Dose-response between frequency of breaks in sedentary time and glucose control in type 2 diabetes: A proof of concept study

Breaks in sedentary time and glucose control

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Tel:01413313357

Word count: 3000/3000

The abstract word count: 250/250

Abstract

Objectives: This study aimed to investigate dose-response between frequency of breaks in sedentary time and glucose control.

Design: Randomised three-treatment, two-period balanced incomplete block trial.

Methods: Twelve adults with type 2 diabetes (age, 60±11 years; body mass index, 30.2±4.7 kg/m^2) participated in two of the following treatment conditions: sitting for 7 hours interrupted by 3 min light-intensity walking breaks every (1) 60 min (Condition 1), (2) 30 min (Condition 2), and (3) 15 min (Condition 3). Postprandial glucose incremental area under the curves (iAUCs) and 21-h glucose total area under the curve (AUC) were measured using continuous glucose monitoring. Standardised meals were provided.

Results: Compared with Condition 1 (6.7±0.8 mmol·L^{-1}·3.5 h^{-1}), post-breakfast glucose iAUC was reduced for Condition 3 (3.5±0.9 mmol·L^{-1}·3.5 h^{-1}, $p<0.04$). Post-lunch glucose iAUC was lower in Condition 3 (1.3±0.9 mmol·L^{-1}·3.5 h^{-1}, $p<0.03$) and Condition 2 (2.1±0.7 mmol·L^{-1}·3.5 h^{-1}, $p<0.05$) relative to Condition 1 (4.6±0.8 mmol·L^{-1}·3.5 h^{-1}). Condition 3 (1.0±0.7 mmol·L^{-1}·3.5 h^{-1}, $p=0.02$) and Condition 2 (1.6±0.6 mmol·L^{-1}·3.5 h^{-1}, $p<0.04$) attenuated post-dinner glucose iAUC compared with Condition 1 (4.0±0.7 mmol·L^{-1}·3.5 h^{-1}). Cumulative 10.5-h postprandial glucose iAUC was lower in Condition 3 than Condition 1 ($p=0.02$). Condition 3 reduced 21-h glucose AUC compared with Condition 1 ($p<0.001$) and Condition 2 ($p=0.002$). However, post-breakfast glucose iAUC, cumulative 10.5-h postprandial glucose iAUC and 21-h glucose AUC were not different between Condition 2 and Condition 1 ($p>0.05$).

Conclusions: There could be dose-response between frequency of breaks in sedentary time and glucose. Interrupting sedentary time every 15 min could produce better glucose control.

Clinical trial registration: Clinicaltrials.gov Identifier: NCT02738996

Keywords: Diabetes mellitus; Glucose; Sedentary lifestyle; Exercise.

1. Introduction

In type 2 diabetes, postprandial glucose control is crucial to reduce the risk of cardiovascular complications and all-cause mortality.[1,2] However, even in people with type 2 diabetes with the recommended HbA1c (glycated haemoglobin) of less than 7% (53 mmol/mol), a high incidence of postprandial hyperglycaemia is detected.[3] The management of postprandial glucose is challenging with standard diet modification and anti-diabetes agents,[2] and new approaches need to be explored.

One potential therapeutic target is the reduction of prolonged sedentary time. Any waking activity in sitting or reclining posture with low energy expenditure is defined as sedentary behaviour.[4] Exposure to sedentary time has deleterious associations with 2-h postprandial glucose, daily glucose, cardiovascular diseases and mortality in healthy people and those with type 2 diabetes.[5–7] The detrimental association between sedentary time and postprandial glucose is independent of physical activity and waist circumference.[5,8] Sedentary time is also related to high insulin concentration and insulin resistance in people with newly diagnosed type 2 diabetes.[9] Persistent increase in insulin concentration and insulin resistance related to prolonged sedentary time indicate overworked pancreatic β cells, which could lead to β cells exhaustion.[10]

In contrast, interrupting sedentary time with short walking breaks (≤3 min) every 20 min or 30 min was shown experimentally to reduce postprandial glucose, daily glucose and insulin resistance compared to uninterrupted sitting (5-7 hours) in healthy people and those with type 2 diabetes.[10–12] Consequently, promoting walking breaks in sedentary time appears to be a simple therapeutic intervention to control postprandial glucose and daily glucose. However, previous studies have always used uninterrupted sitting (5-7 hours) as the reference condition, which is rarely seen in real life, and dose-response between frequency of breaks in sedentary time and glucose control in type 2 diabetes is unknown. The aim of this proof of concept trial is to investigate if there is a dose-response between the frequency of light-intensity

walking breaks in sedentary time and postprandial glucose and daily glucose to understand how often sedentary time should be interrupted to provide therapeutic effects.

2. Methods

This study is an extension of a previously published randomised crossover trial.[13] The study design was a three-treatment, two-period balanced incomplete block trial, which allows us to get evidence efficiently in a short time scale.[14] This study was approved by the Research Ethics Committees of Glasgow Caledonian University and University of Strathclyde. This study conformed to the Declaration of Helsinki, and written informed consent was obtained from all participants.

As previously reported,[13] participants were randomised to two of the following three treatment conditions: sitting for 7 hours interrupted by 3 min light-intensity walking breaks every (1) 60 min (Condition 1), (2) 30 min (Condition 2), and (3) 15 min (Condition 3) (Supplemental Fig. 1). Individuals (aged ≥35 years) with self-reported type 2 diabetes and body mass index (BMI) ≥25 kg/m^2 were recruited between January 2017 and June 2017 from the Glasgow community. Exclusion criteria included pregnancy, alcohol and substance abuse, insulin therapy, liver and renal diseases, cancer, mobility issues and diabetes-related complications such as foot ulcer, foot deformity, peripheral neuropathy and peripheral arterial diseases.

The study protocol lasted 15 days (Supplemental Fig. 2) during which each participant completed two of three treatment conditions on weekdays. Participants were requested to abstain from smoking, alcohol and moderate to vigorous physical activity (MVPA) throughout study period and were additionally requested to avoid caffeine on treatment condition day. Habitual lifestyle and diet were maintained during pre-experimental monitoring period, wash-out period and post-experimental monitoring period. The researcher stayed in touch with participants using participants' preferred mode of communications (phone or email) to issue reminders and to schedule appointments. The details of study protocol were as follows:

Visit 1: On day 1, participants were requested to visit the university laboratory. Anthropometric data (age, gender, height, weight, waist circumference and BMI), blood pressure and drug history were recorded by the researcher. As previously described,[13] a continuous glucose monitoring (CGM, Abbott FreeStyle Libre) and activPAL3 activity monitor (PAL Technologies, Glasgow, UK) were attached. Sleep diary and 24-hour Dietary Recall Form were provided to record bedtime, waking time, dietary intake and medication throughout study period. Participants were instructed to scan glucose every 8 h using the FreeStyle Libre reader throughout study period. Participants then returned home and completed 4 days of pre-experimental monitoring period.

Visit 2: On day 5, participants arrived at the laboratory at 0800 h after an overnight fast to perform the first treatment condition. Participants were seated from 0800 to 0900 h to achieve a physiological steady state. They had access to books, a personal computer and the internet. At 0900 h, participants consumed a standardised breakfast with 50-53.7 g carbohydrate, 9.1-12.6 g fat and 14.7-22.4 g protein. After breakfast, participants performed 3 min light-intensity walking breaks either every 60 min, 30 min or 15 min of sitting depending on the condition they were randomised to. Light-intensity walking breaks were performed on level ground at a pace of 10 meters in 11 seconds (3.2 km/h). The researcher supervised the experiment and gave timing to participants to ensure that participants walked at required pace and performed all breaks in each condition. At 1236 h, a standardised lunch providing 75 g carbohydrate, 14.5 g fat and 28 g protein was served. The laboratory condition was finished at 1600 h. A ready meal dinner with 50.1-55.6 g carbohydrate, 7.3-12.3 g fat and 20.1-30.1 g protein and a snack (bread, apple or plum) with 10-13.1 g carbohydrate, 0.3-0.6 g fat and 0.3-2.6 g protein were also provided for participants to consume at home on that evening.

Visit 3: On day 11, participants performed the second treatment condition and were invited to the laboratory at 0800 h, having fasted overnight. Procedure of the second treatment condition was the same as the first treatment condition; however, a different treatment condition was performed. Participants

consumed identical breakfast, lunch, dinner and snack on both treatment condition days, and meal time and medication time were also standardised. There were five days of wash-out period between the first and second treatment conditions.

Visit 4: The period between day 12 and day 15 was regarded as post-experimental monitoring period. On day 15, participants reported back to the laboratory for the removal of the CGM and activPAL3, and this was the end of participants' participation.

Physical activity and sedentary behaviour were monitored using the activPAL3. This small device was attached to the anterior aspect of the thigh using waterproof plastic tubing and hypoallergenic dressing. This device records the start and duration of sitting, lying, standing and walking for up to two weeks. It is a validated device and has been used in clinical trials and large epidemiological studies.[6,13] Participants were asked to wear the device continuously for 15 days of the experimental period. The data were downloaded using activPAL3^{TM} software (version 7.2.32). For each treatment condition day, estimated energy expenditure (metabolic equivalents, MET) was computed using the 24-h activPAL data. The sleep diary and activPAL files were used to remove sleeping time on treatment condition day, and the laboratory and 24-h sedentary time, standing time and walking time on treatment condition day were then calculated.[13]

Glucose was monitored continuously using the CGM, consisting of a sensor worn on the back of the upper arm and a glucose reader. This is a validated device, which accurately records the interstitial glucose level every 15 minutes for up to two weeks.[15] The glucose data were retrieved wirelessly by holding the reader close to the sensor for a few seconds. The glucose data from this device were downloaded to a personal computer using FreeStyle Libre software (version 1.0).

For primary outcome measures, 3.5-h postprandial glucose incremental area under the curve (iAUC) was calculated for each meal (post-breakfast glucose iAUC, post-lunch glucose iAUC and post-dinner glucose

iAUC) and cumulative 10.5-h postprandial glucose. The glucose iAUCs were calculated by subtracting fasting glucose from overall postprandial glucose levels and using the trapezium rule.[14] Average glucose between 0800 and 0900 h (steady state) was considered as fasting glucose. The period between 0900 h on treatment condition day and 0900 h on the following day was considered as the 24-hour period. Instead of 24-h glucose total area under the curve (AUC), 21-h glucose AUC was calculated using the glucose obtained between 0900 h on treatment condition day and 0600 h on the following day because participants consumed breakfast around 0700 h on the following day.

Block randomisation sequence was generated using SPSS by a blinded researcher, and order was allocated according to this sequence. Participants were then randomised to one of six possible trial-condition orders (Supplemental Fig. 1). Each participant completed two of three treatment conditions and was blinded to trial-condition order until the first treatment condition day.

Sample size calculations have been described previously.[13] Across all experimental conditions and participants, 5.1 % of glucose values (108 of 2136 glucose points) were missing. Within-individual mean substitution was used to deal with missing glucose data.[16] Differences in glucose variables between treatment conditions were assessed by multilevel mixed-effects linear regression with the repeated measurements for the same individuals. Treatment condition and participant were respectively used as a fixed factor and a random factor in these models. The models were adjusted for age (years), sex, waist circumference (cm), anti-diabetes medication dose (mg/day) and carbohydrate intake (g/day), sedentary time (h/day), standing time (h/day), walking time (h/day) and energy expenditure (MET x h/day) on the laboratory condition day. Sedentary time, standing time, walking time, energy expenditure and energy intake differences between treatment conditions were also analysed using multilevel mixed-effects linear regression. Pairwise comparisons between treatment conditions were conducted using *post hoc* Fisher LSD test, which has been recommended to identify differences between three treatment conditions.[17] This test has been shown to produce greater statistical power and control Type I error when there are three

treatment conditions.[17] The level of statistical significance was set at p value ≤0.05. Data are reported as mean±SE unless otherwise stated. IBM SPSS Statistics software (version 24.0) was used to perform statistical analyses.

3. Results

Table 1 reports baseline demographic and anthropometric data. Twelve participants with type 2 diabetes completed treatment conditions and were included in the analysis (Supplemental Fig. 1). The laboratory and 24-h sedentary time, standing time, walking time, energy expenditure and energy intake during treatment condition days are described in Table 2. During the laboratory period, walking time was significantly higher in Condition 3 and Condition 2 than Condition 1. On treatment condition days, the 24-h walking time and energy expenditure were higher in Condition 3 than Condition 2 and Condition 1.

The mean postprandial glucose iAUC and 21-h glucose AUC are reported in Fig. 1. Post-breakfast glucose iAUC was significantly attenuated in Condition 3 (3.5±0.9 mmol·L^{-1}·3.5 h^{-1}, p<0.04) but not in Condition 2 (5.8±0.8 mmol·L^{-1}·3.5 h^{-1}, p=0.418) compared with Condition 1 (6.7±0.8 mmol·L^{-1}·3.5 h^{-1}). There was significant attenuation of post-lunch glucose iAUC in Condition 3 (1.3±0.9 mmol·L^{-1}·3.5 h^{-1}, p<0.03) and Condition 2 (2.1±0.7 mmol·L^{-1}·3.5 h^{-1}, p<0.05) relative to Condition 1 (4.6±0.8 mmol·L^{-1}·3.5 h^{-1}). Compared with Condition 1 (4.0±0.7 mmol·L^{-1}·3.5 h^{-1}), both Condition 3 (1.0±0.7 mmol·L^{-1}·3.5 h^{-1}, p=0.02) and Condition 2 (1.6±0.6 mmol·L^{-1}·3.5 h^{-1}, p<0.04) significantly reduced post-dinner glucose iAUC. Cumulative 10.5-h postprandial glucose iAUC was significantly attenuated in Condition 3 (5.6±2.4 mmol·L^{-1}·10.5 h^{-1}, p=0.02) but not in Condition 2 (9.1±2.0 mmol·L^{-1}·10.5 h^{-1}, p=0.066) relative to Condition 1 (14.8±2.2 mmol·L^{-1}·10.5 h^{-1}). Compared with Condition 1 (153.7±5.7 mmol·L^{-1}·21 h^{-1}), significant reduction in 21-h glucose AUC was observed with Condition 3 (101.5±12.6 mmol·L^{-1}·21 h^{-1}, p<0.001) but not with Condition 2 (136.2±10.6 mmol·L^{-1}·21 h^{-1}, p=0.08). Moreover, 21-h glucose AUC was significantly reduced in Condition 3 relative to Condition 2 (p=0.002). However, no significant

differences between Condition 3 and Condition 2 were observed for the remaining glucose outcome measures ($p \geq 0.089$).

4. Discussion

This study demonstrates that there could be the dose-response between the frequency of light-intensity walking breaks in sedentary time and postprandial glucose and daily glucose in people with type 2 diabetes. This study showed that interrupting sedentary time every 15 min (Condition 3) reduced post-breakfast glucose (48%), cumulative postprandial glucose (62%) and 21-h glucose (34%) relative to interrupting sedentary time every 60 min (Condition 1). Interrupting sedentary time every 15 min (Condition 3) also reduced 21-h glucose (25%) compared with interrupting sedentary time every 30 min (Condition 2). Compared with walking breaks every 60 min, interrupting sedentary time every 30 min and 15 min attenuated post-lunch glucose (54% in Condition 2, 72% in Condition 3) and post-dinner glucose (60% in Condition 2, 75% in Condition 3).

The magnitude of cumulative postprandial glucose reduction (39%) after Condition 2 compared with Condition 1 in this study is similar to a previous study, which reported 39% reduction after 3 min light-intensity walking breaks every 30 min.[12] In this study, Condition 3 performed more breaks and showed an expected larger effect on cumulative postprandial glucose reduction (62%) than previous studies conducted in overweight and obese adults (24% in 2 min walking breaks every 20 min), overweight and obese postmenopausal women (28% in 5 min walking breaks every 30 min), normal weight adults (39% in 1 min 40 s walking breaks every 30 min) and overweight and obese adults with type 2 diabetes (39% in 3 min walking breaks every 30 min).[10,12,14,18]

There is epidemiological evidence that post-breakfast glucose and post-lunch glucose can predict the risk of cardiovascular complications.[1] Post-breakfast glucose has a slightly stronger association with HbA1c than post-lunch glucose, and post-dinner glucose has no effect on HbA1c.[19] Therefore, post-breakfast

glucose and post-lunch glucose are clinically important. However, high post-breakfast is consistently observed in type 2 diabetes.[19,20] It is more common than high post-lunch glucose and post-dinner glucose because the circadian glycaemic profile tends to be the highest after breakfast.[19,20] The present study suggests that interrupting sedentary time every 15 min with light-intensity walking breaks should be recommended to improve post-meal glucose, particularly for post-breakfast glucose, in type 2 diabetes.

This study also demonstrated that interrupting sedentary time every 15 min could produce progressive postprandial glucose reduction, and this is in agreement with a previous study, which observed greater reduction in post-dinner glucose with 10 min post-meal walking than post-breakfast glucose and post-lunch glucose.[21] The effect of interrupting sedentary time every 15 min observed in this study seems comparable to that of anti-diabetes agents such as metformin.[22] Moreover, 21-h glucose reduction in Condition 3 indicates that interrupting sedentary time every 15 min could improve daily glucose control, which is associated with HbA1c.[23] We therefore suggest that interrupting sedentary time every 15 min (Condition 3) in addition to oral anti-diabetes agents might be an effective strategy to improve clinical outcomes.

A strength of this study is to use a much more ecologically valid design compared to previous studies. In previous studies, the reference condition had always been uninterrupted sitting (5-7 hours).[10,12,24] Instead, this study used interrupting sedentary time every 60 min as the reference condition, which is closer to habitual sedentary pattern of people with type 2 diabetes.[25] A recent study investigated postprandial metabolic responses to the frequency of standing breaks in normoglycaemic overweight/obese men; however, the reference condition was uninterrupted sitting for 8 hours.[24] Therefore, the present study provided, for the first time in people with type 2 diabetes, evidence on the dose-response of different frequencies of walking breaks on postprandial glucose and daily glucose. Another strength is that participants were asked to walk on level ground instead of walking on treadmill. It is believed that treadmill walking does not represent habitual walking because its energy expenditure tends to be higher

than habitual walking with the same speed.[26] In addition, the use of CGM and activPAL3 is a strength of this study, and real-time glucose and objectively measured activity/sedentary behaviour were reported.

The present study has some limitations. First, a small sample size was used in this study. However, sample size calculations were conducted, and previous studies with similar study designs had also used the small sample size (n=9-10).[27,28] Using the sample with type 2 diabetes in our study could find greater effect of interrupting sedentary time than previous studies using the sample without type 2 diabetes.[10,24,28,29] Because, it is suggested that the beneficial effects of interrupting sedentary time on glucose control could be proportional to higher underlying levels of insulin resistance.[29] Second, adjusting for a relatively large number of covariates might lead to over-adjustment. However, this could be minimised because covariates used in this study could influence glucose outcomes, and the effect of treatment conditions were observed.[6,13,14,30] Third, time frame of adjusted sedentary time (h/day), standing time (h/day), walking time (h/day) and energy expenditure (MET x h/day) in the models were not the same as glucose outcomes. Fourth, participants were not provided with standardised dinner prior to the laboratory conditions. Fifth, the effect of breaks performed at different times of the day (e.g. morning vs. evening) might be vary,[21] and this should be tested in future studies. Sixth, daily energy requirement for each participant was not calculated, and standardised meals were not based on their energy requirements. Seventh, total sitting time was not matched between treatment conditions. It is not fully understood if it is the frequency of breaks or the reduction of sitting time or change in energy expenditure, which produces the beneficial effects. Nevertheless, statistical analyses were adjusted for carbohydrate intake, sedentary time, total activity time and energy expenditure, and the frequency of breaks in sedentary time could have the dose-response relationship with glucose control. Finally, wearable devices such as the CGM and activPAL3 might influence participants' eating behaviour and physical activity patterns in the evening after treatment condition and during pre-experimental monitoring period, wash-out period and post-experimental monitoring period.

5. Conclusion

This study suggests that more frequent interruption of sedentary time (at least every 15 min) could achieve better postprandial glucose and daily glucose control. The combination of interrupting sedentary time every 15 min and anti-diabetes agents may be effective to reduce HbA1c and diabetes-related complications. The feasibility and clinical effects of interrupting sedentary time every 15 min in free-living settings should be evaluated in future long-term studies.

Practical implications

- There could be a dose-response relationship between the frequency of interruption of sitting time and glucose control.
- Interrupting sitting time every 15 min rather than every 30 min produces better glucose control, particularly for post-breakfast glucose and 21-h glucose.
- Interrupting sitting time at least every 15 min could be used in conjunction with anti-diabetes agents to improve clinical outcomes in type 2 diabetes.

Acknowledgements

The authors would like to thank Prof. Joanne Booth (Glasgow Caledonian University) who provided financial support for participants' meals. The authors also thank participants who volunteered and devoted their time to the study. This work is supported by PAL technologies Ltd (Glasgow, UK) and School of Health and Life Sciences, Glasgow Caledonian University, United Kingdom. The funding agencies did not play any role in the collection of data, in the analysis and interpretation of results, in preparing and writing up manuscript, and in making a decision to submit manuscript.

Declaration of interest: none.

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Figure legends

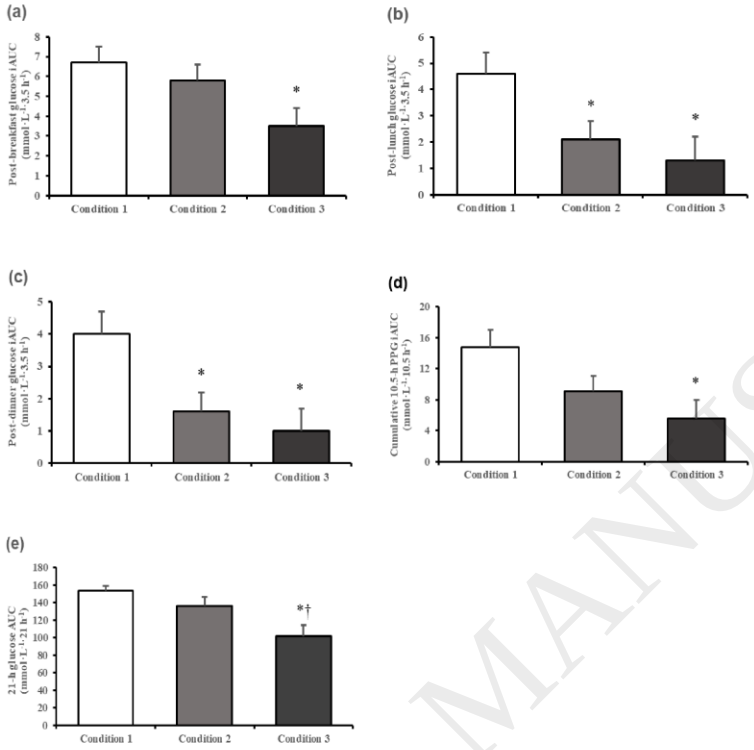

Fig. 1. The effect of three treatment conditions on post-breakfast glucose iAUC (a); post-lunch glucose iAUC (b); post-dinner glucose iAUC (c); cumulative 10.5-h postprandial glucose iAUC (d); and 21-h glucose AUC (e). Data represents means±SE. *Significantly lower compared with Condition 1 ($p<0.05$). †Significantly lower compared with Condition 2 ($p=0.002$). PPG, postprandial glucose.

Baseline characteristics	
Total number of participants (n)	12
Men/Women (n)	8/4
Age (years)	60±11
Waist circumference (cm)	102.1±13.5
BMI (kg/m^2)	30.2±4.7
Duration of diabetes (years)	5.2±2.9
Smoking status, n (%)	
Non-smoker	11 (92%)
Smoker	1 (8 %)
Diabetes management, n (%)	
Diet and lifestyle modification	1 (8.3%)
Metformin	7 (58%)
Metformin + Gliclazide	4 (33%)
Other medications, n (%)	
Statin	5 (41%)
Calcium channel blocker	2 (16%)
ACEI	2 (16%)
ARB	2 (16%)
β blocker	1 (8%)

Data are means±SD or n (%). BMI, body mass index; ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Table 1 Demographic and anthropometric characteristics of participants.

Variables	Condition 1 (n=8)	Condition 2 (n=8)	Condition 3 (n=8)
Laboratory sedentary and activity time			
Sedentary time (h/day)	7.3±0.3	6.9±0.3	6.5±0.3*
Standing time (h/day)	0.4±0.2	0.5±0.2	0.4±0.2
Walking time (h/day)	0.4±0.02	0.7±0.02*	1.2±0.02*†
24-h sedentary and activity time			
Sedentary time (h/day)	12.0±0.3	12.3±0.3	12.7±0.3
Standing time (h/day)	2.4±0.4	2.6±0.5	1.8±0.1
Walking time (h/day)	1.4±0.1	1.6±0.1	2.1±0.1*†
Estimated EE (MET x h/day)	33.3±1.3	33.6±0.3	34.7±0.2*†
Energy intake (kJ/day)	5480.6±37.9	5474.3±31.9	5479.9±37.8
Carbohydrate (energy %)	54.6±0.4	54.7±0.3	54.6±0.3
Fat (energy %)	23.1±0.2	23.1±0.2	23.1±0.2
Protein (energy %)	22.3±0.2	22.3±0.2	22.3±0.2

Data are presented means±SE. *Significantly different from Condition 1 ($p<0.04$).

†Significantly different from Condition 2 ($p\leq0.02$). EE, energy expenditure.

Table 2

Sedentary time, activity time, energy expenditure and energy intake on treatment condition days.