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## **Fitness moderates glyceimic responses to sitting and light activity breaks**

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1 **Fitness moderates glycaemic responses to sitting and light activity breaks**

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24 **ABSTRACT**

25 **Purpose:** Regular engagement in sedentary behaviours can lead to major public health  
26 consequences. This study aimed to experimentally determine whether cardio-respiratory  
27 fitness modifies postprandial glycemia during prolonged sitting and investigated the  
28 potentially blunting influence this may have upon the benefits of interrupting postprandial  
29 sitting time with light activity breaks.

30 **Methods:** Thirty-four adult volunteers (18female; 16male; mean±SD age: 40±9 years, BMI:  
31 24.5±3 kg/m<sup>2</sup>) undertook two 7.5 hour experimental conditions in a randomized order: 1)  
32 Prolonged sitting; 2) Sitting interspersed with 5 minute light walking bouts every 30minutes.  
33 Blood samples were obtained while fasting and throughout the postprandial period following  
34 ingestion of two identical meals. Incremental Area Under the Curve (iAUC) was calculated  
35 for glucose and insulin throughout each experimental condition. Maximal exercise testing  
36 quantified VO<sub>2</sub> peak as a measure of cardiorespiratory fitness (CRF) prior to experimental  
37 conditions. A repeated measures ANOVA investigated whether VO<sub>2</sub> peak modified iAUC  
38 data between conditions. This trial is registered with ClinicalTrials.gov (Reg  
39 no.NCT0493309).

40 **Results:** Interrupting prolonged sitting time with light walking breaks reduced blood glucose  
41 iAUC from 3.89 ± 0.7 to 2.51 ± 0.7 mmol·L<sup>-1</sup>·h (p = 0.015) and insulin iAUC from 241 ± 46  
42 to 156 ± 24 mU·L<sup>-1</sup>·h (p = 0.013) after adjustment for VO<sub>2</sub> peak and sex. A significant  
43 interaction between treatment response and VO<sub>2</sub> peak was observed for glucose (p = 0.035),  
44 but not insulin (p = 0.062), whereby the treatment effect reduced with higher levels of fitness.  
45 Average blood glucose iAUC responses for a man at the 25<sup>th</sup> centile of CRF (42.5 mL·kg<sup>-1</sup>·  
46 min<sup>-1</sup>) within our cohort went from 5.80 to 2.98 mmol·L<sup>-1</sup>·h during the prolonged sitting  
47 and light walking breaks conditions respectively, whereas average responses for a man at the

48 75<sup>th</sup> centile of CRF ( $60.5 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) went from 1.99 to 1.78  $\text{mmol}\cdot\text{L}^{-1}\cdot\text{h}$ . Similar trends  
49 were observed for women.

50 **Conclusions:** Individuals with low levels of CRF gained the most metabolic benefit from  
51 breaking prolonged sitting with regular bouts of light walking. Future interventions aimed at  
52 alleviating the deleterious impacts of sedentary behavior may be optimized by tailoring to  
53 cardio-respiratory fitness levels within the general population.

54 **Key words:**

55 Sedentary Behavior – Type 2 Diabetes – Physical activity – Cardio-respiratory fitness –  
56 Postprandial metabolism

57 **INTRODUCTION**

58 Adults in developed western countries typically spend 50 - 70% of their waking hours sat  
59 down (30), making sedentary behavior the new reference of modern living. Greater time spent  
60 in sedentary behaviors (defined as sitting or reclining with low energy expenditure), has been  
61 associated with an increased likelihood of; metabolic syndrome (10), diabetes, cardiovascular  
62 disease (CVD) and all-cause mortality (4, 27). The evidence of which appears to be the  
63 strongest and most consistent for the risk of type 2 diabetes (T2DM) (4).

64 However, recent epidemiological evidence has suggested that physical activity levels and  
65 cardiorespiratory fitness (CRF) may moderate these associations, such that the association  
66 between sedentary time and markers or outcomes of health may be weaker in those with  
67 higher fitness levels (7, 23, 25), or those undertaking greater physical activity (11). This  
68 suggests that sedentary behavior may be a less important determinant of health in those with  
69 adequate CRF or those that are physically active. While experimental evidence largely  
70 confirms that breaking prolonged bouts of sitting with light-intensity walking can  
71 significantly reduce postprandial blood glucose and insulin in healthy non-obese individuals  
72 (2, 24), in those who are overweight and obese (9, 26), and in those with dysglycemia (15),  
73 no previous experimental trials have investigated whether these responses are modified by  
74 CRF or habitual physical activity levels.

75 CRF in particular is an important candidate for further investigation, as it is one of the  
76 strongest predictors of morbidity and mortality (19). CRF has been shown to moderate the  
77 deleterious impacts of other exposures such as body mass index (BMI), whereby obese  
78 individuals with moderate to high CRF levels have a lower risk of morbidity and mortality  
79 outcomes compared to normal weighted individuals with low CRF levels (12). It is therefore  
80 plausible that high levels of CRF may also protect against the deleterious impacts of  
81 prolonged sedentary behavior. Therefore, we hypothesized that CRF would modify the

82 postprandial glucose response to breaking prolonged sitting with light walking breaks with  
83 lower CRF levels being associated with greater reductions to postprandial plasma glucose.

84

## 85 **METHODS**

### 86 **Study Design**

87 All participants attended the Leicester Diabetes Centre on three separate occasions between  
88 September 2014 and September 2015. The first visit involved consent, familiarization and a  
89 fitness assessment which was followed by two experimental condition visits that were at least  
90 7 days apart. This was a randomized cross-over trial whereby each participant took part in  
91 two experimental treatment conditions in a random order, thereby acting as their own  
92 controls. Order randomization was conducted by a statistician using an online tool. Due to the  
93 nature of the trial, participants were not blinded to their randomized order, however all  
94 outcomes including blood assays were analysed blinded to the experimental condition that  
95 they derived from. Prior to commencing, this study received ethical approval from the  
96 University of Leicester - Health Sciences department and from the local NHS Research and  
97 Development committee.

98 This trial was also registered with ClinicalTrials.gov (NCT0493309).

### 99 **Participants**

100 Thirty six non-obese adults (BMI <30kg/m<sup>2</sup>) aged between 25 – 55 years who worked in a  
101 predominantly seated environment were recruited from the general public via study-specific  
102 information distributed in the community, around the University of Leicester campus and  
103 University Hospitals of Leicester NHS Trust. Two individuals were withdrawn following  
104 enrollment in the study due to a change in personal circumstances (n = 2). This left 34

105 participants who went on to complete the remaining experimental conditions. This is detailed  
106 in **Figure 1**.

107 Exclusion from taking part in this study came under the following circumstances; an inability  
108 to communicate in spoken English, a BMI  $\geq 30\text{kg/m}^2$ , pregnancy, steroid usage, regular  
109 smoking habits, diagnosed T2DM, CVD or psychotic illness. As our study was predicated on  
110 having a broad range of fitness levels, and considering that most of the variance in CRF is  
111 explained by habitual physical activity levels (6), we stratified recruitment by self-reported  
112 leisure time physical activity. Consequently, we enrolled 12 inactive (0 minutes of  
113 MVPA/week), 12 moderately active ( $\geq 75$ minutes -  $<150$ minutes of MVPA/week), and 12  
114 highly active ( $\geq 150$ minutes of MVPA/week) individuals. (See **Table S-1, Supplemental**  
115 **Digital Content 1**, scope of CRF levels captured).

#### 116 **Consent, familiarisation and fitness assessment visit**

117 On arrival, a researcher described in detail all study procedures and written informed consent  
118 was obtained. Participants were then shown the designated experimental area for the study.

119 A venous blood sample was taken to assess HbA1c and confirm absence of T2DM  
120 ( $<6.5\%$  [ $<47.5\text{mmol/mol}$ ]) (29). Body weight (Tanita TBE 611: Tanita, West Drayton, U.K),  
121 waist circumference (midpoint between lower costal margin and iliac crest) and height were  
122 measured to the nearest 0.1kg, 0.5cm and 0.5cm, respectively.

123 In order to assess CRF, participants undertook a maximal incremental exercise test on a  
124 motor driven treadmill (Technogym Excite® 700). Following a three minute warm-up at  
125 4km/h (0% incline), participants would walk or jog at a constant speed that they felt  
126 comfortable with (6, 8, 10, or 12km/h) while elevations in treadmill gradient occurred at a  
127 rate of 0.5% every 30 seconds. All participants received encouragement to continue this  
128 exercise for as long as possible. The test was terminated upon volitional exhaustion.

129 Throughout the test, gas was sampled continuously and analysed using a Metalyser 3B gas  
130 analyser (Cortex 3B, Cortex Biophysik, Leipzig, Germany). Peak oxygen consumption ( $\text{VO}_2$   
131 peak) was calculated using the highest ten second average throughout the testing period.  
132 Before each test, the gas analyser was calibrated according to the manufacturer's  
133 recommendations. As a safety precaution, a 12 lead electrocardiogram was performed by a  
134 cardiac nurse for each participant at rest and during the exercise test.

135 Finally, participants were issued with two activity monitors; an ActiGraph GT3X+  
136 accelerometer (Pensacola, FL) worn on the right anterior axillary line, and an activPAL3  
137 physical activity monitor (PAL Technologies, Glasgow, UK) worn on the midline anterior  
138 portion of the right thigh. Participants were required to wear these for 7 consecutive days,  
139 allowing insight into their habitual sitting and physical activity levels.

#### 140 **Experimental procedure**

141 Participants were asked to avoid alcohol and caffeine for the 48 hours preceding experimental  
142 treatment conditions. As the influence of an acute bout of physical activity on insulin  
143 sensitivity can persist for 48 hours (17), avoidance of moderate and vigorous physical activity  
144 for this timeframe was also instructed. Continuation in this study was subject to participants  
145 being able to confirm their compliance with these restrictions. Following an ethical  
146 amendment to the protocol during this study, a subset of participants were asked to wear an  
147 accelerometer in the 2 days leading up to each experimental condition in order to confirm  
148 adherence to the exercise restriction (See **Table S-2, Supplemental Digital Content 2,**  
149 activity data leading up to experimental conditions).

150 Participants fasted from 10pm the evening before each visit and were asked to keep a record  
151 of all food eaten during the day leading up to their first experimental condition. This could



152 then be replicated prior to their second experimental condition in an attempt to eliminate the  
153 potentially confounding influence of pre-experimental food intake.

154 Participants underwent two separate 7·5 hour experimental treatment conditions:

155 1) Prolonged sitting - participants sat in a designated room (occupied with a desk, books, and  
156 laptop with internet services) while minimising excessive movement. Lavatory breaks were  
157 permitted using a wheelchair to and from the lavatory in order to further reduce unnecessary  
158 movements that could otherwise confound the study.

159 2) Light walking breaks - participants emulated the above, but interrupted sitting time with 5  
160 minute bouts of walking at a light intensity of 3km·hr on the treadmill (Technogym Excite®  
161 700) every 30 minutes. These bouts were performed 12 times, totalling one hour of activity  
162 and 6·5 hours of sitting throughout the course of the experimental day.

163 On arrival, participants had a cannula fitted into an accessible vein from which 10mL  
164 samples were obtained throughout the day. Immediately following the two fasting samples  
165 (depicted at timepoints -1 and 0 in **Figure 2**), participants were given a standardized meal  
166 consisting of 8 kcal per kilogram of body weight, with a macronutrient composition reflective  
167 of co-ingestion in modern western diets (14% protein, 51% carbohydrate and 35% fat). Once  
168 consumed (within  $\leq 15$  minutes), blood sampling commenced at 30, 60, 120, and 180 minutes  
169 thereafter, enabling us to capture the postprandial period. An identical meal was then issued  
170 (time point 3 in **Figure 2**) and sampling continued in a similar fashion at 30, 60, 120, 180,  
171 and 210 minutes following this. Participants were supervised by study staff to ensure  
172 compliance with the protocol and were asked to wear an activPAL monitor to objectively  
173 confirm sitting and walking times during each experimental condition (See **Table S-3**,  
174 **Supplemental Digital Content 3**, sitting and walking data during experimental conditions).  
175 *Ad libitum* water consumption was also noted and made consistent between conditions.

176 **Biochemical analysis**

177 Glucose was analysed on the day of collection by the University Hospitals of Leicester  
178 pathology department, using standard enzymatic techniques with commercially available kits  
179 (Beckman, High Wycombe, U.K).

180 Centrifuged (4°C) plasma samples were stored in -80°C freezers and insulin was analysed  
181 from these collectively at the end of the trial using an electrochemiluminescence assay (Meso  
182 Scale Discovery, Maryland, USA). Each sample was ran in duplicate to ensure reliability of  
183 readings. Duplicate sample values with  $\geq 20\%$  variability were reanalysed. Ambient  
184 conditions of the laboratory were kept consistent in order to reduce variability between  
185 assays.

186 **Free-living activity monitor processing**

187 ActivPAL data were downloaded using the manufacturers software (activPAL Professional  
188 Research Edition, PAL technologies, Glasgow, UK) and 'Event' csv files were processed  
189 using a validated automated algorithm in STATA (StataCorp LP, Texas, USA) described in  
190 detail elsewhere (28).

191 Actigraph data (100Hz) were downloaded using the manufacturer's software (ActiLife  
192 version 6.10.4, Lite Edition), reintegrated into 60 second epoch files and processed using a  
193 bespoke tool (KineSoft, version 3.3.76; KineSoft, New Brunswick, Canada  
194 [[www.kinesoft.org](http://www.kinesoft.org)]). Freedson cut points were used to categorize activity intensities (13).  
195 Non-wear time was defined as a minimum of 60 minutes of continuous zero counts, and  
196 when assessing habitual activity levels, days with at least 10 hours of wear time were  
197 required to be considered valid.

198 The minimum amount of valid days utilised for both ActivPAL and ActiGraph data was three  
199 days.

200 **Statistical analysis**

201 Descriptive characteristics of those who completed this study are summarised overall (n = 34)  
202 and stratified by sex (**Table 1**) for descriptive purposes.

203 Missing glucose and insulin data during the experimental conditions accounted for roughly  
204 2% of overall required samples (34 out of 1,496) (see Table S-4, Supplemental Digital  
205 Content 4, summary of missing glucose and insulin data). These 34 missing data points were  
206 imputed using a regression model previously developed for an acute trial investigating  
207 breaking sedentary behaviour (15). This approach uses key predictors (BMI, ethnicity, age,  
208 fasting values and treatment condition) to derive a regression equation for the glucose and  
209 insulin values at each individual time point, this regression equation is then used to impute  
210 missing values.

211 The iAUC of glucose and insulin was calculated for each experimental condition. Total AUC  
212 was calculated by applying the trapezium rule and further subtraction of fasting levels gave a  
213 single value of iAUC for each participant. Utilising iAUC as opposed to total AUC is  
214 common practice in acute interventions where fasting levels should be unaffected by the  
215 intervention (20). Glucose iAUC was defined *a priori* as the primary outcome.

216 The effect of light walking breaks compared to continuous sitting on outcomes (glucose and  
217 insulin iAUC) and whether CRF modified this response was assessed using a repeated  
218 measures ANOVA. Treatment was entered as a within-person variable, with CRF (as a  
219 continuous variable) entered as a between-subjects covariate. Sex was also entered as a  
220 between-subjects factor. ‘Treatment by CRF’ and ‘treatment by sex’ interaction terms were  
221 investigated to assess the modifying effect of fitness and sex respectively. Sex was included  
222 in the model given that it is a strong determinant of fitness and an important potential  
223 confounder. Treatment by CRF interactions were further explored by calculating the linear

224 regression coefficients within each treatment condition. To highlight the direction of  
225 significant interactions, derived average glucose iAUC values for men and women at the 25<sup>th</sup>,  
226 50<sup>th</sup> and 75<sup>th</sup> centile of the CRF distribution are shown in Figure 3.

227 Two-tailed  $p \leq 0.05$  was considered significant. Analyses were performed with SPSS (version  
228 24). Results are presented as mean  $\pm$  SE or regression coefficient (95% CI) unless stated  
229 otherwise.

## 230 **RESULTS**

231 The key characteristics of those who successfully completed all three study visits are  
232 displayed in **Table 1** (n = 34). Stratification of these characteristics for both males and  
233 females are also presented here.

### 234 **Overall treatment condition effect**

235 The average postprandial concentrations of glucose (**A**) and insulin (**B**) witnessed throughout  
236 the 7.5 hour testing periods for both experimental conditions ('prolonged sitting' and 'light  
237 walking breaks') are depicted in **Figure 2**. There was a significant main effect of treatment  
238 for both glucose ( $F(1, 31) = 6.67, p = 0.015$ ) and insulin ( $F(1, 31) = 7.00, p = 0.013$ ) iAUC  
239 after adjustment for fitness and sex. Interrupting prolonged sitting time with light walking  
240 breaks reduced blood glucose iAUC by 35% (from  $3.89 \pm 0.7$  to  $2.51 \pm 0.7$  mmol·L<sup>-1</sup>·h) and  
241 insulin iAUC by 35% (from  $241 \pm 46$  to  $156 \pm 24$  mU·L<sup>-1</sup>·h).

### 242 **Impact of CRF and sex**

243 There was a significant treatment by CRF interaction for glucose iAUC ( $F(1, 31) = 4.89, p =$   
244  $0.035$ ). The treatment by CRF interaction for insulin iAUC failed to reach significance ( $F(1,$   
245  $31) = 3.76, p = 0.062$ ). There was no treatment by sex interaction for glucose ( $F(1, 31) =$   
246  $1.77, p = 0.194$ ) or insulin ( $F(1, 31) = 1.54, p = 0.223$ ) iAUC.

247 Stratified analysis revealed that each unit increment in CRF (per  $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) was  
248 associated with a lower glucose iAUC ( $-0.21 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{h}$ ; 95% CI  $-0.38, -0.05$ ) ( $p = 0.013$ ) in  
249 the prolonged sitting condition, whereas there was no association between CRF and glucose  
250 iAUC during the light walking breaks condition ( $-0.07 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{h}$ ;  $-0.21, 0.07$ ) ( $p = 0.335$ ).  
251 In contrast, each unit increment in CRF was associated with a lower insulin iAUC ( $-10.93$   
252  $\text{mU}\cdot\text{L}^{-1}\cdot\text{h}$ ;  $-19.48, -2.37$ ) ( $p = 0.014$ ) in the prolonged sitting condition and a lower insulin  
253 iAUC ( $-6.35 \text{ mU}\cdot\text{L}^{-1}\cdot\text{h}$ ;  $-10.90, -1.83$ ) ( $p = 0.007$ ) in the light walking breaks condition.

254

255 **Figure 3** uses the derived regression coefficients to show how the predicted average  
256 difference between conditions for glucose iAUC changes as CRF increases for males and  
257 females. This demonstrates that average blood glucose iAUC response for a man at the 25<sup>th</sup>  
258 centile of CRF within our cohort went from  $5.80$  to  $2.98 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{h}$  (from prolonged sitting  
259 to light walking breaks, respectively), whereas average responses for a man at the 75<sup>th</sup> centile  
260 went from  $1.99$  to  $1.78 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{h}$ . Similar trends were observed for women.

## 261 **DISCUSSION**

262 This study found that interrupting prolonged sitting with regular light walking breaks reduced  
263 postprandial glucose and insulin levels in a healthy cohort. However, CRF modified the  
264 response for glucose such that individuals with lower levels of fitness received incrementally  
265 greater reductions in postprandial glucose. For example, the average response for a man at the  
266 25th centile of CRF within our population ( $\text{VO}_2$  peak of  $42.5 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) demonstrated  
267 relatively high postprandial glucose levels during prolonged sitting ( $5.80 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{h}$ ) but  
268 was able to almost half this level through employing regular light walking breaks. In  
269 contrast, the average response for a man at the 75th centile of fitness ( $\text{VO}_2$  peak of  $60.5$   
270  $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) demonstrated relatively low levels of postprandial glucose during prolonged

271 sitting ( $1.99 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{h}$ ) but only reduced this by a further 11% through employing regular  
272 light walking breaks. The same pattern was demonstrated for women. These results were  
273 supported by further analysis which demonstrated that CRF was inversely associated with  
274 postprandial glucose during prolonged sitting, whereby every unit increment in  $\text{VO}_2$  peak  
275 (per  $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ) was associated with an average reduction of  $0.21 \text{ mmol}\cdot\text{L}^{-1}\cdot\text{h}$  in glucose  
276 iAUC values. Taken together, our results suggest that having high CRF or employing regular  
277 light walking breaks in those with low CRF can both reduce postprandial levels of glucose  
278 during periods of prolonged sitting activity. Elevated postprandial glucose levels are  
279 implicated with the development of T2DM and CVD (5) and therefore strategies to promote  
280 healthy glycaemic responses when sedentary are of high importance.

281 Our observation that those with higher CRF demonstrate less metabolic benefit from light  
282 activity breaks is consistent with previous experimental research that has tended to show  
283 relatively lower metabolic benefits of light activity breaks in healthy cohorts (1, 22)  
284 compared to both those with high risk of chronic disease (9, 15). Our findings also  
285 correspond to cross sectional research that has shown the influence of sedentary time on a  
286 cluster of cardio-metabolic issues to be significantly less pertinent in those with higher fitness  
287 levels (7, 23, 25). The concept that fitter individuals may gain less pronounced health benefits  
288 from lower levels of sitting time is supported by cross-sectional research that have stratified  
289 data by habitual MVPA level, finding that individuals with higher MVPA levels display  
290 significantly weaker associations between sedentary time with HbA1c (3), inflammation  
291 markers (16) and all-cause mortality (11).

292 In contrast, a recent meta-analysis found that the association between sedentary time and  
293 health outcomes persisted in sufficiently active individuals (4). However, this pooled analysis  
294 was predominantly derived from self-reported measures of sedentary time and MVPA which  
295 are prone to bias and consequently may have been insensitive to detecting true interactions. It

296 should also be noted that although observational research linking sedentary behaviour to  
297 health is plentiful, the vast majority have investigated the confounding rather than the  
298 modifying influence of physical activity (4, 27) or fitness (25).

299 The growing observational and experimental data has supported new guidance and  
300 recommendation calling for reductions in sitting time (18). However, if the findings of the  
301 current study continue to be supported by further research, there may be reasonable grounds  
302 to embark upon a more personalized/tailored approach to T2DM prevention. Precision  
303 medicine is important given that a one size fits all recommendation is rarely effective. For  
304 example, interventions to reduce sitting time may be optimized by targeting those with poor  
305 CRF, whereas those with high CRF may be better served by interventions aimed at  
306 maintaining CRF and physical activity levels across the lifespan. However, it should be noted  
307 that median levels of CRF within our population for men and women were 50.3 and 34.0  
308  $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  respectively and that the average reductions in postprandial glucose at this  
309 level of CRF was 41%. As the majority of the general population within the age range  
310 included in this study are estimated to fall below the median levels of fitness within our  
311 population (8), the importance of interrupting sitting time with light activity breaks is likely  
312 to remain generalizable to the majority of the population.

313 This research also suggests that increasing CRF levels may be a viable way to protect against  
314 the potential harms of prolonged sitting. Although there are genetic contributions to fitness,  
315 the largest contributor to an individual's fitness is their time spent in MVPA (6).

316 Participation in regular MVPA outside of seated hours may therefore offer some protection,  
317 particularly in seated occupations such as driving.

318 Our observation that fitter individuals experienced less pronounced postprandial glycaemic  
319 excursions during prolonged sitting may result from favourable physiological adaptations

320 stemming from regular engagement in MVPA (one of the main determinants of fitness), such  
321 as increased skeletal muscle GLUT 4 protein expression (14). This would also leave less  
322 scope for further improvement, potentially explaining why the benefits of interrupting sitting  
323 time with light activity breaks appear to be blunted in those with higher fitness. However,  
324 given that CRF is determined by a mixture of both MVPA engagement and genetics (6), we  
325 cannot distinguish between behavioral and genetic mechanisms driving the results of the  
326 current study.

327 This study has some important limitations. Although this study provides an initial proof-of-  
328 concept from which future research can tailor to alternative study cohorts, findings should not  
329 be generalised outside the population investigated. In particular, given that the population  
330 utilised in this study were healthy, the extent to which CRF modifies responses in high risk or  
331 clinical population remains to be investigated. Our second limitation is that despite  
332 instructions to standardize food intake, and refrain from caffeine and alcohol consumption  
333 leading up to treatment conditions, we did not objectively test participant compliance and  
334 relied on self-reported adherence. In addition, fitness assessments were only conducted at one  
335 time-point, thus direct causality cannot be inferred. Future interventions that actively set out  
336 to manipulate fitness levels and assess prospective change in experimental data are required  
337 to elucidate direct causality. Another concern was that those with higher fitness in this study  
338 were predominantly men and conversely, those with lower fitness were predominantly  
339 women. However, our results were adjusted for sex and it was not found to modify the  
340 treatment effect for glucose which was in contrast to CRF. Therefore the correlation between  
341 sex and CRF is unlikely to be confounding the results of this study.

342 In conclusion, participants with lower fitness had worse postprandial glucose and insulin  
343 responses during prolonged sitting, and were able to gain greater metabolic benefit through  
344 breaking their sitting time with light activities compared to individuals with higher fitness.



345 Future interventions aimed at alleviating the deleterious metabolic impacts of sedentary  
346 behaviour may therefore be optimized by tailoring to cardio-respiratory fitness levels of the  
347 general population.

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378 falsification, or inappropriate data manipulation.

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381 **LIST OF SUPPLEMENTAL DIGITAL CONTENT**

382 **Supplemental Digital Content 1.doc**

383 **Supplemental Digital Content 2.doc**

384 **Supplemental Digital Content 3.doc**

385 **Supplemental Digital Content 4.doc**

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479 **FIGURE CAPTIONS:**

480 **Figure 1** - Trial CONSORT Profile

481 **Figure 2** - Effect of treatment condition on average Blood Glucose and Insulin

482 **Figure 3** – The effect of treatment condition on average iAUC (95% CI) for glucose  
483 categorised by fitness level by the 25<sup>th</sup>, 50<sup>th</sup> and 75<sup>th</sup> centile of the cohort.