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Breaking up of prolonged sitting over three days sustains, but does not enhance, lowering of postprandial plasma glucose and insulin in overweight and obese adults

Short title: Cumulative effect of breaking up sitting time

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ABSTRACT

Aim: To compare the cumulative (three-day) effect of prolonged sitting on metabolic responses during a mixed meal tolerance test (MTT), with sitting that is regularly interrupted with brief bouts of light-intensity walking.

Research design and methods: Overweight/obese adults (n=19) were recruited for a randomized, three-day, outpatient, crossover trial involving: 1) 7-hour days of uninterrupted sitting (SIT); and, 2) 7-hour days of sitting with light-intensity activity breaks [BREAKS; 2-minutes of treadmill walking (3.2 km/hour) every 20 minutes (total: 17 breaks/day)]. On days 1 and 3, participants underwent a MTT (75g carbohydrate, 50g fat), and the incremental area under the curve (iAUC) was calculated from hourly blood samples. GEE models were adjusted for gender, BMI, energy intake, treatment order and pre-prandial values to determine effects of time, condition and time x condition.

Results: The glucose iAUC was 1.3 ± 0.5 and 1.5 ± 0.5 mmol.hr.L^{-1} (mean difference ± SEM) higher in SIT compared with BREAKS on days 1 and 3 respectively (condition effect: P=0.001), with no effect of time (P=0.48) or time x condition (P=0.8). The insulin iAUC was also higher on both days in SIT (Day 1: Δ151 ± 73, Day 3: Δ91 ± 73 pmol.hr.L^{-1}, P=0.01), with no effect of time (P=0.52) or time x condition (P=0.71). There was no between-treatment difference in triglycerides iAUC.

Conclusion: There were significant between-condition effects but no temporal change in metabolic responses to MTT, indicating that breaking up sitting over three days sustains, but does not enhance, the lowering of postprandial glucose and insulin.

INTRODUCTION

For most adults in developed countries, a substantial portion of the waking day is spent sedentary (sitting, reclining, or lying down with low energy expenditure)[1]. The adverse consequences of high durations spent sedentary are potentially manifold, as observational studies indicate that sitting time is adversely associated with cardio-metabolic risk biomarkers, type 2 diabetes, cardiovascular disease, some cancers and premature mortality [2, 3]. Furthermore, these associations generally remain after accounting for total or leisure-time moderate-to-vigorous physical activity (MVPA), suggesting that sedentary time can have detrimental health effects independent of MVPA.

In addition to total sitting time, the manner in which sedentary time is accumulated may also influence disease risk. Observational studies indicate that frequent breaks in sitting time (transitions between sitting to standing/movement) are associated with a more favorable cardio-metabolic risk profile than an equivalent amount of sitting time accumulated in longer, uninterrupted bouts [4, 5]. This is further supported by clinical trial evidence showing that regularly interrupting prolonged sitting with brief (~2-minute) bouts of walking acutely lowered postprandial plasma glucose and insulin concentrations when compared to uninterrupted sitting [6, 7]. Interrupting sitting with light-intensity walking bouts was as effective in reducing postprandial responses as were breaks of moderate-intensity walking [7]. Furthermore, these regular short activity breaks from sitting time were more successful in decreasing postprandial responses, compared to an equivalent duration of sitting preceded by a single bout of continuous physical activity [6]. Although the underlying mechanisms were not assessed in these studies, it is well known that muscle contractions can acutely stimulate glucose uptake via insulin-independent cellular mechanisms [8]. Therefore, it is possible that interrupting sitting time facilitates glucose removal from the circulation through intermittent muscle contractions.

It remains to be experimentally determined whether breaking up sitting time has cumulative effects when performed over a number of days. Previous studies involving varying durations (3 to 56 days) of bed rest, an extreme model of imposed physical inactivity, have shown substantial deterioration in insulin sensitivity and associated elevations in post meal glucose and insulin concentrations, with metabolic derangements observed as early as after three days [9-11]. To our knowledge, there is only one experimental study reporting on the effects of longer-term exposure (>1 day) to increases in sedentary behavior [12]. In the free-living environment, Lyden et al [12] showed that increased sitting time and avoidance of exercise for seven days were associated with increased postprandial insulin concentrations, when compared with an equivalent duration involving usual activity. This study also found that elevated insulin levels were associated with decreases in light intensity activity and longer bouts of uninterrupted sitting time (time >30 minutes and >60 minutes). However, the effect of interrupting sitting with frequent light intensity walking bouts over several days is yet to be investigated experimentally. We hypothesized that consecutive days of prolonged sitting would impair glycemic control and that breaking up sitting with short bouts of activity could potentially mitigate these adverse effects.

In an outpatient laboratory-based study involving overweight/obese sedentary adults, we compared the cumulative (3-day) effects of uninterrupted sitting on postprandial glycemic

control to a mixed meal tolerance test (MTT), with sitting interrupted by brief (2-minute) bouts of light-intensity walking.

METHODS

Study Overview

This randomized crossover trial was undertaken at the Baker IDI Heart and Diabetes Institute (Melbourne, Australia) between December 2010 and May 2012. Participants completed, in random order, three consecutive days of prolonged sitting (SIT) and three consecutive days of sitting with intermittent bouts of light-intensity walking (BREAKS), separated by a minimum 12-day washout period (see **Figure 1**). To eliminate potential bias, condition order was randomly assigned by a third party using computer-generated random numbers (block randomization and balanced block sizes), stratified by gender. Study personnel were blinded to the condition order until the initial informed consent appointment.

Days 1 and 3 of each experimental condition were designated "assessment days" and involved measurements of plasma glucose, insulin and triglyceride concentrations during a MTT. Primary outcomes were changes in area under the curve (AUC) for plasma glucose and insulin, with triglyceride AUC being a secondary endpoint. Based on estimates of population variability (mean post-intervention to baseline glucose AUC ratio 1.1, SD:0.01; mean insulin AUC ratio 1.6, SD:0.3) from our own laboratory and a conservative correlation of 0.50 for repeated measures, it was estimated that 15 participants would provide 80% power (two-sided, 5% level) to detect the smallest clinically meaningful change of the outcome variables. To compensate for potential withdrawals, 24 participants were recruited.

Participants

Sedentary overweight/obese (BMI=25-45 kg/m^2) adults (aged 45-75 years) were recruited from local community advertisements. Participant characteristics are presented in **Table 1.** All were sedentary (self-reported sitting time > 5hours/day), non-diabetic, non-smoking, not taking glucose- or lipid-lowering medications or on anti-coagulant therapy, and not meeting current physical activity guidelines [13]. Of the women who participated, six were postmenopausal and two were perimenopausal. For peri-menopausal women, the menstrual cycle phase could not be determined during the experimental conditions. Written informed consent was obtained from each participant following an explanation of the experimental procedures and the risks involved. The study was carried out in accordance with the principles of the Declaration of Helsinki (2008) and was approved by the Alfred Human Ethics Committee. This trial was registered with the ANZCTR clinical trial registry (ACTRN12610000657022).

Study Protocol

Figure 1 shows the study protocol. Participants were asked to refrain from exercise, caffeine, or alcohol for 48 hours prior to each experimental condition. In the week preceding the experimental conditions, they wore Actigraph GTM1 accelerometers (Actigraph, Pensacola FL) during waking hours for objective measurements of physical activity. The one-minute epoch activity data were processed to derive average sedentary (<100 counts per minute, cpm), light-intensity activity (100-1951 cpm), and MVPA (1952+ cpm) time on valid days

[14]. Weighed/measured food records and Foodworks nutritional software (Xyris, Australia) were used to quantify total energy and macronutrient intakes 48 hours prior to experimental conditions.

To eliminate any diet-induced variability in study outcomes, dietary intakes were strictly controlled starting from the evening meal prior to day 1 to breakfast on day 3. Meal plans were individualized to meet daily estimated energy requirements (Schofield equation, 1.5 physical activity factor) [15] and a target macronutrient profile of 12-15% energy from protein, 55-58% energy from carbohydrate and 29-31% energy from fat. Meals were standardized across conditions and were carefully prepared by research staff in the laboratory kitchen. To accommodate dietary preferences, participants were able to select from a range of meal options, including bran-based cereal or muesli and toast at breakfast, and a meat and salad bread roll at lunch. An evening meal pack, consisting of a commercially available drink, snack and microwave meal, was also provided for participants to prepare at home.

On experimental days, participants were instructed to minimize movement and to drive, or be driven, to the research laboratory. Participants arrived between 0800 and 0900, after a 12-hour overnight fast. They then remained seated for the initial hour to achieve a steady-state before completing one of the following protocols over the next 6 hours:

1) SIT – On all days, participants sat upright in a comfortable chair and had access to a television, DVDs and reading materials (newspapers and magazines). They were required to minimize excessive movement, but were allowed to visit the toilet when necessary. At the completion of the experimental protocol, participants were instructed to return home and limit movements to only activities that were necessary for daily living.

2) BREAKS – The experimental protocol was the same as for SIT, with the exception that participants rose from the seated position every 20 minutes and completed a 2-minute bout of light-intensity walking on a motorized treadmill (level surface) at 3.2km/hr. After each walking bout, they rated their perceived exertion using the standard 6-20 Borg scale to ensure that walking bouts were classified as being light-intensity (acceptable range 6-11).

Meal tolerance test

On assessment days, an indwelling catheter was inserted into an antecubital vein for the hourly collection of blood. Following the steady-state period, participants completed a 4-hour MTT. The mixed meal was provided as a 200mL strawberry-flavoured drink, containing 75g carbohydrate (100% corn maltodextrin) and 50g fat (Calogen: Nutricia, Australia). The MTT was used for the simultaneous measurement of postprandial glucose, insulin and triglyceride responses [16], and as MTTs show strong agreement with oral glucose tolerance test-derived model-based estimates of insulin sensitivity[17, 18]. Blood samples were collected each hour immediately prior to the scheduled walking bout.

Assessment of metabolic outcomes and model-based estimates of insulin sensitivity

Plasma glucose, insulin and triglyceride concentrations were analyzed with a commercial analytical system (Architect ci16200, Abbott Diagnostics). Glucose and triglycerides were

measured on the day of testing using hexokinase (intra-assay CV=1.1%) and glycerol phosphate oxidase methods (intra-assay CV=1.3%), respectively. Insulin samples were collected and stored at -80°C for later testing using a chemiluminescent microparticle immunoassay (intra-assay CV:2.9-4.3%).

Metabolic outcomes from the MTT were assessed using net incremental AUC (iAUC) and total AUC (tAUC) using the trapezoidal method, where net iAUC is calculated from basal levels, and tAUC is taken from a plasma concentration of zero. The HOMA-IR index was calculated using fasting plasma glucose and insulin concentrations ([glucose(mmol/l) × insulin(pmol/ml)] / 135) [19]. Basal insulin secretion was assessed by HOMA-β%, which was calculated as (fasting plasma insulin[pmol/l] × 3.33)/ (fasting plasma glucose[mmol/l] - 3.5). Skeletal muscle insulin sensitivity index (MISI) was assessed from postprandial changes in glucose and insulin concentrations during the MTT. According to Abdul-Ghani et al [20], skeletal muscle insulin sensitivity is reflected by the rate of decline (dG/dt) in postprandial glucose concentrations (mg/L) divided by the mean plasma insulin concentration (mU/L), where dG/dt was calculated as the slope of least squares fit from peak glucose concentration to nadir. This model correlates strongly with muscle insulin sensitivity determined from a hyperinsulinemic euglycemic clamp [20].

Statistical analysis

This study was analyzed on a per-protocol basis. Randomized participants who did not complete the first experimental condition were excluded from the analysis (see **Figure 2**). Reasons for exclusion were: unable to comply with experimental protocol (MTT or treadmill walking, n=3), participant withdrawal (n=1) and diagnosis of type 2 diabetes post screening (n=1), an exclusion criterion.

Generalized estimating equations with exchangeable working correlation were used to evaluate the differential effects of time, experimental condition and the differences in time course between groups (time x condition interaction) using Stata statistical analysis software (Version 12, StataCorp LP). All models were adjusted for potential covariates explaining residual outcome variance (age, gender and BMI), pre-prandial values (iAUC only) and period effects (treatment order). Due to between-condition discrepancies at baseline, models were also adjusted for the experimental change in energy intake and time spent doing light-intensity physical activity.

RESULTS

Potential confounding diet and physical activity variables

According to weighed/measured food records, energy intakes before both experimental conditions were lower than energy intakes during the controlled-feeding period (see **Table 2**). However, a lower energy intake during the pre-experimental period for BREAKS resulted in a greater experimental increase when compared with the SIT condition [+2682 ± 602 kJ (mean ± SEM) vs +1800 ± 592 kJ, P=0.02]. Other dietary changes induced by controlled feeding were increases in carbohydrate intake (P<0.001 for both conditions), and decreases in protein (P<0.001 for both conditions) and fat (P<0.05 for both conditions) intakes.

In the week preceding experimental conditions, monitor-wear time, sedentary time and time spent in MVPA were not different between conditions. However, time spent in light-intensity activities was greater for SIT than BREAKS, indicating a greater experimental decrease in light-intensity physical activity in SIT when compared with BREAKS (-124 ± 18 min vs -59 ± 17 min, $P<0.001$). From habitual levels, participants decreased the time spent in light-intensity physical activity ($P<0.01$ for both conditions) and MVPA ($P<0.05$ for both conditions), whereas sedentary time was not different.

Postprandial responses to the MTT

Mean fasting glucose levels were similar for both conditions on the respective assessment days, with both conditions demonstrating a small decrease over time ($P<0.001$). After adjusting for potential confounders, the iAUC for glucose, which calculates postprandial glycemic response from fasting levels, was significantly lower during BREAKS ($P=0.001$, see **Figures 3a and 3b**), with iAUC being 32% and 31% lower than the prolonged sitting condition on days 1 and 3 respectively. Similarly, tAUC for glucose, which reflects the total exposure to plasma glucose concentrations, was significantly lower in BREAKS than SIT ($P=0.005$, see **Table 3**). Plasma glucose concentrations at 2 hours and dG/dt were also significantly lower in BREAKS than SIT ($P=0.012$ and $P=0.037$ respectively, see **Table 3**). For all measures of postprandial glycemia, there was no effect of time or between-condition differences in the time course (time x condition effect) across the 3-day protocol.

For fasting insulin, there was no effect of time or difference between conditions. On days 1 and 3, iAUC for insulin was 15% lower in BREAKS than the SIT condition ($P=0.01$, see **Figures 3c and 3d**); whereas absolute exposure to plasma insulin (tAUC) was 12% lower on both days in the BREAKS condition than SIT ($P=0.009$, **Table 3**). However, there was no effect of time or difference in the temporal change in postprandial insulinemia. The AUC ratio, which evaluates the overall insulin response to variations in postprandial glycemia, was significantly lower in BREAKS compared with SIT, suggesting less insulin release with breaks in prolonged sitting.

Fasting triglycerides and postprandial triglyceride responses increased in both conditions across time [$P<0.001$ for fasting triglycerides, $P<0.001$ for tAUC (see **Table 3**) and $P=0.049$ for iAUC (see **Figures 3e and 3f**)], with no significant differences between conditions.

Model-based assessments of beta-cell function, hepatic insulin sensitivity and peripheral insulin sensitivity

There was no difference between conditions or in the temporal change in HOMA-IR or MISI. However, there was a significant increase in HOMA-β% over time, but no effect of condition or between-group difference in the time course across the 3-day protocol.

DISCUSSION

Compared to uninterrupted sitting, short bouts of light-intensity walking every 20 minutes attenuated the glycemic response to the MTT, as demonstrated by 31-32% reduction in the incremental glucose response on days 1 and 3. The strongest between-condition difference

was observed between 1 and 3 hours, as plasma glucose concentrations continued to rise after 60 minutes in the sitting condition and were markedly higher than BREAKS at 2 hours. By means of extrapolation based on classifications of hyperglycemia [21], the magnitude of the reduction at 2 hours circumvented plasma glucose concentrations rising to levels consistent with impaired glucose tolerance, a condition that progresses to type 2 diabetes at a rate of 6% per year [22]. Despite significant between-condition effects, no temporal change in the relative hyperglycemia was observed across the 3-day experimental period, suggesting that there was no metabolic maladaption to repeated days of prolonged sitting. Although observational studies indicate that more frequent breaks in sedentary time are associated with more favourable cardio-metabolic biomarker profiles [2, 3], there are no prospective or clinical trials that provide clear evidence of a possible cause-and-effect relationship. Nevertheless, dietary factors and interventions that lower postprandial glycemic responses have been associated with significant reductions in diabetes and cardiovascular risk in long term clinical [23] and prospective observational studies [24], suggesting that lowering postprandial glycemia *per se* may reduce disease risk. Therefore, the relative reduction in postprandial glycemia seen in our study provides a putative mechanism through which breaking up sitting time via short bouts of activity could reduce the long-term risk of developing diabetes and cardiovascular disease.

Our findings concur with those of previous trials that have shown that increasing physical activity during the postprandial period attenuates the glycemic response to meals. However, previous trials vary greatly with respect to the timing (relative to meal), frequency (single vs intermittent), intensity and duration of the physical activity bouts. Nygaard et al [25] demonstrated that 40 minutes of slow post-meal walking produced a similar blood glucose lowering effect as 30 minutes of cycling at 70% of maximal heart rate [26] and 45 minutes of cycling at 57% of maximal oxygen consumption [27], indicating that the blunting effect was not directly proportional to the physical activity intensity level. Presumably, postprandial physical activity duration is another factor likely to affect tissue glucose uptake and utilization. However, Lunde et al [28] showed that doubling the postprandial physical activity time from 20 to 40 minutes did not double the reduction in glycemia (31% vs 39% reduction in iAUC respectively when compared to sitting). Takaishi et al [29] showed that a single 6-minute bout of stair climbing-descending during the absorptive postprandial phase was sufficient to lower the glycemic response when compared to sitting. However, when the 6-minute bout was implemented before the blood glucose peak there was a further rise in postprandial glycemia. In a study similar to our own, Peddie et al [6] demonstrated that interrupting sitting time with frequent short (<2-minute) activity breaks reduced the day-long glycemic response by 37% when compared with a single 30-minute bout of continuous physical activity that preceded prolonged sitting. Importantly, those findings [6] highlight that equivalent amounts of physical activity, accumulated as a single continuous bout or as several short bouts over the course of the day, have distinct effects on blood glucose metabolism.

Clearly, more research is needed on the dose-response of higher frequency and longer duration physical activity, including activity at the lower end of the intensity continuum. In our study, we acknowledge that for those who are unfit and overweight/obese, or for the elderly, that 3.2km/hr is close to the historical threshold of 3 METs, which defines moderate-intensity and increases the propensity for some exercise-like responses. However, our previous study found walking at 5.6-6.2km/hr was no more efficacious than breaks that were 3.2km/hr [7]. Current public health guidelines state that 30 minutes of moderate-intensity physical activity can be accumulated throughout the day in bouts >10-minutes [30]. Although

the total accumulated time was similar (34 minutes) in our study, the relative reduction in postprandial hyperglycemia with shorter and less intense bouts makes this a potentially feasible strategy for those who cannot (or behaviorally will not) tolerate exercise at a higher intensity. Furthermore, as light intensity physical activity manifests in several typical domestic and occupational tasks, such as standing, light yard/house work (eg. cleaning dishes, ironing), and casual walking, this makes it a potentially feasible strategy and may be incorporated into a variety of settings (office, home etc).

The sustained rise in glucose levels with prolonged sitting is most likely related to the redundancy of skeletal muscle contractions, as breaks in sitting would increase muscle glucose uptake by virtue of associated contractile activity. Muscle contractions stimulate blood glucose clearance, independent of insulin, but can also act synergistically with insulin to enhance muscle glucose uptake [8]. These effects may be mediated by metabolic factors within the cell (ie. AMPK and calcium activated proteins) [31], as well as hemodynamic changes (ie. blood volume increment, tissue perfusion and capillary permeability) [32-34] associated with the muscular activity. The metabolic benefit of breaking up sitting in our study is highlighted by the flattening of the glucose curve, evidenced by the lower dG/dt, and the lower insulin to glucose ratio, indicating greater economy in insulin secretion. In contrast, the higher insulin to glucose ratio in SIT suggests that glucose clearance is largely regulated by plasma insulin. This can present a "challenge" to glucose homeostasis between meals when carbohydrate absorption from the gastrointestinal tract decline and persistently high insulin levels cause a rapid decline in blood glucose concentrations. Although not specifically measured, a higher dG/dt may promote reactive hypoglycaemia, counterregulatory hormone secretion and elevated free fatty acid concentrations [35]. Therefore, the negative metabolic consequences of prolonged sitting may include a complex interplay of several factors involving: i) elevated counter-regulatory hormones and free fatty acids, (ii) a redundancy of hemodynamic changes, and (iii) a dependency on insulin-mediated glucose uptake, which in some individuals may be impaired.

Contrary to our proposed hypothesis, we saw no effect on model-based assessments of hepatic and peripheral insulin sensitivity following three days of BREAKS when compared to SIT. One possible explanation is the significant heterogeneity in baseline measures of insulin sensitivity. This study included older overweight/obese individuals, based on observational research suggesting that this demographic is at greatest risk of developing type 2 diabetes. However, it should be noted that this group included both mildly overweight and morbidly obese individuals who are likely to vary in their treatment response. In BREAKS, we observed an inverse association between baseline insulin sensitivity and the day 1-3 change in MISI [r=-0.57, P=0.011 (data not shown)], indicating that over time, breaking up sitting had a greater impact in individuals with poorer muscle insulin sensitivity. This is consistent with evidence suggesting that the metabolic benefit of exercise interventions is greater in type 2 diabetic patients than normal controls [36] and more evident in diabetic patients with greater body weight, fat accumulation and poorer diabetic control [37]. However, no relationship (positive or otherwise) was observed between baseline insulin sensitivity and the temporal change in MISI in the sedentary condition. This might be because we recruited sedentary individuals, and as such, the accumulated sitting time during the experimental conditions was not significantly different from objective measurements taken at baseline. This is contrast to Lyden et al [12], who, in physically active individuals, showed that reducing leisure time physical activity and increasing sitting time for seven days decreased insulin action. Previous

bed rest studies that show worsening insulin sensitivity have failed to report the level of experimental change from habitual levels of physical activity/sedentary behaviors. As these studies have primarily involved trained and healthy individuals, who are likely to be more physically active than the current cohort, a greater impact on metabolic variables and measures of insulin sensitivity would be expected, especially with such an extreme model of physical inactivity.

It is also possible that changes in insulin sensitivity are not seen either until an individual shows clinically significant weight gain or there is significant caloric excess. One of the tenets of the inactivity physiology paradigm is that energy intakes are not normally reduced to match the low energy expenditure associated with prolonged sitting, resulting in a situation of energy surplus [38]. In our study, baseline weights were precisely maintained across protocols as energy intakes approximated the low level of energy expenditure. However, as energy intakes (and meals) were standardized across conditions, small between-condition differences in the energy expenditure were created throughout the day with the addition of the short walking bouts. In an individual weighing 90.5kg, it is estimated that treadmill walking at 3.2km/hour would create a difference in energy expenditure of only 9kCal per bout [39]. We suspect that the net sum of the breaks (~153kCal/day) was not sufficient in magnitude and/or duration to significantly impact on insulin sensitivity. In line with this notion, Stephens et al [40] showed that, when a day of prolonged sitting was accompanied by a 1000kCal/d energy surplus, insulin action determined from glucose infusion technique was 26% lower compared to when energy intakes approximated the energy expenditure of prolonged sitting. Furthermore, compared to a day of minimal sitting (involving standing and ambulation, ~45min/hr), whole-body insulin action was not significantly different from prolonged sitting when both conditions were in approximate energy balance (+162kCal/d vs -30kCal/d respectively). However, there was a trend towards improved insulin sensitivity in the minimal sitting condition, suggesting that factors other than energy surplus might be involved in the decline in insulin action.

Interestingly, in both of our experimental conditions there were changes over time in certain outcome variables (fasting glucose, fasting triglycerides, postprandial triglycerides and HOMA-%β), suggesting that these variables do not reflect the dynamic circumstances associated with breaking up sitting time. Another aspect of the experimental protocol could also account for these changes; however, a number of potentially relevant variables were not measured (eg. NEFA and markers of de novo lipogensis). Thus, we can only speculate as to the underlying mechanisms. The strictly controlled diet, specifically the increase in the relative contribution of carbohydrates to total energy, may be relevant. High carbohydrate diets can increase fasting and postprandial triglycerides via hepatic de novo lipogenesis [41-43], an effect that can occur within three days of feeding [42]. However, we cannot exclude the possibility that the small reduction in baseline levels of physical activity may have contributed to a reduction in lipoprotein lipase activity and peripheral clearance of triglycerides [38]. HOMA-%β also increased in both conditions over time, characterized by a reduction in fasting glucose. Reasons for the change in HOMA-%β remain unknown, but may relate to a role of postprandial hypertriglyceridemia on beta cell function [44]. However, HOMA-β% was assessed in the basal state and dynamic measures of beta-cell function may be more appropriate in the context of the current experimental design.

Strengths of our study include: the strict supervision of experimental conditions; the statistical adjustment for baseline discrepancies in diet and physical activity; and controlled feeding during experimental conditions, which avoided the confounding influence of large changes in energy balance. However, there are limitations. First, the simultaneous implementation of diet and activity protocols meant that we could not dissociate time-specific effects of diet from that of the experimental conditions. Second, we suspect that the small sample size and/or the inherent natural variation in the sample population hindered our ability to detect between-treatment differences in insulin sensitivity. Other considerations include the use of surrogate measures of insulin sensitivity and the basal-state assessment of beta-cell function.

In conclusion, our findings are consistent with our previous single-day study and the theoretical suggestion of metabolic activation with intermittent bouts of gentle walking during prolonged sitting. Although there were no cumulative short-term effects on metabolic outcomes, the between-condition differences further highlight the need to examine the chronic effects of repetitive (daily) exposure to breaking up prolonged sitting on postprandial hyperglycemia and hyperinsulinemia, as well as the potential benefit to certain patient populations (eg. type 2 diabetes, morbidly obese) and the mechanisms and pathways that connect acute observations to a reduction in the development of chronic disease.

CLINICAL PERSPECTIVES

Recent experimental studies have demonstrated that regularly breaking up a single bout of prolonged sitting, even with short duration, light-intensity physical activity breaks, acutely lowers postprandial hyperglycemia. However, this effect has not been tested over multiple days in experimental studies. This manuscript reports novel findings from a randomized, controlled laboratory trial in which regularly breaking up prolonged sitting over three days sustains, but does not enhance, the lowering of postprandial glucose and insulin. Given that postprandial hyperglycemia (together with related hyperinsulinemia) have been implicated in the development of chronic metabolic diseases such as type 2 diabetes and cardiovascular disease, breaking up sitting time with short bouts of activity might be a long-term potential strategy to help reduce disease risk, particularly for at-risk populations (eg. obese, elderly) or when more intense forms of physical activity cannot be tolerated.

AUTHOR CONTRIBUTIONS

DWD, BAK, RNL, EC, JES, GNH, MTH and NO were involved in the concept and design of the study. RNL, CR and LH were involved in participant recruitment, data collection and ensured that trial was conducted according to the specified protocol. EC was responsible for the statistical analysis of the data. RNL wrote the manuscript and editing of the manuscript for important intellectual content was done by BAK, NO, EC and DWD. Final manuscript revisions were done by JES, GNH and MTH. DWD obtained funding and takes full responsibility for the work as a whole, including the study design, access to data and the decision to submit and publish the manuscript.

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DECLARATION OF CONFLICTING INTERESTS

None declared.

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Table 1: Participant characteristics

Demographics	
Age, yr	56.7 (1.5)*
Male No. (%)	11 (58%)
Occupation (%)	
Retired	8 (42%)
Unemployed	2 (11%)
Housewife	2 (11%)
Working part time	2 (11%)
Clerical/administrative work	2 (11%)
Public service/health work	3 (11%)
Anthropometrics	
BMI, kg/m^2	32.7 (1.0)
Waist circumference, cm	108.3 (2.7)

*Data are expressed as means (SEM) or number (%) where specified.

Table 2: Dietary and activity variables during the pre-experimental period and the change observed with implementation of experimental protocols

	SIT	BREAKS
Dietary intakes		
Energy, kJ per day		
Pre-experimental period*	9360 (556)	8478 (561) §
Experimental change†	+1800 (592)	+2682 (602) §
Carbohydrate, % of total kJ		
Pre-experimental period	49.1 (1.8)	51.6 (1.6)
Experimental change	+8.3 (1.8)	+5.7 (1.6)
Protein, % of total kJ		
Pre-experimental period	17.0 (0.6)	17.1(0.6)
Experimental change	-3.3 (0.6)	-3.3 (0.6)
Fat, % of total kJ		
Pre-experimental period	34.3 (1.6)	31.6 (1.3)
Experimental change	-4.4 (1.8)	-1.8 (1.5)
Accelerometer data		
Daily wear time, min		
Pre-experimental period‡	821 (22)	797 (25)
Experimental change	-101 (26)	-54 (22)
Sedentary, min/day		
Pre-experimental period	538 (14)	540 (18)
Experimental change	+36 (19)	+17 (16)
Light-intensity, min/day		
Pre-experimental period	254 (16)	229 (17) §
Experimental change	-124 (18)	-59 (17) ¶
Moderate-vigorous, min/day		
Pre-experimental period	29 (6)	28 (7)
Experimental change	-13 (4)	-12 (5)

*Dietary intakes were assessed from weighed/measured food records during the 48-hour period before the trial

†Defined as the change from the pre-experimental period

‡Data from the week preceding the experimental condition. No differences were observed during the habitual and restricted periods.

Paired t-test revealed significant between condition differences, §P<0.05 ¶P<0.01

Data are expressed as means (SEM)

Table 3: Basal and postprandial responses to the MTT and model-based estimates of insulin sensitivity and beta-cell function

	SIT		BREAKS		*P**		
	Day 1	Day 3	Day 1	Day 3	Condition	Time	Condition x Time
Weight (kg)	94.6 (14.5)†	94.6 (14.4)	94.5 (14.4)	94.5 (2.9)	0.922	0.971	1.000
Fasting glucose (mmol/l)	5.1 (0.5)	5.0 (0.4)	5.2 (0.4)	5.0 (0.4)	0.955	<0.001	0.425
2 hour glucose (mmol/l)	8.1 (2.8)	7.9 (3.0)	7.1 (2.6)	7.2 (2.6)	0.012	0.849	0.739
dG/dt ($mg.l^{-1}.hr^{-1}$)[1]	83.4 (8.1)	85.0 (8.1)	68.7 (8.1)	70.2 (8.1)	0.037	0.808	0.150
Glucose tAUC ($mmol.hr.l^{-1}$)	30.4 (1.2)	29.8 (1.2)	29.0 (1.2)	28.5 (1.2)	0.005	0.148	0.793
Fasting insulin (pmol/l)	71.4 (5.4)	75.2 (5.7)	67.9 (5.1)	71.4 (5.4)	0.326	0.246	0.740
Insulin tAUC ($pmol.hr.l^{-1}$)	1474 (162)	1484 (163)	1301 (143)	1309 (144)	0.009	0.859	0.879
$AUC_{ins/glu}$	48.8 (4.8)	50.0 (4.9)	44.3 (4.4)	45.3 (4.5)	0.037	0.526	0.983
Fasting triglycerides (mmol/l)	1.5 (0.1)	1.8 (0.1)	1.5 (0.1)	1.8 (0.1)	0.828	<0.001	0.384
Triglyceride tAUC ($mmol.hr.l^{-1}$)	8.2 (0.7)	10.2 (0.8)	8.1 (0.7)	10.1 (0.8)	0.786	<0.001	0.522
Muscle insulin sensitivity index	2.41 (0.32)	2.40 (0.32)	2.17 (0.32)	2.16 (0.32)	0.276	0.969	0.353
HOMA-IR	2.73 (0.21)	2.77 (0.22)	2.61 (0.21)	2.65 (0.21)	0.404	0.744	0.691
HOMA-β%	150 (12)	179 (14)	138 (11)	166 (13)	0.121	<0.001	0.955

Abbreviations: MTT = meal tolerance test, dG/dt = rate of decline in plasma glucose concentration, tAUC= Total area under the curve (from a plasma concentration of zero), $AUC_{ins/glu}$ = ratio of the total areas under the curve for insulin and glucose, HOMA-IR= homeostasis model assessment of insulin resistance, HOMA-%β = homeostasis model assessment of beta-cell function

*GEE models were adjusted for age, sex, BMI, treatment order, pre-condition light-intensity physical activity time and the experimental change in energy intake.

†Data are expressed as estimated marginal means (SEM)

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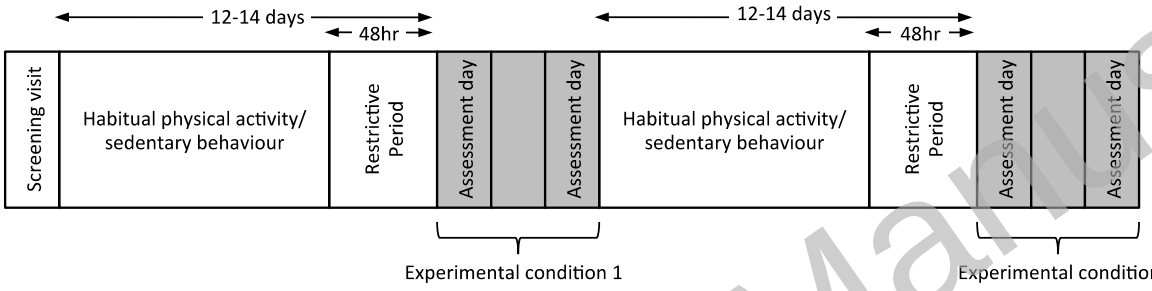

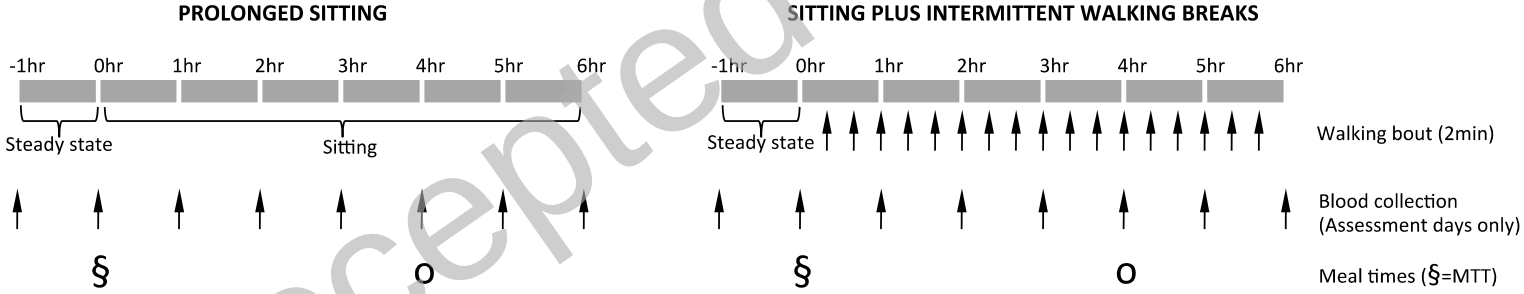

FIGURE 2: Participant flow diagram

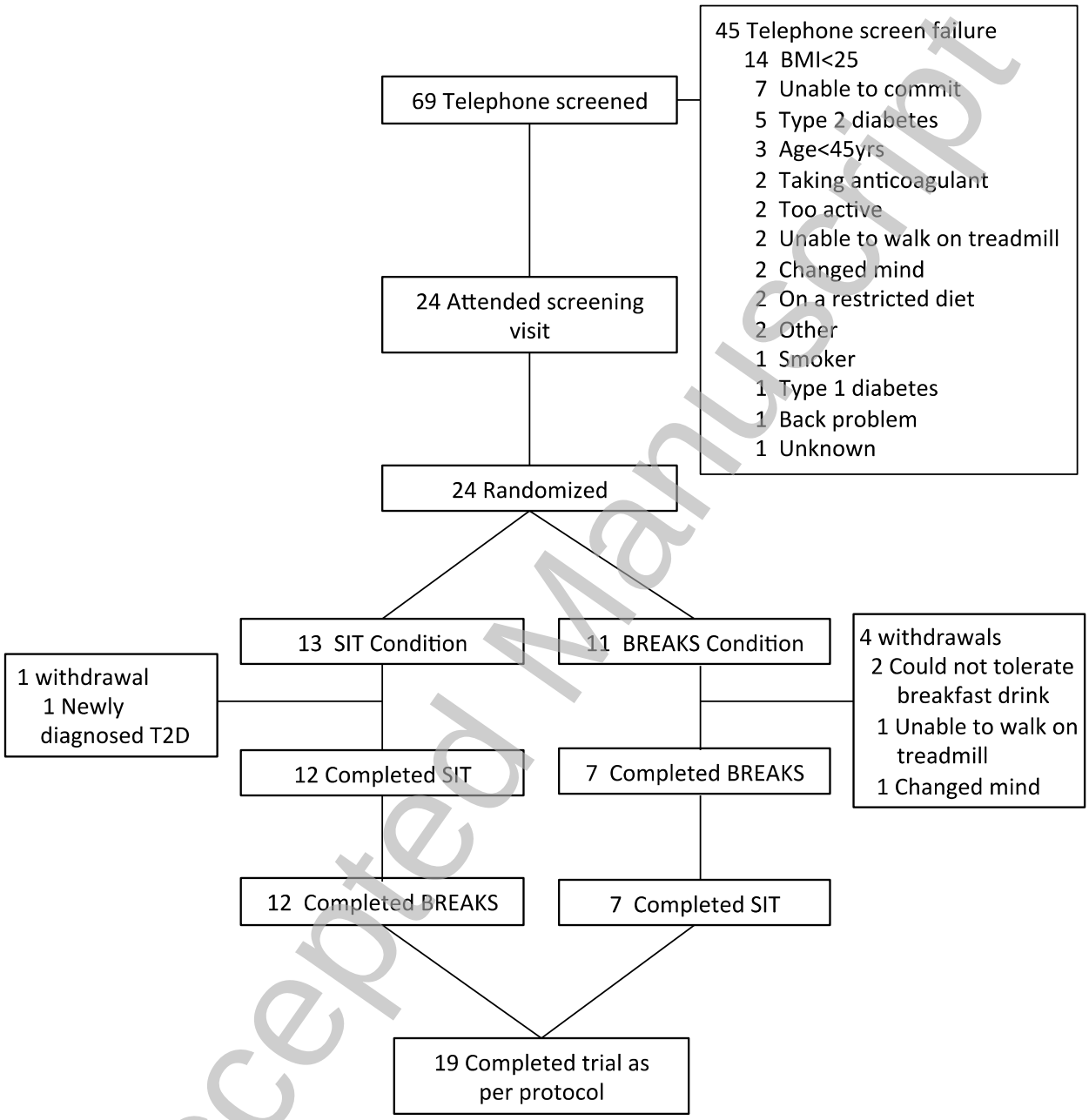

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FIGURE 3: (a) Unadjusted postprandial glucose responses to the MTT on day 1 and day 3 of experimental protocols. Circles = BREAKS; Squares = SIT; Solid marker + solid line = Day 1; Open marker + broken line = Day 3

(b) Estimated marginal means for glucose iAUC (adjusted for age, sex, BMI, treatment order, fasting glucose, pre-condition light-intensity physical activity time and the experimental change in energy intake) and error bars SEM. Circles = BREAKS; Squares = SIT

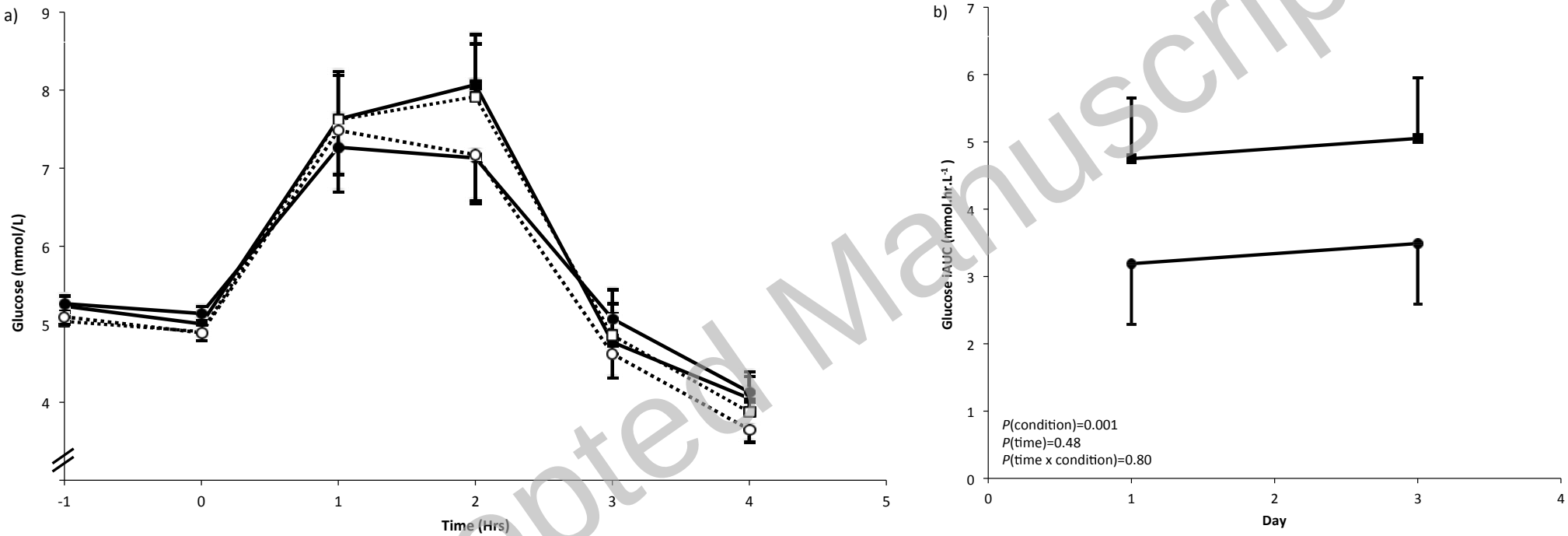
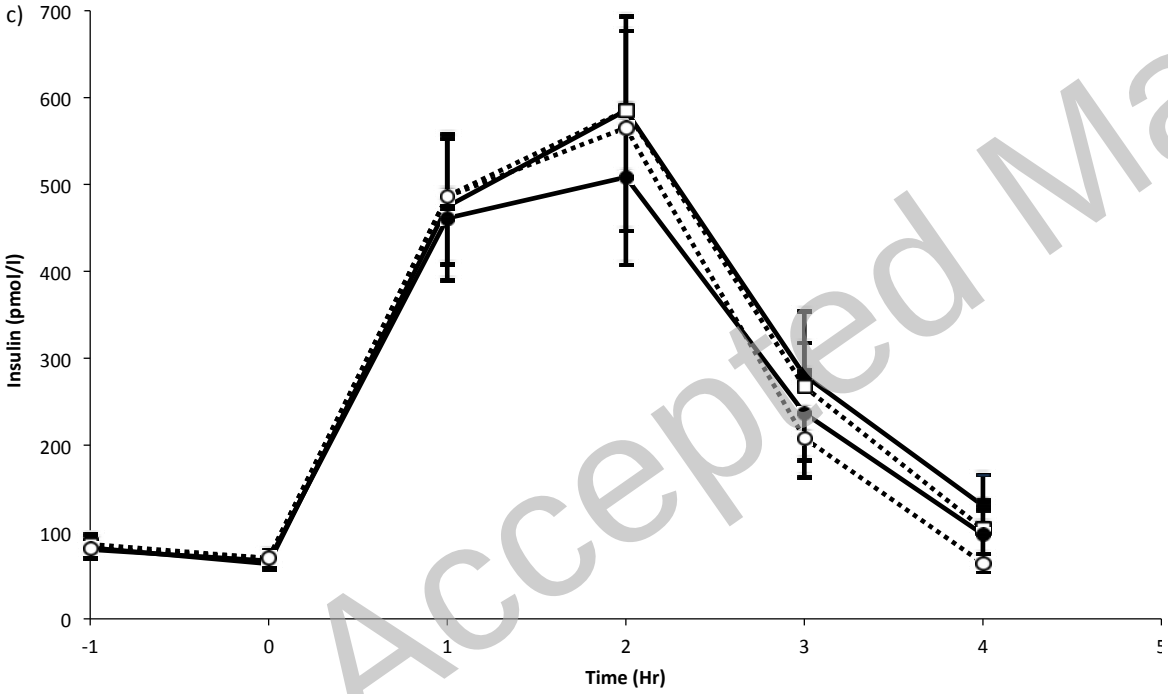


FIGURE 3: (c) Unadjusted postprandial insulin responses to the MTT on day 1 and day 3 of experimental protocols. Circles = BREAKS; Squares = SIT; Solid marker + solid line = Day 1; Open marker + broken line = Day 3
(d) Estimated marginal means for insulin iAUC (adjusted for age, sex, BMI, treatment order, fasting insulin, pre-condition light-intensity physical activity time and the experimental change in energy intake) and error bars SEM. Circles = BREAKS; Squares = SIT



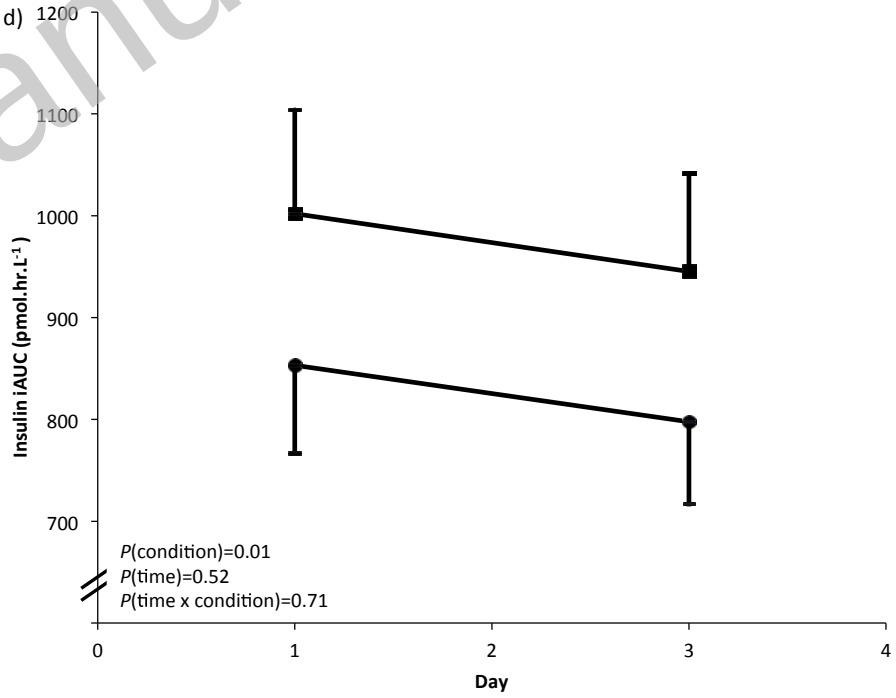
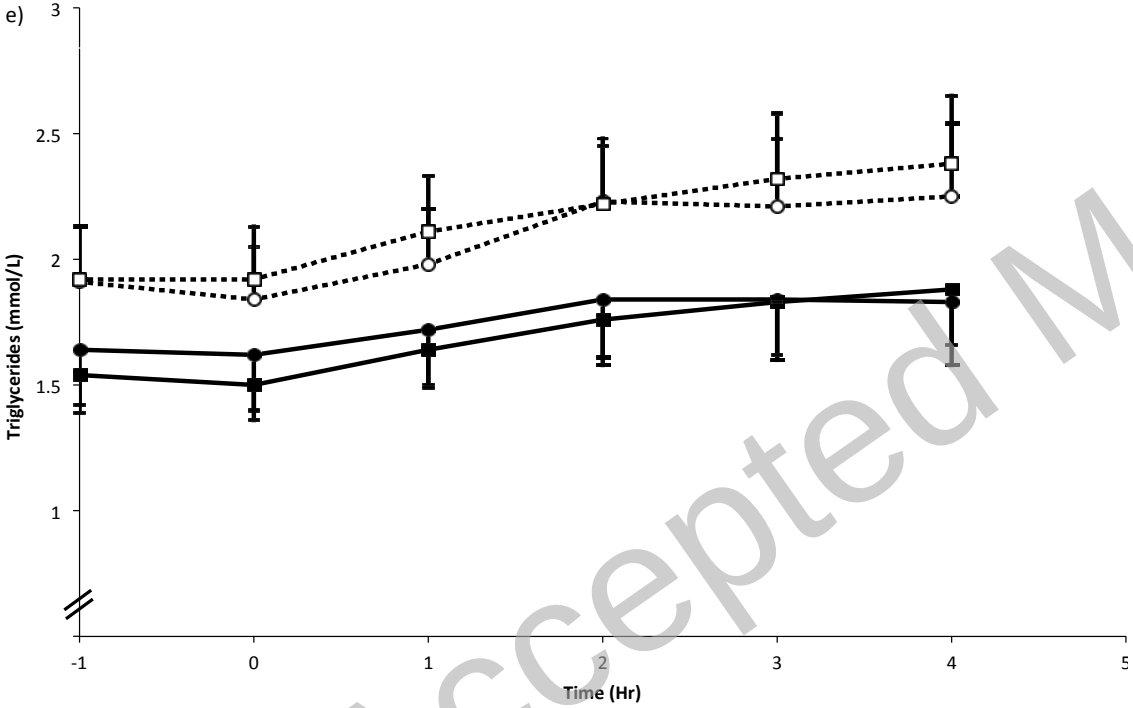
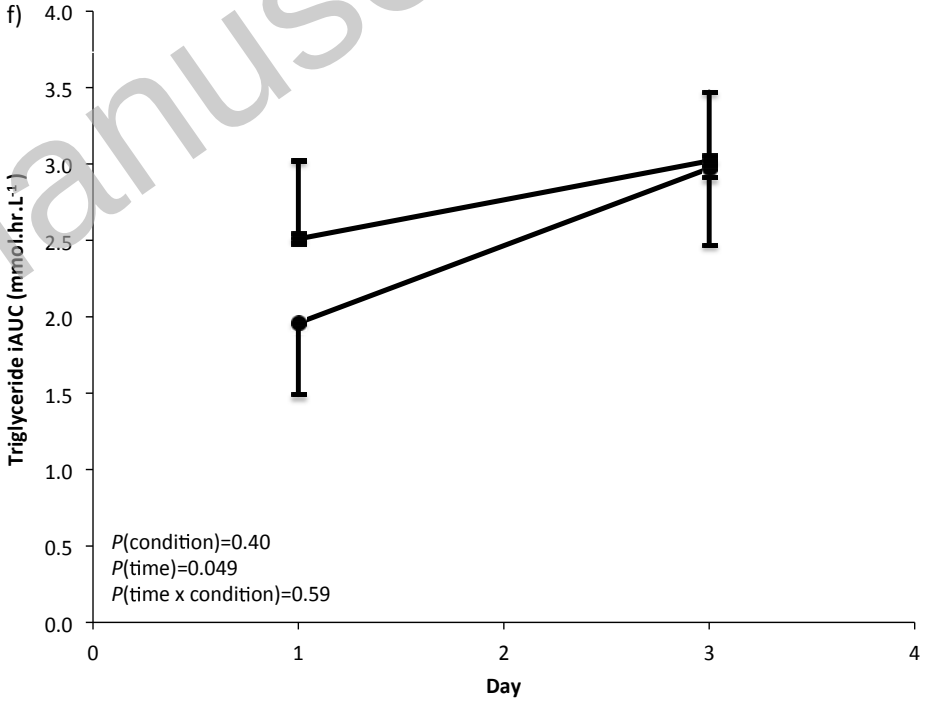


FIGURE 3: (e) Unadjusted postprandial triglyceride responses to the MTT on day 1 and day 3 of experimental protocols. Circles = BREAKS; Squares = SIT; Solid marker + solid line = Day 1; Open marker + broken line = Day 3
(f) Estimated marginal means for triglyceride iAUC (adjusted for age, sex, BMI, treatment order, fasting triglycerides, pre-condition light-intensity physical activity time and the experimental change in energy intake) and error bars SEM. Circles = BREAKS; Squares = SIT





SUMMARY STATEMENT

Breaking up prolonged sitting with frequent short breaks of gentle walking over three days sustains, but does not enhance, the lowering of post-meal glucose and insulin responses in overweight and obese individuals.