Manuscript Title:

**Prolonged Uninterrupted Sitting Elevates Postprandial Hyperglycaemia
Proportional to Degree of Insulin Resistance**

Running title: Sitting, insulin resistance & postprandial dysglycaemia

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NON-STRUCTURED ABSTRACT

Prolonged uninterrupted sitting is related adversely to cardiometabolic risk markers and postprandial hyperglycaemia, relative to sitting interrupted by regular brief activity breaks. However, whether the magnitude of hyperglycaemic responses to prolonged sitting is dependent upon the underlying degree of insulin resistance remains unclear. Data were pooled from three randomised cross-over laboratory-based trials (n=62) that examined the postprandial blood glucose- and insulin-lowering effects of prolonged sitting versus sitting interrupted by regular brief activity breaks in overweight/obese adults who had normal or impaired glucose metabolism (two trials), or type 2 diabetes not treated by insulin (one trial). Corrected for study effects, the magnitude of differences in postprandial glucose and insulin responses between the two conditions was significantly exacerbated with poorer baseline levels of fasting glucose, insulin and/or surrogate markers of β -cell function and insulin resistance. This suggests that those with higher underlying levels of insulin resistance may derive greater metabolic benefits from regularly interrupting prolonged sitting than their counterparts. If these findings can be replicated, they will have implications for future targeting and optimisation of physical activity/sedentary behaviour interventions in the prevention and management of type 2 diabetes.

INTRODUCTION

Postprandial hyperglycaemia increases cardiometabolic risk (1, 2), even in those who have not developed type 2 diabetes (T2D) (1, 3). Indeed, chronic postprandial glucose excursions and their variability have been shown to contribute to pancreatic β -cell failure and progression to late stage diabetes (4). Furthermore, postprandial glucose elevation is a key predictor of microvascular and macrovascular complications and cardiovascular mortality, and is more tightly correlated with these endpoints than are either HbA1c or fasting glucose (5, 6). Accordingly, identifying safe, effective and pragmatic ways to manage postprandial glycaemia remains an important consideration for both T2D prevention and management.

The 2016 American Diabetes Association's (ADA) Position Statement on physical activity/exercise and diabetes now includes specific recommendations to reduce and interrupt prolonged sitting (7). This is based in part on population-level evidence that high volumes of daily sitting time are associated with poorer cardiometabolic health outcomes (8). The ADA Position Statement also draws upon evidence from acute experimental studies showing that regular brief interruptions to prolonged sitting time can improve cardiometabolic risk markers in healthy and overweight/obese adults, in those with impaired glucose regulation, and in those with overt T2D (7). However, whether the magnitude of postprandial dysglycaemia in response to prolonged sitting is dependent upon the underlying degree of glycaemic control, β -cell function, or insulin resistance remains unclear (9, 10).

Three similar laboratory-based controlled trials involving participants across the diabetes risk continuum examined the difference in postprandial glucose and insulin responses between two acute conditions: prolonged uninterrupted sitting and sitting interrupted via brief walking breaks. We pooled data from these trials and tested whether these effects were proportional to participants' underlying degree of dysglycaemia and insulin resistance.

METHODS

Overview of studies

Three separate randomised cross-over trials (participant total $n=62$) were performed at the Baker Heart & Diabetes Institute (Melbourne, Australia) between April 2009 and November 2014 (11-13). Each study examined the acute effects on postprandial plasma glucose and insulin (primary outcomes) of prolonged uninterrupted sitting, compared to sitting interrupted by regular brief light-intensity walking breaks. All three studies involved overweight/obese (BMI 25–45 $\text{kg}\cdot\text{m}^2$) adults aged 45–75 years, who were physically inactive (self-reported not meeting physical activity guidelines of 150 min/week) and sedentary (self-reported sitting >5 hours/day), with one study (Study 3) involving participants with T2D who were diet or metformin controlled (23/24 participants taking metformin) and not taking insulin nor any other hypoglycaemic agents (11).

Lead-in periods and trial conditions

As previously described (11-13), participants refrained from structured exercise, alcohol and caffeine 48-h prior to each trial condition. Dietary and accelerometer-derived physical activity data 48-h before each of the trial conditions, and anthropometric and biochemical

data on the morning of each trial condition, did not differ significantly between conditions (11-13).

On the trial days, participants reported to the laboratory between 0700 and 0800 h, having fasted overnight. Upon arrival, they had a catheter inserted in an antecubital fossa vein for blood sampling, then commenced a 'sitting steady-state' period. Following the steady-state phase, they consumed either a liquid (12, 13) or a regular (11) mixed-meal (see Table 1). They then interrupted prolonged sitting time with light-intensity walking breaks on a treadmill ($3.2 \text{ km}\cdot\text{h}^{-1}$, zero gradient) for either 2-min every 20 min (12, 13) or 3-min every 30 min (11), accumulating 24-min activity ($6 \text{ min}\cdot\text{h}^{-1}$) over 4 hours. Hourly blood samples were collected and analysed for plasma glucose and serum insulin concentrations by an independent laboratory using standardised methods (11-13).

Data handling and statistics

Postprandial glucose and insulin responses and the insulin/glucose ratio over 4 h during each trial were summarised as total area under the curve (tAUC), calculated using the trapezoidal method from a baseline concentration of zero. Intervention-induced changes were assessed as the prolonged uninterrupted sitting minus the walking breaks condition values. Baseline fasting values were calculated as the average of two fasting blood samples taken an hour apart, across the two conditions. Surrogate indices of insulin sensitivity (%S), insulin resistance (IR) and steady-state β -cell function (% β) were then calculated based on fasting glucose and insulin concentrations (average of the first two fasting samples) using the homeostatic model assessment (HOMA2) online calculator (<https://www.dtu.ox.ac.uk/homacalculator/>) version 2.2.3. Similarly, the quantitative insulin sensitivity check index (QUICKI) (14) was calculated as $1/(\log \text{ fasting glucose } [\text{mg}\cdot\text{dL}^{-1}] + \log \text{ fasting insulin } [\text{mU}\cdot\text{L}^{-1}])$. The above indices are accurate predictors of insulin sensitivity/resistance in normal, obese and diabetic individuals when compared to hyperinsulinaemic-euglycaemic glucose clamp methods (15).

Using Stata 14 (StataCorp LP), mixed models examined whether key fasting parameters of dysglycaemia and insulin resistance were associated with intervention-induced changes in glucose, insulin and insulin/glucose ratio and tAUC. Models included a random intercept for study (correcting for study differences), and adjusted for individual-level covariates potentially explaining residual outcome variance (age, waist circumference and gender), treatment order, and meal glycaemic load [(glycaemic index * grams of carbohydrates)/100] per kg body weight. All models met assumptions of linearity and normality of residuals. Statistical significance was set at $P < 0.05$.

RESULTS

Participant characteristics are in Table 1. There were no significant differences between studies in baseline BMI or waist circumference. Consistent with the sampling, Study 3 participants were significantly older, had significantly higher fasting glucose concentrations, and poorer β -cell function than their counterparts. Each study had near identical activity

conditions, but the mixed-meal provided in Study 3 was relatively higher in carbohydrate and glycaemic load than in the other studies.

INSERT TABLE 1 ABOUT HERE

Table 2 shows the relationships of the various fasting parameters of dysglycaemia and insulin resistance with the intervention-induced changes in tAUC for postprandial glucose, insulin and insulin/glucose ratio. The prolonged sitting condition generally elicited adverse changes that were of greater magnitude in the tAUC outcomes when the participant's baseline values of fasting glucose, insulin, and insulin/glucose ratio, insulin resistance (HOMA-IR) and insulin output (HOMA-% β) were higher, and when insulin sensitivity was lower (HOMA-%S, QUICKI). The exceptions were that there were no large or significant relationships seen between HOMA-%S, HOMA-IR or QUICKI and changes in glucose tAUC. For example, the impact prolonged uninterrupted sitting had on glucose tAUC relative to the walking breaks condition was greater by 1.23 mmol hL⁻¹ for each 1 mmolL⁻¹ higher fasting glucose. Most of these associations were statistically significant, except that only HOMA-% β and fasting glucose were significantly associated with postprandial glucose. The additional adjustment for meal glycaemic load made little difference to results. Removal of waist circumference from the models also did not alter the associations to any meaningful degree (data not shown). Associations did not vary significantly by age, waist circumference or gender.

INSERT TABLE 2 ABOUT HERE

DISCUSSION

To date it has been difficult to estimate whether the effect of prolonged sitting is dependent upon underlying glycaemic control, insulin resistance and/or β -cell function. Meaningful comparisons and preliminary meta-analyses can be hampered due to extensive heterogeneity in sub-populations, data accessibility, and between-study differences in experimental design, methods, dietary exposures and quantification of glycaemic responses (9, 10). We were able to provide rigorous estimates by evaluating this topic using data pooled from three laboratory-based interventions that used very similar methods, and controlling for the limited differences between the studies.

Our results for a very brief, modest, regularly-delivered activity stimulus are interesting when considered alongside what has been reported regarding immediate glucose responses to structured exercise (16). Knudsen et al (16) recently demonstrated that an hour of continuous exercise increased postprandial glucose responses (relative to rest) in those with normal glycaemic tolerance, but not in those with impaired glucose tolerance and T2D. Both studies indicated a relevance of pre-existing glucose metabolism for postprandial dysglycaemia, but such that metabolic dysfunction appeared to dampen the benefits of continuous exercise and enhance the benefits of brief, regular walking breaks. With the recent inclusion of recommendations to reduce and interrupt prolonged sitting time in the ADA physical activity guidelines (7), our findings may be informative for future person-specific precision medicine

approaches, and the optimisation of physical activity/sedentary behaviour interventions in relation to T2D prevention and management.

The cross-over design is a strength of the studies that we have included in our analysis, since it provides control for fixed person-specific factors and affords smaller sample sizes. However, there are several limitations that should be considered when interpreting and generalising our findings. First, an *a posteriori* hypothesis was used on a convenience sample of previous studies. Second, indirect estimates that are more reflective of hepatic insulin resistance/sensitivity were used in the absence of more direct measures of insulin sensitivity/resistance, which may limit comparability between studies. Third, despite the adjustments made for between-study differences, and meal dose (varying at the individual and study level), it is possible that there some residual confounding may still be present. Finally, we could not rigorously compare participants with T2D to their counterparts, since they all came from one study. However, results based on the continuous biomarkers (which were between and within studies) were consistent with prolonged sitting being especially detrimental (acutely) in those with T2D.

The exaggerated postprandial glucose and insulin responses to prolonged sitting associated with poorer underlying glycaemia, β -cell function and higher insulin resistance may be attributable to differential intervention effects on insulin sensitivity, intracellular signalling, vascular hemodynamics, or modulation of sympathetic activity (9). Further studies could explore these as important mediators or modifiers of the associations that we have reported. The use of glucose and insulin clamp techniques to quantify underlying determinants of glycaemic control and differential rates of insulin secretion and glucose release/uptake peripherally, or at various target sites such as the liver during trial conditions (e.g., using oral and intravenous stable isotope glucose tracers), would also be informative. Finally, long-term interventions in both healthy and chronic disease populations would be needed to evaluate non-acute effects.

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CONFLICT OF INTEREST

No potential conflicts of interest relevant to this paper were reported by any author.

AUTHOR CONTRIBUTIONS

PCD conceived and designed the research question, analysed and interpreted the data, and wrote the manuscript. RNL, EW, NO, BAK and DWD assisted in the concept and design of the research question and participated in critical revision of the manuscript for intellectual content. All authors approved the final version of this manuscript.

Table 1. Participant and study characteristics

	Study 1	Study 2	Study 3
Sample size	19	19	24
<i>Participant characteristics</i>			
Gender (male/female)	11/8	11/8	14/10
Age (y)	56.4 ± 6.6	53.8 ± 4.9	62.1 ± 6.2 ^{a,b}
BMI (kg·m ⁻²)	32.7 ± 4.2	31.2 ± 4.1	33 ± 3.4
Waist circumference (cm)	108.3 ± 11.7	105 ± 13.8	112.6 ± 9.7
Fasting glucose (mmol·L ⁻¹)	5.1 ± 0.5	5.2 ± 0.5	8 ± 1.3 ^{a,b}
Fasting insulin (mU·L ⁻¹)	10.4 ± 5.1	7.5 ± 4.0	12.1 ± 7.4
HOMA-%β	99.0 ± 30.1	75.3 ± 16.7 ^a	49.5 ± 25.2 ^{a,b}
HOMA-%S	106.6 ± 53.7	148.0 ± 65.5 ^{a,c}	86.2 ± 40.5
HOMA-IR	1.2 ± 0.6	0.9 ± 0.5 ^{a,c}	1.5 ± 0.9
QUICKI	0.34 ± 0.03	0.36 ± 0.03	0.32 ± 0.02 ^{a,b}
HbA1c (%)	n/a	n/a	7.2 ± 0.7
Normal glucose tolerance	11 (58%)	10 (53%)	nil
Impaired glucose tolerance	8 (42%)	9 (47%)	nil
Type 2 diabetes	nil	nil	24 (100%)
<i>Study characteristics</i>			
Trial condition meal			
Energy (kcal)	763 ± 0	763 ± 0	796 ± 114
Carbohydrate (g·kg ⁻¹)	0.8 ± 0.1	0.9 ± 0.2	1.2 ± 0.1*†
Fat (g·kg ⁻¹)	0.5 ± 0.1	0.6 ± 0.1	0.3 ± 0.0*†
Protein (g·kg ⁻¹)	nil	nil	0.3 ± 0.0*†
Glycaemic load·kg ⁻¹	0.8 ± 0.1	0.8 ± 0.2	0.7 ± 0.1*†
Prolonged condition sitting (min) ^d	240	240	240
Breaks condition sitting (min) ^d	216	216	216
Breaks condition walking (min) ^d	24	24	24
Break duration (min) ^d	2	2	3

Data are expressed as mean ± SD or n (%).

^a Differs from Study 1 ($P < 0.05$).

^b Differs from Study 2 ($P < 0.05$).

^c Differs from Study 3 ($P < 0.05$).

^d Fixed for each participant; not statistically compared between the studies.

Note: In line with WHO/IDF and ADA criteria, normal glucose tolerance = 2 hour plasma glucose < 7.8 mmol·L⁻¹ and impaired glucose tolerance = 2 hour plasma glucose ≥ 7.8 mmol·L⁻¹ but not formally diagnosed with type 2 diabetes.

Table 2. Relationships of intervention-induced changes (Δ ; prolonged uninterrupted sitting – walking breaks condition) in postprandial glucose, insulin and insulin/glucose ratio responses, with baseline levels of indices of dysglycaemia and insulin resistance.

Parameter	Δ Glucose tAUC (mmol·h·L ⁻¹)	P-value	Δ Insulin tAUC (mU·h·L ⁻¹)	P-value	Δ Ins:Glu tAUC (mU:mmol·h·L ⁻¹)	P-value
Fasting glucose and/or insulin concentrations ^a						
<i>Model 1</i>	1.29 (0.49, 2.08)	$\leq .001$	12.37 (8.08, 16.66)	$\leq .001$	6.89 (3.72, 10.06)	$\leq .001$
<i>Model 2</i>	1.23 (0.43, 2.04)	$\leq .003$	12.35 (8.07, 16.64)	$\leq .001$	6.81 (3.64, 9.97)	$\leq .001$
HOMA-% β						
<i>Model 1</i>	-0.07 (-0.11, -0.04)	$\leq .001$	1.39 (0.41, 2.38)	.006	0.96 (0.32, 1.59)	.003
<i>Model 2</i>	-0.07 (-0.11, -0.04)	$\leq .001$	1.39 (0.40, 2.37)	.006	0.93 (0.30, 1.56)	.004
HOMA-%S						
<i>Model 1</i>	0.00 (-0.02, 0.02)	.806	-0.87 (-1.36, -0.39)	.006	-0.51 (-0.82, -0.20)	.001
<i>Model 2</i>	0.00 (-0.02, 0.02)	.818	-0.87 (-1.35, -0.38)	.006	-0.50 (-0.81, -0.19)	.002
HOMA-IR						
<i>Model 1</i>	-0.39 (-2.17, 1.39)	.665	114.11 (80.18, 148.04)	$\leq .001$	65.12 (41.61, 88.63)	$\leq .001$
<i>Model 2</i>	-0.34 (-2.12, 1.44)	.706	114.53 (80.71, 148.35)	$\leq .001$	65.02 (41.66, 88.38)	$\leq .001$
QUICKI						
<i>Model 1</i>	-0.13 (-1.43, 1.18)	.850	-56.53 (-83.30, -29.77)	$\leq .001$	-30.88 (-47.65, -14.10)	$\leq .001$
<i>Model 2</i>	-0.16 (-1.45, 1.14)	.811	-56.20 (-83.04, -29.36)	$\leq .001$	-30.25 (-47.24, -13.25)	$\leq .001$

Data are expressed as unstandardised coefficients (95% CI) per one unit of each exposure. For QUICKI, one unit is arbitrary; therefore, effects are presented per 0.03 units (approximately 1 SD).

Model 1: random intercept for study (1/2/3), adjusts for treatment order, waist circumference, age and gender as fixed effects

Model 2: as per model 1 with further adjustment for meal glycaemic load per kg body weight

tAUC, total area under the curve.

QUICKI, quantitative insulin sensitivity check index.

^a Δ Glucose tAUC model examines fasting glucose ($\text{mmol}\cdot\text{L}^{-1}$); Δ Insulin tAUC model examines fasting insulin ($\text{mU}\cdot\text{L}^{-1}$); Δ Ins:Glu tAUC model examines the ratio of fasting insulin ($\text{mU}\cdot\text{L}^{-1}$) / fasting glucose ($\text{mmol}\cdot\text{L}^{-1}$)

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