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

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ABSTRACT

The effect of light to moderate intensity exercise, such as that used as a mode of transport, on glycaemic response (GR) testing is unclear. The aim was to investigate the effect of acute exercise (walking and cycling) simulated to act as a mode of transport, prior to GR testing on the intra-individual variability of blood glucose and insulin. Eleven male participants visited the laboratory four times. Initially they undertook a VO_2max and two submaximal exercise tests. For the other three visits they either rested (25 min), cycled or walked 5km followed by a two hour GR test after consuming a glucose drink (50g available carbohydrate). The mean CV of each transport group was below the International Organisation for Standardisation cut off of 30%. The highest mean coefficient of variation (CV) of glucose area under the curve (GAUC) was between the rest and walking trials (30%) followed by walking and cycling (26%). For insulin AUC (IAUC) the highest mean CV was between walking and cycling (28%) followed by rest and walking (24%). The lowest GAUC and IAUC were between rest and cycling (25% and 14%, respectively). The current study also did not find differences (p >.05) between the conditions for GAUC (rest: 134.5 ± 104.6; walking: 115.5 ± 71.7; cycling: 142.5 ± 75 mmol·120min·L^{-1}) and IAUC (rest: 19.45 ± 9.12; walking: 16.49 ± 8.42; cycling: 18.55 ± 9.23 µmol·120min·mL^{-1}). The results indicate no difference between the tests undertaken however further research should ensure the inclusion of two rest conditions.

Keywords: glycaemic index, exercise, standardization

INTRODUCTION

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

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

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

METHODS

Eleven men (age 26 ± 4 years; weight 74.1 ± 8.1 kg; height 177 ± 7 cm; BMI 23.8 ± 3.1 $kg \cdot m^{-2}$; VO_2max 40.6 ± 6.6 $ml \cdot kg^{-1} \cdot min^{-1}$) participated in the present study. Eligibility criteria included being male, exercising ≤150 min per week, aged between 18-40 years, and free of metabolic disorders. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the University Research Ethics Committee at Oxford Brookes University.

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

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

Then participants completed two submaximal exercise tests followed by a VO_2max test. The first submaximal test consisted of walking at a steady pace of 5 $km \cdot h^{-1}$ and 1% gradient for 10 min while taking gas measurements to determine the oxygen uptake (VO_2) using an automated gas analysis system (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 $l \cdot min^{-1}$ (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 $km \cdot h^{-1}$ with 1% inclination equivalent to
92 37 ± 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

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

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

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

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

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

RESULTS

All eleven participants completed the trial. Participants exercised at 50% VO_2max which equates to a workload of 96 ± 31W during the cycling trial. During the walking trial they exercised at 37 ± 8 %VO_2max. Mean energy expenditure during cycling and walking were 30 ± 8 and 93 ± 9 kcal, respectively.

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 $mmol \cdot L^{-1}$) and insulin (rest: 51 ± 17; walking: 53 ± 14; cycling 57 ± 21 $nmol \cdot mL^{-1}$) 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

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

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

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

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

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

CONCLUSION

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

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

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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.

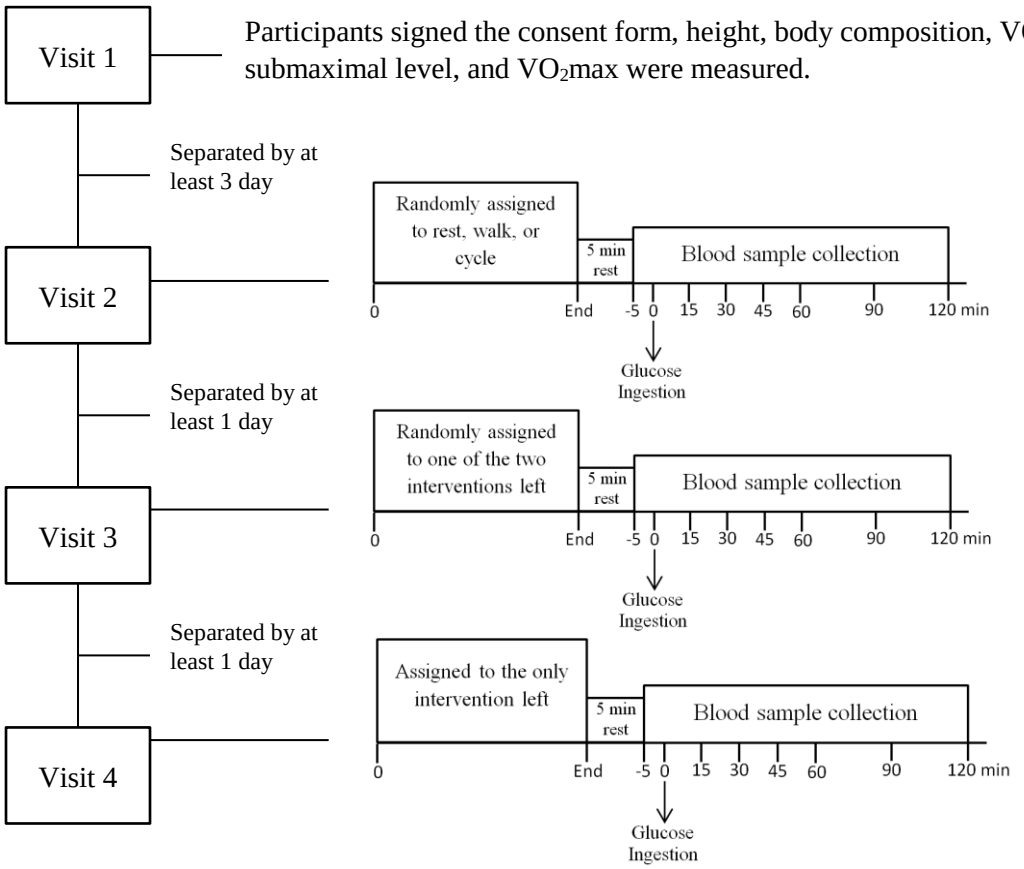
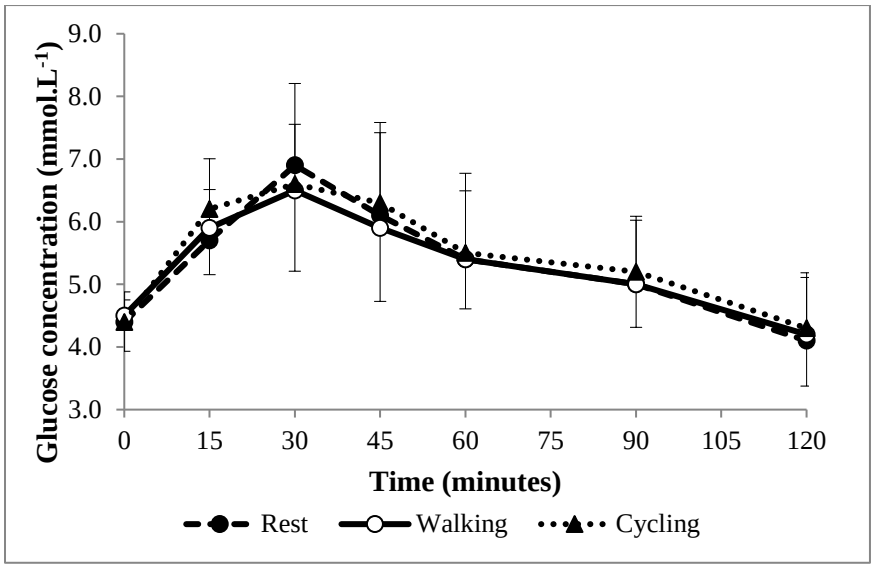


Figure 1: Summary of the experimental protocol and study design.



342

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 ± SD.

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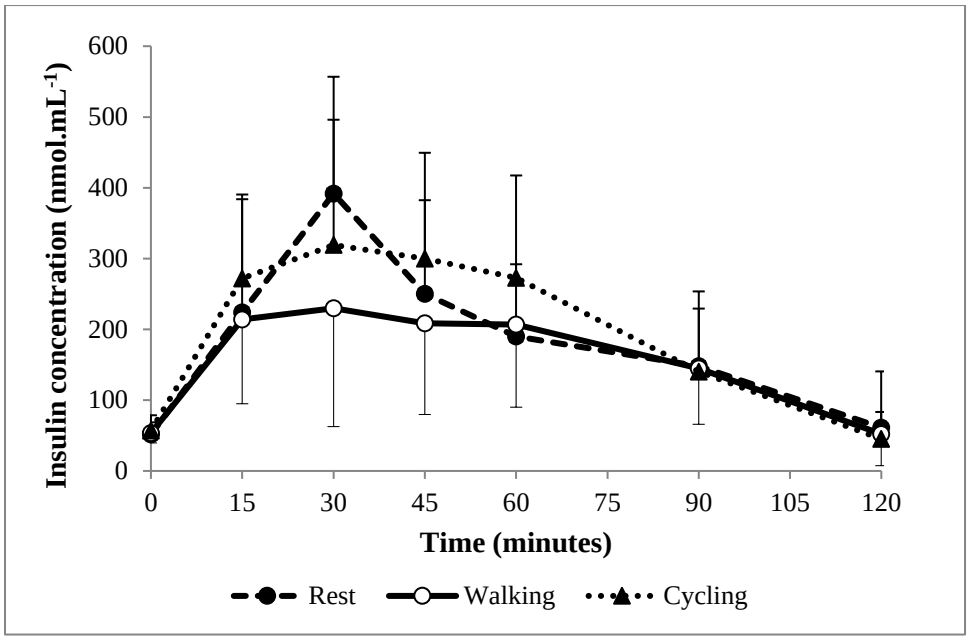

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347 Figure 3: Temporal plasma insulin response curves following rest, walking or cycling simulated to act as

348 a mode of transport. Data indicates the median ± SD.

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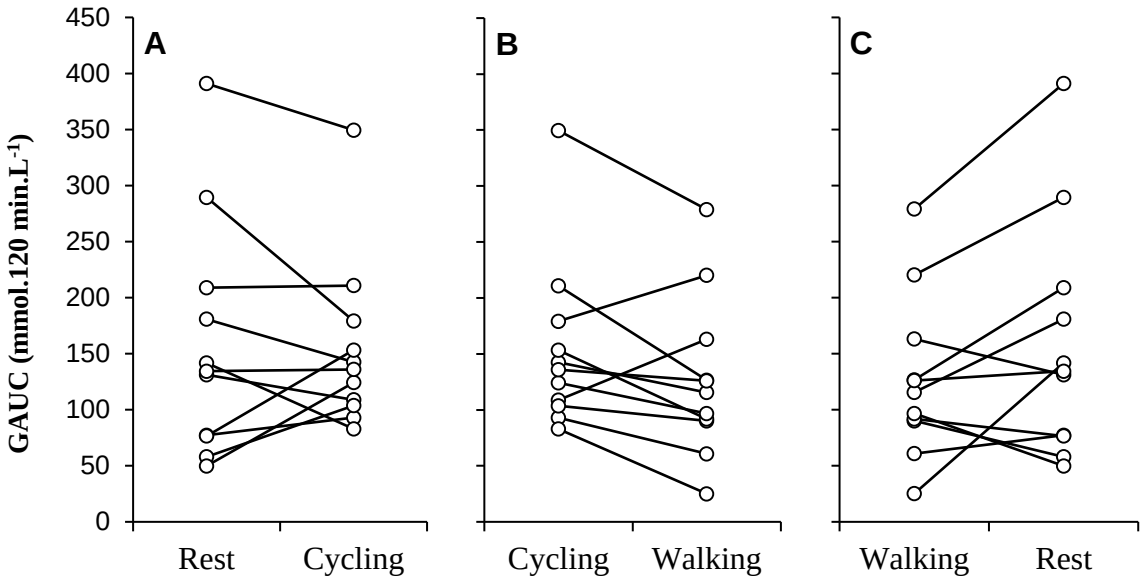

350

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.