

The effect of mode of transport on intraindividual variability in glycemic and insulinemic response testing

Article

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1 ABSTRACT

2 The effect of light to moderate intensity exercise, such as that used as a mode of transport, on glycaemic
3 response (GR) testing is unclear. The aim was to investigate the effect of acute exercise (walking and
4 cycling) simulated to act as a mode of transport, prior to GR testing on the intra-individual variability of
5 blood glucose and insulin. Eleven male participants visited the laboratory four times. Initially they
6 undertook a VO₂max and two submaximal exercise tests. For the other three visits they either rested (25
7 min), cycled or walked 5km followed by a two hour GR test after consuming a glucose drink (50g
8 available carbohydrate). The mean CV of each transport group was below the International Organisation
9 for Standardisation cut off of 30%. The highest mean coefficient of variation (CV) of glucose area under
10 the curve (GAUC) was between the rest and walking trials (30%) followed by walking and cycling (26%).
11 For insulin AUC (IAUC) the highest mean CV was between walking and cycling (28%) followed by rest
12 and walking (24%). The lowest GAUC and IAUC were between rest and cycling (25% and 14%,
13 respectively). The current study also did not find differences ($p > .05$) between the conditions for GAUC
14 (rest: 134.5 ± 104.6 ; walking: 115.5 ± 71.7 ; cycling: 142.5 ± 75 mmol·120min·L⁻¹) and IAUC (rest: 19.45
15 ± 9.12 ; walking: 16.49 ± 8.42 ; cycling: 18.55 ± 9.23 μmol·120min·mL⁻¹). The results indicate no
16 difference between the tests undertaken however further research should ensure the inclusion of two rest
17 conditions.

18

19 Keywords: glycaemic index, exercise, standardization

20 INTRODUCTION

21 In 2010, the International Organisation for Standardisation (ISO) published the first edition of guidelines
22 for standardising the determination of glycaemic index (GI) of foods for practice and research purposes
23 (International Standards Office, 2010). According to the report, subjects should avoid vigorous exercise
24 on the morning of the test, as it has been shown to raise whole body glucose uptake and glucose area
25 under the curve (GAUC) (Rose et al., 2001). This will result in an increase in coefficient of variation
26 (CV) between trials and possibly exceed the acceptable level of variability for the reference food of 30%
27 (International Standards Office, 2010). The large within-subject variability for the reference food can
28 decrease the accuracy, precision, and reproducibility of GI (Brouns et al., 2005). People may not exercise
29 in the morning before the test but may walk and cycle in order to commute to these research studies.
30 There has been little agreement on the effect of low to moderate intensity exercise on glycaemic response
31 (GR) testing. Some studies have shown no effect on GR after aerobic exercise (Ben-Ezra et al., 1995;
32 Roberts, Desbrow et al., 2013). Whereas, others have observed a decrease (Bonen et al., 1998) or an
33 increase in GAUC (Knudsen et al., 2014; Rose et al., 2001). Either way, whether it is an increase or a
34 decrease, the CV of the measurement will be affected by this change. Regardless of whether this change
35 leads to a CV >30% or not, researchers should always aim to minimise it in order to improve precision. It
36 should be noted that none of the abovementioned studies reported the CV between trials.

37
38 Understanding the effect of walking and cycling at an average pace of commuting, on blood glucose and
39 insulin levels is particularly important. Subjects may come by bus (rest) to one visit and cycle or walk to
40 another visit. In Oxford Brookes University a significant portion (33%) of staff and students walk or cycle
41 to the university (Oxford Brookes University, 2016) and any alteration in the type, duration or intensity of
42 this activity may add noise to the results and hence reduce their reproducibility (Brouns et al., 2005).
43 Therefore, the aim of this study was to investigate the effect of acute exercise (walking and cycling) prior
44 to testing on the intra-individual variability of blood glucose and insulin responses. We hypothesised that

45 performing acute exercise before blood glucose and insulin response testing will increase the intra-
46 individual variability between trials.

47

48 **METHODS**

49 Eleven men (age 26 ± 4 years; weight 74.1 ± 8.1 kg; height 177 ± 7 cm; BMI 23.8 ± 3.1 kg·m⁻²; VO₂max
50 40.6 ± 6.6 ml·kg⁻¹·min⁻¹) participated in the present study. Eligibility criteria included being male,
51 exercising ≤ 150 min per week, aged between 18-40 years, and free of metabolic disorders. This study was
52 conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were
53 approved by the University Research Ethics Committee at Oxford Brookes University.

54

55 Participants visited the laboratory four times during the study. They undertook a preliminary test at visit
56 1, then rest, cycling and walking tests followed by a five minute rest and a two hour GR test for the three
57 subsequent visits given in random order. The GR was measured using the protocol adapted by Brouns and
58 colleagues (2005) and by following the ISO guidelines (International Standards Office, 2010). A
59 summary of the experimental protocol and study design is presented in Figure 1.

60

61 For the preliminary test participants arrived at the laboratory after fasting for three hours, avoiding
62 caffeine consumption for 10 hours, having avoided the consumption of alcohol and refrained from any
63 strenuous physical activity for 24 hours. Their height was taken using a stadiometer (Seca, Birmingham,
64 UK) and body composition was measured using Tanita BC-418 (Tanita, Middlesex, UK) body
65 composition analysis.

66

67 Then participants completed two submaximal exercise tests followed by a VO₂max test. The first
68 submaximal test consisted of walking at a steady pace of 5 km·h⁻¹ and 1% gradient for 10 min while
69 taking gas measurements to determine the oxygen uptake (VO₂) using an automated gas analysis system
70 (Metalyzer 3B, Cortex, Germany). This test was used to determine the intensity of walking as a

71 percentage of VO_2max . Subjects rested for 10 minutes before they completed the second submaximal test
72 which consisted of five stages of cycling exercise. The work load started at 40 Watt (W) and
73 progressively increased by 15 W every 4 minutes until it reached 100 W at stage 5. This was used to
74 determine the work load of the cycling condition. Following the submaximal tests, subjects took a 15
75 minute rest and then performed an incremental ramp exercise test (VO_2max) on an electromagnetically
76 braked cycle ergometer (Corival, Lode, The Netherlands). The incrementation rate was 5 W every 15s
77 corresponding to ramp slope of 20 W per minute from a baseline of 20 W. The test was terminated and
78 VO_2max was considered reached when two of the following criteria were met: 1) the participant could no
79 longer sustain a pedalling cadence of at least 60 rpm, 2) a respiratory exchange ratio ≥ 1.1 , 3) an increase
80 in oxygen uptake $< 0.2 \text{ l}\cdot\text{min}^{-1}$ (Howley et al., 1995). For this study, the maximal test was preceded by a
81 submaximal test for practical reasons. It has been shown that maximal oxygen uptake is not affected by
82 varying stages of exhaustion at the beginning of the test (Stamford et al., 1978).

83
84 During the three experimental visits participants arrived at the laboratory after 10-hours overnight fasting,
85 having avoided the consumption of alcohol and caffeine and refrained from any strenuous physical
86 activity for the last 24 hours as recommended in the ISO guidelines (International Standards Office,
87 2010). They were also instructed to come to the laboratory by bus or driving to avoid exercising prior to
88 testing and standardise physical activity. Participants were asked if they followed the instructions given
89 on their arrival to measure compliance. None of our participants were excluded based on this. Participants
90 were randomised to one of the three following conditions: 1) rest on a chair for 25 min (rest), 2) cycle 5
91 km at 50% VO_2max (cycling), and 3) walk 5 km at a speed of $5 \text{ km}\cdot\text{h}^{-1}$ with 1% inclination equivalent to
92 $37 \pm 7\%$ VO_2max (walking). These conditions were applied to simulate a 5 km commute to the
93 laboratory. The exercises were not matched for work but instead the distance was fixed to simulate a
94 realistic scenario where participants commute from home which is always at a fixed distance from the
95 laboratory. All conditions were followed by 5 min rest and by a two hour glucose test.

96

97 The chosen distance for walking and cycling was set at 5 km which was based on two criteria: 1) the
98 average distance travelled to work by trip length and mode in Great Britain (Department for Transport,
99 2013) and 2) the distance required to apply for a parking permit at Oxford Brookes University (Oxford
100 Brookes University, 2014). According to the national travel survey conducted in 2012, the highest
101 percentage of people (24%) who used walking, cycling, or taking the bus as mode of transport commuted
102 an average distance of 5.6 km (Department for Transport, 2013). However, as the distance required to
103 apply for a parking permit at Oxford Brookes University is >5 km (Oxford Brookes University, 2014), 5
104 km was set as the average distance where most people will most likely use the bus, bicycle, or walking as
105 mode of transport. The speed of $5 \text{ km}\cdot\text{h}^{-1}$ was set based on the preferred walking speed of normal weight
106 adults (Browning et al., 2006).

107
108 Capillary blood samples were taken at -5, 0, 15, 30, 45, 60, 90, and 120 min following the glucose drink.
109 The glucose drink was ingested at 0 min and consisted of 250 ml of water mixed with 50 g of available
110 carbohydrate (Myprotein, Cheshire, UK). Blood glucose was immediately measured using an automatic
111 blood glucose analyser (Glucose 201+, Hemocue, Sweden) who has a CV% of 1.3% (APPN, 2015). The
112 accuracy of the analyser was checked daily using a control solution. Following the measurement of blood
113 glucose, 300 μl of blood was collected in a microtainer and held on ice until centrifuged at 4000 rpm for
114 10 min (MicroCentaur, MSE, UK). Blood plasma was pipetted and stored at -40°C where plasma insulin
115 was later analysed using electrochemiluminescence immunoassay using an automated analyzer (Cobas
116 E411, Roche Diagnostics, USA) who has a CV% of 2% (Roche Diagnostic USA).

117
118 In order to standardise food intake, participants received pre-packaged meals that were consumed on the
119 day before each trial with an unlimited access to water. The diet given was subject-specific, covering their
120 daily energy and nutrient requirements. The pre-packaged meals consisted of cornflakes, whole milk,
121 bread, cheese, butter, tomato, pasta, tomato sauce, apple, and banana. On average the diets provided $50 \pm$
122 2% carbohydrate, $15 \pm 1\%$ protein, and $35 \pm 1\%$ fat of the total energy intake. The energy requirement for

123 each participant was calculated using a predictive equation (Harris & Benedict, 1918) and a physical
124 activity questionnaire (IPAQ, 2002). Participants were asked to bring all the leftovers the following day in
125 order to measure their compliance to the diet given before they were cleared to start. Lack of compliance
126 was defined as a %CV above 3% for energy, carbohydrate, and protein and above 6% for fat between
127 trials (El-Chab et al., 2016). None of our participants exceeded these values. The average %CV for
128 energy, carbohydrate, protein and fat intakes between the three test days were 0.4%, 1.1%, 1.0% and
129 0.7%, respectively.

130
131 Statistical analyses were performed using SPSSv.22 (IBM, NY, USA). The GAUC and IAUC were
132 calculated using the trapezoidal model (Food and Agriculture Organization and World Health
133 Organization, 1998). The CV of the AUC values obtained for each condition were calculated ($CV = 100 \times$
134 mean/SD). Shapiro–Wilk statistic was used to determine the normality of the data. Repeated measure
135 ANOVA with Bonferroni correction was performed to test differences in GAUC and IAUC between the
136 three conditions. Where data were skewed, the Friedman test was used. This study was primarily powered
137 based on the ISO guidelines which recommends the inclusion of a minimum of 10 participants
138 (International Standards Office, 2010). The sample size required to compare the CV was calculated using
139 the equation published by Hopkins (2000) which suggests that 10 participants were needed. Statistical
140 significance was set at $p < .05$. All values are mean \pm standard deviation unless stated otherwise.

141

142 **RESULTS**

143 All eleven participants completed the trial. Participants exercised at 50% $VO_2\text{max}$ which equates to a
144 workload of $96 \pm 31W$ during the cycling trial. During the walking trial they exercised at 37 ± 8
145 % $VO_2\text{max}$. Mean energy expenditure during cycling and walking were 30 ± 8 and 93 ± 9 kcal,
146 respectively.

147

148 The highest mean CV of GAUC was observed between rest and walking (30%) followed by walking and
149 cycling conditions (26%), while the variability between rest and cycling was the lowest (25%). On the
150 other hand, the highest mean CV of the plasma IAUC was observed between walking and cycling (28%)
151 followed by rest and walking (24%), while the variability between rest and cycling was the lowest (14%).

152
153 There was no difference between blood glucose (rest: 4.4 ± 0.5 ; walking: 4.5 ± 0.6 ; cycling 4.4 ± 0.3
154 $\text{mmol}\cdot\text{L}^{-1}$) and insulin (rest: 51 ± 17 ; walking: 53 ± 14 ; cycling $57 \pm 21 \text{ nmol}\cdot\text{mL}^{-1}$) values at baseline p
155 $>.05$. Temporal blood glucose and insulin response curves following either rest, walking or cycling are
156 presented in Figure 2 and 3, respectively. The GAUC and IAUC at 60 and 120 minutes for all conditions
157 can be found in Table 1. No statistically significant effect was detected between all conditions. Figure 4
158 presents the paired data between all three conditions.

159

160 DISCUSSION

161 The present study was designed to determine the effect of acute exercise (walking and cycling) prior to
162 GR testing on within-subject variability of blood glucose and insulin responses and found that the CV of
163 GAUC between rest and cycling was 26% while the CV between cycling and walking was 25%. These
164 values are below the 30% cut-off set by ISO (International Standards Office, 2010) and borderline intra-
165 individual variability of 22-25% seen in previous studies (Clegg et al., 2011; Williams et al., 2008;
166 Wolever et al., 1985). However, the CV between rest and walking (30%) matched the acceptable level of
167 variability for reference food as outlined by ISO, however it exceeded the intra-individual variability as
168 seen in previous studies by at least 5%.

169

170 However, this data also needs to be considered on an individual basis. In the situation where walking was
171 used as a mode of transport during one test day and rest during another test day the mean CV was 30%.
172 However 5 participants had a CV of greater than 30%. In this situation, researchers will either 1) need to
173 perform a third test of the reference food in the case where only two were completed, 2) repeat the test

174 that is inconsistent with the other two tests in the case where three were completed, or 3) exclude subjects
175 with large variability (International Standards Office, 2010). So in this case 5 participants would need to
176 repeat the test. What is interesting is that in the rest /cycle comparison where the mean CV was 26%, the
177 number of people with a CV of greater than 30% was also 5. This implies that different types of exercise
178 have similar effects and implications for GI testing. Furthermore this highlights the major limitation of the
179 current study in not having a second rest trial which can be used as a baseline from which to make
180 comparisons. Without this it is difficult to make any conclusions about whether exercise per se has an
181 effect on GR variability.

182
183 In the ISO guidelines, the reference food, usually glucose or white bread, serves as a reference point
184 which other foods (test food) are measured against. Large intra-individual variability for the reference
185 food can decrease the accuracy, precision, and reproducibility of the GI (Brouns et al., 2005). Due to the
186 small numbers of subjects included in GI studies (n= 8-12) (Brouns et al., 2005; Foster-Powell et al.,
187 2002; Wolever, Jenkins et al., 1988) poor standardisation can have implications even if the CV is less
188 than 30%. If we were to consider a hypothetical scenario where 180 mmol·L⁻¹ is the GAUC of the
189 reference food in one of the subjects, a CV of +25% (equivalent to 257 mmol·L⁻¹) will lead to a GI of 70
190 whereas a CV of +30% (equivalent to 277 mmol·L⁻¹) will lead to a GI of 65 (GI = GAUC of reference
191 food divided by GAUC of test food multiplied by 100). In this hypothetical example, walking to the
192 laboratory before the test food and taking the bus before the reference food testing can contribute to an
193 additional 5 point discrepancy in GI. Without the a rest/rest comparison we cannot tell if this discrepancy
194 would be the same following two similar standardisation protocols but it does indicate some limitations in
195 the GI methodology.

196
197 Median GAUC and mean IAUC after 120 min were approximately 16% lower after walking compared to
198 rest, although this difference was not statistically different. These results therefore need to be interpreted
199 with caution given the lack of rest/rest comparison. Bonen and colleagues (1998) showed similar

200 reduction of 16% in GAUC but no difference in IAUC after low intensity exercise compared to rest.
201 These findings are supported by previous studies who showed that a single bout of exercise improves
202 insulin sensitivity in muscles (Hayashi et al., 2005; Nazar et al., 1987) leading to a reduced insulin
203 response (Ben-Ezra et al., 1995; Hayashi et al., 2005) and improved glucose tolerance (Bonen et al., 1998;
204 Nazar et al., 1987). However, other studies have also shown contradictory results mainly after high
205 intensity exercise (Braun et al., 1995; King et al., 1995). The differences in GAUC were less pronounced
206 between cycling and rest. A possible explanation for this might be that although cycling had a higher
207 intensity than walking (50% vs. $37 \pm 8\%$ VO_2max , respectively); it was significantly shorter (14min for
208 cycling and 60min for walking). It could be argued that the total energy expenditure which was higher
209 during walking (93 ± 9 kcal) compared to cycling (30 ± 8 kcal) led to the pronounced effect of walking. It
210 has been shown that energy expenditure rather than intensity has more impact on insulin sensitivity
211 (Braun et al., 1995). A 30 ± 8 kcal of energy expenditure during cycling may not be significant enough to
212 alter glucose response. The large standard deviation might explain the lack of statistically significant
213 difference between walking and the two other conditions.

214
215 As outlined above a major limitation of this study is lack of a second rest trial. This would have allowed
216 the calculation of baseline intra-individual variability from which comparisons could have been made
217 within our study group. Another limitation of this study is that we did not measure participants' energy
218 expenditure on the day preceding each visit to make sure it does not vary significantly. However, we did
219 provide instruction to our participants to keep their physical activity level as close as possible the day
220 before each visit and compliance was measured on their arrival to the laboratory. This study did not
221 include female participants; however, we do not consider it as a limitation as there is no difference in GR
222 or impact of exercise on GR between genders (Wolever et al., 2003; Bonen et al., 1998).

223

224 **CONCLUSION**

225 The current study did not find any differences in blood glucose following three different modes of
226 transport prior to GR test. We also found that the mean CV of each transport group was below the ISO cut
227 off of 30%. Differences in CV can have implications for GI values as demonstrated above and future
228 studies should include two rest conditions to allow the calculation of baseline intra-individual variability.
229 This will allow for conclusions to be made as to the possible impact of exercise on GI values.

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231 The authors' contributions are as follows: AE and MC contributed to the study design and interpretation
232 of the findings; AE was the principal investigator and contributed to the data collection, data analyses,
233 and writing the manuscript. All authors read and approved the final version of the manuscript. No external
234 financial support was gained for this study.

235

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- 320

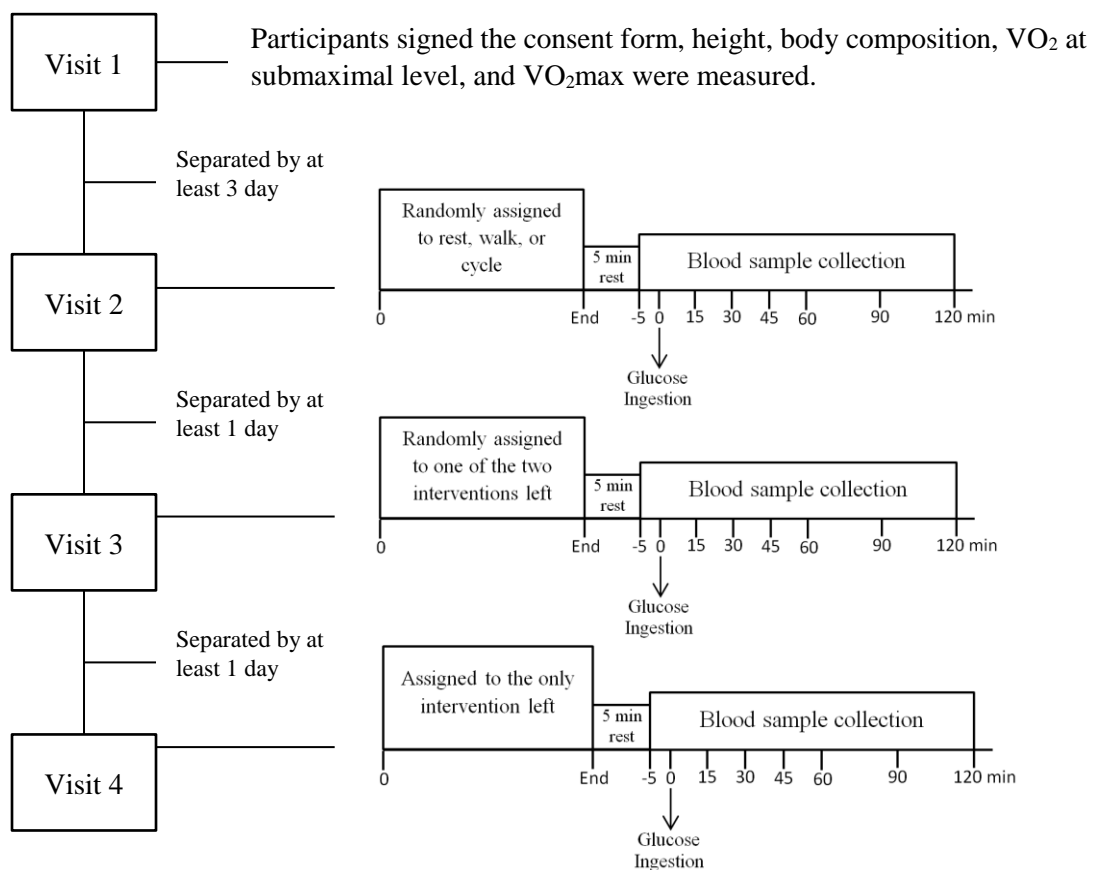
321 Table 1. Plasma glucose and insulin areas under the curve during 60 min and 120 min of the 2h glucose
 322 test following either rest, walking or running

	Rest	Walking	Cycling	P value	
GAUC	mmol.60min.L ⁻¹	102.4 ± 56.5	85.9 ± 44.8	109.8 ± 41.8	.78
	mmol.120min.L ^{-1*}	134.5 ± 104.6	115.5 ± 71.7	142.5 ± 75	.10
IAUC	μmol.60min.mL ⁻¹	13.14 ± 5.76	10.72 ± 5.34	12.64 ± 5.42	.23
	μmol.120min.mL ⁻¹	19.45 ± 9.12	16.49 ± 8.42	18.55 ± 9.23	.29

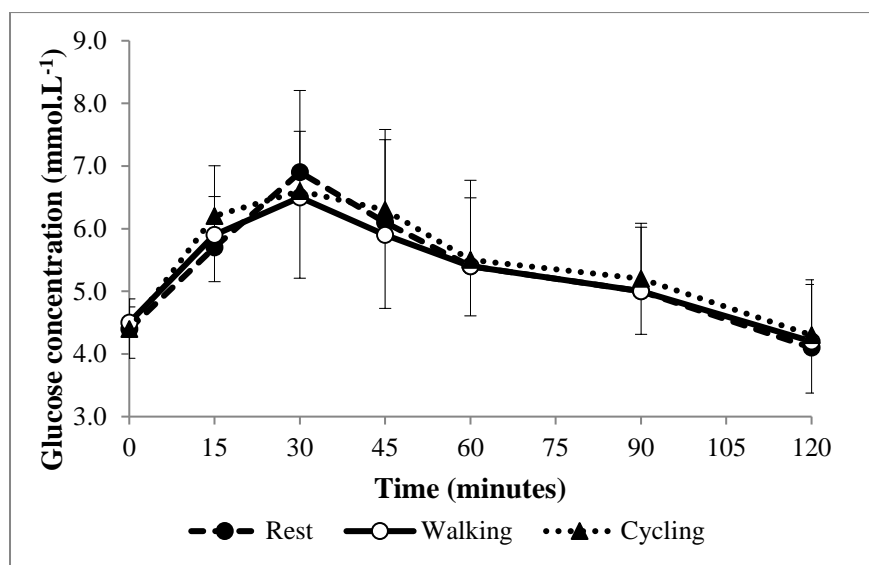
323 Values are mean ± SD.

324 * Values are median ± SD.

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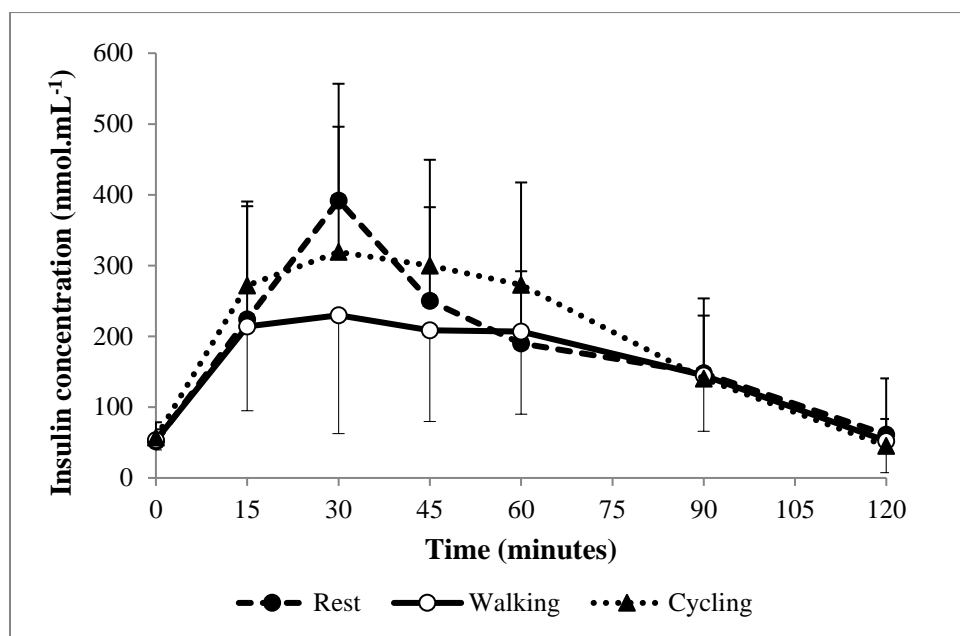
341 Figure 1: Summary of the experimental protocol and study design.



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343 Figure 2: Temporal blood glucose response curves following either rest, walking or cycling simulated to
344 act as a mode of transport. Data indicates the median \pm SD.

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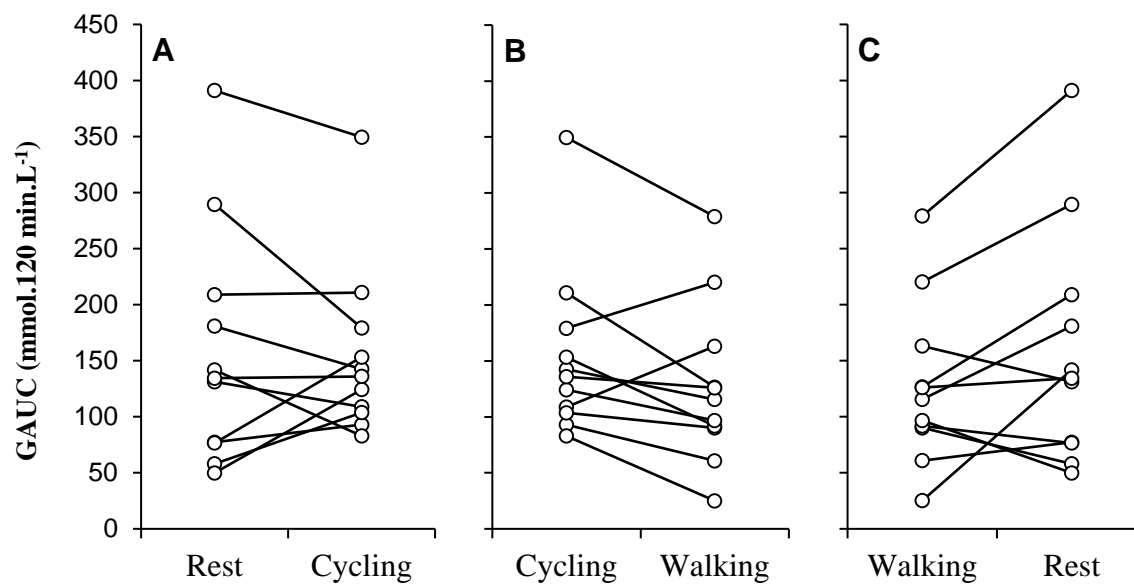


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347 Figure 3: Temporal plasma insulin response curves following rest, walking or cycling simulated to act as

348 a mode of transport. Data indicates the median \pm SD.

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351 Figure 4. Paired data of the incremental blood glucose area under the curve between all conditions. (A)

352 Rest vs. cycling. (B) Cycling vs. walking. (C) Walking vs. rest.