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ARTICLE

Interrupting prolonged sitting in type 2 diabetes: nocturnal persistence of improved glycaemic control

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Abstract

Aims/hypothesis We aimed to examine the effect of interrupting 7 h prolonged sitting with brief bouts of walking or resistance activities on 22 h glucose homeostasis (including nocturnal-to-following morning hyperglycaemia) in adults with type 2 diabetes.

Methods This study is an extension of a previously published randomised crossover trial, which included 24 inactive overweight/obese adults with type 2 diabetes (14 men; 62 ± 6 years) who completed three 7 h laboratory conditions, separated by 6–14 day washout periods: SIT: (1) prolonged sitting (control); (2) light-intensity walking (LW): sitting plus 3 min bouts of light-intensity walking at 3.2 km/h every 30 min; (3) simple resistance activities (SRA): sitting plus 3 min bouts of simple resistance activities (alternating half-squats, calf raises, brief gluteal contractions and knee raises) every 30 min. In the present study, continuous glucose monitoring was performed for 22 h, encompassing the 7 h laboratory trial, the evening free-living period after leaving the laboratory and sleeping periods. Meals and meal times were standardised across conditions for all participants.

Results Compared with SIT, both LW and SRA reduced 22 h glucose [SIT: 11.6 ± 0.3 mmol/l, LW: 8.9 ± 0.3 mmol/l, SRA: 8.7 ± 0.3 mmol/l; $p < 0.001$] and nocturnal mean glucose concentrations [SIT: 10.6 ± 0.4 mmol/l, LW: 8.1 ± 0.4 mmol/l, SRA: 8.3 ± 0.4 mmol/l; $p < 0.001$]. Furthermore, mean glucose concentrations were sustained nocturnally at a lower level until the morning following the intervention for both LW and SRA (waking glucose both −2.7 ± 0.4 mmol/l compared with SIT; $p < 0.001$).

Conclusions/interpretation Interrupting 7 h prolonged sitting time with either LW or SRA reduced 22 h hyperglycaemia.

Electronic supplementary material The online version of this article (doi:10.1007/s00125-016-4169-z) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

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The glycaemic improvements persisted after these laboratory conditions and nocturnally, until waking the following morning. These findings may have implications for adults with relatively well-controlled type 2 diabetes who engage in prolonged periods of sitting, for example, highly desk-bound workers.
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Keywords Cardiometabolic risk · Diabetes · Glycaemic control · Glycaemic variability · Nocturnal glycaemia · Physical activity · Resistance exercise · Sedentary behaviour · Sitting · Walking

Abbreviations

AUC_{total}	Total area under the curve
CGM	Continuous glucose monitor
CONGA-1	Continuous overall net glycaemic action at 1 hour
iAUC	Incremental AUC
LW	Sitting interrupted with 3 min bouts of light-intensity walking
MAGE	Mean amplitude of glycaemic excursion
$SD_{glucose}$	SD of glucose
SIT	Prolonged sitting
SRA	Sitting interrupted with 3 min bouts of simple resistance activities

Introduction

Postprandial hyperglycaemia is linked to an increased risk of micro- and macro-vascular complications, particularly in individuals with type 2 diabetes [1, 2]. Even with anti-hyperglycaemic medications, adults with type 2 diabetes can spend between 25 and 40% of the day, and approximately 2 h on average nocturnally, in a state of hyperglycaemia (blood glucose >10 mmol/l) [3]. Moreover, the frequency and magnitude of glucose fluctuations and oscillations throughout the day (glycaemic variability) may also increase the risk of diabetic and cardiovascular complications independently of overall blood glucose levels [4–6]. Therefore, identifying safe and effective ways to manage postprandial glucose homeostasis is imperative within type 2 diabetes management.

Despite the multitude of benefits of regular moderate-to-vigorous exercise for improving glycaemic control and insulin sensitivity in type 2 diabetes patients [7], many adults with type 2 diabetes do not meet physical activity recommendations [8]. Indeed, population studies demonstrate that adults can spend over 65% of their waking hours in sedentary behaviours (involving prolonged sitting), while only ~5% of waking hours are spent in moderate-to-vigorous physical activity [9, 10]. These large volumes of sedentary time are associated with higher type 2 diabetes risk, even after controlling for leisure-time spent in moderate-to-vigorous physical activity [11, 12].

Recent experimental evidence suggests that reducing and interrupting prolonged sitting with brief bouts (<5 min) of standing or light ambulation acutely lowers postprandial glucose and insulin concentrations in healthy, overweight/obese adults and in those with impaired glucose regulation [13–15]. We recently expanded upon these findings, providing the first laboratory evidence in patients with type 2 diabetes that regular brief interruptions to high amounts of prolonged sitting (7 h) with light activities (3 min bouts every 30 min) significantly reduced concurrent postprandial glucose, insulin and C-peptide responses following standardised mixed-meals [16]. Further, in healthy-active young adults, a day of light-intensity physical activity and minimal sitting (<6 h) improved whole body insulin action the following morning, compared with a day of prolonged (16 h) sitting [17]. Altogether, these studies highlight the detrimental effects of prolonged sitting and the potential benefits of reducing and interrupting overall sitting time. However, it remains unclear whether benefits in type 2 diabetes patients: (1) persist beyond the immediate 7 h intervention period (i.e. nocturnally until waking the subsequent morning); and (2) extend to reductions in glycaemic variability (i.e. the frequency and magnitude of glucose oscillations and fluctuations).

Using continuous glucose monitoring technology to better characterise and understand meal-to-meal and temporal glucose homeostasis, glycaemic variability, and potential carry-over effects beyond the controlled-laboratory setting [16], we compared the impact of 7 h prolonged sitting with 7 h sitting interrupted with brief bouts of light-intensity walking or simple resistance activities on 22 h glucose homeostasis in adults with type 2 diabetes. We hypothesised that interrupting prolonged sitting time over 7 h would lower postprandial glucose responses, 22 h hyperglycaemia and glycaemic variability, and that improvements in glycaemic control would be sustained nocturnally, until the morning following the intervention.

Methods

Participants As previously reported [16], non-smoking men and women (BMI 25–40 kg/m^2; aged 35–75 years; with type 2 diabetes [diet or metformin-controlled, ≥3 months duration, based on American Diabetes Association diagnostic criteria [18]]) were recruited. Participants were excluded if they self-

reported sitting <5 h/day and/or were meeting physical activity guidelines (≥150 min/week of moderate-intensity exercise). The study was approved by the Institutional Human Research Ethics Committee and all participants provided written informed consent.

Study design This randomised crossover trial was undertaken at the Baker IDI Heart and Diabetes Institute between October 2013 and November 2014. Detailed screening and testing procedures have been described previously [16]. The continuous glucose monitor (CGM) data reported here were a prespecified secondary outcome of the study and have not been previously reported. In brief, participants attended the laboratory on five separate occasions: visit 1, medical screening visit; visit 2, familiarisation visit; and visits 3–5, three acute 8 h trial condition visits in a randomised order, each separated by a 6–14 day washout period. Trial condition order was randomised by a third party (block randomisation with balanced block sizes) and stratified by sex.

Experimental protocol and laboratory conditions As previously reported [16], on trial condition days, participants arrived at the laboratory at ~07:00 hours, after a 12 h fast. For 48 h prior to trial condition days, participants were asked to abstain from caffeine, alcohol and structured moderate-to-vigorous physical activities (i.e. no physical activity beyond that of daily living). Each laboratory condition was a total duration of 8 h (~08:00–16:00 hours; Fig. 1) and commenced with a 60 min 'steady-state' period (from −1 h to 0 h), after which participants consumed standardised breakfast (at 0 h) and lunch (at 3.5 h) meals, with the time taken to consume (<20 min per meal) replicated in subsequent conditions. Participants began the following experimental protocols, in a randomised order, after the breakfast meal: (1) prolonged sitting (SIT); (2) sitting interrupted with 3 min bouts of light-intensity walking (LW; 3.2 km/h) every 30 min; and (3) sitting interrupted with 3 min bouts of simple resistance activities (SRA) every 30 min (comprising 20 s body weight half-squats, 20 s calf raises, 20 s gluteal contractions and knee raises; repeated three times in sequential order while mimicking a standardised video recording).

Participants sat upright in a comfortable chair throughout each 8 h laboratory condition and were instructed to minimise excessive movement, only rising from the chair to void. Standardised lavatory visits were incorporated into the protocol to minimise unscheduled physical activity; however, additional lavatory visits were permitted. Participants complied with the respective laboratory 8 h condition protocols under direct supervision from research staff.

At the end of each 8 h laboratory visit (~16:00 hours), participants returned home and were asked to consume their standardised evening meal between 19:00 and 20:00 hours that evening and sleep at their usual time, keeping these timings as consistent as possible for subsequent trial conditions. As per the 48 h prior to each trial condition, participants were

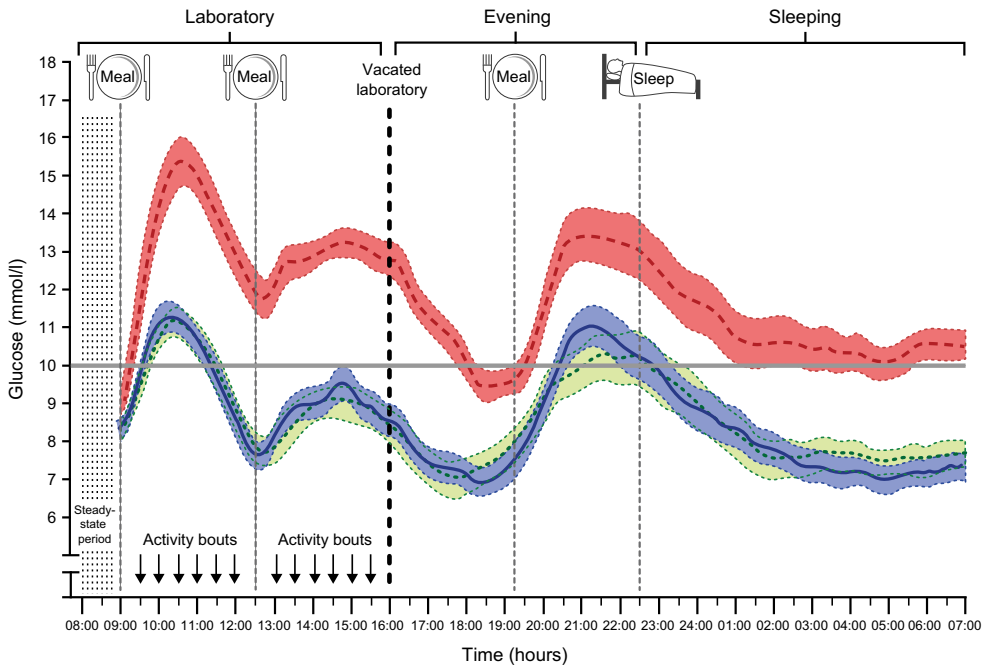

Fig. 1 Mean glucose profiles (± SEM) over 22 h during and following each trial condition. The shaded area (prior to 09:00 hours) denotes the 1 h sitting, steady-state period prior to commencing each trial condition. The 3 min activity bouts every 30 min during the LW and SRA interventions are illustrated by the arrows. Standardised meals in the laboratory were consumed at 09:00 hours and 12:30 hours, while mean dinner and bed times were at 19:15 hours and 22:28 hours, respectively (grey vertical dashed lines). The dashed black vertical line denotes when participants vacated the laboratory, at ~16:00 hours. The solid grey horizontal line at 10 mmol/l glucose represents the hyperglycaemic threshold. Red shaded area and dashed line, SIT; blue shaded area and solid line, LW; green shaded area and dotted line, SRA

asked to continue to abstain from caffeine and alcohol until after removing the CGM the following morning.

Standardisation of diet, medications and physical activity To minimise diet-induced variability during testing periods, meals were standardised between conditions and individualised to meet 33% of daily estimated energy requirements using the Schofield equation and a physical activity factor of 1.5 [19]. The target macronutrient profile was 12–15% energy from protein, 55–58% from carbohydrate and 29–31% from fat [16]. Evening meal packs were provided for participants to consume at home (between 19:00 and 20:00 hours) on the evening of, and prior to each experimental condition. Prescribed medications were continued throughout the study.

Participants were instructed to maintain their normal physical activities after leaving the laboratory, but refrain from any structured moderate-to-vigorous physical activity until after the removal of the CGM (see below) the following morning. To objectively measure any possible postural compensatory behaviour during the evening of the test day that may have occurred as a result of the trial condition, an activPAL3 triaxial physical activity monitor (PAL-technologies, Glasgow, Scotland, UK) was worn on the right thigh during each condition for 22 h for objective measurements of time spent sitting, standing and stepping, both inside and outside the laboratory [20]. As previously described [16], anthropometric, biochemical and dietary data and accelerometer-derived physical activity data were not significantly different 48 h before each trial condition.

Continuous glucose monitoring For the present study, a CGM (iPro2 CGM with Enlite sensors; Medtronic, Northridge, CA, USA) was inserted immediately upon arrival at the laboratory (07:00–07:15 hours) by trained research personnel into the subcutaneous fat in the lumbar region, and secured using a thin clear film according to the manufacturer's instructions. Once inserted, the CGM recorded interstitial fluid glucose concentrations every 5 min for 22 h (data collection occurred from 09:00 hours on the trial day until 07:00 hours the following morning). For subsequent conditions, new sensors were inserted within approximately 2 cm of the initial insertion site. To calibrate the CGM, capillary (fingerstick) blood glucose samples were collected at six standardised times during the 22 h period (three in the laboratory and three at home) according to the manufacturer's instructions, using a commercial, time-stamped glucometer (Abbott Freestyle Optium, Witney, Oxfordshire, UK). Participants were provided with verbal and written instructions for the collection of capillary measurements at home and the times of collection (which were later confirmed in the laboratory using the glucometer's stored memory function). Validation studies have demonstrated good agreement between individual glucose measurements derived via Enlite sensors and venous blood [21, 22], along with test-retest reliability [23].

Data handling and statistical analyses Physical activity monitor data (activPAL events files) were processed in SAS 9.4 (SAS Institute, Cary, NC, USA) to analyse time spent sitting, standing and stepping for both the trial condition (laboratory), and the post-trial condition until bedtime (evening) periods. A modified algorithm was used to identify participant sleep time as ≥20 min of continuous sitting/lying occurring at or following self-reported bedtime [24]. Invalid/non-wear days were identified as containing <10 h of waking wear, ≥95% of waking wear time spent in any one activity or <500 steps [25].

CGM data were analysed using the R statistical software package, version 3.1.2 (www.r-project.org, accessed 17 Feb 2016). Data were summarised into three different time periods: (1) overall (waking and nocturnal hours over the 22 h trial period); (2) meal times; and (3) nocturnal. To summarise the overall CGM data, we calculated 22 h mean glucose and total area under the curve (AUC_{total}) using the trapezoidal method from a baseline concentration of zero. Time in hyperglycaemia was quantified as time spent with glucose >10 mmol/l. A number of common indices of glycaemic variability (CV [%], SD of glucose [$SD_{glucose}$], mean amplitude of glycaemic excursion [MAGE] and continuous overall net glycaemic action at 1 hour [CONGA-1]) were also calculated for the 22 h period (see electronic supplementary material (ESM) Methods for details).

Meal times for breakfast, lunch and dinner were defined as 15 min before the meal until 3 h after the end of the meal. The data period during which participants were eating the meal was excluded from analysis. We calculated the baseline glucose concentration before each meal (mean of glucose during the 15 min before the meal). To summarise each meal response we calculated the net incremental area under the curve (iAUC) because it has been shown to be more reflective of the glucose response to a meal than AUC_{total} [26]. Net iAUC was calculated for each meal as total incremental area below the curve, subtracting the area below each pre-meal baseline glucose concentration from that above. Finally, time in hyperglycaemia was calculated for all meal periods.

Nocturnal glucose was defined as the period beginning with activPAL-derived sleep time until self-reported wake time the next day. Nocturnal glucose was quantified using mean glucose, AUC_{total} and time in hyperglycaemia. Waking glucose was defined as the average of the final 15 min of the 22 h continuous glucose monitoring period for all participants.

Generalised linear mixed-models with random intercepts were used to evaluate the differential effects of the experimental conditions on all summary outcome variables using Stata 12 (StataCorp, College Station, TX, USA). Residuals were examined for serial correlation, heteroscedasticity and

normality. Substantial departures from model assumptions were not observed. A two-tail probability level of 0.05 was adopted. Data are expressed as mean ± SEM in text unless otherwise stated. All models were adjusted for potential covariates explaining residual outcome variance (age, BMI and sex), including preprandial values and period effects (treatment order) for glucose outcomes. Glycaemic variability outcomes were additionally adjusted for mean glucose concentrations. Meal-by-condition, sex-by-condition and BMI-by-condition interaction tests were also performed for mean glucose, iAUC and time in hyperglycaemia.

Results

Participant characteristics Twenty-four participants (see Table 1 for participant characteristics) were randomised and completed all trial conditions. Aside from BMI (31.5 kg/m^2 vs 35.2 kg/m^2 for men vs women; $p = 0.005$), there were no significant differences in sex-related baseline variables or medications [16].

Table 1 Participant characteristics

Demographics	Baseline
Sex (male/female)	14/10
Age (y)	62 ± 6
BMI (kg/m^2)	33.0 ± 3.4
Waist circumference (cm)	112.6 ± 9.7
Diabetes duration (y)	6.8 ± 5.1
Ethnicity	
European	20 (83%)
Asian	4 (17%)
Medications, *n* (%)	
Metformin	23 (96%)
Statin	15 (63%)
Anti-hypertensive	16 (67%)
Metabolic and cardiovascular risk factors	
HbA_{1c} (%)[b]	7.2 ± 0.7
HbA_{1c} (mmol/mol)[b]	55.1 ± 8.0
eGFR (ml min^{-1} [1.73 m^2]$^{-1}$)[b]	86.7 ± 8.1
Fasting glucose (mmol/l)[a]	8.2 ± 1.4
Fasting insulin (pmol/l)[a]	85.9 ± 54.7
Fasting triacylglycerol (mmol/l)[a]	1.9 ± 1.0
Fasting total cholesterol (mmol/l)[a]	4.4 ± 0.8
Fasting LDL-cholesterol (mmol/l)[a]	2.5 ± 0.8
Fasting HDL-cholesterol (mmol/l)[a]	1.1 ± 0.3
Systolic blood pressure (mmHg)[b]	123 ± 14
Diastolic blood pressure (mmHg)[b]	77 ± 9

Data are expressed as mean ± SD, or number (%) where specified

[a] Measured at the screening visit

[b] Measured at the beginning of the first trial condition

eGFR, estimated GFR

Postural allocation and meal/sleep periods Data from the activPAL are shown in Table 2. By design, the LW and SRA conditions saw greater proportions of the laboratory period spent standing or stepping compared with SIT. In turn, LW and SRA were characterised by greater allocations of time to stepping and standing, respectively. During the evening period, there were no significant differences in time spent seated, standing or stepping between trial conditions.

Recorded dinner, bedtime and waking times were between 18:15 and 20:30 hours (mean: 19:15 hours), 20:38 and 02:21 hours (mean: 22:28 hours), and 05:45 and 09:20 hours (mean: 07:01 hours), respectively (data not shown). Mean (± SD) within-participant differences in dinnertime (23 ± 20 min), bedtime (48 ± 32 min) and waking time (24 ± 16 min) were not significantly different between trial conditions, nor were mean sleep durations (SIT: 8 h 12 min ± 55 min; LW: 7 h 55 min ± 57 min; SRA: 8 h 13 min ± 56 min; $p > 0.1$ for all; data not shown).

Glucose homeostasis and glycaemic variability over 22 h An overview of the mean 22 h glycaemic profiles for all participants by trial condition is presented in Fig. 1. Over the entire 22 h period, mean glucose concentrations, cumulative AUC_{total} and time spent in hyperglycaemia (>10 mmol/l) were all significantly reduced during the LW and SRA conditions compared with SIT (Table 3). Measures of glycaemic variability (MAGE, CONGA-1 and $SD_{glucose}$) were significantly reduced for the LW and SRA conditions compared with SIT when adjusting for baseline glucose levels and other covariates, but not after additionally adjusting for mean 22 h glucose levels (ESM Table 1, Model 1 vs Model 2). Similarly, CV (%) was not significantly different between conditions. No significant differences were observed between LW and SRA for any glycaemic outcomes. No hypoglycaemic episodes (i.e. glucose <3.9 mmol/l) were observed during any of the trial conditions (data not shown).

Postprandial glycaemic control Mean glucose, iAUC, and time spent in hyperglycaemia were all significantly lower for the LW and SRA conditions compared with SIT for each meal (see Fig. 2). A significant meal-by-condition interaction effect was observed for mean glucose and glucose iAUC responses (Fig. 2a,b), but not time in hyperglycaemia (Fig. 2c). While both LW and SRA reduced glucose concentrations for each meal period compared with SIT, the mean glucose and glucose iAUC reductions for breakfast were significantly larger than lunch and dinner ($p < 0.001$; Fig. 2a,b). Further, mean glucose and iAUC reductions following the dinner meal were significantly greater for the SRA condition compared with both LW ($p < 0.05$; Fig. 2a,b) and SIT ($p < 0.05$). No significant sex-by-

Table 2 Total time spent sitting, standing or stepping during the trial conditions, as derived from activPAL

Condition	SIT (min)	LW (min)	SRA (min)
Laboratory			
Wear time	504 ± 3	504 ± 3	502 ± 3
Sitting	499 ± 3	449 ± 3*	453 ± 3*
Standing	4 ± 1	9 ± 1*	30 ± 1*‡
Stepping	2 ± 1	46 ± 1*	19 ± 1*‡
Evening			
Wear time	417 ± 24	418 ± 25	375 ± 24
Sitting	279 ± 11	265 ± 11	262 ± 11
Standing	108 ± 21	119 ± 22	79 ± 21
Stepping	30 ± 2	34.6 ± 3	35 ± 2
Laboratory + Evening			
Wear time	919 ± 29	900 ± 30	839 ± 30*
Sitting	777 ± 17	700 ± 18*	682 ± 17*
Standing	110 ± 21	123 ± 22	105 ± 21
Stepping	32 ± 3	79 ± 3*	52 ± 3*‡

Data are expressed as mean ± SEM

Total time in each activity during the laboratory condition period ('Laboratory'), the period after the laboratory condition ('Evening') and over the entire period until bedtime ('Laboratory + Evening')

*$p < 0.05$ vs SIT; ‡$p < 0.05$ vs LW

condition or BMI-by-condition interaction effects were observed for any of the glycaemic variables (data not shown).

Nocturnal glycaemic control Mean glucose concentrations, AUC_{total} and time spent in hyperglycaemia were all significantly reduced during the sleeping period in the LW and SRA test conditions vs the SIT condition (see Table 3). Mean glucose concentrations were also significantly lower the morning following the intervention for both LW and SRA compared with SIT (−2.7 ± 0.4 mmol/l for both; $p < 0.001$; Table 3). No significant differences were observed between LW and SRA.

Table 3 Glycaemic control over 22 h and nocturnal glucose levels for each trial condition

Variable	SIT	LW	SRA
22 h			
Mean glucose (mmol/l)	11.6 ± 0.3	8.9 ± 0.3†	8.7 ± 0.3†
AUC_{total} (mmol/l × h)	254.9 ± 6.7	194.7 ± 6.6†	191.5 ± 6.6†
Time in hyperglycaemia (h)	14.7 ± 0.9	6.3 ± 0.8†	6.3 ± 0.9†
Sleeping[a]			
Mean glucose (mmol/l)	10.6 ± 0.4	8.1 ± 0.4†	8.3 ± 0.4†
AUC_{total} (mmol/l × h)	86.9 ± 3.7	64.6 ± 3.6†	68.0 ± 3.7†
Time in hyperglycaemia (h)	4.7 ± 0.4	1.4 ± 0.4†	1.8 ± 0.4†
Waking glucose (mmol/l)	10.3 ± 0.3	7.6 ± 0.3†	7.6 ± 0.3†

Data are expressed as mean ± SEM

[a] Sleeping is the time period from bedtime until the end of the 22 h period

†$p < 0.001$ vs SIT

Discussion

The novel finding in this study is that interrupting high levels of prolonged sitting (7 h) with brief bouts of light-intensity walking and simple resistance activities (3 min every 30 min) significantly lowered 22 h hyperglycaemia, including nocturnal hyperglycaemia, in inactive overweight/obese adults with type 2 diabetes. Of particular note, while reductions in postprandial glucose were observed during the 7 h controlled laboratory period, improved glycaemic control persisted into the subsequent free-living evening and sleeping periods until the following morning. An average waking glucose reduction of 2.7 mmol/l was observed for both the LW and SRA conditions compared with SIT.

The use of continuous glucose monitoring facilitated the tracking of glucose homeostasis both within the laboratory conditions, and during subsequent free-living and nocturnal periods outside the laboratory (see Fig. 2). Exposure to postprandial hyperglycaemia (>10 mmol/l) was highly prevalent during SIT for the observed 22 h period. Indeed, participants spent some 57% more time in hyperglycaemia over the 22 h for SIT compared with both LW and SRA. For perspective, this duration of time in hyperglycaemia equates to approximately twice that previously reported in individuals with type 2 diabetes on standardised diets while observed in a free-living environment [3, 27, 28]. In addition, time spent in nocturnal hyperglycaemia was some 60% greater for prolonged sitting compared with the activity conditions. These data highlight both the detrimental and persistent nature of high levels of prolonged sitting in type 2 diabetes patients, but also the glycaemic benefits of regularly interrupting high levels of prolonged sitting.

Concordant with the second-meal phenomenon [29], glucose responses across all conditions were lower following the lunch and dinner meals compared to breakfast. Mean reductions in time spent in hyperglycaemia were slightly lower for the dinner meal (though not statistically significant) compared with breakfast and lunch, which could be a consequence of the breakfast and lunch meals being closer together than lunch and dinner, allowing less time for glucose clearance. Importantly, while all postprandial glucose responses were lower during the SRA and LW conditions compared to SIT, the most marked reductions were observed following the breakfast meal. This may be attributable to the overall glucose responses being highest for breakfast, but is also suggestive that the timing of activity-breaks, or prolonged sitting, around meals – particularly the first meal of the day coming off an overnight fast – may be an important consideration for reducing glycaemic excursions.

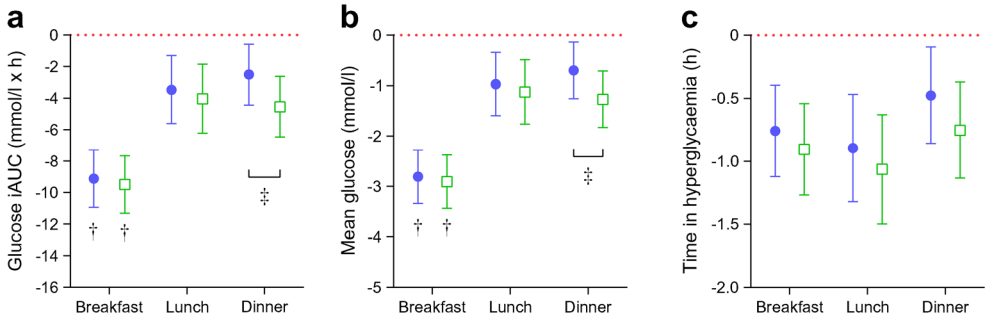

Fig. 2 Difference relative to SIT in (**a**) glucose net incremental AUC (iAUC), (**b**) mean glucose and (**c**) time in hyperglycaemia by meal. Blue circles, LW−SIT, green squares, SRA−SIT. Data are expressed as mean ± 95% CI. Mean glucose, iAUC, and time spent in hyperglycaemia were all significantly lower for the LW and SRA conditions compared with SIT for each meal ($p < 0.05$). $^{\dagger}p < 0.001$ vs lunch and dinner meals; $^{\ddagger}p < 0.05$, SRA−SIT vs LW−SIT

Interestingly, while postprandial glucose excursions were improved with both LW and SRA conditions compared with SIT for each of the three post-meal periods, the postprandial glucose responses following the dinner meal were significantly lower for SRA compared with LW. While speculative, this finding could be related to the nature of the activity-break intervention (different modality and/or increased intensity/energy expenditure of SRA bouts relative to LW) [16] and/or differential effects on hepatic glucose output or peripheral insulin sensitivity with varying exercise modes [30, 31]. However, such factors would not fully explain why glucose concentrations were generally similar between LW and SRA conditions during both the laboratory and sleeping periods.

This is the first study to report data on glycaemic variability when comparing a bout of prolonged sitting with sitting frequently interrupted with brief bouts of activity in type 2 diabetes. Although the prognostic value of glycaemic variability in type 2 diabetes remains contentious [32] (largely due to the relatively recent advent of continuous glucose monitoring technology, inconsistent findings, and a lack of prospective data) there is evidence to suggest that greater glycaemic variability may be adversely associated with endothelial dysfunction, oxidative stress and diabetic complications [4–6]. In the absence of a gold-standard measure to assess glycaemic variability, and because different indices assess distinct aspects of glycaemic variability, we computed a range of commonly used measures. While significant reductions in MAGE, CONGA-1 and $SD_{glucose}$ were observed with LW and SRA interventions compared with SIT, these effects were not apparent following statistical adjustment for mean glucose levels. These findings, together with the lack of between-condition differences in per cent CV (which directly normalises for mean glucose), point to a similar *relative* magnitude of glucose fluctuations around lower means for the LW and SRA conditions, rather than less variability *per se*.

The measures used in this study do not permit conclusions on the putative mechanisms responsible for the improvements in glycaemic control. However, in the same participants, we previously reported concurrent attenuations in venous glucose, insulin and C-peptide during the LW and SRA conditions, relative to SIT [16]. The lowering of venous glucose concentrations in spite of lower insulin and C-peptide (indicative of reduced endogenous insulin secretion) with light activity is suggestive of either enhanced insulin sensitivity and/or a greater reliance on insulin-independent contraction-mediated glucose disposal [30, 33]. Recent investigations appear to offer more support for the role of the skeletal muscle contraction-mediated glucose uptake pathway in improved postprandial glucose metabolism during acute (one day) interventions examining frequent ambulatory interruptions in sitting time [33]. However, it is possible that these signalling pathways are differentially altered in type 2 diabetes patients.

A key strength of this study is the randomised crossover design, which incorporated both controlled-laboratory, free-living and nocturnal elements. Participants were their own controls, which enhances both the internal validity and reliability of our data and permitted a smaller sample size. The laboratory trial and subsequent free-living phases were examined with the use of objective, posture-discriminating devices, while participants consumed a standardised, ecologically valid, western-type diet [34]. The continuous activity measurements, alongside CGM use, enabled us to account for these key activity and dietary behaviours, thereby increasing the experimental rigor of our findings.

When interpreting the findings of this study, it is important to emphasise that while continuous glucose monitoring offers many clinical and research advantages, it is an imperfect surrogate for venous blood glucose. CGM data accuracy may be influenced by numerous factors, including signal stability, physiological differences in interstitial glucose homeostasis, physiological/device lag-times and individual differences. These limitations remain relatively under-researched [21, 35]. While we attempted to best account for the known measurement issues where possible, we note that our CGM data appear to have over-estimated (by about twofold) the quantitative differences between the sitting and active conditions when compared with our previously reported venous glucose

data over 8 h [16]. Reasons for this are not readily apparent; however, we do acknowledge that it would have been more desirable to insert the CGM the night prior to each intervention to allow the sensors to fully equilibrate. Consideration of this and the above factors would be prudent in future research.

We also acknowledge that both the prescribed activity/sedentary behaviour during the laboratory phase and the dietary profiles (e.g. macronutrient profile, glycaemic index, meal frequency and size) may not reflect habitual behaviours in sedentary individuals in real-world settings and could have exaggerated the glycaemic differences we observed between trial conditions. With this in mind, an important caveat for this study is that the two activity-break conditions (6 min or ~10% activity per hour) were closer to activity patterns typically observed for sedentary free-living individuals (i.e. ~30–35% activity per hour) [9, 10] compared with the imposed experimental arrangements of our prolonged sitting condition. Although it was important to first establish 'proof-of-concept' in a controlled-laboratory setting and to accurately describe dose–response variables, 7 h prolonged sitting with only 1–2 toilet breaks, while plausible under some circumstances (e.g. during extended automobile/plane journeys or in those who may be required to carry out prolonged desk work to meet deadlines), is likely to be an extreme scenario for much of the population. Consequently, it remains uncertain whether adding additional activity breaks on top of a control (sitting) condition that could be aligned more closely to free-living variations in activity would elicit meaningful glycaemic benefits. Therefore, this requires further investigation.

It will be important to establish the efficacy of these interventions in type 2 diabetes patients with more advanced disease, particularly as such patients are more likely to have poorer glycaemic control, are more likely to experience hypoglycaemic episodes, and may be less responsive to exercise-mediated glucose reductions [36]. Nevertheless, our findings have relevance to a majority of those with type 2 diabetes (~80–85%), who are not treated with insulin or insulin combined with other oral glucose-lowering agents [37]. Further, it was also encouraging that no hypoglycaemic events were observed despite marked reductions in postprandial hyperglycaemia during the activity-break conditions used in this study.

As mentioned, future studies should examine the glycaemic effects of interventions that interrupt prolonged sitting in more ecologically relevant, free-living environments (e.g. the workplace) that would be more reflective of habitual sitting patterns. It will also be important to determine the effects of these interventions over longer time-periods (i.e. multiple days or weeks) and the specific mechanisms by which different light-intensity activities improve glycaemic control. Finally, it would be relevant to compare, but also combine, strategically placed frequent interruptions in sitting with structured bouts of morning or evening exercise. This would assist in identifying the doses, timing and patterning of physical activity that are required to optimize 24 h and postprandial glycaemic control for type 2 diabetes management. In these contexts, a further consideration would be the impact of energy balance, which was not strictly controlled for in this study.

In conclusion, this study demonstrates that interrupting high levels of prolonged sitting time with brief light-intensity walking or simple resistance activity bouts over 7 h reduces concurrent postprandial glucose responses in adults with type 2 diabetes, with glycaemic improvements persisting until the next morning. Although generalisability and longer term efficacy, practicality, and suitability for the workplace and home environment still need to be established, these findings may have implications for adults with relatively well-controlled type 2 diabetes who engage in prolonged periods of sitting, such as in a workplace setting.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Contribution statement PCD conceived, designed and conducted the study, analysed and interpreted the data, and wrote the manuscript. JMB, PS and EC assisted with data cleaning or management and statistical analyses/interpretation. NDC provided clinical support during data collection. JMB, RNL, JWS, NES, NDC, GWL, PS, EC, NO, BAK, DWD assisted in the concept and design of the study and participated in critical revision of the manuscript for intellectual content. All authors approved the final version of this manuscript. PCD and DWD are the guarantors of this work and, as such, had full access to all the study data and take responsibility for data integrity and accuracy of the data analysis.

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