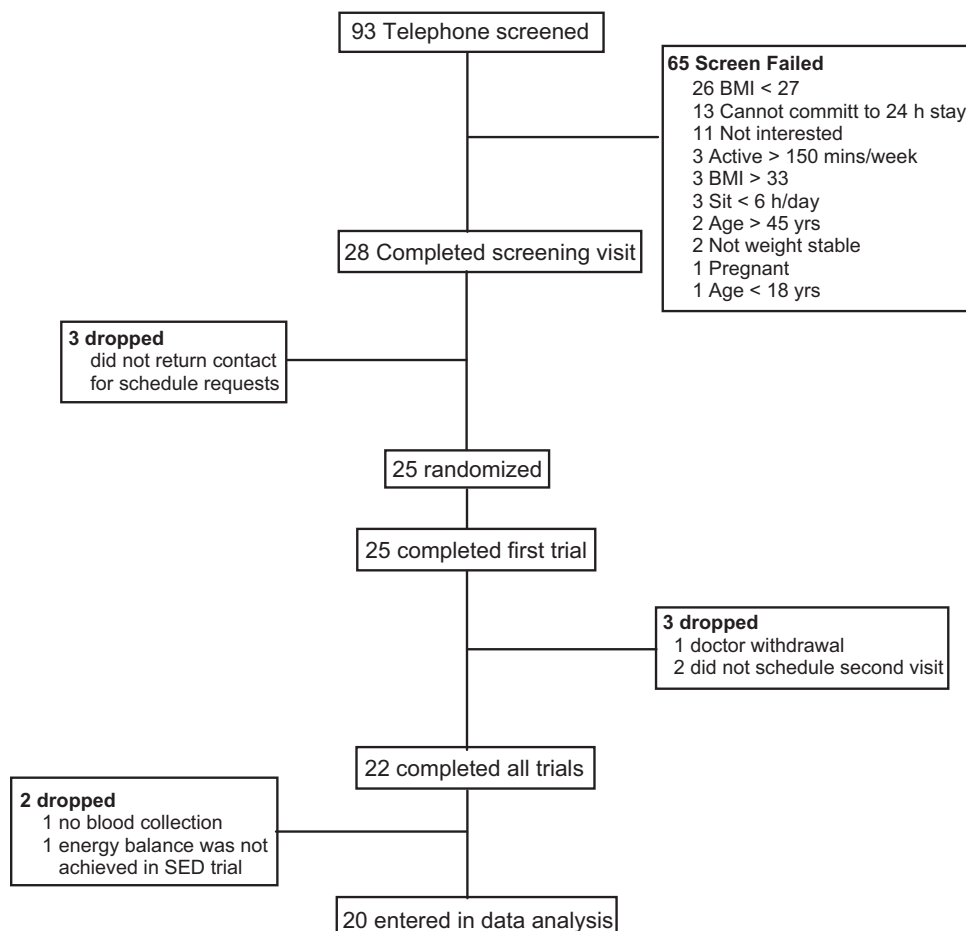


Fig. 1. Trial Consolidated Standards of Reporting Trials diagram.

Study Design

Following a screening visit, each eligible subject completed three separate 4-day trial conditions that consisted of 3 days in free-living conditions followed by 24 h in a whole-room calorimeter. The three trial conditions were administered in random order:

Sedentary. During the 3-day free-living period, subjects were asked to maintain usual levels of daily activity and were asked to refrain from structured exercise. On *day 4*, subjects remained sedentary (SED) in the whole-room calorimeter.

Sedentary + 1 continuous bout of activity. During the 3-day free-living period, subjects were instructed to perform 45 min of moderate-intensity walking once per day in the morning and maintained usual levels of daily activity the rest of the day. On *day 4*, participants remained sedentary in the whole-room calorimeter except to perform one bout (ONE) of 45-min moderate-intensity treadmill walking at 10:00AM.

Sedentary + microbouts of activity. During the 3-day run-in period, subjects performed 5 min of moderate intensity walking bouts each hour for nine consecutive hours in daily life and maintained usual levels of daily activity the rest of the time. On *day 4*, participants performed 5 min of moderate-intensity treadmill walking every hour (MICRO) for nine consecutive hours from 1000 to 1800 and remained sedentary the rest of the day.

The study conditions were separated by a 28-day washout period, and women were studied in the follicular phase of the menstrual cycle. All of the visits were conducted at the Clinical and Translational Research Center of University of Colorado Hospital (CTRC). This study was approved by the Colorado Multiple Institutional Review Board (COMIRB) and was in accordance with the Declaration of Helsinki.

Screening Visit

At screening, written consent was obtained, and participants were screened for exclusion criteria. This included a medical history and physical examination. The short version of the International Physical Activity Questionnaire (IPAQ) (6) was completed to assess eligibility on the basis of inclusion criteria for habitual physical activity and time spent sedentary. Subjects then performed a test on a motorized treadmill to determine the walking pace that was prescribed for the ONE and MICRO conditions. The walking test started at a pace of 2.4 mph, and the pace increased by increments of 0.3 mph every 2 min. At each level, subjects rated their perceived effort on a Borg scale from 0 (very light) to 20 (maximal exertion). The aim was to identify the speed that participants associated with a level of effort reaching 13 (somewhat hard). The walking test was stopped when the participant rated the speed of the treadmill with an rate of physical exertion (RPE) of 16 (hard to very hard). The treadmill speed associated with 13 RPE was the pace that was prescribed on *day 4* for ONE and MICRO. For the 3 days of the run-in period, subjects were instructed to walk at a pace similar to what was established with the walking test, i.e., the participants perceived moderate-intensity walking pace, an RPE of 13.

Randomization

Participants were randomized to one of three possible trial-condition orders using balanced blocks prepared for male and female participants. The study statistician (Z. Pan) prepared the computer-generated randomization lists and sealed envelopes for randomization. Once informed consent was obtained, a study member opened the sealed randomization envelope revealing the trial-condition order.

Run-in Diet and Physical Activity

A 3-day standard diet was provided by the CTRC metabolic kitchen during the run-in to each inpatient study visit. The macronutrient composition of the diet was 30% fat, 55% carbohydrate (CHO), and 15% protein of total energy intake. Daily energy needs were calculated on the basis of an estimate of resting metabolic rate (RMR) derived from the

average of 1) direct measurement by hood indirect calorimetry, and 2) an estimate using the following equation: $[(23.9 \times \text{FFM in kg}) + 372]$, where fat-free mass (FFM) was measured by dual energy X-ray absorptiometry (DXA, Hologic Delphi-W, Bedford, MA) (20, 35). The estimated RMR was then multiplied by an activity factor (1.4–1.7) based on the time spent physically active self-reported in the IPAQ. Participants were instructed to eat all food and bring back leftovers to the metabolic kitchen. They were also asked to abstain from consuming alcohol and to consume the same amount of caffeine (number of cups) for 24 h before each whole-room calorimeter stay. Daily energy needs in the chamber were estimated using the same equations as described above but applying an activity factor of 1.3. Compliance with the activity prescriptions (SED, ONE, or MICRO) during the 3-day run in period was objectively assessed with the use of an inclinometer (ActivPAL, Glasgow, UK) and accelerometer (Actigraph GT3X+, Fort Walton Beach, FL). Results on physical activity during the run-in diet were reported elsewhere (8).

Inpatient Study Day

Figure 2 depicts the inpatient study protocol. Participants reported to the CTRC at 0730, voided, and were weighed. An intravenous catheter was placed into the antecubital vein for blood sampling. Subjects then entered the whole-room calorimeter for a 23-h stay. Subjects remained seated for the first hour to achieve a steady state within the individual and the whole-room calorimeter. A fasting blood sample was obtained at 0855. Breakfast, lunch, dinner, and snack were given at 0900, 1300, 1830, and 2130 and contained 30%, 35%, 25%, and 10% of daily energy needs, respectively. The macronutrient composition of each meal was 65% CHO, 20% fat, and 15% protein of total energy intake; a moderately high-carbohydrate diet was used to optimize our ability to observe differences in fuel utilization between the three conditions. $1\text{-}^{13}\text{C}$ oleic acid (20 mg/kg of FFM, 99% enrichment, Cambridge Isotopic Laboratories, MA) was mixed and administered with the liquid breakfast meal. Breath and blood samples were then collected every hour for 14 h from 0900 to 2200, and at 0300. Final blood and breath samples were collected at 0700 the following day. In MICRO, blood samples were collected prior to each activity bout for 9 bouts. Blood samples were obtained by having subjects extend their arm through a porthole imbedded in the wall of the whole-room calorimeter. Lights-out and a sleep opportunity were scheduled from 2230 to 0630. Urine was collected in one jug for the “waking time” from the start of the study day to 2230 (bedtime), and in a second jug during “sleep time” from 2230 (bedtime) to 0700 the following morning. Study participants exited the whole-room calorimeter at 0700.

Energy Expenditure and Substrate Oxidation

Twenty-three-hour respiratory gas exchange data were extrapolated to 24-h values. Total energy expenditure (TEE) and substrate oxidation were determined using O_2 consumption, and CO_2 production was determined from the flow rates and differences in gas concentrations between air entering and air exiting the calorimeter and nitrogen excretion in the urine, as previously described (25). Energy expenditure (EE) and substrate oxidation (13) were determined over 24 h, waking time and sleeping time. Twenty-four hour energy balance was calculated as the difference between 24-h energy intake and 24-h TEE. Activity energy expenditure (AEE) was calculated as $\text{TEE} - 10\% \text{ of TEE} - \text{SMR}$, where 10% TEE is the thermic effect of food and SMR (sleep metabolic rate) was the sleep metabolic rate measured from 0100 to 0300 (30).

Dietary Fatty Acid Oxidation

Participants collected an hourly breath sample for $^{13}\text{CO}_2$ by blowing through a tube into two 15-ml Vacutainer tubes. Breath CO_2 was sampled directly from the Vacutainer with a syringe, and $^{13}\text{CO}_2/^{12}\text{CO}_2$ was measured with isotopic ratio mass spectrometer (IRMS, Delta V, Thermo Electron, Bremen, Germany). The average baseline enrichment value was subtracted from the subsequent values for each subject, and each time point was expressed as the increase in enrichment relative to the subject's

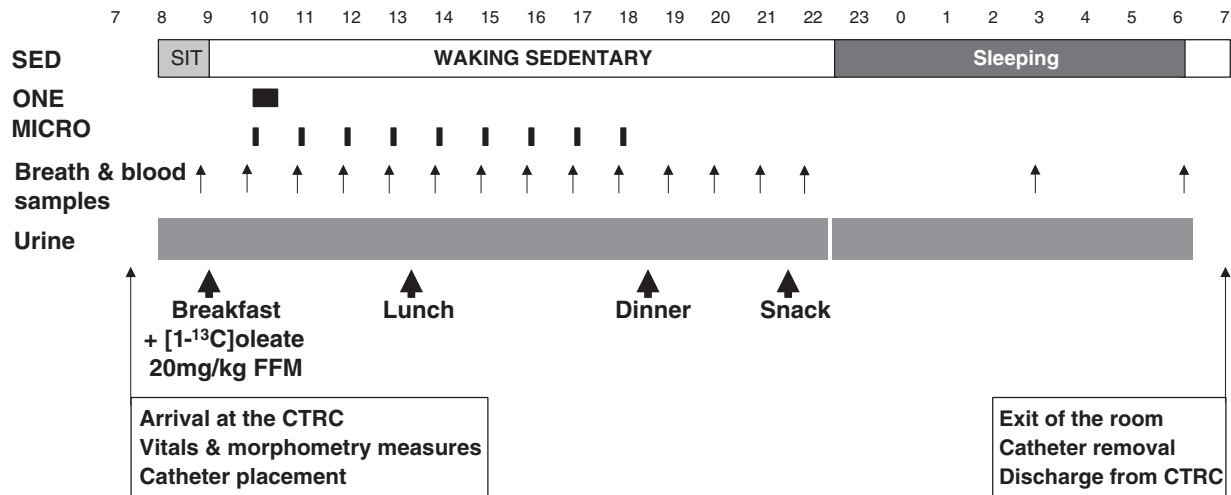


Fig. 2. Protocol of the study day in the whole-room calorimeter. SED, sedentary condition in which participants remained sedentary (no exercise); ONE, one bout condition in which participants remained sedentary for the day except for completing one bout of moderate-intensity treadmill walking for 45 min; MICRO (microbouts) condition in which participants remained sedentary for the day except for completing nine bouts of 5-min moderate-intensity treadmill walking once an hour for nine consecutive hours. CTSC, Center of University of Colorado Hospital; FFM, fat-free mass. ■ indicates 45-min bout of walking; ▮ indicates 5-min walking bout; ↑ indicates blood collection; and solid arrowhead indicates meals.

own baseline. By using time-matched CO_2 production rates from the whole-room calorimeter, $1\text{-}^{13}\text{C}$ oleic acid oxidation was calculated as the instantaneous percentage recovery of ^{13}C in expired CO_2 per hour for 14 h, and after 24-h cumulative oxidation, rates were also calculated over 24 h, as previously described (4) and, after correction for CO_2 entrapment in the bicarbonate pool and TCA cycle (1).

Plasma Analysis

Whole blood was added to a preservative (3.6 mg EDTA plus 2.4 mg glutathione in distilled water). Plasma and serum were separated after spinning and stored at -80°C until analyzed. EDTA plasma samples were assayed for triglycerides (TG), glucose, and insulin. Insulin concentrations were measured using a standard double-antibody radioimmunoassay (EMD Millipore, St. Charles, MO). Serum glucose concentrations were determined using the hexokinase method, TGs were measured using the enzymatic assay, and nonesterified fatty acids (NEFA) was measured by using commercial enzymatic assay (Wako Diagnostics, Mountain View, CA); all samples were run on the Beckman Coulter AU480 Chemistry Analyzer (Brea, CA).

Data and Statistical Analysis

The sample size was calculated using data from a previous study (11). In that study, the authors observed in 19 overweight men that interruptions of prolonged sitting with moderate-intensity activity (2 min every 20 min) reduced 5-h glucose and insulin incremental area under the curve (iAUC) by 29% and 23%, respectively, compared with uninterrupted sitting. Assuming the effect sizes between conditions would be similar to this study, we estimated that 20 paired observations were needed to achieve an 80% power to detect a direct treatment effect, while adopting a two-tailed testing and $\alpha < 0.05$.

iAUCs were calculated with the trapezoidal rule for plasma metabolites and insulin. Linear mixed models (LMM) were used to test differences in total substrate use, TG, FFA, glucose, and insulin iAUCs and dietary fat oxidation with intervention as a repeated effect, sequence, period, and intervention as fixed effects and subjects as a random effect with a compound symmetry covariance. Energy balance was taken into account as a covariate when necessary. Least significant difference (LSD) post hoc tests were used to examine between-condition differences. Carryover effects were expected to be minimal because of the minimum 28-day washout period between consecutive interventions. Pearson cor-

relation coefficients were calculated to examine the relationships among the outcomes, i.e., TEE, AEE, energy balance, substrate use, dietary fatty acid oxidation, plasma metabolites, and insulin iAUCs. Data are expressed as means $\pm$ SD, unless otherwise stated. All statistical analyses were performed with SPSS (v22.0, IBM, SPSS Statistics, Chicago, IL).

RESULTS

Participant Characteristics

The CONSORT diagram is shown as Fig. 1. Twenty-five sedentary overweight adults ($n = 12$ men/13 women; 31.6 ± 6.5 yr; $\text{BMI} = 30.5 \pm 2.7 \text{ kg/m}^2$) were recruited to participate in the study. Data are shown for 20 participants (10 men/10 women; 32.4 ± 6.3 yr; $\text{BMI}, 30.6 \pm 2.9 \text{ kg/m}^2$) who completed all the procedures. Participant's characteristics are displayed in Table 1.

Table 1. Participant characteristics

	Men	Women	Total
<i>N</i>	10	10	20
Age, yr	31.5 ± 7.4	33.2 ± 5.1	32.4 ± 6.3
BMI, kg/m^2	28.9 ± 3.0	31.9 ± 2.0	30.6 ± 2.9
Body mass, kg	$88.0 \pm 13.6^{**}$	83.5 ± 7.2	85.7 ± 10.8
FFM, kg	$63.2 \pm 10.0^{***}$	48.3 ± 3.6	55.8 ± 10.5
FM, kg	$24.6 \pm 4.4^{***}$	35.2 ± 4.4	29.9 ± 6.9
Fat mass, %	$28.1 \pm 2.5^{***}$	42.0 ± 2.3	35.0 ± 7.5
Fasting glucose, mg/dl	88.0 ± 4.8	87.6 ± 4.3	87.8 ± 4.4
Fasting triglyceride, mg/dl	$154.6 \pm 107.7^*$	83.5 ± 14.7	117.2 ± 81.2
Fasting insulin, $\mu\text{IU/ml}$	5.4 ± 2.7	5.15 ± 1.3	5.2 ± 2.1
HOMA-IR (SED day fasting)	1.5 ± 0.8	1.4 ± 0.4	1.4 ± 0.6
IPAQ self-reported sitting time, h/day	9.6 ± 2.9	11.3 ± 3.5	10.5 ± 3.3

Data are presented as the means $\pm$ SD. *n*, number of subjects; BMI, body mass index; FFM, fat-free mass; FM, fat mass; HOMA-IR, homeostatic model assessment of insulin resistance; IPAQ, international physical activity questionnaire. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.0001$ vs. women.

Twenty-Four Hour Energy Intake, Expenditure, Balance, and Nutrient Oxidation

Daily patterns of EE and respiratory quotient (RQ) are presented in Supplemental Fig. S1 (available at <https://doi.org/10.5281/zenodo.2399298>). Twenty-four-hour energy intake, expenditure, and balance are shown in Fig. 3A. By design, both TEE and AEE were matched between the two active conditions and significantly higher than SED ($P < 0.0001$ for both). Because total energy intake was the same across all three study conditions, participants were in negative energy balance in both ONE (-1.50 ± 0.17 MJ/day, $P < 0.01$) and MICRO (-1.64 ± 0.17 MJ/day, $P < 0.01$) compared with SED. Twenty-four-hour non-protein respiratory quotient (NPRQ) was lower in ONE compared with both SED and MICRO conditions (0.881 ± 0.006 vs. 0.900 ± 0.006 and 0.900 ± 0.009 , respectively, $P < 0.05$ for both; Fig. 3D), even when accounting for differences in energy balance. MICRO was associated with higher 24-h carbohydrate oxidation compared with both ONE ($P < 0.01$) and SED ($P < 0.001$; Fig. 3C). In contrast, ONE was associated with higher 24-h total fat oxidation compared with SED ($P < 0.001$) and higher 24-h dietary fat oxidation compared with both SED and MICRO ($P < 0.05$ for both; Fig. 3, C and E). When taking energy balance into account, both 24-h total and dietary fat oxidation were higher and 24-h carbohydrate oxidation lower in ONE compared with MICRO ($P < 0.05$ for all). When expressing nutrient oxidation as a percentage of TEE, we observed that ONE had greater reliance on fat as fuel and less on carbohydrate compared with SED. Nutrient oxidation as a percentage of TEE was not altered in MICRO (Fig. 3B).

In ONE, changes in both 24-h total and dietary fat oxidation were positively correlated with the increase in 24-h EE ($R^2 = 0.66$, $P < 0.001$; $R^2 = 0.58$, $P < 0.01$, respectively), but these associations were not observed in MICRO, even though the increase in 24-h EE was similar. In both active conditions, changes in 24-h fat oxidation were negatively correlated with changes in 24-h carbohydrate oxidation (ONE: $R = -0.79$, $P < 0.0001$; MICRO: $R = -0.84$, $P < 0.0001$).

Substrate Oxidation During Waking and Sleeping Periods

As expected, EE was significantly higher in ONE and MICRO compared with SED ($P < 0.0001$ for both; Fig. 4C) during the waking time when physical activity was performed. Waking NPRQ was lower in ONE compared with SED and MICRO (0.894 ± 0.006 vs. 0.911 ± 0.005 and 0.914 ± 0.009 , respectively, $P < 0.05$ for both). During sleep, NPRQ was lower in both ONE and MICRO compared with SED (0.847 ± 0.009 and 0.852 ± 0.008 vs. 0.875 ± 0.011 , respectively, $P < 0.05$ for both). In both active conditions, carbohydrate oxidation decreased during the night. Compared with SED, this was associated with greater fat oxidation in ONE, but increased protein oxidation, in MICRO ($P < 0.05$ for all; Fig. 4E).

Plasma Metabolites and Index of Insulin Sensitivity

Twenty-four-hour profiles of glucose, TG, insulin, and FFA are shown in Supplemental Fig. S2 (available at <https://doi.org/10.5281/zenodo.2399364>). There were no differences between conditions in fasting concentrations or 24-h iAUC values for

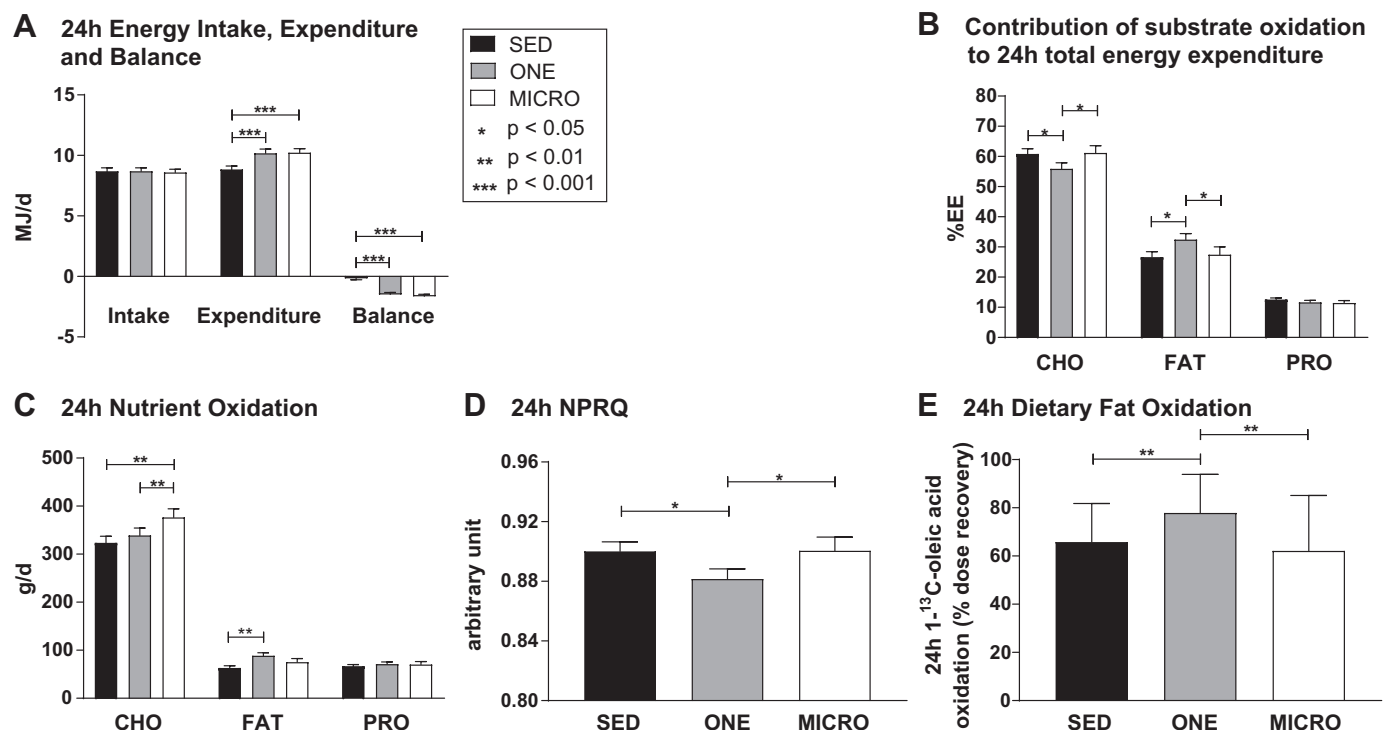


Fig. 3. Absolute and relative 24 h nutrient oxidation, balance, and dietary fat oxidation. A: energy intake (MJ/day), energy expenditure (MJ/day), and energy balance (MJ/day) during the study day in the whole room calorimeter. B: relative contribution (%) of carbohydrate (CHO), fat (FAT), and protein (PRO) oxidation to total 24-h energy expenditure. C: absolute nutrient oxidation (g/day) for CHO, FAT, and PRO. D: 24-h nonprotein respiratory quotient for each study condition: sedentary (SED), one bout (ONE), and microbouts (MICRO). E: 24-h dietary fat oxidation (percent dose recovery from 1-¹³C oleic acid stable isotope tracer) for each study condition: SED, ONE, and MICRO. Data are presented as means $\pm$ SE. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

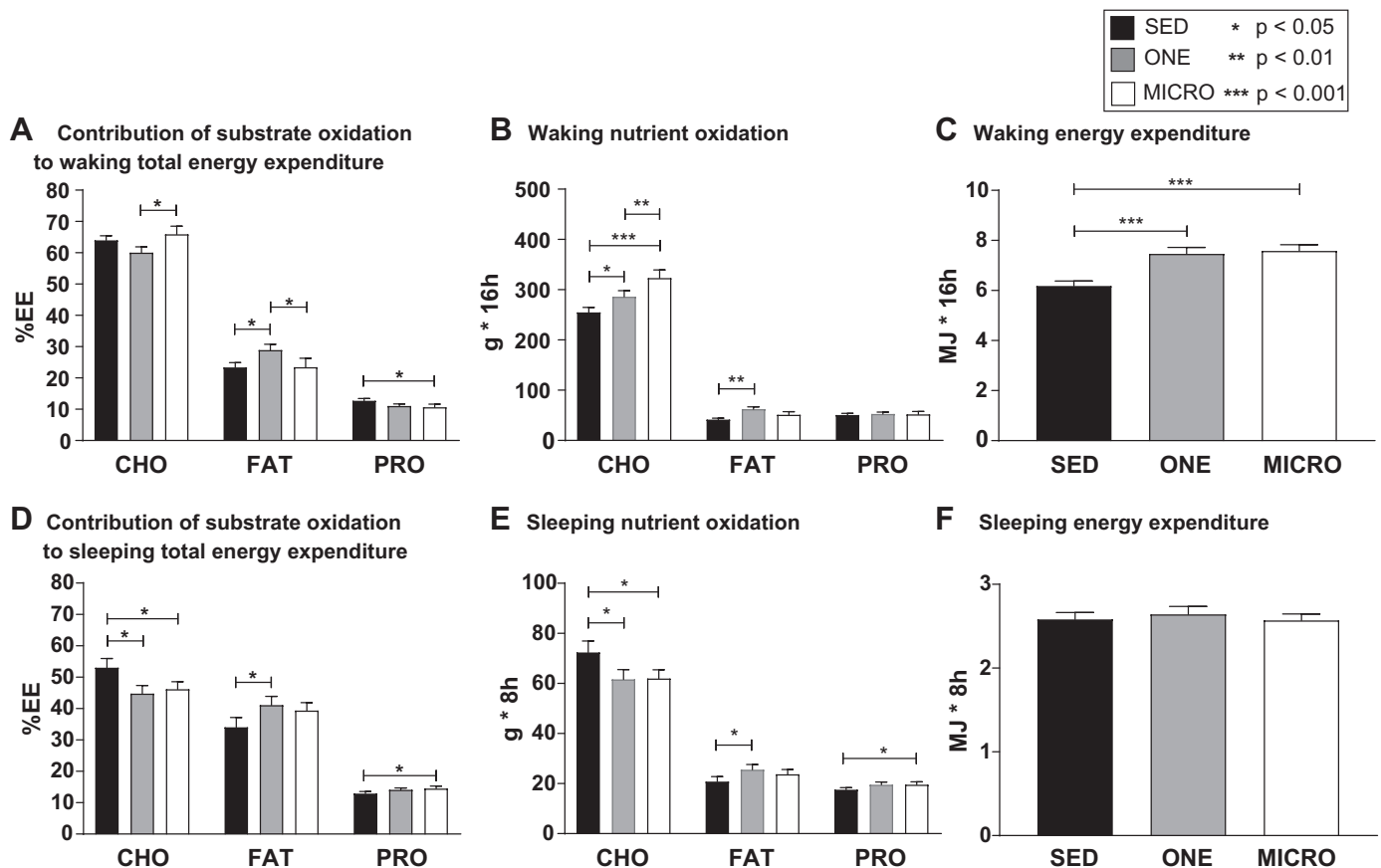


Fig. 4. Waking and sleeping substrate oxidation. Absolute and relative substrate oxidation during waking (0800–2230) and sleeping (2230–0630) time. A: relative contribution (%) of carbohydrate (CHO), fat (FAT), and protein (PRO) oxidations to waking energy expenditure (EE). B: waking absolute nutrient oxidation (grams) of CHO, FAT, and PRO oxidations for waking energy expenditure. C: waking energy expenditure. D: relative contribution (%) of CHO, FAT, and PRO oxidations to sleeping energy expenditure. E: sleeping absolute nutrient oxidation (grams) of CHO, FAT, and PRO oxidations for sleeping energy expenditure. F: sleeping energy expenditure. %EE, percent contribution to energy expenditure. Data are presented as means $\pm$ SE. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

plasma glucose, insulin, FFA, and TG (Fig. 5). After 4 days of both active conditions HOMA-IR was decreased compared with SED (SED = 1.67 ± 0.87 , ONE = 1.20 ± 0.52 , MICRO = 1.42 ± 0.70 ; $P < 0.05$ for both), indicating an improvement in fasting insulin sensitivity. In addition, insulin iAUC measured from the first to the last microbout of activity (1000–1800) was reduced in ONE (ONE = $-16599 \pm 10871 \mu\text{IU}\cdot\text{ml}^{-1}\cdot 9 \text{ h}^{-1}$; $P < 0.0001$) and MICRO (MICRO = $-11,570 \pm 8,866 \mu\text{IU}\cdot\text{ml}^{-1}\cdot 9 \text{ h}^{-1}$; $P < 0.01$) compared with SED (SED = $-5,283 \pm 10,667 \mu\text{IU}\cdot\text{ml}^{-1}\cdot 9 \text{ h}^{-1}$) (Fig. 5). No differences were noted over this same time period in plasma glucose, TG, and FFA.

DISCUSSION

In this cross-over study, we showed in sedentary, overweight, or obese adults that four days of frequent interruptions in sedentary time with short moderate-intensity walking breaks every hour for 9 h leads to greater reliance upon carbohydrate as fuel compared with a sedentary control condition. In contrast and to our surprise, a single isoenergetic continuous bout of moderate-intensity walking led to greater total and dietary fat oxidation. These findings suggest that when energy expenditure is equal between the two active conditions, breaking up sedentary time impacts daily patterns in fuel utilization differ-

ently than when exercise is performed as a single bout in the morning.

Breaking up sedentary behavior with short bouts of activity resulted in improved postprandial glucose and insulin metabolism in several previous acute and short intervention studies (2, 5, 9–11, 18, 21, 27, 34). In the present study, we observed a significant reduction in plasma insulin iAUC when participants were performing microbouts of activity, but postprandial glucose concentration was not reduced over the 9-h active period. There are several possible reasons for the discrepancy in postprandial glucose responses in response to short frequent breaks of prolonged sitting compared with sedentary control. Previous studies supplied liquid meal replacement shakes as the study day energy intake. While the use of liquid meal replacement shakes ensures accurate standardization of macronutrient intake, it does not reflect the postprandial responses of foods that are regularly consumed by the target population. The current study administered food preference questionnaires and provided meals composed of whole foods including dietary fiber. Dietary fiber has been shown to attenuate postprandial plasma glucose responses (22, 32). Additionally, the current study did not include participants with impaired glucose tolerance or Type 2 diabetes, as has been done in previous studies (10, 11, 21). Along this line, Blankenship et al. (5) studied

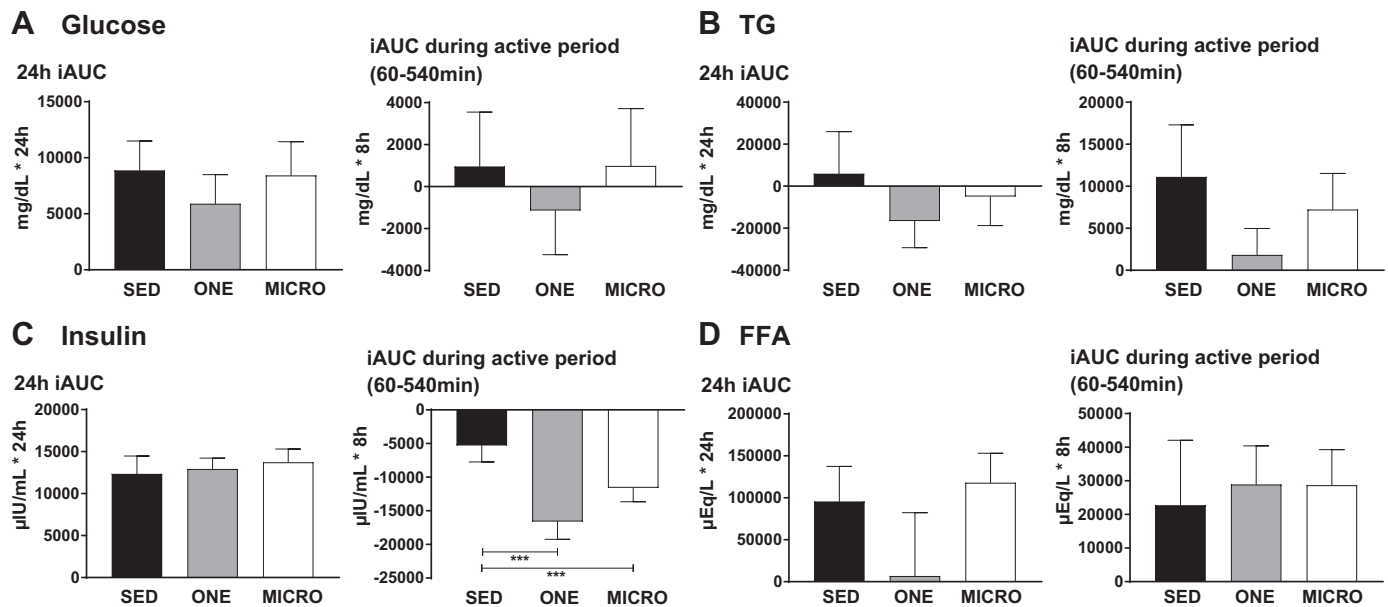


Fig. 5. Twenty-four hour- and active-period incremental area under the curves (iAUCs) for plasma metabolites. iAUCs for plasma glucose, triglycerides (TG), insulin, and free fatty acids (FFAs) measured during the inpatient study day. A: 24-h and active-period plasma glucose iAUCs. B: 24-h and active-period plasma TG iAUCs. C: 24-h and active-period plasma insulin iAUCs. D: 24-h and active-period FFA iAUCs. Active period iAUC measured from the first to the last microbout of activity (1000–1800). SED, sedentary exposure; ONE, one bout (45 min of moderate intensity at 1000) walking exposure; MICRO, microbout exposure (5-min walking per hour for nine consecutive hours at moderate intensity from 1000 to 1800). Data are presented as means $\pm$ SE. *** $P < 0.001$.

overweight and obese participants similar to the present study, but also did not observe differences in plasma glucose responses to a meal tolerance test administered at the end of a day after a protocol involving either frequent long or short activity breaks (conditions matched for EE). However, glycemic variability measured by continuous glucose monitoring was reduced in the two frequent break conditions, indicating improved glucose control (5). Peddie et al. (27) showed a reduction in postprandial glucose iAUC in young healthy male adults compared with sedentary condition when interrupting sitting with walking breaks of 1 min and 40 s every 30 min (30 min of total walking) over 9 h. Similarly, Bailey et al. (2) showed in overweight young adults a beneficial effect on postprandial glycemia when sitting was interrupted by 2-min bouts of light walking every 20 min (28 min total) for 6 h. In the current study, independent of changes in substrate use, both modalities of physical activity improved insulin sensitivity after 4 days, as indicated by the decrease in HOMA-IR in the fasted state the following morning and the decrease in postprandial insulin iAUC during the active period. Taken together, both exercise interventions improved indexes of insulin sensitivity but through a differential metabolic response to varying frequencies of activity bouts. These studies suggest that while less apparent in nondiabetic populations, frequent interruptions of sedentary time help control glucose metabolism via a better insulin sensitivity and greater use of carbohydrate as fuel in postprandial state and over 24 h, thus lowering glycemia mean and variability. The magnitude of the effects may be related to the frequency of the interruptions. Interestingly, these effects seem to be independent of energy balance and are likely related to the frequent interruptions of sedentary time.

To distinguish the effect of energy expenditure from those resulting from the frequent interruptions of sedentary time, we included an isoenergetic single continuous bout of moderate-

intensity walking in the study design. At the same energy expenditure and energy deficit, frequent interruptions of sedentary activity with short bouts of moderate intensity of physical activity primarily rely upon carbohydrate substrate to maintain energetic homeostasis over 24 h, while a single bout of moderate-intensity activity favors the oxidation of both 24-h total and dietary fatty acids, as previously reported (4). One could assume that the greater use of fat oxidation observed with physical activity performed as a continuous long bout may result over the long run in a greater weight loss than what could be attained by performing multiple short bouts of activity. Jackicic et al. (19) showed that multiple short bouts and time-matched long continuous bouts of activity induced similar weight loss after 18 mo of intervention in sedentary overweight women. It is, however, important to note that the short bouts were of 10-min duration, which could be long enough to trigger fat oxidation compared with a 5-min bout of activity. Future studies looking at the long-term impact of microbouts of activity versus long bouts of activity on body weight regulation will be needed to further test this hypothesis.

The differential effects between these two active conditions were also observed over the waking and sleeping periods when examined separately. During the waking period, frequent interruptions in sedentary time were associated with greater carbohydrate oxidation, while the performance of a single bout of walking led to an increase in lipid oxidation. As suggested by the tight correlation between carbohydrate and fat oxidation observed over 24 h for each active condition, nutrient oxidation is likely the result of competition between substrates entering the TCA cycle (28). One may assume the potential following scenario. Glucose was preferentially used with the short bouts of activity because it was readily available as skeletal muscle glycogen, especially during the first minutes of the bout of activity. The regular muscle contractions spread across the day

with the frequent interruptions of sedentary time may have further stimulated the translocation from the cytoplasm to the membrane of the glucose transporter GLUT4, as previously shown (3). This triggered the uptake and oxidation of glucose by the cell to provide energy. Because the microbouts of activity were performed in the postprandial state only, glucose was constantly available and competing against fat. When performing 45 min of walking, glycogen storage was at least partly depleted, thus allowing fat to be oxidized for energy expenditure and glycogen pools were refilled. Because of the close relationship between energy and fat balances (14, 33), we observed that at the equal energy expenditure, fat oxidation was increased but only when physical activity was performed as one single bout.

During the sleeping periods, carbohydrate oxidation was reduced in the two active conditions compared with the sedentary condition. Interestingly, while this was in favor of increased fat oxidation after 45 min of moderate-intensity walking performed in the morning, it was associated with an increase in protein oxidation following a day performing microbouts of activity. However, measurement of protein oxidation via urinary nitrogen excretion is not a direct measure of protein oxidation but an assessment of protein deamination. The greater disappearance of protein may rather reflect a use of protein for gluconeogenesis to replenish muscle glycogen than a use of protein as fuel for the body. Over 24 h, the microbouts of activity likely trigger the use of glycogen stores and its replenishment, thus enhancing glycogen turnover; future studies will be needed to test this hypothesis. These differences and changes in nocturnal nutrient metabolism are important given the growing body of data pointing toward a key role of sleep in the regulation of energy homeostasis and metabolism (29). For example, we showed that higher rates of nocturnal fat oxidation are associated with lower weight gain over 5 years in adults (31). Future research is needed to better understand the changes induced by the two different types of physical activity interventions on the changes observed in waking and sleeping nutrient metabolism.

A major strength of this study is that it was a randomized controlled trial testing energy-matched active conditions to isolate the respective effects of the frequency of interruptions of sedentary time from energy expenditure. Also, diet, alcohol, and caffeine consumption were controlled, and nutrient metabolism was measured over 24 h in a whole-room calorimeter. There are some methodological factors that limit generalizability of the present findings. Comparing the active conditions to the sedentary conditions in stable energy balance would have been more rigorous and most likely representative of what happened in daily life in chronic situations. However, we mathematically and statistically accounted for differences in energy balance, and the two active conditions were in similar energy imbalance. Another limitation was the artificial elevation of dietary carbohydrate oxidation that may have potentialized the use of carbohydrate as fuel during the MICRO condition. Additionally, while dietary fat oxidation was measured, we cannot comment on the source of carbohydrate that was oxidized during the study day. Future studies using both fat- and carbohydrate-stable isotope tracers will be needed.

Conclusions and Future Directions

In conclusion, we showed that while 4 days of frequent interruptions in sitting time primarily relies upon carbohydrate as fuel, a single long bout of activity primarily influences lipid metabolism. This suggests that the beneficial effect of interrupting sedentary time on glucose control that was previously reported is likely related to a greater reliance upon carbohydrate as fuel. This effect does not appear to be related to energy expenditure and balance, but rather to increasing the frequency of muscle contractions spread across the day. Underlying mechanisms at play, as well as the role of moderating factors, such as weight status, insulin resistance, and sex need to be examined in the future.

ACKNOWLEDGMENTS

The authors thank study volunteers for their time and participation, as well as the medical, nutrition, and administrative staff of the University of Colorado Clinical and Translational Research Center. This study is currently in clinical trials (see www.clinicaltrials.gov; NCT: NCT02258438).

GRANTS

This work was supported by the National Institutes of Health grant numbers K99DK100465 (A. Bergouignan) and UL1 TR001082 (A. Bergouignan), K01DK113063 (C. A. Rynders) and P30DK048520. This publication was also supported by Grant Number T42OH009229-10 (A. Bergouignan) from Centers for Disease Control and Prevention National Institute for Occupational Safety and Health Mountain and Plains Education and Research Center.

DISCLAIMERS

The contents are solely the responsibility of the authors and do not represent the views of the U.S. Department of Veterans Affairs, the United States Government, the Center for Disease Control, National Institute of Occupational Health and Safety and the Mountain and Area Plains Education and Research Center.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

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

REFERENCES

1. Antoun E, Momken I, Bergouignan A, Villars C, Platat C, Schoeller DA, Blanc S, Simon C. The [$1-^{13}\text{C}$] acetate recovery factor to correct tracer-derived dietary fat oxidation is lower in overweight insulin-resistant subjects. *E Spen Eur E J Clin Nutr Metab* 5: e173–e179, 2010. doi:10.1016/j.eclnm.2010.05.002.
2. Bailey DP, Locke CD. Breaking up prolonged sitting with light-intensity walking improves postprandial glycemia, but breaking up sitting with standing does not. *J Sci Med Sport* 18: 294–298, 2015. doi:10.1016/j.jsams.2014.03.008.
3. Bergouignan A, Latouche C, Heywood S, Grace MS, Reddy-Luthmoodoo M, Natoli AK, Owen N, Dunstan DW, Kingwell BA. Frequent interruptions of sedentary time modulates contraction- and insulin-stimulated glucose uptake pathways in muscle: Ancillary analysis from randomized clinical trials. *Sci Rep* 6: 32044, 2016. doi:10.1038/srep32044.
4. Bergouignan A, Momken I, Lefai E, Antoun E, Schoeller DA, Platat C, Chery I, Zaharieva A, Vidal H, Gabert L, Normand S, Freyssenet D, Laville M, Simon C, Blanc S. Activity energy expenditure is a major

- determinant of dietary fat oxidation and trafficking, but the deleterious effect of detraining is more marked than the beneficial effect of training at current recommendations. *Am J Clin Nutr* 98: 648–658, 2013. doi:10.3945/ajcn.112.057075.
5. Blankenship JM, Granados K, Braun B. Effects of subtracting sitting versus adding exercise on glycemic control and variability in sedentary office workers. *Appl Physiol Nutr Metab* 39: 1286–1293, 2014. doi:10.1139/apnm-2014-0157.
 6. Booth M. Assessment of physical activity: an international perspective. *Res Q Exerc Sport* 71: 114–120, 2000. doi:10.1080/02701367.2000.11082794.
 7. Buman MP, Winkler EA, Kurka JM, Hekler EB, Baldwin CM, Owen N, Ainsworth BE, Healy GN, Gardiner PA. Reallocating time to sleep, sedentary behaviors, or active behaviors: associations with cardiovascular disease risk biomarkers, NHANES 2005–2006. *Am J Epidemiol* 179: 323–334, 2014. doi:10.1093/aje/kwt292.
 8. De Jong NP, Debauche I, Pan Z, Garnotel M, Lyden K, Sueur C, Simon C, Bessesen DH, Bergouignan A. Breaking up sedentary time in overweight/obese adults on work days and non-work days: results from a feasibility study. *Int J Environ Res Public Health* 15: 2566, 2018. doi:10.3390/ijerph15112566.
 9. Dempsey PC, Blankenship JM, Larsen RN, Sacre JW, Sethi P, Straznicki NE, Cohen ND, Cerin E, Lambert GW, Owen N, Kingwell BA, Dunstan DW. Interrupting prolonged sitting in type 2 diabetes: nocturnal persistence of improved glycaemic control. *Diabetologia* 60: 499–507, 2017. doi:10.1007/s00125-016-4169-z.
 10. Dempsey PC, Larsen RN, Sethi P, Sacre JW, Straznicki NE, Cohen ND, Cerin E, Lambert GW, Owen N, Kingwell BA, Dunstan DW. Benefits for Type 2 diabetes of interrupting prolonged sitting with brief bouts of light walking or simple resistance activities. *Diabetes Care* 39: 964–972, 2016. doi:10.2337/dc15-2336.
 11. Dunstan DW, Kingwell BA, Larsen R, Healy GN, Cerin E, Hamilton MT, Shaw JE, Bertovic DA, Zimmet PZ, Salmon J, Owen N. Breaking up prolonged sitting reduces postprandial glucose and insulin responses. *Diabetes Care* 35: 976–983, 2012. doi:10.2337/dc11-1931.
 12. Duviols BM, Schaper NC, Bremers MA, van Crombrughe G, Menheere PP, Kars M, Savelberg HH. Minimal intensity physical activity (standing and walking) of longer duration improves insulin action and plasma lipids more than shorter periods of moderate to vigorous exercise (cycling) in sedentary subjects when energy expenditure is comparable. *PLoS One* 8: e55542, 2013. [Erratum in *PLoS One* 9: e105135, 2014.] doi:10.1371/journal.pone.0055542.
 13. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol* 55: 628–634, 1983. doi:10.1152/jap.1983.55.2.628.
 14. Galgani J, Ravussin E. Energy metabolism, fuel selection and body weight regulation. *Int J Obes* 32, Suppl 7: S109–S119, 2008. doi:10.1038/ijo.2008.246.
 15. Healy GN, Dunstan DW, Salmon J, Cerin E, Shaw JE, Zimmet PZ, Owen N. Breaks in sedentary time: beneficial associations with metabolic risk. *Diabetes Care* 31: 661–666, 2008. doi:10.2337/dc07-2046.
 16. Healy GN, Dunstan DW, Salmon J, Cerin E, Shaw JE, Zimmet PZ, Owen N. Objectively measured light-intensity physical activity is independently associated with 2-h plasma glucose. *Diabetes Care* 30: 1384–1389, 2007. doi:10.2337/dc07-0114.
 17. Healy GN, Matthews CE, Dunstan DW, Winkler EA, Owen N. Sedentary time and cardio-metabolic biomarkers in US adults: NHANES 2003–06. *Eur Heart J* 32: 590–597, 2011. doi:10.1093/eurheartj/ehq451.
 18. Holmstrup M, Fairchild T, Keslacy S, Weinstock R, Kanaley J. Multiple short bouts of exercise over 12-h period reduce glucose excursions more than an energy-matched single bout of exercise. *Metabolism* 63: 510–519, 2014. doi:10.1016/j.metabol.2013.12.006.
 19. Jakicic JM, Winters C, Lang W, Wing RR. Effects of intermittent exercise and use of home exercise equipment on adherence, weight loss, and fitness in overweight women: a randomized trial. *JAMA* 282: 1554–1560, 1999. doi:10.1001/jama.282.16.1554.
 20. Jankowski CM, Gozansky WS, Van Pelt RE, Wolfe P, Schwartz RS, Kohrt WM. Oral dehydroepiandrosterone replacement in older adults: effects on central adiposity, glucose metabolism and blood lipids. *Clin Endocrinol (Oxf)* 75: 456–463, 2011. doi:10.1111/j.1365-2265.2011.04073.x.
 21. Larsen RN, Kingwell BA, Robinson C, Hammond L, Cerin E, Shaw JE, Healy GN, Hamilton MT, Owen N, Dunstan DW. Breaking up of prolonged sitting over three days sustains, but does not enhance, lowering of postprandial plasma glucose and insulin in overweight and obese adults. *Clin Sci (Lond)* 129: 117–127, 2015. doi:10.1042/CS20140790.
 22. Lunde MSH, Hjellset VT, Holmboe-Ottesen G, Høstmark AT. Variations in postprandial blood glucose responses and satiety after intake of three types of bread. *J Nutr Metab* 2011: 437587, 2011. doi:10.1155/2011/437587.
 23. Matthews CE, George SM, Moore SC, Bowles HR, Blair A, Park Y, Troiano RP, Hollenbeck A, Schatzkin A. Amount of time spent in sedentary behaviors and cause-specific mortality in US adults. *Am J Clin Nutr* 95: 437–445, 2012. doi:10.3945/ajcn.111.019620.
 24. Matthews CE, Keadle SK, Troiano RP, Kahle L, Koster A, Brychta R, Van Domelen D, Caserotti P, Chen KY, Harris TB, Berrigan D. Accelerometer-measured dose-response for physical activity, sedentary time, and mortality in US adults. *Am J Clin Nutr* 104: 1424–1432, 2016. doi:10.3945/ajcn.116.135129.
 25. Melanson EL, Ingebrigtsen JP, Bergouignan A, Ohkawara K, Kohrt WM, Lighton JR. A new approach for flow-through respirometry measurements in humans. *Am J Physiol Regul Integr Comp Physiol* 298: R1571–R1579, 2010. doi:10.1152/ajpregu.00055.2010.
 26. Mummery WK, Schofield GM, Steele R, Eakin EG, Brown WJ. Occupational sitting time and overweight and obesity in Australian workers. *Am J Prev Med* 29: 91–97, 2005. doi:10.1016/j.amepre.2005.04.003.
 27. Peddie MC, Bone JL, Rehner NJ, Skeaff CM, Gray AR, Perry TL. Breaking prolonged sitting reduces postprandial glycemia in healthy, normal-weight adults: a randomized crossover trial. *Am J Clin Nutr* 98: 358–366, 2013. doi:10.3945/ajcn.112.051763.
 28. Randle PJ. Regulatory interactions between lipids and carbohydrates: the glucose fatty acid cycle after 35 years. *Diabetes Metab Rev* 14: 263–283, 1998. doi:10.1002/(SICI)1099-0895(199812)14:4<263::AID-DMR233>3.0.CO;2-C.
 29. Reutrakul S, Van Cauter E. Sleep influences on obesity, insulin resistance, and risk of type 2 diabetes. *Metabolism* 84: 56–66, 2018. doi:10.1016/j.metabol.2018.02.010.
 30. Rising R, Harper IT, Fontvielle AM, Ferraro RT, Spraul M, Ravussin E. Determinants of total daily energy expenditure: variability in physical activity. *Am J Clin Nutr* 59: 800–804, 1994. doi:10.1093/ajcn/59.4.800.
 31. Rynders CA, Bergouignan A, Kealey E, Bessesen DH. Ability to adjust nocturnal fat oxidation in response to overfeeding predicts 5-year weight gain in adults. *Obesity (Silver Spring)* 25: 873–880, 2017. doi:10.1002/oby.21807.
 32. Sadakiyo T, Ishida Y, Inoue S-I, Taniguchi Y, Sakurai T, Takagaki R, Kurose M, Mori T, Yasuda-Yamashita A, Mitsuzumi H, Kubota M, Watanabe H, Fukuda S. Attenuation of postprandial blood glucose in humans consuming isomaltodextrin: carbohydrate loading studies. *Food Nutr Res* 61: 1325306, 2017. doi:10.1080/16546628.2017.1325306.
 33. Schrauwen P, Lichtenbelt WD, Saris WH, Westerterp KR. Fat balance in obese subjects: role of glycogen stores. *Am J Physiol Endocrinol Metab* 274: E1027–E1033, 1998. doi:10.1152/ajpendo.1998.274.6.E1027.
 34. van Dijk JW, Venema M, van Mechelen W, Stehouwer CD, Hartgens F, van Loon LJ. Effect of moderate-intensity exercise versus activities of daily living on 24-hour blood glucose homeostasis in male patients with type 2 diabetes. *Diabetes Care* 36: 3448–3453, 2013. doi:10.2337/dc12-2620.
 35. Villalon KL, Gozansky WS, Van Pelt RE, Wolfe P, Jankowski CM, Schwartz RS, Kohrt WM. A losing battle: weight regain does not restore weight loss-induced bone loss in postmenopausal women. *Obesity (Silver Spring)* 19: 2345–2350, 2011. doi:10.1038/oby.2011.263.