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The effect of frequency of activity interruptions in prolonged sitting on postprandial glucose metabolism: A randomized crossover trial☆

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ABSTRACT

Objective: The primary objective was to test the hypothesis that increased frequency of interruptions in prolonged sitting reduces postprandial glycemia independent of energy intake and expenditure.
Materials/Methods: Healthy, sedentary, centrally obese men (n = 14; age*, 28.2 (23.4; 38.3) years; BMI, 31.9 ± 6.7 kg/m^2; VO_2max*, 39.5 (38.8; 40.9) ml/min/kg; HbA1c, 5.3 ± 0.4% (34.1 ± 4.2 mmol/mol); mean ± SD (*median (25th; 75th percentile)) completed four 8-h interventions in randomized order: 1) uninterrupted sitting (SIT), 2) sitting interrupted by 2 min of walking (~30% of VO_{2max}) every 20th minute (INT20), 3) sitting interrupted by 6 min of walking every hour (INT60), and 4) sitting interrupted by 12 min of walking every second hour (INT120). A standardized test drink was served at the beginning of and 4 h into the intervention (total of 2310 ± 247 kcal; 50% energy from carbohydrate, 50% energy from fat). Outcomes included the difference in the 8-h total area under the curve (tAUC) for primarily plasma glucose, and secondarily plasma insulin and C-peptide during INT20, INT60, and INT120 compared to SIT.
Results: No difference [95% CI] was observed in the primary outcome, the 8-h tAUC for the plasma glucose, during INT20, INT60, and INT120 compared to SIT (−65.3 mmol/l*min [−256.3; 125.7], +53.8 mmol/l*min [−143.1; 250.8], and +18.6 mmol/l*min [−172.4; 209.6], respectively).
Conclusions: Interrupting sitting with increasing frequency did not reduce the postprandial plasma glucose response to prolonged sitting in healthy, sedentary, centrally obese men.

1. Introduction

Sedentary behavior is associated with increased risk of cardiovascular disease, type 2 diabetes, and mortality independent of volume of physical activity (PA) [1], although very high PA volumes may attenuate the negative effects of excessive sedentary behavior [2]. Attaining these volumes of PA may not be feasible for the majority of the population, thus studies investigating minimal volume PA that may mitigate the health consequences of sedentary behaviors are needed.

Observational cross-sectional studies suggest that frequent interruptions in prolonged sitting may be associated with reduced cardiometabolic risk, independent of sitting time and PA volume [3,4]. Moreover, by statistically substituting prolonged sitting time with interrupted sitting time, beneficial associations with cardiometabolic risk factors have been observed [5–7]. This is broadly supported by existing experimental evidence suggesting that higher PA volumes

Abbreviations: CGM, Continuous glucose monitoring; ECG, Electrocardiogram; EE, Energy expenditure; EI, Energy intake; HOMA-IR, Homeostasis model assessment of insulin resistance; iAUC, Incremental area under the curve; INT20, Sitting interrupted by 2 min of walking every 20th minute; INT60, Sitting interrupted by 6 min of walking every hour; INT120, Sitting interrupted by 12 min of walking every second hour; LPA, Low-intensity physical activity; MET, Metabolic equivalent of task; NEFA, Non-esterified fatty acid; OGTT, Oral glucose tolerance test; PA, Physical activity; REE, Resting energy expenditure; RER, Respiratory exchange ratio; SIT, Prolonged sitting; tAUC, Total area under the curve; $tAUC_{insulin/glucose}$, Ratio of insulin tAUC/glucose tAUC; $tAUC_{C\text{-}peptide/glucose}$, Ratio of C-peptide tAUC/glucose tAUC; $tAUC_{insulin/glucagon}$, Ratio of insulin tAUC/glucagon tAUC; $tAUC_{C\text{-}peptide/glucagon}$, Ratio of C-peptide tAUC/glucagon tAUC; VCO_2, Carbon dioxide elimination; VO_2, Oxygen consumption; VO_{2max}, Maximal oxygen consumption.

☆ Trials registration: NCT02951624, ClinicalTrials.gov.

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during interruptions in prolonged sitting may elicit larger effects on postprandial metabolic parameters than lower PA volumes [8]. Similarly, increasing the frequency of interruptions increased fat oxidation among young, physically inactive men [9]. The health benefits may however be explained by a higher energy expenditure (EE) observed with increasing frequency of interruptions and not by the frequency of interruptions per se. Therefore, the role of the frequency of interruptions in prolonged sitting on cardiometabolic risk factors, independent of the intensity and total duration of PA are yet to be investigated in an isocaloric experimental design to infer causality.

Thus, the primary aim of the study was to test the hypothesis that increasing the frequency of interruptions in prolonged sitting will reduce postprandial glycemia independent of energy intake (EI) and EE in healthy, sedentary, centrally obese men. Secondary aims were to investigate the effects of increasing the frequency of interruption on other markers of postprandial glucose and lipid metabolism, as well as 24-h post-intervention glucose homeostasis and postprandial carbohydrate and fat oxidation.

2. Research Design and Methods

2.1. Study Design, Randomization, Allocation and Blinding

The study was a randomized crossover trial, including four 8-h interventions separated by a minimum wash-out period of four days for each participant with a median [25th; 75th percentile/min-max] of 6 [6; 8/4–34] days. Each possible sequence of the four interventions were written on a paper, separated into opaque envelopes, and stored in a locked closet. By random drawing, participants were allocated to one of 24 possible sequences, which was then unblinded to the researcher, but blinded to the participant until arrival at the lab on the morning of each intervention. The sequential delivery of interventions is presented in eFigure 1.

Participants were asked to maintain their habitual level of PA and their habitual diet throughout participation in the study.

2.2. Participants

All participants were recruited between November 2016 and November 2017 through advertisement at the University of Copenhagen and www.forsøgsperson.dk. The recruitment process is depicted in eFigure 1. Inclusion criteria were: 1) males, 2) 20–50 years of age, and 3) waist-to-height ratio ≥0.5 and/or waist circumference ≥102 cm. Exclusion criteria were: 1) clinically diagnosed diabetes, 2) uncontrolled hypertension, 3) use of glucose- and/or lipid-lowering medication, 4) smoking, 5) evidence of thyroid-, liver-, lung-, heart- or kidney disease, 6) non-sedentary occupation, 7) contraindications to participation in maximal oxygen consumption (VO_{2max}) measurement, and 8) VO_{2max} above average according to age [10]. Participant eligibility was based on preliminary screening, including standard medical history and examination, electrocardiogram (ECG), blood chemistry screen, two-hour oral glucose tolerance test (OGTT), standard anthropometric measures, and estimation of VO_{2max}.

All participants provided written and oral consent. The study was approved by the scientific ethical committee of the Capital Region of Denmark (53476) and registered at clinicaltrials.org (NCT02951624).

2.3. Intervention

Participants were instructed to refrain from caffeine and alcohol 24 h prior to and following each intervention, as well as participation in moderate- to vigorous PA 48 h prior to and 24 h following each intervention. If a participant was ill during the 48 h prior to the intervention, the intervention was rescheduled. If a participant became ill during the 24 h following the intervention, data obtained during this period were excluded from the analyses. On each of the test days, participants reported to the lab between 7.30 and 9.00 a.m. after an overnight fast (>10 h).

The interventions are depicted in eFigure 2. One of the interventions was designed as a control intervention, while the three remaining interventions were similar regarding total duration of sedentary time (7 h and 12 min) and low-intensity PA (LPA) time (48 min). Sedentary time was spent sitting in a chair, restricted to sedentary behaviors (working on a computer, watching movies, reading, etc.). LPA time was spent walking on a treadmill with a target intensity of 30% of VO_{2max} (corresponding to 2.0 metabolic equivalent of task (MET) [11]). Additional PA was restricted to standing and walking in order to go to the restroom and was enforced by continuous observation. The frequency and duration of interruptions in sitting time differed between interventions as follows: (1) Sitting (Control; SIT), participants spent 8 h sedentary; (2) INT20, participants were sedentary in bouts of 18 min with 2 min of LPA between each bout; (3) INT60, participants were sedentary in bouts of 54 min with 6 min of LPA between each bout; and (4) INT120, participants were sedentary in bouts of 108 min with 12 min of LPA between each bout.

2.4. Outcomes

The primary outcome was the difference in 8-h total AUC (tAUC) for postprandial glucose concentrations between the active interventions (INT20, INT60, and INT120) and SIT.

Other outcomes included the differences in 8-h tAUC and incremental AUC (iAUC), and 4-h tAUC and iAUC following the first and second test drink, respectively, for postprandial glucose (except 8-h tAUC), insulin, C-peptide, glucagon, triglyceride, non-esterified fatty acid (NEFA), total cholesterol, HDL and LDL cholesterol concentrations between the active interventions (INT20, INT60, and INT120) and SIT. Moreover, other outcomes included the difference in 24-h and nocturnal post-intervention plasma glucose homeostasis (CGM) (i.e. mean, coefficient of variation, minimum, maximum, tAUC and iAUC), the difference in estimated 8-h rate of carbohydrate and fat oxidation, as well as the differences in Matsuda, insulinogenic, and disposition indices [12], and the ratios of insulin tAUC/glucose tAUC ($tAUC_{insulin/glucose}$), C-peptide tAUC/glucose tAUC ($tAUC_{C\text{-}peptide/glucose}$), insulin tAUC/glucagon tAUC ($tAUC_{insulin/glucagon}$), and C-peptide tAUC/glucagon tAUC ($tAUC_{C\text{-}peptide/glucagon}$) [13] between the active interventions (INT20, INT60, and INT120) and SIT.

3. Measurements

3.1. Body Composition

Height, weight, and waist circumference was measured using standard procedures. Participants underwent a dual x-ray absorptiometry scan (Prodigy Advance, GE Medical Systems – Lunar, Madison, WI, USA) for determination of fat percentage and lean mass.

3.2. Oral Glucose Tolerance Test

During screening, an OGTT was performed as previously described in detail [14], with the exception that blood samples were only drawn at fasting and 2 h following ingestion of the glucose solution. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated based on fasting plasma insulin and glucose concentrations [12].

3.3. Resting Energy Expenditure

During screening, resting energy expenditure (REE) was assessed as previously described in detail [15], with the exception that the duration of acclimatization and measurement was 15 min each.

3.4. Estimation of VO_{2max}

During screening, VO_{2max} was estimated using a graded treadmill test (Technogym Runrace, Gambettola, Italy). The test included two warm-up periods of 3 min of walking at a velocity of 2 and 4 km/h, respectively (inclination 0%), followed by an increment in velocity of 2 km/h every 2 min until a velocity of 10 km/h was reached (inclination 0%). Thereafter, the inclination was increased by 3% every minute until exhaustion. Achievement of VO_{2max} was evaluated based on the following criteria: 1) plateauing of oxygen consumption (VO_2) with incremental workloads, 2) respiratory exchange ratio (RER) >1.1, and 3) a subjective evaluation of maximal effort by the assessor. VO_2 was assessed using continuous indirect calorimetric measurements (Quark CPET, Cosmed, Italy). Walking velocity targeting 30% of individual VO_{2max} was estimated by performing linear regression using the VO_2 at walking velocities of 0.8 and 2 km/h, 2 and 4 km/h, or 4 and 6 km/h, whichever interval included 30% of individual VO_{2max}.

3.5. Physical Activity, Posture Allocation and Energy Expenditure

From 24 h prior to through 24 h following the interventions, PA level was assessed using the accelerometer-based activity monitor, activPAL3 micro (PalTechnologies, UK). The activPAL3 micro was worn on the front of the right thigh, medially between knee and hip joint. The activPAL3 micro allows for estimation of EE, quantification of PA into sitting, standing and stepping time, as well as number of steps during free-living [16].

3.6. Food Intake Prescription

Participants were provided standardized meals for the 24 h prior to and following each intervention. The total energy content of the meals was identical for all participants, and matched the daily energy requirements of an adult male, weight of 90 kg, aged 18–59.9 years and with a PA level factor of 1.45 (≈2900 kcal; 24 h before: 53% carbohydrate, 34% fat, 13% protein; 24 h after: 56% carbohydrate, 33% fat, 11% protein) [17]. The participants were instructed not to ingest anything besides these meals during the period. On arrival at the lab, participants were asked whether they followed the food intake prescription. No deviations were reported.

3.7. Mixed Meal Tolerance Test

A standardized test drink was served at the beginning of and 4 h into the intervention. Each test drink covered one third of the participant's individual daily energy need (PA level factor of 1.69), and consisted of 50% energy from carbohydrate (Fantomalt; Nutricia, England; per 100 g; carbohydrate, 96.0 g; total sugars, 6.0 g) mixed with water in a 1:1.15 scale, and added 50% energy from fat (Calogen; Nutricia, England; per 100 g; fat, 50.0 g; saturated fat, 5.3 g). The PA level factor used to estimate energy needs during the interventions was determined to 1.69 rather than 1.45 (the PA level factor used 24 h prior to and following each intervention) based on the assumption that participants were more physically active during interventions compared to free-living, as they spent 10% of the duration of the active interventions engaged in walking. EI during the interventions amounted to a total of 2310 ± 247 kcal. Participants were instructed to ingest the test drink within 10 min. For one participant, ingestion of the second test drink during SIT lasted 30 min, and thus this trial was then postponed by 30 min.

3.8. Blood Sampling and Analysis

An intravenous catheter was inserted into an antecubital vein for blood sampling. Blood samples were collected 10 min before and 30, 60, 120, and 180 min after each test drink, as well as 240 min after the last test drink (total of 11 samples per day). Blood samples were centrifuged (15 min, 4 °C, 3500 rev/min) immediately after sampling. For the analysis of plasma glucose, insulin, C-peptide, triglyceride, total cholesterol, HDL and LDL cholesterol concentrations, blood samples were stored at 4 °C, and analyzed at the Department of Clinical Biochemistry at Rigshospitalet the following morning. For analyses of plasma glucagon (drawn using chilled aprotinin-coated tubes) and NEFA concentrations, blood samples were pipetted into Eppendorf tubes immediately following centrifugation and stored at -80 °C until analysis. Plasma glucagon concentrations were analyzed using radioimmunoassay kits (EMD Millipore Corporation, MA, USA), and plasma NEFA concentrations were analyzed using NEFA-HR(2) assay kits (Wako Chemicals GmbH, Germany).

3.9. Continuous Glucose Monitoring

For measurement of blood glucose concentrations during the 24 h following each intervention, a continuous glucose monitoring (CGM) system (iPro2 recorder, Medtronic, CA, USA) was installed by insertion of a glucose sensor (Enlite sensor, Medtronic, CA, USA) into the abdominal subcutaneous tissue (using Enlite serter, Medtronic, CA, USA) before the intervention was started. For retrospective calibration, the participants measured blood glucose concentration through finger pricks four times per day during the period of monitoring (total of seven).

3.10. Energy Expenditure and Substrate Oxidation

VO_2 and carbon dioxide elimination (VCO_2) were measured during the last 2 h of the interventions using indirect calorimetry measurements (Quark CPET, Cosmed, Italy). Fasting VO_2 and VCO_2 were assessed during a period of 10 min before the beginning of the intervention in order to adjust subsequent measures of 8-h EE, carbohydrate and fat oxidation for daily variations in fasting rates [18]. Urine produced during the 8 h of the intervention was collected in a plastic container (LX Container; RPC-Promens, UK), weighed, mixed, and 3 ml were pipetted into a non-coated tube (VACUETTE® Blood Collection Tubes; Greiner Bio-One, Austria). The sample was stored at 4 °C and analyzed for concentration of urine urea at the Department of Clinical Biochemistry at Rigshospitalet the following morning. Heart rate was monitored throughout the intervention (Polar V800; Polar, Denmark).

Based on volume of urine and concentrations of urine urea, urinary nitrogen excretion was calculated, which together with indirect calorimetry measurements was used for calculation of EE [19], as well as carbohydrate and fat oxidation [20].

3.11. Statistical Analyses

Sample size calculation was based on a previous study on the effects of interrupting prolonged sitting with LPA in our lab [14]. Hence, we expected a 10% difference in the postprandial glucose response (tAUC) between the active interventions (INT20, INT60, and INT120) and SIT with a within group SD of 10%. Assuming a correlation coefficient of 0.6 between repeated measures, we estimated that with at least 10 participants, the study would have >90% power at an alpha level of 5% to detect a significant difference in postprandial plasma glucose response between interventions. Based on this, the sample size was determined to at least 10 participants, whereas resources allowed for a maximum of 15 participants. Recruitment was terminated on November 24th 2017, when 14 participants had been included, and constituted the full analysis set. Sample size calculation was performed using Stata IC/SE 13.1 (Statacorp, Tx, USA).

The tAUC and iAUC were calculated using the trapezoidal method [21], and tAUC was defined as the area above a concentration of zero, whereas iAUC was defined as the area above fasting concentration. Postprandial glucagon and NEFA concentrations decreased, and thus the iAUC was negative, defined as the area between fasting concentration and the curve.

All outcomes were analyzed using repeated measures one-way ANCOVA implemented in a mixed effects model, having intervention as fixed factor and participant as random effect. All outcomes were adjusted for the respective fasting level except for the index, ratio, and CGM outcomes. Contrast analyses between the groups were performed if indicative by the Wald test comparing all four interventions ($p < 0.05$). All secondary outcomes were considered exploratory, thus no post hoc corrections of p-values were performed. Model assumptions were investigated through the predicted values and the standardized residuals. Data were analyzed as observed, i.e. no imputations were used to replace missing data. A total of four participants had missing insulin and glucagon values (due to hemolysis or similar problems with blood sampling) at a total of seven and two time points for three and two participants, respectively.

The significance level was set to $p < 0.05$ (two-tailed) and data are presented as mean and 95% confidence interval unless otherwise specified. All statistical calculations were performed using Stata IC/SE 13.1 (Statacorp, Tx, USA). A statistical analyses plan was developed prior to commencing the statistical analyses.

4. Results

Participant characteristics are presented in Table 1. All participants completed each of the four interventions. EE and PA (i.e. sitting, standing, and stepping time, as well as no. of steps) before, during, and after each of the interventions are presented in Table 2. EE and PA before, during and after the interventions were similar, as were fasting concentrations of all measured plasma parameters. The pre-specified intensity of 30% VO_{2max} during walking bouts in INT20, INT60, and INT120 was achieved (Table 2). Response curves for HR are presented in eFigure 3.

4.1. Glucose Metabolism

For the primary outcome, the 8-h postprandial glucose response, no difference from SIT was observed during INT20, INT60, or INT120 (tAUC: −65.3 mmol/l∗min [−256.3; 125.7], +53.8 mmol/l∗min [−143.1; 250.8], and +18.6 mmol/l∗min [−172.4; 209.6] for INT20, INT60, and INT120, respectively) (Fig. 1). However, during INT20, the postprandial response was generally higher for glucagon, and lower for glucose, insulin, and in particular for the exploratory outcome, the 8-h postprandial C-peptide response (tAUC: −150,568 pmol/l∗min [−286,962; −14,174]; iAUC: −149,817 pmol/l∗min [−285,959; −13,674]). Although none were significant according to the Wald test ($p > 0.05$). (Fig. 1). The individual 4-h postprandial response following the first and second test drink generally resembled the 8-h response (eFigure 4A–B). Response curves for plasma glucose, insulin, C-peptide and glucagon are presented in eFigure 5A–D.

Table 1
Participant characteristic.

Age (years)†	28.2 (23.4; 38.3)
Height (cm)†	182.1 (177.2; 186.2)
Body mass (kg)	104.3 ± 20.1
Waist circumference (cm)	109.8 ± 16.1
BMI (kg/m^2)	31.9 ± 6.7
Fat content (%)†	34.2 (31.8; 38)
Lean mass (kg)	63.7 ± 8.2
VO_2max (ml/min)	3878 ± 511
VO_2max (ml/min/kg)†	39.5 (38.8; 40.9)
Fasting glucose (mmol/l)	4.9 ± 0.4
Fasting insulin (pmol/l)†	74 (63; 115)
2-h glucose (mmol/l)	6.2 ± 1.8
HbA1c (mmol/l)	34.1 ± 4.2
HbA1c (%)	5.3 ± 0.4
HOMA-IR†	2.3 (2.1; 4.0)

Data are presented as mean ± SD († median (25th; 75th percentile)), VO2max = maximal oxygen uptake; HbA1c = glycated hemoglobin; HOMA-IR = homeostasis model assessment of insulin resistance.

Exploratory outcomes including the difference in indices for the glucose metabolism during INT20, INT60, and INT120 compared to SIT are presented in eFigure 6. No difference in the insulinogenic and disposition indices, as well as the $tAUC_{insulin/glucose}$, $tAUC_{insulin/glucagon}$, and $tAUC_{C\text{-}peptide/glucagon}$ were observed during INT20, INT60, and INT120 compared to SIT (eFigure 6). The Matsuda index was lower during INT60 compared to SIT (−0.6 [−1.1; −0.1]) and the $tAUC_{C\text{-}peptide/glucose}$ was lower during INT20 compared to SIT (−38.3 [−68.1; −8.6]), although not significantly according to the Wald test ($p > 0.05$) (eFigure 6).

Compared to SIT, total carbohydrate oxidation was higher during INT20, INT60, and INT120, respectively (+189.9 kcal [121.8; 258.0], +215.1 kcal [147.7; 282.4], and +212.2 kcal [144.5; 279.9], respectively) (Fig. 2A+C).

Unadjusted analyses (post hoc analyses) are presented in eTable 1.

4.2. Fat Metabolism

Exploratory outcomes including 8-h postprandial response in lipid parameters were generally not affected during INT20, INT60, and INT120 compared to SIT (eFigure 7). However, the 8-h postprandial NEFA response was reduced during INT60 and INT120 compared to SIT (tAUC: −6.6 mmol/l∗min [−12.3; −0.9] and −5.9 mmol/l∗min [−11.6; −0.2] for INT60 and INT120, respectively; negative iAUC: −6.2 mmol/l∗min [−10.8; −1.6] and −7.3 mmol/l∗min [−11.9; −2.7] for INT60 and INT120, respectively), but not during INT20 compared to SIT (eFigure 7). The individual 4-h postprandial response following the first and second test drink generally resembled the 8-h response (eFigure 8A–B). Response curves for plasma triglyceride and NEFA are presented in eFigure 5E–F.

Total fat oxidation was higher during INT20 compared to SIT (+95.8 kcal [38.2; 153.4]), whereas no difference was observed for INT60 and INT120 compared to SIT (+38.9 kcal [−18.7; 96.6] and +19.6 [−38.8; 78.0] for INT60 and INT120, respectively) (Fig. 2B+D).

Unadjusted analyses (post hoc analyses) are presented in eTable 1.

4.3. Twenty-Four-Hour Post-Intervention Glucose Homeostasis (CGM)

Exploratory outcomes that include parameters quantifying the 24-h post-intervention glucose homeostasis are presented in eFigure 9A. There were no differences in any of these parameters following INT20, INT60, and INT120 compared to SIT (eFigure 9A). When the nocturnal period following the interventions was considered, the iAUC for the blood glucose response was lower during INT20 and INT120 compared to SIT (−167.4 mmol/l∗min [−286.3; −48.6] and −130.9 mmol/l∗min [−249.7; −12.2], respectively) (eFigure 9B).

5. Discussion

The main finding of this study was that, independent of EI and EE, postprandial glycemia was not reduced by increases in frequency of interruptions in prolonged sitting among healthy, sedentary, centrally obese men.

This experimental study does not directly support previous observational cross-sectional studies suggesting that frequently interrupting prolonged sitting lowers the postprandial glucose response independent of PA level [3,4]. Moreover, the main finding is not in agreement with the previous observation that postprandial glycemia decreases when prolonged sitting is interrupted by PA bouts of different durations, intensities, and frequencies [14,22,23]. This discrepancy may predominantly be explained by the volume of PA, particularly intensity and duration, used during interruptions in prolonged sitting, which generally were higher in previous studies compared to the present study. This indicates that increasing the intensity and/or duration of PA may be more effective in reducing postprandial glycemia than increasing the frequency [14,22,23]. Participants in this study had a

Table 2
Energy expenditure and physical activity 24-h pre, during, and 24-h post interventions.

	SIT	INT20	INT60	INT120
Pre-intervention	(n = 12)	(n = 13)	(n = 14)	(n = 12)
Energy expenditure (kcal)	2807 ± 364	2879 ± 363	2794 ± 339	2813 ± 388
Sitting (%)	80.9 ± 7.8	81.0 ± 12.8	83.6 ± 11.8	81.8 ± 15.3
Standing (%)	12.3 ± 4.4	12.5 ± 11.2	11.5 ± 8.7	13.3 ± 14.1
Stepping (%)	6.9 ± 4.7	6.4 ± 3.2	4.9 ± 3.6	4.9 ± 3.1
Steps (no.)	4219 ± 3188	3747 ± 1884	2957 ± 2177	3021 ± 2065
During intervention	(n = 14)	(n = 14)	(n = 14)	(n = 14)
Energy expenditure (kcal)	966 ± 145	1250 ± 186	1214 ± 155	1188 ± 155
Fasting energy expenditure (kcal/10 min)	17.6 ± 3.2	18.1 ± 3.5	17.8 ± 2.4	18.1 ± 3.6
Intensity during walking (%VO_2max)	–	28.4 ± 2.8	29.5 ± 2.4	29.4 ± 1.9
Mean heart rate (bpm)[a]	78.6 ± 8.0	82.1 ± 7.4	82.0 ± 7.0	81.1 ± 5.6
Stepping (%)[b]	0.4 ± 0.2	11.5 ± 0.3	10.6 ± 0.4	10.4 ± 0.3
Post-intervention	(n = 12)	(n = 13)	(n = 14)	(n = 13)
Energy expenditure (kcal)	2790 ± 263	2933 ± 289	2831 ± 374	2775 ± 375
Sitting (%)	80.7 ± 6.2	79.5 ± 11.7	84.3 ± 11.9	83.4 ± 9.3
Standing (%)	12.9 ± 5.5	13.6 ± 9.8	10.1 ± 9.1	11.0 ± 6.3
Stepping (%)	6.5 ± 2.2	6.9 ± 4.2	5.6 ± 4.2	5.7 ± 3.5
Steps (no.)	3967 ± 1523	4225 ± 2403	3533 ± 2651	3344 ± 1969

Data are presented as mean ± SD. SIT = continuous sitting (control); INT20 = sitting interrupted every 20th minute; INT60 = sitting interrupted every hour; INT120 = sitting interrupted every second hour.
[a] n = 14 for SIT and INT20, n = 13 for INT60 and INT120.
[b] n = 12 for SIT and INT120, n = 13 for INT20, n = 14 for INT60.

normal glucose tolerance, indicating an ability to regulate postprandial glycemia and thus maintaining a normal glucose concentration, possibly explaining why postprandial glycemia was not affected in this study. This point is further strengthened by the non-significant trend over the reduction in postprandial insulin secretion when prolonged sitting was interrupted every 20th minute indicating that the regulation of glucose homeostasis per se may be improved. This trend is in agreement with previous studies observing a lower postprandial insulin or C-peptide response when prolonged sitting is interrupted by PA of different durations, intensities, and frequencies [14,22,24].

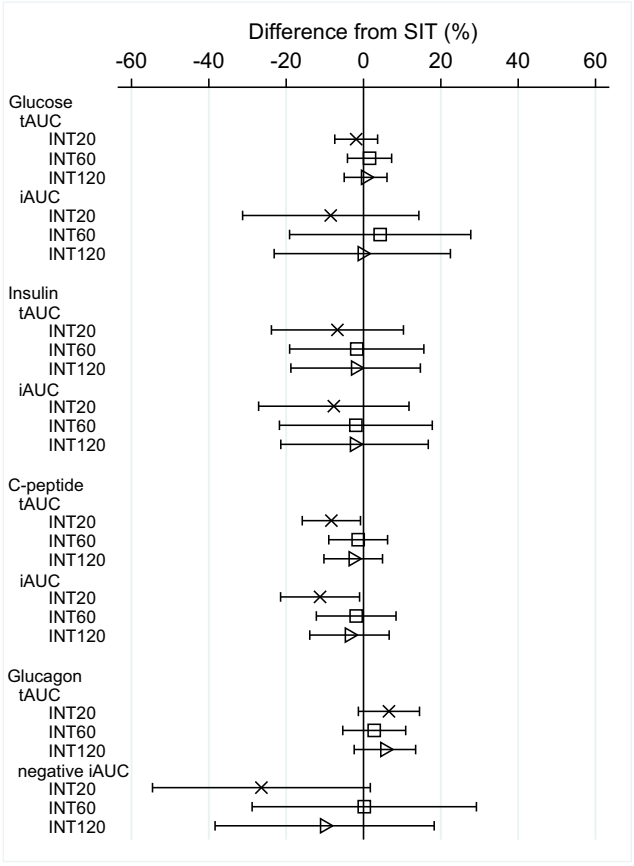

Fig. 1. Difference (%) in 8-h total and incremental area under the curve (tAUC and iAUC, respectively) for plasma glucose, insulin, C-peptide, and glucagon during INT20 (cross marks), INT60 (squares), and INT120 (triangles) compared to SIT. Data are presented as relative difference and 95% confidence intervals. SIT = continuous sitting (control); INT20 = sitting interrupted every 20th minute; INT60 = sitting interrupted every hour; INT120 = sitting interrupted every second hour.

In line with the present study, previous studies have observed no difference in postprandial triglyceride response [9,14,23], and an increase in fat oxidation [9,25] when prolonged sitting was interrupted by PA. In the present study these findings are extended with the observation that this is independent of EI and EE. According to post hoc analysis the relative contribution of fat to total oxidation was not increased, although indicating a shift towards higher fat contribution when prolonged sitting was interrupted every 20th minute compared to every hour or less. Regarding the effect of interrupting prolonged sitting on the postprandial NEFA response, previous findings are few and inconsistent [26,27]. In the present study, the fat metabolism seems to be affected differently when prolonged sitting is interrupted by walking in different intervals. The decrease in postprandial NEFA response, observed when prolonged sitting is interrupted every hour or less, may indicate that the largely unchanged fat oxidation is supported more by NEFA and less by other lipids. The maintained postprandial NEFA concentration combined with an increase in fat oxidation, observed when prolonged sitting is interrupted every 20th minute, may indicate a higher degree of lipolysis contributing to maintenance of postprandial NEFA concentrations. As insulin suppress lipolysis [28], an increase in lipolysis would be consistent with the non-significant trend over the reduction in insulin secretion when prolonged sitting was interrupted every 20th minute. However, adequate measures (e.g. muscle biopsies and direct measures of lipolysis) are needed to confirm this.

In the present study, interventions were matched regarding EI and EE, as well as intensity of PA, and hence any observed differences between trials may be attributed to the frequency of interruptions in prolonged sitting, independent of these factors. Interrupting prolonged sitting with increasing frequency involves an increase in the number of contractions of muscles involved in the transition between sitting and standing, as well as a decrease in the duration of contractile inactivity during sitting bouts. This may be essential in the differential regulation of metabolism suggested by observations in this study. Lack of contractile activity versus contractile inactivity has previously been proposed to entail different effects on mechanisms involved in metabolic regulation [22,29]. Hence, increasing the intensity of walking during interruptions

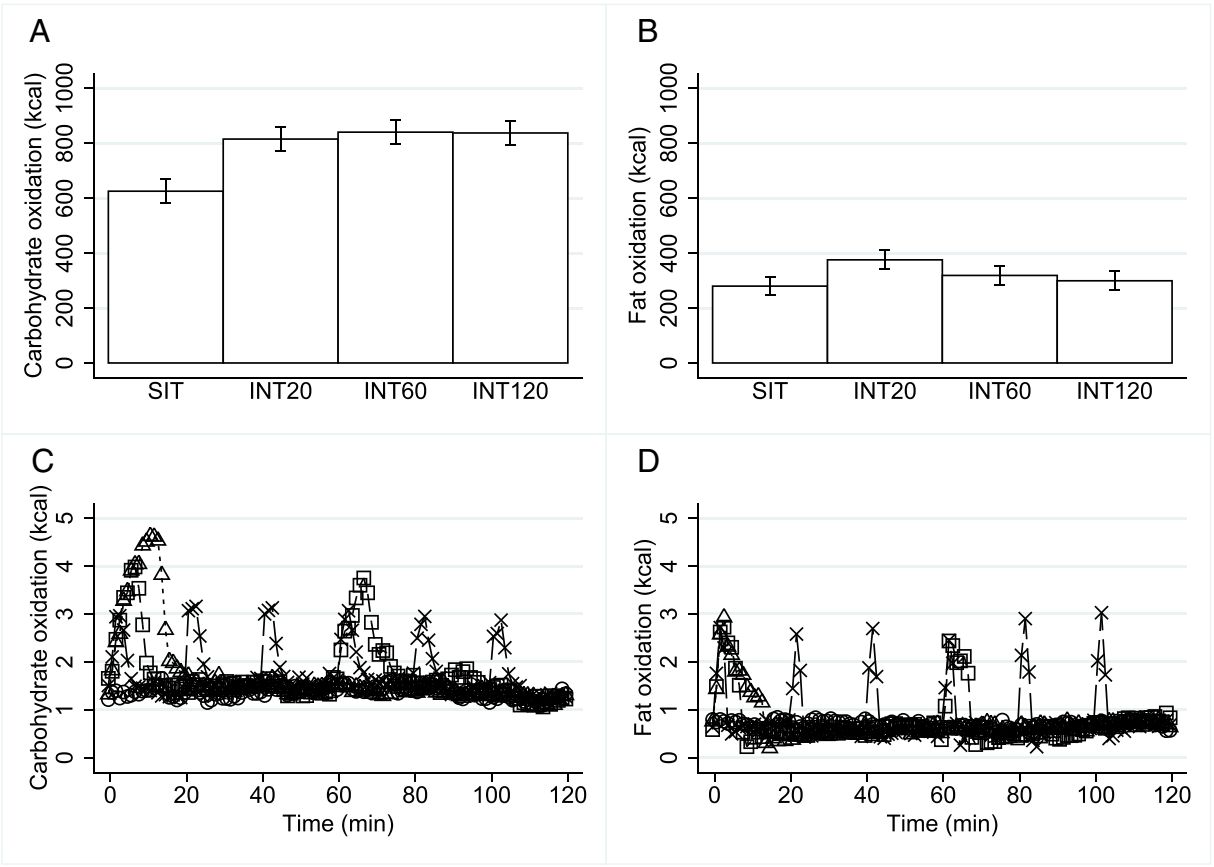

Fig. 2. Substrate oxidation: (A) Total 8-h carbohydrate and (B) fat oxidation (kcal) during SIT, INT20, INT60, and INT120 (estimated based on 2-h indirect calorimetry). Data are presented as mean and 95% confidence intervals. Rate of (C) carbohydrate and (D) fat oxidation (kcal) during the last 2 h of SIT (circles), INT20 (cross marks), INT60 (squares), and INT120 (triangles). Data are presented as mean per minute. SIT = continuous sitting (control); INT20 = sitting interrupted every 20th minute; INT60 = sitting interrupted every hour; INT120 = sitting interrupted every second hour.

in prolonged sitting from 3.2 km/h to 5.8–6.4 km/h yielded no further decrease in postprandial glucose and insulin response [22], although the mechanisms underlying these effects nevertheless appeared to be different [30]. Common to these interventions was an indication that contraction-induced glucose uptake is increased when prolonged sitting is interrupted rather often. To fully understand the mechanisms involved in the effects of the frequency of interruptions in prolonged sitting on postprandial metabolic regulation, studies directly investigating the underlying mechanism are warranted.

Limitations of the present study include the exploratory nature of secondary outcome analyses. If all exploratory outcomes were subjected to statistical testing, independent of the global test statistics, this would increase the type 1 error rate [31]. Thus, it was decided *only* to present the 95% confidence intervals for interpretational purposes. To make valid statistical inferences on the exploratory outcomes these findings should be confirmed in experimental studies designed specifically for that purpose. Moreover, the acute design precludes extrapolation of the findings to long-term effects on metabolic health, and the inclusion of a somewhat homogeneous group of participants precludes generalization of the observations to other subpopulations. The order of each intervention in the sequence of delivery was not equally distributed as randomization was not performed in blocks. The sequence was however blinded to the participant until the morning of each intervention, and was separated by a washout period, and thus carryover effects of the preceding intervention are unlikely, which is further supported by similar EI and EE before the interventions. The meals served during the interventions were liquid and lacked protein, possibly causing increased rate of gastric emptying, affecting the postprandial plasma responses [32], which limits extension of observations outside the lab. During trials, participants were subject to energy surplus because of overestimation of the daily EE, leading to energy supply exceeding the energy needs of the participants. Energy surplus may reduce insulin action [33], however as this was similar across interventions, any reduction in insulin action may be similar across interventions and thus should not affect the findings of this study.

Although the primary hypothesis was not confirmed, interesting findings in this study provide initial experimental indication that the frequency of interruptions in prolonged sitting may after all affect metabolic health. The non-significant trend over the reduction in insulin secretion may indicate an insulin sparing effect of frequently interrupting sitting time, which may, if repeated daily, lead to preservation of pancreatic β-cell function, and thereby decreased risk of development of metabolic diseases, such as type 2 diabetes [34]. Further studies are needed to confirm the effects on insulin secretory capacity. Moreover, investigating this using a long-term study design could contribute to support the introduction of frequent interruptions in prolonged sitting.

To conclude, increasing the frequency of interruptions in prolonged sitting did not acutely affect postprandial glycemia.

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Disclosure Statement

The authors declare no conflict of interest.

Author Contributions

IKT and MR-L designed the trial and KK, FB, DWD and BKP contributed to the trial design. IKT collected the data with support from MYJ, NSP, NZJ and CFB. IKT planned and conducted the statistical analysis, and wrote the first draft of the manuscript under supervision of MR-L. All authors reviewed and revised the manuscript and approved the final version. IKT and MR-L are guarantors of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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