Breaking up prolonged sitting with standing or walking attenuates the postprandial metabolic response in post-menopausal women: A randomised acute study

Running title – Breaks in sitting time and metabolic risk

Authors and Affiliations -

Joseph Henson[1,2] PhD, Melanie J. Davies[1,2] MD, Danielle H. Bodicoat[1,2,3] PhD, Charlotte L. Edwardson[1,2] PhD, Jason M.R. Gill[4] PhD, David J. Stensel[2,5] PhD, Keith Tolfrey[2,5] PhD, David W. Dunstan[6,7] PhD, Kamlesh Khunti[3] MD, Thomas Yates[1,2] PhD

[1] Diabetes Research Centre, University of Leicester, UK

[2] NIHR Leicester-Loughborough Diet, Lifestyle and Physical Activity Biomedical Research Unit, UK

[3] NIHR Collaborations for Leadership in Applied Health Research and Care (CLAHRC) East Midlands, UK and Diabetes Research Centre, University of Leicester, UK

[4] Institute of Cardiovascular and Medical Sciences, University of Glasgow, UK

[5] School of Sport, Exercise and Health Sciences, Loughborough University, UK

[6] Baker IDI Heart and Diabetes Institute, Melbourne, Victoria, Australia

[7] Mary MacKillop Institute of Health Research, Australian Catholic University, Melbourne, Victoria, Australia

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Corresponding Author:
Joseph Henson
Leicester Diabetes Centre
Leicester General Hospital
Leicester
LE5 4PW
UK

Email address: jjh18@le.ac.uk
Tel: +44 116 258 4389.
Fax: +44 116 258 4053.

Objective

To determine whether breaking up prolonged sitting with short bouts of standing or walking improves post-prandial markers of cardio-metabolic health in women at high risk of type 2 diabetes.

Research Design and Methods

Twenty-two overweight/obese, dysglycaemic, postmenopausal women (mean age ± SD: 66.8±4.6 years) each participated in two of the following treatments; prolonged, unbroken sitting (7.5 hours) or prolonged sitting broken up with either standing or walking at a self-perceived light-intensity (for 5 minutes every 30 minutes). Both allocation and treatment order were randomised. The incremental area under the curves (iAUC) for glucose, insulin, non-esterified fatty acids (NEFA) and triglycerides were calculated for each treatment condition (mean ± SEM). The following day, all participants underwent the 7.5 hours sitting protocol.

Results

Compared to a prolonged bout of sitting (iAUC 5.3±0.8mmol/L•h), both standing (3.5±0.8) and walking (3.8±0.7) significantly reduced the glucose iAUC (both $p<0.05$). When compared with prolonged sitting (548.2±71.8mU/L•h), insulin was also reduced for both activity conditions (standing: 437.2±73.5; walking: 347.9±78.7; both $p<0.05$). Both standing (-1.0±0.2mmol/L•h) and walking (-0.8±0.2) attenuated the suppression of the NEFA compared with prolonged sitting (-1.5±0.2); both $p<0.05$. There was no significant effect on triglyceride iAUC. The effects on glucose (standing and walking) and insulin (walking only) persisted into the following day.

Conclusions

Breaking up prolonged sitting with 5-minute bouts of standing or walking at a self-perceived light-intensity reduced postprandial glucose, insulin and NEFA responses in women at high risk of type 2 diabetes. This simple, behavioural approach could inform future public health interventions aimed at improving the metabolic profile of post-menopausal, dysglycaemic women.

Sedentary behaviour, now commonly conceptualised as sitting during waking hours with low energy expenditure (1), has recently emerged as an independent determinant of morbidity (particularly type 2 diabetes) and mortality (2-4). Multiple observational studies have also demonstrated a positive association between objectively measured sedentary time and markers of diabetes risk, independent of the amount of moderate-to-vigorous physical activity (MVPA) undertaken (5-7). This suggests that sedentary behaviour is likely to be a distinct risk factor for type 2 diabetes and a potential target for lifestyle intervention. This is important as individuals at high risk of type 2 diabetes spend around 70% of their waking time sedentary, with 25% in light activity and <5% engaged in MVPA (6). Moreover, the inverse correlation between sedentary behaviour and MVPA is weak (7), further suggesting these are independent behaviours. However, experimental data are needed to determine whether a causal relationship exists between modifications to sedentary time and metabolic health.

Recently, experimental studies which have broken up prolonged sitting with short periods of light or moderate intensity activity have been shown to reduce postprandial glucose and insulin concentrations in both healthy and overweight adults (8-11). These studies suggest that important health-related metabolic processes occur when individuals transition from sitting to movement (light and moderate intensity). However, it is unclear whether moving from sitting to standing provides a sufficient stimulus to elicit metabolic benefits. Whilst there is emerging evidence that sustained bouts of standing may improve glucose regulation (12, 13), it is not clear whether breaking up prolonged sitting with intermittent short bouts of standing might improve the metabolic health of individuals at high risk of chronic disease.

Therefore, the aim of this study was to establish whether breaking up prolonged sitting through frequent short bouts of standing or walking activity modulates postprandial metabolic responses in individuals at high risk of type 2 diabetes.

Research Design and Methods

Study design

A balanced incomplete block design was utilised for this study (14). Such designs have been used in pharmaceutical trials and reduce participant burden whilst minimising the intra-subject effect, thus increasing the sensitivity of the outcome (15, 16). With this design, participants were randomised to two of the three following treatment conditions: 1) prolonged, unbroken sitting (7.5 hours); 2) prolonged sitting broken up with standing for 5 minutes every 30 minutes or 3) prolonged sitting broken up with walking for 5 minutes every 30 minutes (Supplemental Table S1). Regardless of the treatment condition carried out on day 1, all participants underwent the prolonged sitting protocol on day 2, thus each treatment condition was carried out over two consecutive days. As an acute bout of physical activity may enhance insulin sensitivity for up to 48 hours (17), we used a minimum wash-out period of 7 days between each condition (the maximum wash-out was 22 days).

Participants attended five separate visits to the Leicester Diabetes Centre, Leicester, UK. Supplemental Figure 1 describes the study design. One to two weeks after an initial familiarisation visit, participants were randomised by an independent third party to one of six sequences, prepared by the study statistician prior to recruitment of the first participant (Supplemental Table S1).

The study is registered with clinicaltrials.gov (NCT02135172). Informed consent was obtained from all eligible participants and ethical approval was obtained from the Northampton Research Ethics Committee.

Participants

A total of 34 participants were recruited between January 2014 and October 2014. Post-menopausal women at high risk of developing type 2 diabetes were identified from studies previously conducted within the Leicester Diabetes Centre (18, 19). This cohort was included in order to negate the impact of hormone variations and as associations between sedentary behaviour and markers of cardio-metabolic health have previously been shown to be stronger in women (20).

Eligibility criteria included: overweight or obese (BMI ≥27.5 kg/m^2 or ≥25 kg/m^2 if south Asian), post-menopausal women (12 consecutive months without menstruation (21)), aged 50-75 years with screen detected impaired glucose regulation (IGR) identified within the 12 months prior to the invitation letter being sent. IGR was defined as 2 hour post-challenge glucose ≥7.8mmol/L to <11.1mmol/L following a standard oral glucose tolerance test (22), or HbA1c between 5.7-6.4% (39-46mmol/mol) inclusive (23). Exclusion criteria were regular purposeful exercise (≥150 minutes of objectively measured MVPA over a typical week), inability to communicate in spoken English, steroid use, known type 2 diabetes, or currently taking hormone replacement medication.

In total, 30 participants were randomised (Figure 1). Causes of drop out between familiarisation and randomisation are detailed in Figure 1. A further 8 individuals were

excluded after randomisation, due to cessation of the venous cannula line which resulted in less than 50% of data collection (n=5), illness (n=2), or a change in personal circumstance (n=1). This left 22 participants that were included in the analysis. There were no significant differences in BMI, age or HbA1c between those who dropped out or were excluded and those who were included in the study.

Familiarisation visit

Before participating in the experimental protocol, all participants visited the Leicester Diabetes Centre for a familiarisation visit where they provided informed consent. This allowed participants to become accustomed to the walking speed and also familiarize themselves with the Borg rating of perceived exertion (RPE) scale (24). A venous blood sample was also taken for HbA1c, lipid profile, and non-esterified fatty acids (NEFA) analysis.

Body mass (Tanita TBE 611, Tanita, West Drayton, UK), waist circumference (midpoint between the lower costal margin and iliac crest), and height were measured, to the nearest 0.1kg, 0.5cm and 0.5cm respectively.

Participants also wore an accelerometer (placed on the right anterior axillary line) for seven days after familiarisation (Actigraph GT3X+, Pensacola, FL, USA) to measure time spent engaged in sedentary, light or MVPA, under free-living conditions.

Experimental regimen overview

Participants were asked to record all food and drink consumed the day before the first experimental condition. They were then asked to replicate this diet before subsequent treatments. Participants were also requested to avoid alcohol, caffeine and any MVPA for two days prior to each experimental condition.

Participants arrived at the laboratory by car (08:00) after a 10 hour fast and had a cannula fitted into an accessible vein. A fasting blood sample (9ml) was then taken (time point: -1 h) for the quantification of glucose, insulin, NEFA and triglycerides. Participants were asked to sit quietly for 60 minutes and a further 9ml blood sample was taken. A standardised mixed-meal breakfast (croissant, butter, cheese, double cream, skimmed milk and a meal replacement drink (Complan, Nutricia Limited, Wiltshire, UK)) was consumed (09:00; 0 h) providing 0.66g fat, 0.66g carbohydrate and 0.4g protein per kg of body mass (58% fat, 26% carbohydrate and 16% protein). The time taken to consume the meal (≤15 minutes) was recorded and replicated in subsequent conditions. Blood was sampled again at 30, 60, 120 and 180 minutes postprandially. Lunch, with an identical nutrient composition to breakfast, was consumed at 12:00 with blood samples at 30, 60, 120, 180 and 210 minutes postprandially. The research staff supervised participants throughout each study cycle to ensure full compliance with the trial protocols. Participants consumed water *ad libitum* during the first of the experimental conditions and were then asked to replicate the volume ingested in subsequent conditions.

Experimental Regimens – Day 1

Experimental Condition: Prolonged sitting (7.5 hours)

During the prolonged sitting condition, walking and standing was restricted (lavatory visits were conducted via a wheelchair). Participants sat in a designated room equipped with a chair, desk and access to books, magazines and internet services.

Experimental Condition: Sitting (total 6.5 hours) + Standing (total 60 minutes)

This followed the same procedure as the sitting condition except that participants were instructed to break their sitting time by standing close to their chair for 5 minutes, every 30 minutes. Individuals were asked to stand in the same, fixed position. In total, individuals accumulated 12 bouts (60 minutes) of standing.

Experimental Condition: Sitting (total 6.5 hours) + Walking (total 60 minutes)

This was similar to the standing condition, but sitting time was punctuated with 5 minute bouts of walking at a self-perceived light intensity on a treadmill (Spazio Forma Folding Treadmill, TechnoGym UK Ltd, Bracknell, UK). During the first bout of walking, participants were gradually taken up to a speed that registered between 10 and 12 on the Borg RPE scale (24), up to a maximum of 4.0 km/h. This speed was fixed and replicated for all other intervals. In total, individuals accumulated 12 bouts (60 minutes) of walking.

The average treadmill speed during the walking condition was 3.0km/h (range =1.5-4.0km/h) with an average RPE score of 10 (range 8-12).

Experimental Regimens – Day 2 (Prolonged sitting – 7.5 hours)

To determine whether any acute effects of standing and walking persisted into the next day, participants returned to the laboratory (08:00) following another 10 h fast to undergo the prolonged sitting protocol (including the same standardised meals and timings). They were asked to consume exactly the same meal as the previous evening – whilst again avoiding alcohol, caffeine and MVPA.

Sedentary, physical activity and posture data

Participants were asked to wear an accelerometer (Actigraph GT3X+, Pensacola, FL, USA) and an activPAL professional physical activity monitor (PAL Technologies, Glasgow, Scotland), during experimental conditions and an accelerometer for 7 days before each experimental condition (Supplemental Figure 1).

ActivPAL proprietary software (activPAL Professional V5.9.1.1) was used to create processed csv files.

For accelerometer data collected over each 7 day period, non-wear time was defined as a minimum of 60 minutes of continuous zero counts and days with at least 10 h of wear time were considered valid (5, 6). Valid data required at least three valid days (25). Freedson cut points were used to categorise activity intensity (26). Accelerometer data were analysed using

a bespoke tool (KineSoft version 3.3.76, KineSoft, New Brunswick, Canada; www.kinesoft.org).

Biochemical analysis

Plasma glucose and serum triglyceride concentrations were determined using standard enzymatic techniques with commercially available kits (Beckman, High Wycombe, UK). The measurement of plasma NEFA involved a three stage colorimetric assay using a commercially available kit (RX Monza, Randox Laboratories, County Antrim, UK). Glucose, triglycerides and NEFA were analysed on the day of collection.

Insulin samples underwent centrifugation to separate plasma within 15 minutes of collection. Plasma was stored at $-80^{\circ}C$ and analysed at the end of data collection using an enzyme immuno-assay (Mercodia, Sweden). All measurements and analysis were undertaken by individuals blinded to experimental condition and independent of the scientific advisory team.

Sample size

The primary outcome was incremental postprandial area under the glucose curve (iAUC) on day 1. Allowing for an intervention effect of a 20% change in glucose iAUC, a standardised difference of 1 (where the SD is equivalent to the anticipated intervention effect), a within-person correlation of 0.3, 90% power, and an alpha of 0.025 (allowing for two primary comparisons against control conditions), we estimated that we would require 12 participants for a complete 3-treatment, 3-period crossover design. Twice as many participants were

required for the 3-treatment, 2-period balanced incomplete block design (27), and a 20% drop-out rate was allowed for; therefore we aimed to recruit 30 participants with 24 needed to complete the trial. Estimates were based on previous experimental research (8), and with consideration given to the high risk nature of our cohort where a greater effect was anticipated.

Statistical Analyses

In line with best practice for acute studies where fasting physiology does not change, outcomes were calculated as iAUC rather than total AUC (28). Values were determined using the trapezium rule and by subtracting fasting levels from the overall postprandial response.

Participants were excluded if they had over 50% of blood samples missing across any treatment condition (n=5). Missing outcome data for remaining participants were imputed using a regression model with key predictor variables (BMI, age, fasting values, ethnicity and treatment) for each time point and outcome. Imputation was used to correct for verification bias (29). Across all experimental conditions, 11% of data values (378/3472) were missing and imputed (Supplemental Table S2)On average, participants were missing 2 (1-4) (median (IQR) values across all experimental days and biochemical variables.

Multilevel mixed-effects linear regression was used to look at the difference between groups in the continuous outcome measures (glucose, insulin, NEFA, triglycerides) allowing for repeated measurements from the same individuals. In these models, treatment was modelled as a fixed factor and participant as a random factor. The primary analysis involved comparing standing and walking against the control (prolonged sitting) condition. Tests between

treatment conditions (standing vs. walking) were conducted for exploratory purposes and form a secondary outcome for the study.

All data were analysed using STATA (version 13.0; StataCorp, College Station, TX). A p-value of <0.05 was considered statistically significant. Descriptive data are reported as mean ± SD in text and tables, unless otherwise stated, and as mean ± SEM in Figure 2, Figure 3 and Supplemental Tables S3-S6.

In order to aid interpretation of the results, a sensitivity analysis was conducted to investigate whether results were affected by analysing the total AUC (including fasting values). Furthermore, we also investigated whether fasting values differed between day 1 and day 2 (Supplemental Table S7).

Results

Anthropometric, biochemical and demographic information of the included participants are displayed in Table 1.

Experimental Regimens – Day 1

Biochemical results collected on day 1 (for each experimental condition) are presented in Figure 2, with the corresponding numerical values displayed in Supplemental Table S3.

The mean glucose iAUC response (iAUC) was 5.3±0.8mmol/L•h in the prolonged sitting condition. Breaking sitting time with 5 minutes of standing, every 30 minutes, reduced the

glucose iAUC by 34% (3.5±0.8mmol/L•h, p=0.022) compared with prolonged sitting. Similarly, walking reduced the glucose iAUC by 28% (3.8±0.7mmol/L•h, p=0.009) compared with prolonged sitting.

A similar pattern of results were observed for insulin and NEFA on day 1. The insulin iAUC was reduced by 20% (437.2±73.5mU/L•h, p=0.045) when breaking sitting time with standing and by 37% (347.9±78.7mU/L, p=0.008) when it was broken with walking compared with prolonged sitting (548.2±71.8mU/L•h). Breaking sitting time with standing attenuated the suppression of the NEFA iAUC by 33% (-1.0±0.2mmol/L•h, p=0.024), and with walking by 47% (-0.8±0.2mmol/L•h, p=0.003) compared with prolonged sitting (-1.5±0.2mmol/L•h).

There were no significant differences between the standing and walking conditions for any of these outcomes (glucose p=0.717, insulin p=0.376, NEFA p=0.398).

Conversely, neither standing (6.2±0.8mmol/L•h) nor walking (6.1±0.8mmol/L•h) significantly reduced the triglyceride iAUC compared with the sitting condition (5.6±0.7mmol/L•h) on day 1.

Experimental Regimens – Day 2 (Prolonged sitting – 7.5 hours)

17 participants completed the second day due to problems with intravenous cannulation. Biochemical results for day 2 are presented in Figure 3 with the corresponding numerical values displayed in Supplemental Table S4.

Day 2 yielded a mean net glucose response of 4.8±0.6mmol/L•h if participants had undertaken the sitting condition on day 1. Breaking sitting time with standing on day 1 elicited a response of 3.9±0.8mmol/L•h on day 2 (19% reduction in iAUC compared to sitting, p=0.039). Similarly, walking carried out on day 1 reduced the glucose iAUC by 17% on day 2 (4.0±0.7mmol/L•h, p=0.027). There was no significant difference between the standing and walking conditions (p=0.877).

The mean net insulin response was 464.6±70.2mU/L•h if participants had undertaken the sitting condition on day 1. The significant results for standing on day 1 did not persist into the second day (363.5±57.5mU/L•h, p=0.325). In contrast, results for walking persisted into day 2 (354.3±57.3U/L•h p=0.038). There was no significant difference between the standing and walking conditions (p=0.529).

There was no difference in triglyceride response between the prolonged sitting (7.2±0.5mmol/L•h) and standing conditions (7.2±0.8mmol/L•h, p=0.603) on day 2. Results for the walking condition (6.0±0.7mmol/L•h, p=0.077) neared significance compared to prolonged sitting.

The effects of standing and walking on NEFA were no longer significant on day 2 (standing: -1.0±0.3mmol/L•h, p=0.161; walking: -1.0±0.3mmol/L•h, p=0.144) when compared to prolonged sitting (-1.5±0.2mmol/L•h).

Sensitivity analysis

The pattern of results and significance levels were largely unaffected if the data were analysed using total AUC on day 1 (Supplemental Table S5). However, total AUC on day 2 failed to reach significance for both glucose (standing and walking condition) and insulin (standing only) (Supplemental Table S6). Conversely, results for NEFA became significant for both standing and walking. There were no significant differences between any fasting values on day 1 or day 2 (Supplemental Table S7).

Sedentary and physical activity data

Free-living accelerometer data collected after the familiarisation visit (n=22) (Supplemental Table S8) showed that participants spent 594±80 minutes per day sedentary (71.5% of total wear time) and only engaged in modest amounts of MVPA (19±10 minutes per day; 2% of total wear time); there was no difference in these behaviours for the 7-days prior to each experimental conditions ($p>0.05$).

The Actigraph and activPAL monitor data recorded during the experimental conditions confirmed that compliance to the protocol was high (Supplemental Tables S8 and S9). Participants took an average 6±2 steps and 252±18 steps during each 5-minute standing and walking bout respectively.

Conclusions

In overweight, post-menopausal women with dysglycaemia we observed that interrupting periods of prolonged sitting with 5 minutes of standing every 30 minutes elicits similar changes to postprandial glucose metabolism as breaking up sitting with identical periods of self-perceived light-intensity walking. Compared with uninterrupted sitting, standing reduced the postprandial rise in glucose by 34% (compared with a 28% reduction for walking) and the postprandial rise in insulin concentrations by 20% (37% for walking) on the day of the intervention. Moreover, the observations for glucose (standing and walking) and insulin (walking only) persisted into the next day.

These data build on previous work in overweight men and women (8) reporting similar glucose and insulin postprandial responses after light and moderate intensity walking. The present findings extend these observations by suggesting that metabolic benefits are also accrued when regularly breaking up prolonged sitting by moving from a sitting to a stationary upright posture.

To date, four other studies have examined the acute effect of standing on postprandial glucose and insulin responses (12, 13, 30, 31). Two of these found that breaking prolonged sitting with regular standing breaks had no impact on postprandial glucose (30, 31) and insulin (30) in young healthy men. In contrast, alternating 30 minute bouts of sitting and standing throughout the day has been shown to significantly reduce the iAUC between trial conditions for postprandial glucose (11% reduction compared to prolonged sitting) (12). A non-randomised office-based study also found that glucose levels were reduced by 43% following an afternoon of standing compared with seated computer work (13). The fact that

our study reported effects that were towards the upper end of those reported in previous studies, whilst employing substantially smaller doses of standing, is likely to be driven by differences in sample characteristics and potentially the increased frequency in interruptions to prolonged sitting. Other studies have been conducted in groups that are broadly representative of the general population (age <50 years, BMI <30kg/m^2), whereas our participants were older with existing dysglycaemia who represent those likely to be referred into diabetes prevention pathways. This is particularly important given the prominence of national and international strategies highlighting the need for identification and subsequent referral of individuals at high risk of type 2 diabetes (32, 33).

Another novel finding was that reductions in glucose and insulin responses following the breaking up of prolonged sitting were maintained into the second observation day. Glucose remained 19% lower after the standing condition and 17% lower after the walking condition. Similarly, insulin remained 24% lower after the walking condition. These findings are consistent with a previous experimental study carried out in obese adults showing that a single bout of modest exercise (50% VO$_2$ peak on a stationary cycle ergometer) increased insulin sensitivity into the next day (11). A similar study also demonstrated that the morning after a prolonged bout of sitting (17 hours), participants exhibited a significant reduction (39%) in whole-body insulin action compared to upright light-intensity activity (10). Our findings indicate that an even lower activity stimulus (e.g.: standing) may yield metabolic advantages for a minimum of 24 hours.

The mechanisms underpinning the effects of standing and walking on glucose and insulin levels requires further elucidation. Acute and chronic light-intensity physical activity training studies have consistently demonstrated improvements in markers of glycaemic control in

those with dysglycaemia, with similar effects observed between light and moderate intensity exercise training regimes, when matched for total volume (34). However, it has not been established whether the specific mechanisms involved in enhancing peripheral glucose uptake that have been shown for MVPA, primarily through the translocation and turnover of GLUT-4 (35), are observed with walking at a self-perceived light intensity or standing.

The attenuated postprandial suppression in plasma NEFA concentration observed on day 1 of this study for both the standing and walking is likely to reflect an increase in the lipolysis of triglycerides stored in adipose tissue in order to supply the working muscle. Moreover, the reduction of insulin in the standing and walking conditions suggests that suppression of lipolysis, driven by the antilipolytic properties of insulin (36), may have been reduced in these conditions. Previous studies have shown that during low-intensity exercise, adipose tissue lipolysis increases four-to fivefold above resting levels (37). Others have also reported that lipolysis and mobilization of NEFA resulting from exercise are related to, and may be enhanced by, hormonal changes, particularly increased catecholamines levels (38).

We found no change in the triglyceride iAUC for the standing and walking conditions on either day 1 or day 2 of the experimental regimens. The non-significant results on day 1 are consistent with previous studies that have shown no effect (9, 12). Decreased triglyceride levels were observed on day 2 following the walking condition, although the changes were not statistically significant (17% reduction compared to sitting, p=0.077). However, the magnitude of the effect for walking on day 2 was consistent with previous studies demonstrating that walking (both intermittent and continuous) elicited reductions in the postprandial triglyceride levels the following day (16-23% reduction). Our results corroborate

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with other findings suggesting that standing is not a sufficient stimulus to reduce postprandial triglyceride levels (12, 30).

This study has a number of strengths. Firstly, we studied postmenopausal women at high risk of type 2 diabetes, so the findings are directly relevant for public health guidance and interventions for metabolic risk reduction. Secondly, this is the first study to directly compare the effects of breaking up prolonged sitting with standing and walking, demonstrating that they both induce cardio-metabolic benefits. Moreover, by employing a two day protocol we were able to determine that the acute effects of standing and walking persisted into the following day. Our study also highlights the importance of reporting both iAUC and total AUC in experimental studies that assess outcomes over several days. Although results on day 1 were unaffected by the analysis method there were small differences in interpretation on day 2. Notwithstanding the non-significant differences in mean fasting levels on day 2, it is possible that the intervention conditions had a subtle effect on fasting pathophysiology that subsequently influenced total AUC. As such, results should be interpreted in relation to the method used; for this study the primary focus was on the postprandial response (iAUC). Finally, all measurements were performed by the same team of trained staff, following identical standard operating procedures and analysis was conducted by individuals blinded to treatment allocation.

This study has several important limitations. Firstly, the acute nature of the trial prohibits inferences about longer-term chronic effects. Secondly, the test meals used were relatively high in fat (58% of total energy) and further studies are needed in order to determine whether the findings persist when meals with a macronutrient composition more representative of dietary recommendation are consumed. However, the macronutrient composition of food was

almost identical to that which may be plausibly consumed by the general population through a meal or as a snack. For example, based on an 80kg individual the standardised meal used in this study is equivalent to 46g brown bread, 6g butter, 100g bacon and a 59g chocolate bar (39). Studies have also indicated that the recommended daily intake of fat is often exceeded by many adults (40). We also relied upon participants to record and standardise their own food intake the day before and in-between each experimental conditions for practical reasons, therefore misreporting is possible. Similarly, no physical activity data was recorded between day 1 and day 2. Thirdly, the prolonged nature of the sitting condition may not reflect habitual behaviour for many individuals where some standing or light movement would be expected over an 8 hour period. Nonetheless, it was important to initially establish a proof of concept where standing and walking effects are observed compared to a prolonged standardised bout of sitting. Future studies should also focus on whether the effects observed in this study are replicated under free living scenarios. The reduced sample size (and subsequent underpowered comparison) particularly pertaining to comparisons on day 2 increased the risk of a type 2 error and thus limits the conclusions that can be drawn over the second day. Furthermore, the study was not designed to assess differences between the standing and walking conditions which were included as a secondary outcome. Finally, further research is needed to determine whether the effects can be generalized to men and premenopausal women.

In conclusion, this study demonstrates that breaking up prolonged sitting with 5-minute bouts of standing or walking at a self-perceived light intensity reduces postprandial glucose, insulin and NEFA responses in post-menopausal women at high risk of type 2 diabetes. This simple, behavioural approach could inform future public health interventions aimed at improving the metabolic profile of dysglycaemic individuals. Habitual standing and light-intensity physical

activity are behaviourally more ubiquitous than MVPA and may therefore provide appealing interventional targets in the promotion of metabolic health. However, future behavioural intervention studies are needed to investigate the most effective methods of reducing habitual sedentary behaviour within a prevention context and to assess generalizability beyond post-menopausal women.

Conflict of Interest

K.K. (Chair), M.J.D. and T.Y. are members of the NICE Public Health Guidance (PH38) Preventing type 2 diabetes: risk identification and interventions for individuals at high risk.

Author contributions

J.H., M.J.D., D.H.B., C.L.E., J.M.R.G., D.J.S., K.T., D.D., K.K. and T.Y. made significant contributions to the concept and subsequent design of the study; all authors made substantial

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Figure 1. Study *CONSORT* Diagram

121 participants invited from previous type 2 diabetes prevention projects

70 replies

36 negative responses

28 not interested
3 diagnosed with type 2 diabetes
3 unable to contact
2 other health problems

3 screen failures

1 did not meet inclusion criteria
(BMI <25 kg·m^{-2})
2 diagnosed with type 2 diabetes

34 attended familiarisation visit

1 withdrawn; change in circumstance

30 randomised

Block A	Block B	Block C	Block D	Block E	Block F
Sitting + Standing	Standing + Walking	Walking + Sitting	Sitting + Walking	Standing + Sitting	Walking + Standing
N= 5	N= 6	N= 5	N= 5	N= 4	N= 5
Dropout N=2	Dropout N=1	Dropout N=2	Dropout N=2	Dropout N=1	

22 completed study (day 1; primary outcome)

	Incomplete data N=2		Incomplete data N=1	Incomplete data N=1	Incomplete data N=1

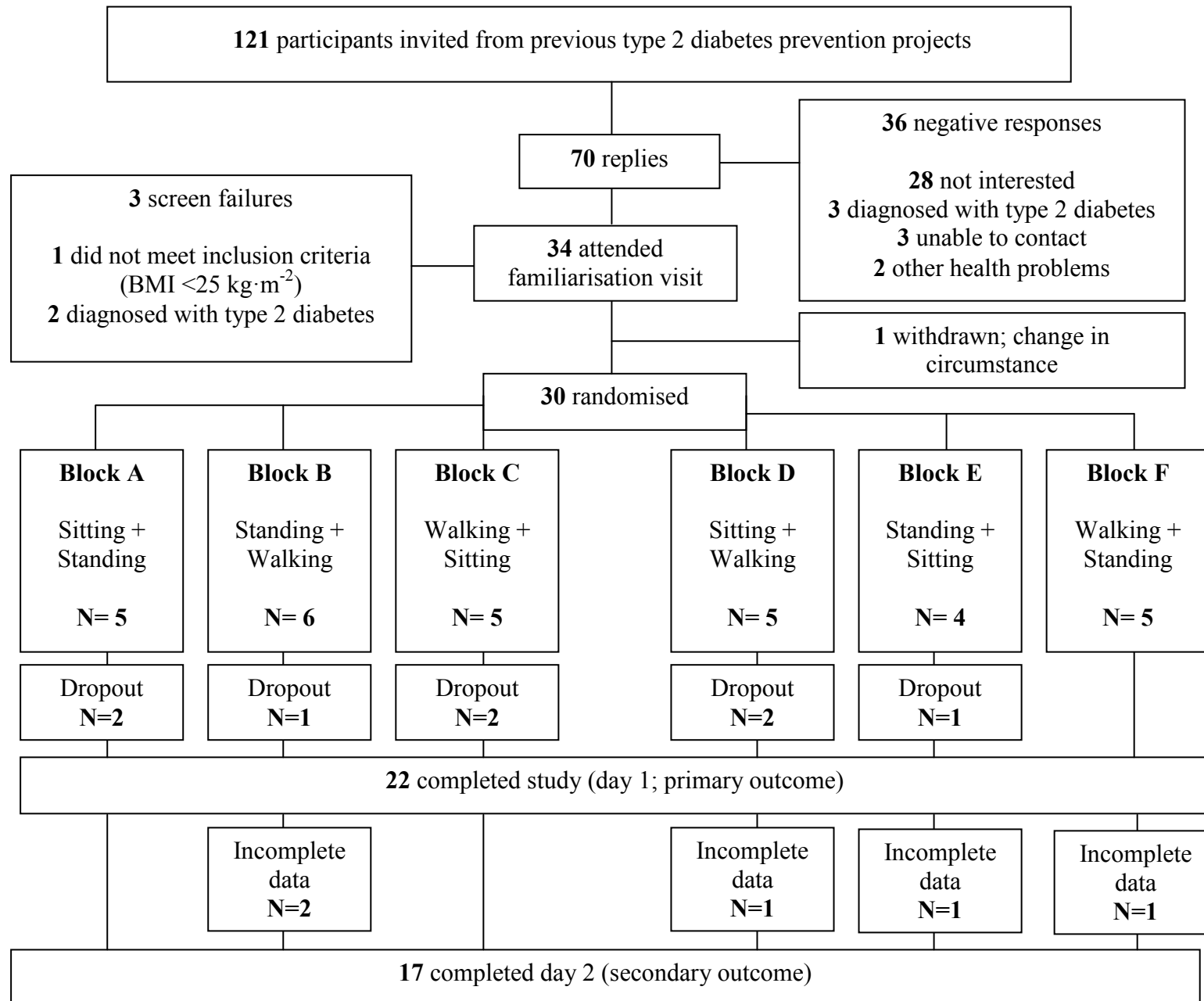17 completed day 2 (secondary outcome)

27

Table 1. Metabolic, demographic and anthropometric characteristics at baseline and dietary and physical activity variables during the study (n=22)

Baseline characteristics	
Age (years)	66.6 ± 4.7
Current smoker	1 (4.5)
BMI (kg/m^2)	32.9 ± 4.7
Waist circumference (cm)	102 ± 9.0
Body mass (kg)	83.6 ± 11.7
Total cholesterol (mmol/L)	5.60 ± 0.87
Triglycerides (mmol/L)	2.17 ± 0.86
Non-esterified fatty acids (mmol/L)	0.44 ± 0.24
HbA1c (%)	5.8 ± 0.2
HbA1c (mmol/mol)	40 ± 2.3
Fasting plasma glucose (mmol/L)	5.4 ± 0.4
Lipid lowering medication	5 (22.7)
Beta-blockers	5 (22.7)
ACE Inhibitors	3 (13.6)
Ethnicity White European Black and minority ethnic	20 (90.9) 2 (9.1)
In study characteristics	
Diet Total energy intake (kcal/day) Total fat (energy %) Total carbohydrate (energy %) Total protein (energy %)	1717 ± 234 58 ± 0.2 26 ± 0.1 16 ± 0.2
Walking speed (km/h)	3.0 (1.5-4.0)
Borg rate of perceived exertion score	10 (8-12)

Data are presented as mean±standard deviation, number (%) or mean (range)

Figure 2. The effect of sitting, standing and walking upon glucose (A), insulin (B) NEFA (C) and triglyceride (D) levels on day 1 (n=22)
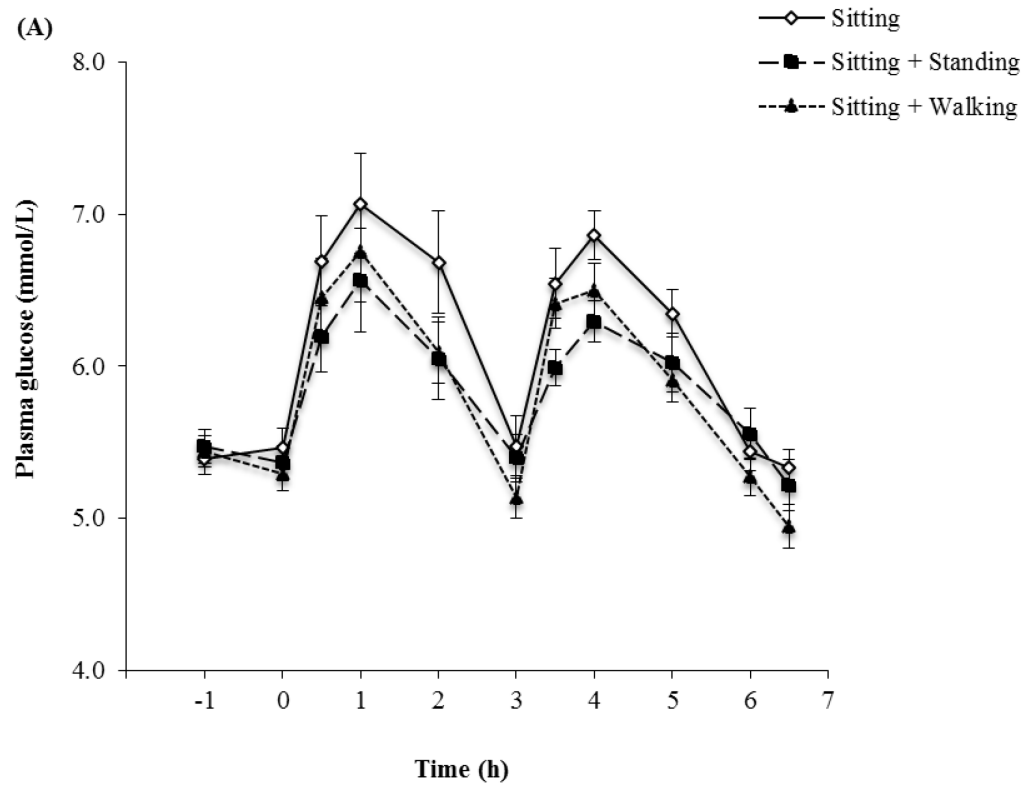
iAUC: Standing vs. sitting p=0.022; Walking vs. sitting p=0.009
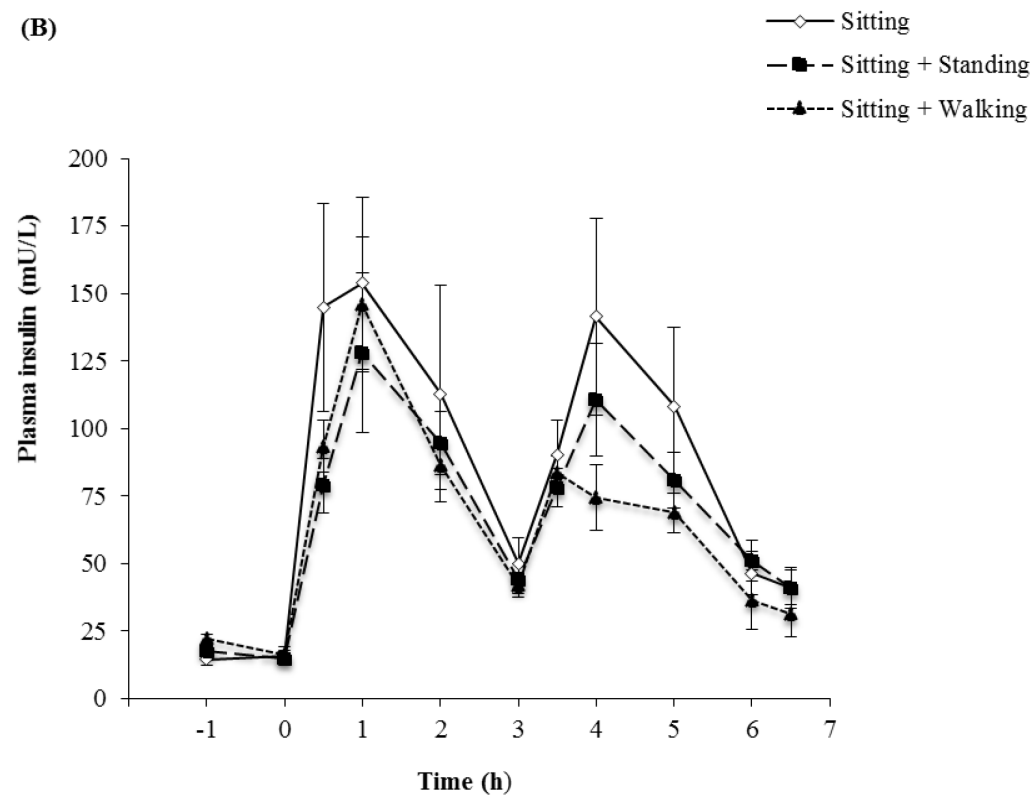
iAUC: Standing vs. sitting p=0.045; Walking vs. sitting p=0.008

(C)
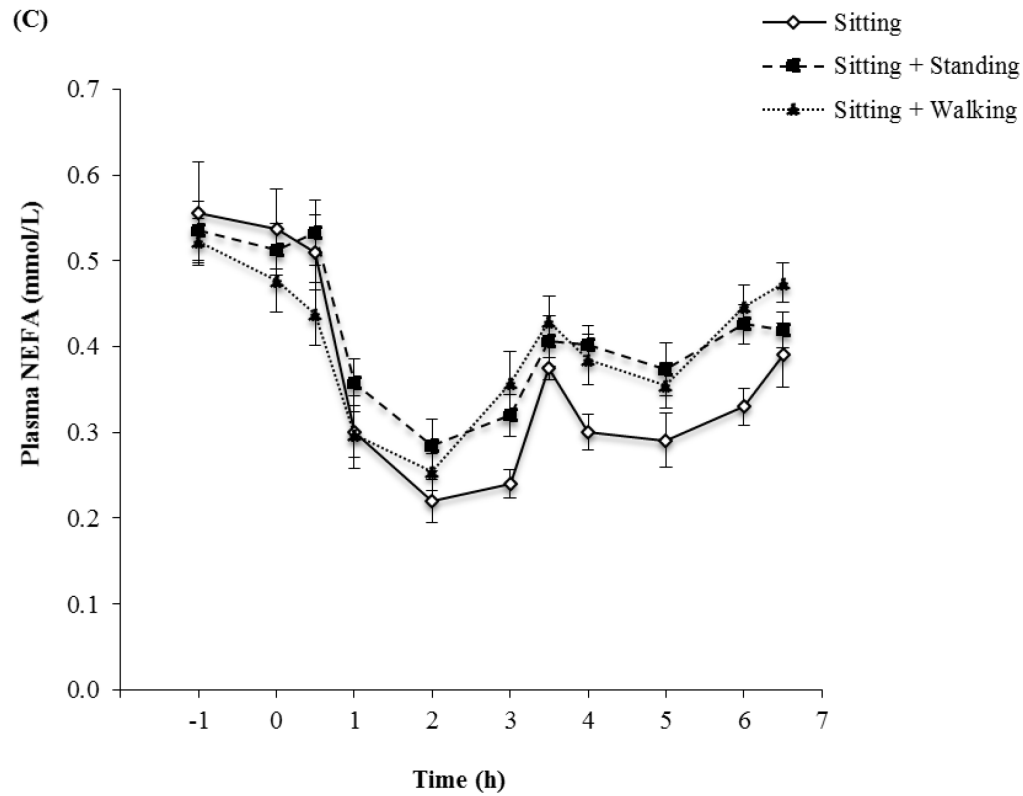
iAUC: Standing vs. sitting p=0.024; Walking vs. sitting p=0.003

(D)
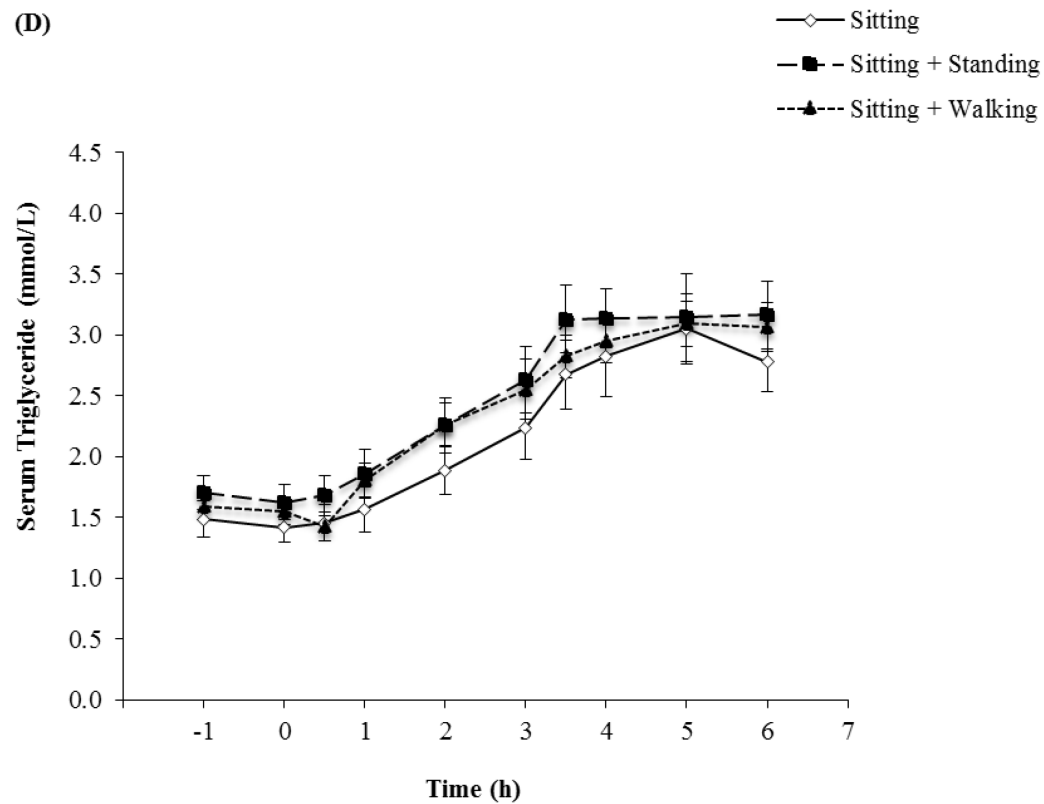
iAUC: Standing vs. sitting p=0.493; Walking vs. sitting p=0.673

Figure 3. The effect of sitting, standing and walking upon glucose (A), insulin (B) NEFA(C) and triglyceride (D) levels on day 2 (n=17)

(A)
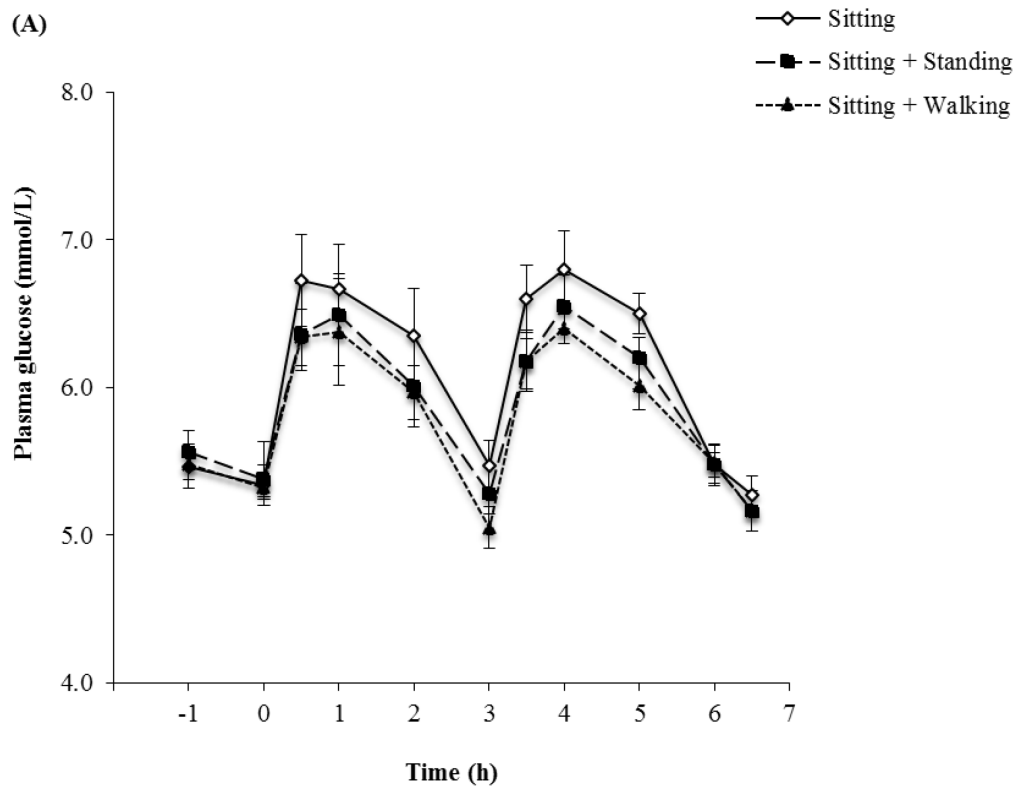
iAUC: Standing vs. sitting p=0.039; Walking vs. sitting p=0.027

(B)
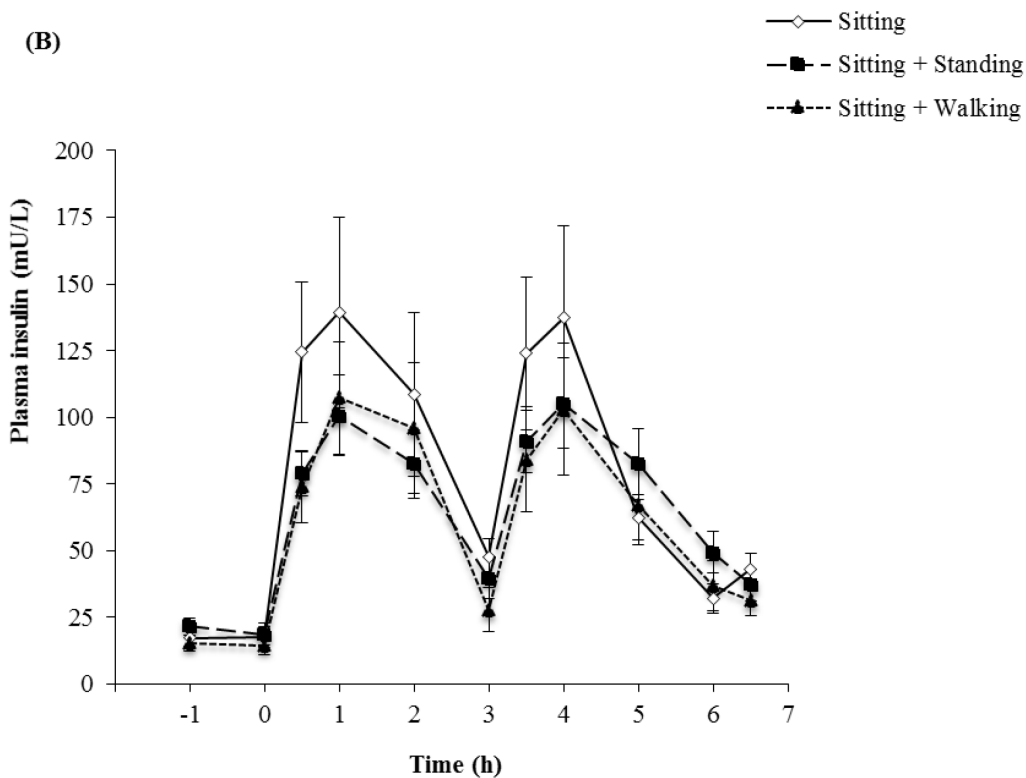
iAUC: Standing vs. sitting p=0.325; Walking vs. sitting p=0.038

31

(C)
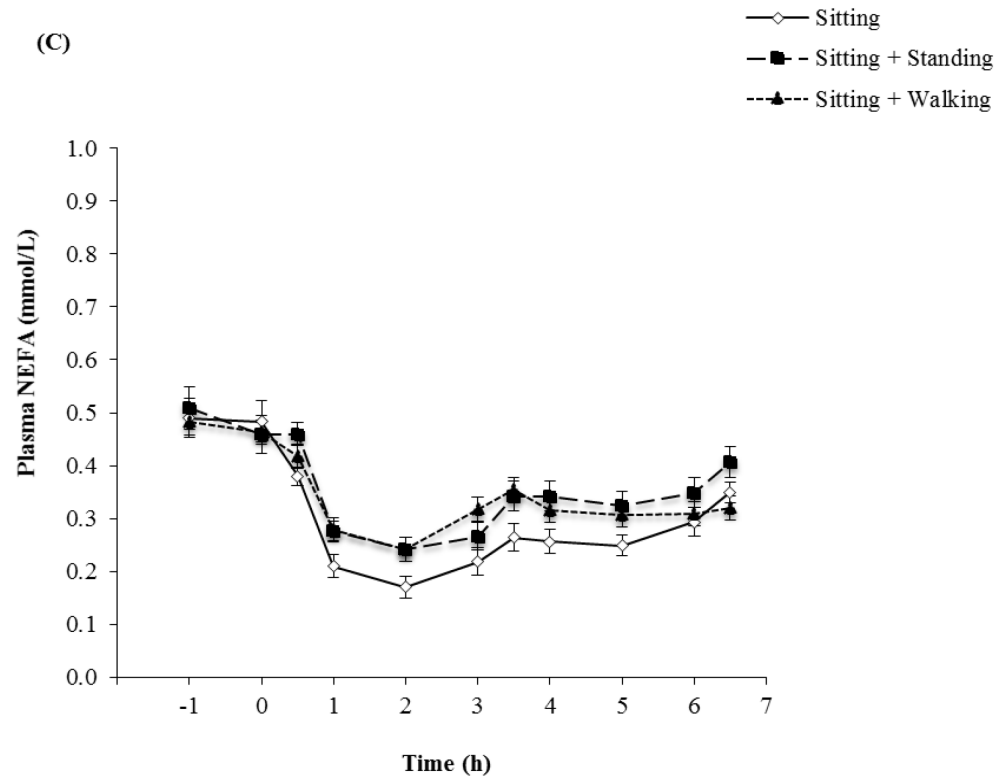
iAUC: Standing vs. sitting p=0.161; Walking vs. sitting p=0.144

(D)
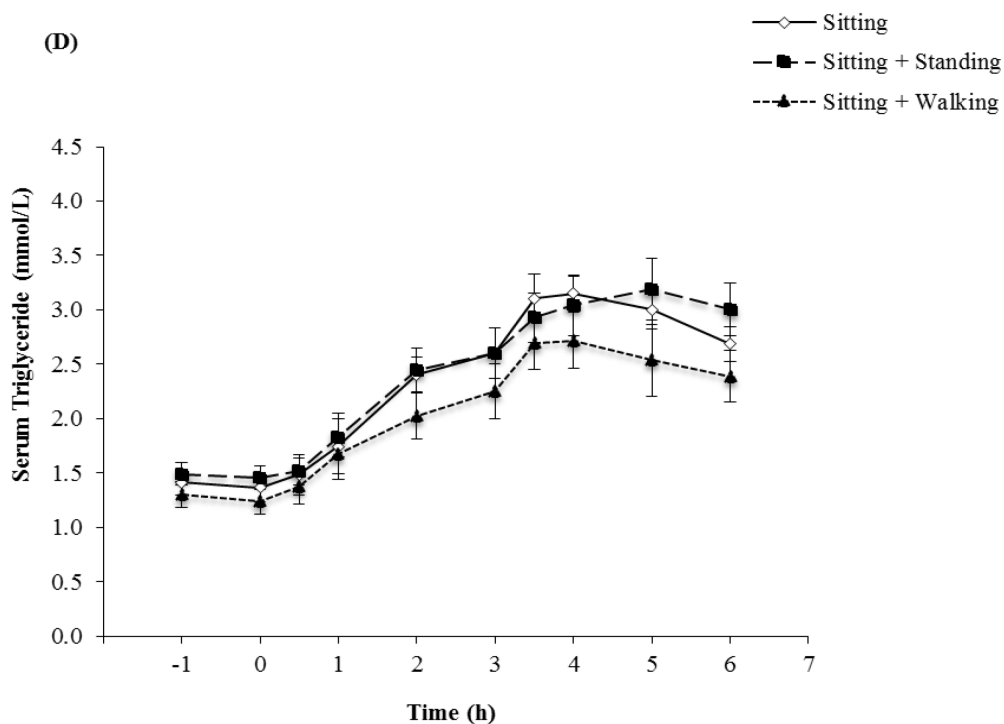
iAUC: Standing vs. sitting p=0.603; Walking vs. sitting p=0.077

Mean (± SEM) glucose, insulin, NEFA and triglycerideson day 1 (Figure 2; A, B, C, D) and day 2 (Figure 3; A, B, C, D) measured over a 6.5-h period during the prolonged sitting, sitting and standing and sitting and walking conditions. Standardised meals provided at 0h and 3h. iAUC; incremental area under the curve, SEM; standard error of the mean, NEFA; non-esterified fatty acids

Supplemental Table S1. Balanced incomplete block design sequence. Each participant was randomised to one of the sequences A-F.

Sequence	Period 1		Period 2	
	Day 1	**Day 2**	**Day 1**	**Day 2**
A	Sitting	Sitting	Standing	Sitting
B	Standing	Sitting	Walking	Sitting
C	Walking	Sitting	Sitting	Sitting
D	Sitting	Sitting	Walking	Sitting
E	Standing	Sitting	Sitting	Sitting
F	Walking	Sitting	Standing	Sitting

Supplemental Figure 1. Study protocol for treatment conditions

<------------7-14 days ------------> <---------------- 2 days ------------> <------------7-14 days ----------><---------------- 2 days -------------->

<--------24hr--------> <-------24hr------>

| Familiarisation visit | Habitual physical activity | *Restrictive Period (no alcohol and MVPA 48 hrs before)

* Record all food and drink consumed | Day 1: Experimental condition | *Restrictive Period

* Consume identical amounts of the same food and drink as the previous evening | Day 2: Sitting protocol | Habitual physical activity | *Restrictive Period (no alcohol and MVPA 48 hrs before)

* Consume identical amounts as the previous experimental condition | Day 1: Experimental condition | *Restrictive Period

* Consume identical amounts of the same food and drink as the previous evening | Day 2: Sitting protocol |

Standardised meals

Standardised meals

Standardised meals

Standardised meals

Actigraph GT3X+ worn
Actigraph GT3X+ and activPAL worn

34

Supplemental Table S2. Number of imputed values for each participant included across both experimental days

Participant Number	Day 1: Missing values across both randomised experimental conditions (total scheduled samples = 22)				Day 2: Missing values across both randomised experimental conditions (total scheduled samples = 22)			
	Glucose	Insulin	Triglycerides	NEFA	Glucose	Insulin	Triglycerides	NEFA
1	2 (9)	7 (32)	6 (28)	7 (32)				
2	0	0	1 (5)	2 (9)	0	1 (5)	4 (18)	6 (28)
3	0	0	2 (9)	2 (9)	4 (18)	5 (23)	5 (23)	7 (32)
4	0	3 (14)	3 (14)	3 (14)	1 (5)	1 (5)	1 (5)	2 (9)
5	2 (9)	5 (23)	3 (14)	4 (18)				
6	1 (5)	5 (23)	3 (14)	1 (5)	1 (5)	1 (5)	2 (9)	1 (5)
7	2 (9)	7 (32)	2 (9)	3 (14)	0	1 (5)	1 (5)	2 (9)
8	0	2 (9)	1 (5)	3 (14)	2 (9)	2 (9)	2 (9)	3 (14)
9	3 (14)	6 (28)	6 (28)	6 (28)	0	2 (9)	2 (9)	1 (5)
10	1 (5)	2 (9)	1 (5)	2 (9)	0	0	0	1 (5)
11	0	2 (9)	0	0	0	0	0	2 (9)
12	0	0	0	0				
13	0	4 (18)	3 (14)	8 (36)	3 (14)	3 (14)	4 (18)	4 (18)
14	1 (5)	4 (18)	4 (18)	4 (18)	4 (18)	5 (23)	5 (23)	5 (23)
15	0	0	1 (5)	8 (36)				
16	3 (14)	3 (14)	3 (14)	4 (18)	4 (18)	4 (18)	4 (18)	4 (18)
17	2 (9)	3 (14)	5 (23)	5 (23)	4 (18)	5 (23)	5 (23)	4 (18)
18	1 (5)	3 (14)	2 (9)	2 (9)	3 (14)	4 (18)	4 (18)	4 (18)
19	1 (5)	3 (14)	1 (5)	1 (5)	2 (9)	2 (9)	2 (9)	2 (9)
20	0	2 (9)	0	0				
21	0	5 (23)	2 (9)	2 (9)	1 (5)	1 (5)	2 (9)	3 (14)
22	1 (5)	5 (23)	1 (5)	1 (5)	1 (5)	2 (9)	2 (9)	4 (18)

Data presented as number (percentage of total available samples)

Supplemental Table S3. Incremental area under the curve (iAUC) results on day 1 for biochemical measurements for each experimental condition (n = 22)

	Mean ± SEM			P-value	
	Sitting	**Standing**	**Walking**	**Standing vs. sitting**	**Walking vs. sitting**
Glucose (mmol/L•h)	5.3±0.8	3.5±0.8	3.8±0.7	0.022	0.009
Insulin (mU/L•h)	548.2±71.8	437.2±73.5	347.9±78.7	0.045	0.008
Triglycerides (mmol/L•h)	5.6±0.7	6.2±0.8	6.1±0.8	0.493	0.673
NEFA (mmol/L•h)	-1.5±0.2	-1.0±0.2	-0.8±0.2	0.024	0.003

SEM; standard error of the mean, NEFA; non-esterified fatty acids

Supplemental Table S4. Incremental area under the curve (iAUC) results on day 2 for biochemical measurements for each experimental condition (n = 17)

	Mean ± SEM			P-value	
	Sitting	**Standing**	**Walking**	**Standing vs. sitting**	**Walking vs. sitting**
Glucose (mmol/L•h)	4.8±0.6	3.9±0.8	4.0±0.7	0.039	0.027
Insulin (mU/L•h)	464.6±70.2	363.5±57.5	354.3±57.3	0.325	0.038
Triglycerides (mmol/L•h)	7.2±0.5	7.2±0.8	6.0±0.7	0.603	0.077
NEFA (mmol/L•h)	-1.5±0.2	-1.0±0.3	-1.0±0.3	0.161	0.144

SEM; standard error of the mean, NEFA; non-esterified fatty acids

Supplemental Table S5 Total area under the curve (AUC) results on day 1 for biochemical measurements for each experimental condition (n = 22)

	Mean ± SEM			P-value	
	Sitting	**Standing**	**Walking**	**Standing vs. sitting**	**Walking vs. sitting**
Glucose (mmol/L•h)	46.5±1.2	44.0±1.1	44.1±1.1	0.004	<0.001
Insulin (mU/L•h)	661.7±81.6	560.9±80.1	475.2±85.9	0.048	0.018
Triglycerides (mmol/L•h)	16.5±1.4	18.7±1.5	17.8±1.5	0.641	0.571
NEFA (mmol/L•h)	-2.5±0.2	-3.0±0.2	-2.9±0.2	0.019	0.046

SEM; standard error of the mean, NEFA; non-esterified fatty acids

Supplemental Table S6. Total area under the curve (AUC) results on day 2 for biochemical measurements for each experimental condition (n = 17)

	Mean ± SEM			P-value	
	Sitting	Standing	Walking	Standing vs. sitting	Walking vs. sitting
Glucose (mmol/L•h)	44.9±0.9	43.9±1.0	43.7±1.0	0.102	0.082
Insulin (mU/L•h)	595.2±79.9	505.2±58.0	474.6±54.9	0.323	0.013
Triglycerides (mmol/L•h)	17.7±1.2	18.3±1.2	15.5±1.2	0.653	0.084
NEFA (mmol/L•h)	-2.2±0.1	-2.6±0.2	-2.5±0.2	0.009	0.033

SEM; standard error of the mean, NEFA; non-esterified fatty acids

Supplemental Table S7. Fasting biochemical values for each experimental condition (Day 1 and Day 2)

	Day 1	**Day 2**	**P value (Day 1 vs. Day 2)**
Glucose (mmol/L) – Sitting	5.4 ± 0.4	5.5 ± 0.4	0.878
Glucose (mmol/L) - Standing	5.5 ± 0.4	5.6 ± 0.4	0.902
Glucose (mmol/L) - Walking	5.4 ± 0.4	5.5 ± 0.3	0.898
Insulin (mu/L) - Sitting	15.6 ± 9.6	17.4 ± 11.1	0.471
Insulin (mu/L) - Standing	16.0 ± 8.6	20.1 ± 11.8	0.183
Insulin (mu/L) - Walking	16.4 ± 5.6	16.8 ± 10.7	0.823
NEFA (mmol/L) - Sitting	0.55 ± 0.16	0.49 ± 0.13	0.163
NEFA (mmol/L) – Standing	0.53 ± 0.13	0.48 ± 0.15	0.219
NEFA (mmol/L) –Walking	0.51 ± 0.11	0.47 ± 0.09	0.378
Triglycerides (mmol/L) – Sitting	1.54 ± 0.49	1.39 ± 0.36	0.363
Triglycerides (mmol/L) – Standing	1.66 ± 0.47	1.47 ± 0.37	0.121
Triglycerides (mmol/L) - Walking	1.56 ± 0.47	1.27 ± 0.43	0.093

Data presented as mean ± standard deviation

Supplemental Table S8. Physical activity data before and during each experimental condition (n = 22)

Experimental regimens	Mean (range) Days accelerometer worn	Wear-time (Minutes)	Sedentary time (Minutes)	Light activity (Minutes)	MVPA (Minutes)	Steps
			Mean ± SD			
Familiarisation visit	6 (4-7)	831±85	594±80	218±52	19±10	5024±2021
7 days prior to experimental condition 1	5 (4-6)	821±89	569±91	234±59	18±14	4731±2667
2 days prior to experimental condition 1	2	826±87	597±98	225±56	4±3	3399±2027
7 days prior to experimental condition 2	5 (4-6)	829±86	588±91	223±54	18±9	4822±1659
2 days prior to experimental condition 2	2	838±102	600±108	233±62	5±2	3612±1983
Day 1 - Sitting Condition	1	478±27	475±11	3±1	Nil	121±50
Day 1 - Standing Condition	1	475±11	448±13	27±5	Nil	197±64
Day 1 - Walking Condition	1	477±9	415±9	41±11	21±13	4818±1550
Day 2 – Sitting Condition	1	480±11	476±11	4±1	Nil	116±45

SD; standard deviation, MVPA; moderate-to-vigorous physical activity

Supplemental Table S9. ActivPAL results during each experimental condition

	Minutes ± SD		
	Sitting	**Standing**	**Walking**
Day 1 – Sitting condition	479.7±4.3	1.5±0.7	0.8±0.1
Day 1 – Standing condition	419.1±6.9	62.9±2.2	1.4±0.2
Day 1 - Walking condition	412.7±7.3	4.8±0.8	64.2±0.3
Day 2 – Sitting condition	481.1±4.0	1.7±0.4	0.5±0.1

SD; standard deviation