Sitting and metabolic risk in children

TITLE: Prolonged sitting and markers of cardiometabolic disease risk in children and youth: a randomized crossover study

ABBREVIATED TITLE: Sitting and metabolic risk in children

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Structured Abstract

**Objective:** Recent evidence suggests that short bouts of uninterrupted sedentary behavior reduce insulin sensitivity and glucose tolerance while increasing triglyceride levels in both healthy and overweight/obese adults. To date no study has examined the acute impact of uninterrupted sitting in children and youth. The objective of the present study was to determine whether 8 hours of uninterrupted sitting increase markers of cardiometabolic disease risk in healthy children and youth, in comparison to 8 hours of sitting interrupted by light intensity walk breaks or structured physical activity.

**Materials/Methods:** 11 healthy males and 8 healthy females between the ages of 10 and 14 years experienced 3 conditions in random order: (1) 8 hours of uninterrupted sitting (Sedentary); (2) 8 hours of sitting interrupted with a 2-minute light-intensity walk break every 20 minutes (Breaks); and (3) 8 hours of sitting interrupted with a 2-minute light-intensity walk break every 20 minutes as well as 2 x 20 minutes of moderate-intensity physical activity (Breaks+Physical Activity).

Insulin, glucose, triglyceride, HDL and LDL cholesterol area under the curve were calculated for each condition.

**Results:** We observed no significant differences in the area under the curve for any marker of cardiometabolic disease risk across the 3 study conditions (all \( p > 0.09 \)).

**Conclusions:** These results suggest that in comparison to interrupted sitting or structured physical activity, a single bout of 8 hours of uninterrupted sitting does not result in measurable changes in circulating levels of insulin, glucose, or lipids in healthy children and youth.

**Key Terms:** Sedentary behavior, insulin sensitivity, glucose tolerance, pediatric population
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Abbreviations

BMI: Body mass index
REE: Resting Energy Expenditure
iAUC: Incremental area under the curve
HDL: High density lipoprotein
LDL: Low density lipoprotein
Introduction

Prolonged bouts of uninterrupted sedentary behavior (sitting or reclining while expending ≤ 1.5 metabolic equivalents [1]) result in deleterious changes in insulin sensitivity, glucose tolerance, and plasma triglyceride levels in both healthy and overweight/obese adults [2–8]. Although initial studies in this area focused primarily on long-term bed rest and other restrictive forms of sedentary behavior [8], more recent studies have found that prolonged sitting may also result in significant reductions in insulin sensitivity and glucose tolerance in adult participants [3–5]. Dunstan and colleagues have recently reported that insulin and glucose responses to a standardized meal were elevated by nearly 25% following 7 hours of uninterrupted sitting in overweight and obese adults, in comparison to sitting with periodic light-intensity walk breaks [5]. Moreover, Stephens et al [4] reported that a single day of sitting reduced insulin action by 39% among a group of recreationally active young adults.

Despite the recent findings in adults, to date the effects of uninterrupted sitting in the pediatric population remain unexamined. Epidemiological studies have reported consistent associations between sedentary behavior and metabolic dysfunction in children and youth [9–11], suggesting that prolonged sitting may have a measurable health impact in this population. Given that the average child in North America spends more than half their waking hours sitting down [12–14], any cardiometabolic disease risk resulting from uninterrupted sedentary behavior in this age group would be of great public health importance. The objective of the present randomized crossover study was to determine whether 8 hours of uninterrupted sitting would result in increased concentrations of common markers of cardiometabolic disease risk in healthy children and youth, in comparison to a day of sitting interrupted by light intensity walk breaks, with and without structured physical activity.
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Methods

Participants

Nineteen healthy children and youth (11 male, 8 female) aged 10-14 years were recruited for this study. There were no limits placed on body weight or physical activity levels prior to study entry. Written consent was obtained from the parents of all participants prior to participation. Oral assent was obtained from participants aged 10-13 years, while participants aged 14 years provided written consent. This study conformed to the ethical standards outlined in the Declaration of Helsinki and was approved by the Research Ethics Boards at the Children’s Hospital of Eastern Ontario Research Institute and the University of Ottawa.

Baseline Testing Session

Participants visited the Behavioral and Metabolic Research Unit at the University of Ottawa on 4 occasions – 1 baseline session and 3 experimental sessions – each separated by at least one week. Participants arrived for all sessions at 07:30, and were instructed to fast and abstain from structured exercise for 12 hours prior to each visit. The baseline session included measurements related to anthropometry, physical activity, sedentary behavior, cardiorespiratory fitness (VO₂peak), and resting energy expenditure (REE). Weight was measured to the nearest 0.1 kg using a calibrated electronic scale. Standing height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured at the midpoint between the lower border of the last rib and the upper border of the iliac crest after a gentle expiration. Pubertal development was assessed using self-reported Tanner stages as previously validated by Taylor et al. [15].
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REE and VO\textsubscript{2} peak were assessed using an Ultima PF/PFX (MedGraphics, St Paul, USA) metabolic cart. VO\textsubscript{2} peak was measured using the Dubowy graded treadmill protocol [16]. Participants wore an Actical accelerometer (Philips Respironics, Andover, USA) on their right hip for a total of five week days and two weekend days following baseline testing. Accelerometer data were processed using standardized reduction procedures [12] in SAS version 9.2 (SAS Institute, Cary, USA) and were used to assess baseline levels of physical activity and sedentary behavior. Pediatric accelerometer cut-points of 100, 1,500 and 6,500 counts per minute were used to identify light-, moderate- and vigorous-intensity physical activity, respectively [17]. Daily energy requirements were estimated as the sum of REE and average daily physical activity-related energy expenditure as calculated using the Actical 2.12 software (Philips Respironics, Andover, USA).

**Experimental Sessions**

The 3 experimental conditions were performed in random order, as determined by TJS using a random number generator in Microsoft Excel (Microsoft Corporation, Redmond, USA) (Figure 1). Participants were blinded to the order of conditions, and only told which condition they would experience upon arrival in the lab each morning. Upon arrival to the lab at 07:30 a catheter was inserted into an antecubital vein for blood sampling. During the Sedentary condition, participants remained seated at all times from 07:30 until 15:30 (when necessary, participants were transported to the washroom via wheelchair). The Sedentary With Breaks (Breaks) condition was similar to the Sedentary condition, with the exception that participants walked for 2 minutes on a treadmill at an intensity equivalent to 30\% of VO\textsubscript{2} peak every 20 minutes beginning at 8:40 (i.e. 08:40, 09:00, 9:20, etc). Finally, the Sedentary With Breaks and Physical Activity
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(Breaks+PA) condition was similar to the Breaks condition, but in addition to walking at a light-intensity every 20 minutes, participants also performed two 20-minute bouts of moderate-intensity physical activity by walking or jogging on a treadmill at 60% of VO\(_2\)\(_{\text{peak}}\) from 08:40-09:00 and from 12:40-13:00. During all 3 conditions, participants engaged in a standardized set of common sedentary behaviors in identical order – 4 hours of watching movies and television programs, 2 hours of puzzles and other forms of mental work, and 1.5 hours of video games.

**Standardized Meals**

Standardized meals were provided at breakfast (08:15) and lunch (12:00), using a menu developed for the pediatric population. Breakfast consisted of white bread, butter, peanut butter, cheddar cheese, and orange juice, while lunch included chicken strips, tortilla chips, grapes, baby carrots, 2% milk, lemonade, ketchup, and Oreo cookies [18]. Both meals were standardized relative to estimated daily energy requirements (rather than macronutrient intake) with breakfast and lunch respectively providing 25% and 40% of estimated daily needs. The mean±SD intake at breakfast and lunch were 2322±410 and 3669±799 kJ, respectively. The proportion of calories from carbohydrate, fat, and protein respectively at breakfast were 52±5%, 36±5% and 12±1% while at lunch they were 57±2%, 31±3% and 12±3%. Participants with allergies or food intolerances (n=3) had individual food items replaced. However, each participant received identical meals at each of their 3 visits, and was asked to consume all food that was provided.

**Markers of Cardiometabolic Disease Risk**

Six blood draws were performed during each experimental day, with each sample requiring approximately 6 ml of blood. The first blood draw occurred at 08:00, and further draws were performed every 90 minutes until 15:30. All markers of cardiometabolic disease risk were
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assessed in duplicate using heparinized plasma, which was stored at -80 °C prior to analysis.

There were no missing samples for any participant or variable of interest. Insulin was assayed by enzyme-linked immunosorbent assay (ALPCO Diagnostics, Salem, USA). Glucose, triglycerides, HDL and LDL cholesterol were assessed on the Ortho Vitros 5.1FS (Ortho-Clinical Diagnostics, Rochester, NY). The inter-assay precision for each test was as follows: insulin 11%; glucose 2%; triglycerides 2%; HDL-Cholesterol 3%, LDL-Cholesterol 3%. Net incremental area under the curve (iAUC) was calculated for all cardiometabolic disease risk factors using the trapezoid rule [19]. This approach was used rather than the positive iAUC since HDL- and LDL-Cholesterol curves were expected to have negative values [19].

Statistical Analyses

Sample size calculations were based on a recent study using a similar crossover design in overweight and obese adults [5]. Although this differs from the current study population, it is the only human study with a design similar in nature to the present study [5]. We estimated that 13 paired observations would provide 90% power to detect an absolute difference as small as 3,000 pmol/L∙min in our primary outcome of insulin incremental area under the curve (iAUC) across conditions with a standard deviation of 3,000 pmol/L∙min at an alpha level of \( p=0.05 \) and a two-tailed distribution.

Insulin, triglyceride, HDL and LDL cholesterol iAUC were non-normally distributed, and therefore transformed using a Box-Cox transformation. To determine if males and females could be combined into one analysis, sex-by-condition interactions were assessed for all dependent variables. No significant interactions were detected, therefore males and females were combined for all analyses to maximize statistical power. A linear mixed-model was fitted for the iAUC of
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each risk factor, with effects for condition, age, sex, BMI, waist circumference, Tanner stage, and baseline physical activity and sedentary behavior. Statistical significance was defined as a $p$ value of 0.05 or less, and a Bonferroni correction was used to adjust for multiple comparisons in post hoc tests following the mixed-effect model. A similar linear mixed-model for raw levels of each risk factor over time was also fitted to assess temporal differences between conditions. This model included effects for condition, time, time-by-condition interaction, age, sex, BMI, waist circumference, Tanner stage, and baseline physical activity and sedentary behavior. Data are presented as mean ± standard deviation. All statistical tests were performed in SAS 9.2.

Results

Subject characteristics are presented in Table 1. In comparison to female participants, males were significantly older and more sedentary (all $p < 0.01$). However, there were no differences between males and females in terms of BMI, self-reported Tanner stage, moderate-to-vigorous physical activity, or any marker of cardiometabolic disease risk at baseline (all $p \geq 0.15$) There were no significant differences in baseline markers of cardiometabolic disease risk across the 3 experimental conditions (all $p > 0.25$).

iAUC values for the 3 experimental conditions are presented in Table 2. We did not observe significant differences for any marker of cardiometabolic disease risk (all $p > 0.09$). This finding remained consistent with or without adjustment for age, sex, Tanner stage, BMI, waist circumference, and baseline physical activity and sedentary behavior. Separating analyses by sex did not materially change these results (data not shown).
When examining temporal changes in markers of cardiometabolic disease risk across conditions, we observed a significant time-by-condition interaction for plasma glucose concentrations only ($p = 0.001$). Post-hoc tests determined that the glucose concentrations were significantly greater at Time 2 during the Breaks+PA condition than during the Breaks condition ($p=0.004$) but not the Sedentary condition ($p=0.051$) (Figure 2). Glucose levels were not significantly different across conditions at any other time point (all $p>0.40$).

**Discussion**

The results of the present randomized crossover study suggest that an acute prolonged bout of uninterrupted sitting does not result in deleterious changes in traditional markers of cardiometabolic disease risk in healthy children and youth. Although we observed a small increase in glucose levels following breakfast in the Breaks+PA condition, we observed no other differences across the three study conditions for any outcome of interest. These results are in contrast to those reported in both healthy and overweight/obese adults, where uninterrupted sitting has been reported to result in acute and deleterious changes in insulin sensitivity and glucose tolerance [5].

There are several factors which could explain the discrepancy between the current findings and those observed in adults [2-5]. The current study focused on healthy boys and girls who were more physically active than the average Canadian youth [12]. Girls, but not boys, were also less sedentary than the national average [12]. The participants were also aerobically fit and metabolically healthy at baseline, which is underscored by the relatively small insulin and glucose responses following both breakfast and lunch. It is probable that a similar investigation in physically inactive youth, a highly sedentary population, or those with obesity or other...
elevated cardiometabolic disease risk factors, may produce results more similar to those observed in adults. Employing a more sensitive measure of metabolic disease risk (i.e. continuous glucose measurements), or a larger food challenge (i.e. liquid meal high in fat and/or sugar) may also have more clearly differentiated between the study conditions. However, these techniques are substantially more burdensome than those used in the current study, which may impede their use in studies of the pediatric population. Examining the expression of genes related to carbohydrate and lipid metabolism may also have revealed differences between conditions, and is worth exploring in future studies in this population. However, given the present results it seems likely that the acute impact of a single bout of uninterrupted sitting on cardiometabolic disease biomarkers is simply smaller (or absent) in the pediatric population, as compared to adults.

It is not immediately clear why a transient increase in plasma glucose levels was observed at 9:30 during the Breaks+PA condition, but not during the Sedentary or Breaks conditions in the present study. It is worth noting that this increase occurred at the blood draw following the first 20-minute bout of exercise at 60% of VO$_2$peak. Aerobic exercise has been reported to increase hepatic glucose production and plasma glucose levels in adults [20], and it is possible that this was the cause of the increase observed in the present study. However, there is unlikely to be any clinical significance to this brief and relatively small increase in glucose levels.

The current study has several strengths and limitations that warrant mention. This is the first investigation into the acute impact of uninterrupted sedentary behavior in the pediatric population, and employed a rigorous randomized crossover design. Further, in contrast to the liquid meals that are sometimes used in adult studies of this nature [5], the standardized meals employed in the current study were similar to the food eaten by children on a normal basis,
increasing the ecological validity of our study. However, it is also possible that a liquid meal that is high in sugar and fat may also have provided a greater metabolic stimulus, which may have more effectively differentiated the impact of our three study conditions. A longer exposure to uninterrupted sitting may also have resulted in different results, although the ecological validity of such an approach would be questionable. All participants in the current study were healthy at baseline, and the majority were both lean and physically active. It is therefore unclear whether similar results would be observed among a population of overweight or obese youth, or those showing signs of metabolic dysfunction. The present findings are also limited by our small sample size, and by the relatively small number of outcomes which were examined. Finally, the present study did not examine whether fluctuations in energy balance across the three conditions may have influenced metabolic risk [4].

It is possible that the present findings may have differed had our study included a large number of participants, although our sample size calculation suggests that we had sufficient power for our primary outcome of insulin iAUC [5]. It is also worth noting that there were no consistent trends across the various outcomes measured, regardless of statistical significance. Although the insulin iAUC during the Sedentary condition was the highest of the 3 conditions, this was not the case for any other risk marker. This lends support to our conclusion that prolonged sitting does not result in significant increases in markers of cardiometabolic risk in this age group, and suggests that our results would not have been appreciably different with a larger sample size.

In conclusion, our findings suggest that in comparison to light walk breaks with or without structured physical activity, 8 hours of uninterrupted sitting do not result in measurable changes in circulating levels of insulin, glucose, or lipids in healthy children and youth. This suggests
that the relationship between sedentary behavior and increased health risk observed in epidemiological studies may be due to the behaviors children engage in while seated, rather than any direct metabolic impact of sitting per se [21-23]. Future research should involve children at risk of cardiometabolic abnormalities to determine whether the results are comparable.

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Disclosure Statement

The authors have no potential conflicts of interest to report.

Author Contributions

Study design: TJS, JPC, RCC, GSG, GPK, ED, MST; data collection: TJS, JPC, ED; Data analysis and interpretation: TJS, JPC, MST; manuscript writing and editing: TJS, JPC, RCC, GSG, GPK, ED, MST.
References


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### Table 1. Baseline characteristics of study participants.

<table>
<thead>
<tr>
<th></th>
<th>Male (n=11)</th>
<th>Female (n=8)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>12.9 (0.8)</td>
<td>11.3 (0.7)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>18.7 (4.5)</td>
<td>17.4 (2.9)</td>
<td>0.49</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>66.6 (15.8)</td>
<td>59.8 (5.7)</td>
<td>0.26</td>
</tr>
<tr>
<td>Tanner Stage</td>
<td>1.9 (1.0)</td>
<td>1.5 (0.8)</td>
<td>0.41</td>
</tr>
<tr>
<td>Sedentary behavior (min/day)</td>
<td>539.4 (48.3)</td>
<td>461.1 (66.0)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MVPA (min/day)</td>
<td>66.8 (28.5)</td>
<td>59.5 (23.8)</td>
<td>0.56</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>42.6 (27.7)</td>
<td>33.1 (18.8)</td>
<td>0.42</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.7 (0.4)</td>
<td>4.6 (0.3)</td>
<td>0.71</td>
</tr>
<tr>
<td>LDL-Cholesterol</td>
<td>1.9 (0.6)</td>
<td>2.0 (0.7)</td>
<td>0.91</td>
</tr>
<tr>
<td>HDL-Cholesterol</td>
<td>1.3 (0.3)</td>
<td>1.3 (0.4)</td>
<td>0.15</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.7 (0.2)</td>
<td>0.7 (0.2)</td>
<td>0.81</td>
</tr>
</tbody>
</table>

BMI: body mass index; MVPA: moderate-and-vigorous physical activity; LDL: low density lipoprotein; HDL: high density lipoprotein. Date are presented as mean (SD). Significance was assessed using an independent measures t test.
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Table 2. Net incremental area under the curve (iAUC) values for biomarkers of cardiometabolic disease risk during 8 hours of prolonged sitting, with or without interruptions or physical activity (n=19).

<table>
<thead>
<tr>
<th></th>
<th>Sedentary</th>
<th>Breaks</th>
<th>Breaks + Physical Activity</th>
<th>p for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (pmol/L·min)</td>
<td>80,559.3 (66380.3)</td>
<td>78,707.3 (83074.5)</td>
<td>64,270.8 (42272.4)</td>
<td>0.552</td>
</tr>
<tr>
<td>Glucose (mmol/L·min)</td>
<td>185.3 (171.7)</td>
<td>163.2 (148.8)</td>
<td>248.0 (220.4)</td>
<td>0.091</td>
</tr>
<tr>
<td>Triglycerides (mmol/L·min)</td>
<td>99.8 (114.4)</td>
<td>101.5 (69.0)</td>
<td>65.2 (66.4)</td>
<td>0.106</td>
</tr>
<tr>
<td>HDL-Cholesterol (mmol/L·min)</td>
<td>-38.7 (39.9)</td>
<td>-32.2 (40.2)</td>
<td>-52.3 (41.4)</td>
<td>0.431</td>
</tr>
<tr>
<td>LDL-Cholesterol (mmol/L·min)</td>
<td>-62.9 (67.2)</td>
<td>-50.0 (28.9)</td>
<td>-76.5 (51.4)</td>
<td>0.400</td>
</tr>
</tbody>
</table>

Sedentary: 8 hours of uninterrupted sitting.
Breaks: 8 hours of sitting interrupted with a 2-minute light-intensity walk break every 20 minutes.
Breaks + Physical Activity: 8 hours of sitting interrupted with a 2-minute light-intensity walk break every 20 minutes as well as 40 minutes of moderate-intensity physical activity.
Data are presented as mean (standard deviation). Significance was assessed by a linear mixed-model with effects for condition, age, sex, BMI, waist circumference, Tanner stage, and baseline physical activity and sedentary behavior. Although raw values are presented above, all statistical analyses have been performed using normalized data.
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Figures

Figure 1. Overview of the study protocol.

Sedentary

Breaks

Breaks+PA

Blood Draw (BD)  BD  BD  BD  BD  BD  BD
Participant arrives in lab, begins sitting

07:00 08:00 09:00 10:00 11:00 12:00 13:00 14:00 15:00 16:00

Participant returns home

20-minute walk at 60% VO₂peak
2-minute walk at 30% VO₂peak
Figure 2. Insulin (A) and glucose (B) concentrations during 8 hours of prolonged sitting, with or without interruptions or physical activity (n=19).

Sedentary: 8 hours of uninterrupted sitting.

Breaks: 8 hours of sitting interrupted with a 2-minute light-intensity walk break every 20 minutes.

Breaks + Physical Activity: 8 hours of sitting interrupted with a 2-minute light-intensity walk break every 20 minutes as well as 40 minutes of moderate-intensity physical activity.

Data are presented as mean and standard deviation. Significance was assessed by a linear mixed-model with effects for condition, time, time-by-condition interaction, age, sex, BMI, waist...
circumference, Tanner stage, and baseline physical activity and sedentary behavior. A Bonferroni correction was used to adjust for multiple comparisons in post hoc tests following the mixed-effect model. Only plasma glucose showed a significant time-by-condition interaction ($p = 0.001$). * = significant difference between Breaks and Breaks + PA condition, $p<0.01$. 
