Fuel Use during Exercise at Altitude in Women with Glucose–Fructose Ingestion

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Short Title: Substrate oxidation in women at high altitude

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Abstract

Purpose: This study compared the co-ingestion of glucose and fructose on exogenous and endogenous substrate oxidation during prolonged exercise at terrestrial high altitude (HA) versus sea level, in women. **Method:** Five women completed two bouts of cycling at the same relative workload (55% W_{max}) for 120 minutes on acute exposure to HA (3375m) and at sea level (~113m). In each trial, participants ingested 1.2 g.min^{-1} of glucose (enriched with ^{13}C glucose) and 0.6 g.min^{-1} of fructose (enriched with ^{13}C fructose) before and every 15 minutes during exercise. Indirect calorimetry and isotope ratio mass spectrometry were used to calculate fat oxidation, total and exogenous carbohydrate oxidation, plasma glucose oxidation and endogenous glucose oxidation derived from liver and muscle glycogen. **Results:** The rates and absolute contribution of exogenous carbohydrate oxidation was significantly lower at HA compared with sea level (ES>0.99, P<0.024), with the relative exogenous carbohydrate contribution approaching significance (32.6±6.1 vs. 36.0±6.1%, ES=0.56, P=0.059) during the second hour of exercise. In comparison, no significant differences were observed between HA and sea level for the relative and absolute contributions of liver glucose (3.2±1.2 vs. 3.1±0.8%, ES=0.09, P=0.635 and 5.1±1.8 vs. 5.4±1.7 grams, ES=0.19, P=0.217), and muscle glycogen (14.4±12.2% vs. 15.8±9.3%, ES=0.11, P=0.934 and 23.1±19.0 vs. 28.7±17.8 grams, ES=0.30, P=0.367). Furthermore, there was no significant difference in total fat oxidation between HA and sea level (66.3±21.4 vs. 59.6±7.7 grams, ES=0.32, P=0.557). **Conclusion:** In women, acute exposure to HA reduces the reliance on exogenous carbohydrate oxidation during cycling at the same relative exercise intensity.

Keys Words: Acute Hypoxia, Carbon Isotope, Exogenous Carbohydrate Oxidation, Liver Glycogen, Muscle Glycogen, Plasma Glucose Oxidation

1 **Introduction**
2
3 Endogenous stores of carbohydrate and fat are utilized as substrates to meet the metabolic demands

4 of the working muscle, as well as exogenous sources of carbohydrate if provided. The contribution

5 of carbohydrate and fat oxidation to energy expenditure at sea level is primarily related to the

6 intensity and duration of exercise, as well as the type and amount of carbohydrate ingested.

7 However, exposure to high altitude (HA) and the reduction in arterial oxygen saturation, is likely

8 to alter substrate oxidation during exercise compared with sea level. The complete oxidation of

9 carbohydrate requires less oxygen per mole of ATP synthesized compared with the oxidation of

10 free fatty acids (1) potentially leading to a greater reliance on carbohydrate oxidation in hypoxia.

11 However, the literature is inconclusive when comparing substrate oxidation during exercise

12 between hypoxia and normoxia (2). This heterogeneity may be explained by variations in study

13 design, such as participants' sex, method of exercise intensity determination (absolute vs. relative)

14 and whether carbohydrate was ingested or not (2).

15

16 Greater dependency on plasma glucose has been shown in men when comparing acute hypoxia

17 with normoxia, using the same absolute exercise intensity (3, 4). Conversely, when the exercise

18 intensity is normalized to the same relative exercise intensity the literature for men is equivocal

19 (2). These data either show an increase (5, 6) or no change in the respiratory exchange ratio (RER)

20 (7, 8). When an exogenous source of carbohydrate is provided on acute exposure to HA, men have

21 shown an increased reliance on endogenous carbohydrate oxidation (9), as well as a reduced

22 reliance on muscle glycogen and increased fat oxidation (10) during exercise at the same relative

23 intensity. However, it is known that sex influences substrate oxidation during exercise at sea level,

24 with women generally relying more on fat than carbohydrate as a fuel source compared with men

25 (11, 12). This has been attributed to women having a higher percentage of body fat (12), a greater

26 lipid content in muscles fibers (11) and better mobilization of fatty acids from subcutaneous

27 adipose tissue linked to cyclic changes in estrogen and progesterone (13). However, following the

28 provision of an exogenous source of glucose during exercise at sea level, most studies show no

29 statistical sex-differences in exogenous carbohydrate oxidation during exercise (14, 15) with one

30 exception (16). In the study by Riddell et al. (16), higher exogenous carbohydrate oxidation was

31 accompanied by a greater reduction in endogenous carbohydrate oxidation during exercise in

32 women compared with men. This is in contrast to other studies, which showed no difference (17)

33 or smaller reductions in endogenous or carbohydrate oxidation compared with men (14). Despite

34 this, there have only been limited studies assessing whole body carbohydrate or fat oxidation in

35 women at altitude.

36

37 In contrast to men, women have a lower RER, with increased free fatty acid availability when

38 exercising at a moderate relative exercise intensity (70% of altitude-related maximal oxygen

39 uptake ($\dot{V}O_{2\,max}$)) on acute exposure to hypoxia (4,300m) compared with normoxia (18). These

40 women also showed no differences in substrate oxidation at HA between the early-follicular and

41 mid-luteal menstrual cycle phases when levels of estradiol and progesterone are high. Conversely,

42 a study, which recruited men and women, showed no change for either sex in RER during acute

43 hypoxic exposure (4100m) compared with normoxia when exercising at a matched relative

44 exercise intensity (50% of altitude-related $\dot{V}O_{2\,max}$) (19). However, whole body carbohydrate

45 oxidation was increased at HA in both men and women following hypoxic exposure when using

46 the same absolute exercise intensity.

47   Considering the potential differences in fuel use between women and men at sea level, and what

48   has been documented for men at altitude, it is surprising that the fuel use responses to carbohydrate

49   ingestion during exercise at acute hypoxia have yet to be established in women. Thus, the purpose

50   of the present study was to compare the effects of co-ingesting ^{13}C glucose and ^{13}C fructose during

51   120 minutes of moderate intensity cycling exercise on exogenous and endogenous fuel use during

52   acute hypoxia (terrestrial high altitude) and normoxia (sea level) in women.

53

54   **Methods**
55   *Participants*

56   Seven women from the British military were recruited for this study, providing 86% power to

57   detect differences in the rate of exogenous carbohydrate oxidation, with an expected mean

58   difference of 0.2 g.min^{-1} between high altitude and sea level, assuming a standard deviation of 0.17

59   g.min^{-1} at an alpha of 0.05. However, only five completed the study due to attrition at points where

60   it was not possible to recruit additional participants. The five participants (age 25 ± 2 years, body

61   mass 61.7 ± 5.6 kg), were engaged in regular physical training (3-5 training days per week) and

62   considered to be physically fit but not elite athletes. Participants provided written informed consent

63   before the study, which was approved by the Ministry of Defence Research Ethics Committee

64   (Protocol 412/13). This cohort of participants were part of a larger research project and the design

65   of this study and the associated protocols have been described previously (10).

66

67   *Preliminary Testing*

68   Participants completed two maximal incremental cycle tests to volitional exhaustion to determine

69   their individual maximal workload (W_{max} (20)) and $\dot{V}O_{2max}$ on a bicycle affixed to a bicycle trainer

70   (Compu Trainer® Pro Lab, Racer Mate, USA), calibrated according to the manufacturer's

71 instructions. The first test was performed at sea level (altitude ~113m) with the second test

72 performed a week later during acute exposure to normobaric hypoxia (FiO$_2$ ~13.4% (considering

73 water vapour partial pressure (21) and daily fluctuations of barometric pressure) equivalent to

74 3375m (the reported altitude for the New Refuge Torino in the Italian Alps; PiO$_2$ 95.2 mmHg).

75 Oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) measurements were made

76 throughout using an online gas analysis system (Metalyser, Cortex, Germany), calibrated

77 following the manufacturer's instructions and were calculated using standard metabolic algorithms

78 (22) using the Haldane transformation. FiO$_2$ and FiCO$_2$ were measured continuously, rather than

79 assuming constants, thus correcting for changes in ambient conditions. This is important in a

80 normobaric chamber where the FiO$_2$ is reduced to simulate a given altitude. At sea level and

81 normobaric hypoxia participants achieved W_{max} values of 208.5 ± 34.4 W and 190.9 ± 31.3 W, and

82 $\dot{V}O_{2max}$ values of 44.7 ± 7.1 ml.kg^{-1}.min^{-1} and 37.4 ± 5.9 ml.kg^{-1}.min^{-1}, respectively.

83

84 ***Design of the Study***

85 Following the assessment of W_{max}, participants completed two experimental cycling trials for 120

86 minutes at 55% $W_{max.,}$ one at terrestrial HA (barometric pressure 506.7 ± 1.4 mmHg, PiO$_{2:}$ $96.3 \pm$

87 0.3 mmHg (New Refuge Torino, Alps, Italy)), as described previously (10) and another at sea level

88 seven weeks later. To control for menstrual cycle phases, the women were tested in either the

89 early-follicular or mid-luteal phase, which has no effect on fuel use at HA (18). Each cycling test

90 involved the ingestion of 1.8 g.min^{-1} of carbohydrate (1.2 g.min^{-1} of glucose (D-glucose, Thornton

91 and Ross Ltd, Huddersfield, UK) and 0.6 g.min^{-1} of fructose (D-fructose, Danisco, Oy, Oktka,

92 Finland) at regular intervals during exercise. Stock glucose (natural δ^{13}C abundance = -32.58 ‰)

93 and fructose (natural δ^{13}C abundance = -30.04 ‰), was enriched using 0.24g of U-^{13}C$_6$ D-glucose

94   (Cambridge Isotope Laboratories, Inc, Tewksbury, MA, USA), and 0.12g of U-$^{13}C_6$ D-fructose

95   (Cambridge Isotope Laboratories, Inc), achieving a combined enrichment of $\delta^{13}C$ = +115.88 ‰.

96   All $\delta^{13}C$ measurements are quoted with reference to the internationally accepted standard for

97   carbon isotope measurements, Vienna Pee Dee Belemnite (VPDB). The ^{13}C abundance of stock

98   glucose and fructose and ^{13}C enrichment of spiked glucose and fructose was determined using

99   liquid chromatography coupled to isotope ratio mass spectrometry (LC-IRMS; Isoprime, Cheadle,

100   UK), using L-Fucose as an isotopic internal standard as previously described (23).

101

102   *Diet and physical activity before testing*

103   Participants recorded their food intake and activity patterns during the 72 hours before the first

104   experimental trial and were instructed to repeat the same diet and activity pattern before the

105   subsequent trial. Participants were required to refrain from any intense and/or prolonged physical

106   activity, alcohol or caffeine consumption in the 36 hours before each experimental trial. In

107   addition, they were asked to refrain from ingesting carbohydrates derived from plants which utilize

108   the C_4 photosynthetic cycle, in which there is higher natural abundance of ^{13}C (e.g. maize derived

109   sugars) for the duration of the study. This precaution ensured that background $^{13}CO_2$ abundance

110   was less likely to be perturbed from oxidation of endogenous and dietary substrate stores from

111   naturally "enriched" C4 origin. A standardized evening meal was consumed 12 hours before each

112   experimental trial (total 1443 kcal; 53% carbohydrate, 17% fat, 30% protein).

113

114   *Experimental Trials*

115   Each experimental trial was performed at 19 to 21°C following an overnight fast. Participants

116   repeated their trials at the same time of day, to avoid any influence of circadian variance. On arrival

117 a catheter (20 gauge Introcan Safety®, B. Braun Medical Ltd, Sheffield, UK) was inserted into an

118 antecubital vein for regular blood sampling. After 20 minutes of acute exposure to each

119 environmental condition, peripheral oxygen saturation (SpO_2 (Nellcor N-20, Covidien, Dublin,

120 Ireland) was measured and resting blood samples were drawn for the analysis of plasma glucose,

121 serum insulin, serum free fatty acids, plasma lactate, plasma metanephrine and normetanephrine

122 concentrations, as well as plasma ^{13}C glucose enrichment.

123

124 Participants then rested for 10 minutes whereby $\dot{V}O_2$ and $\dot{V}CO_2$ measurements were made using

125 an online gas analysis system (Metalyser, Cortex, Germany). For the measurement of $^{13}C/^{12}C$ in

126 expired CO_2, 12 ml samples of expired gas were collected in duplicate in Labco Exetainers®

127 (SerCon Ltd, Crewe, UK) via a mixing chamber (Jaeger, Germany).

128

129 After a 5 minute standardized warm up, which included the calibration of the bicycle trainer

130 (Compu Trainer Pro Lab, Racer Mate, USA), an initial bolus of the carbohydrate solution was

131 consumed (397ml). Participants then completed 120 minutes of cycling; 5 minutes at 40% W_{max},

132 5 minutes at 45% W_{max}, 5 minutes at 50% W_{max}, 105 minutes at 55% W_{max}. These workloads were

133 calculated from participants' sea level and normobaric hypoxic W_{max} for the sea level and HA

134 environments, respectively. Additional boluses (229ml) of the carbohydrate solution were

135 provided every 15 minutes throughout the 120 minute exercise period. Expired gas breath samples

136 were collected and measurements of $\dot{V}O_2$ and $\dot{V}CO_2$ were made every 15 minutes during exercise.

137 Samples of expired gas for $^{13}CO_2$ analysis were collected during the final 60 seconds of each

138 collection period. Samples for the analysis of plasma glucose, serum insulin, serum free fatty acids

139 and plasma lactate were drawn every 15 minutes, those for plasma ^{13}C glucose enrichment were

140   drawn at 60, 90 and 120 minutes and those for plasma metanephrine and normetanephrine

141   concentrations were drawn at 60 and 120 minutes. Heart rate, rating of perceived exertion (RPE)

142   and SpO_2 was measured every 15 minutes during cycling exercise.

143

144   *Analyses*

145   Plasma and serum samples collected at HA were initially stored at $-20^{\circ}C$ until they were

146   transported back to the United Kingdom, where they were then stored at $-80^{\circ}C$ until analysis, as

147   per the samples collected at sea level. All samples were analysed in accordance with the procedures

148   described previously (10).

149

150   The $^{13}C/^{12}C$ ratio in expired CO_2 was determined using isotope ratio mass spectrometry (IRMS;

151   AP2003, GVI Instruments Ltd, Manchester, UK). The isotopic ratio $^{13}C/^{12}C$ is derived against

152   laboratory CO_2 (itself calibrated against VPDB) from the ion beam area ratio measurements with

153   correction of the small contribution of $^{12}C^{16}O^{17}O$ at m/z 45; the Craig correction (24). The $^{13}C/^{12}C$

154   ratio in plasma glucose was determined using LC-IRMS as described previously (23).

155

156   Oxidation rates of total fat, total carbohydrate, endogenous carbohydrate (liver and muscle),

157   plasma glucose and exogenous carbohydrate derived from glucose and fructose ingestion

158   combined, were calculated by indirect calorimetry ($\dot{V}O_2$ and $\dot{V}CO_2$) and stable isotope

159   measurements ($^{13}C/^{12}C$ ratio in expired CO_2 and plasma glucose), as detailed below.

160

161

162

163 *Calculations*

164 Total CHO and fat oxidation (g·min^{-1}) were computed from $\dot{V}O_2$ (L·min^{-1}) and $\dot{V}CO_2$ (L·min^{-1})

165 using stoichiometric equations (25), with the assumption that protein oxidation during exercise

166 was negligible.

167

168 The isotopic enrichment of the ingested glucose and fructose, (R_{exo}), was expressed in standard

169 $\delta^{13}C$ units (‰) relative to VPDB (26). Exogenous carbohydrate oxidation derived from the

170 combined ingestion of glucose and fructose (G_{exo}, grams) was computed by using equation 1 (27).

171

172   Exogenous Carbohydrate Oxidation (g·min^{-1}) = $\dot{V}CO_2$ [($R_{exp} - R_{ref}$)/ ($R_{exo} - R_{ref}$)] /k (1)

173

174 where $\dot{V}CO_2$ is in litres per minute, R_{exp} is the measured isotopic composition in expired CO_2, R_{ref}

175 is the isotopic composition of expired CO_2 at rest before exercise and carbohydrate ingestion, R_{exo}

176 is the measured isotopic composition of the exogenous glucose and fructose ingested, and k

177 (0.7426 l·g^{-1}) is the rate adjusted value for the complete oxidation of glucose (28). The use of R_{ref}

178 from expired CO_2 at rest is typical of studies in this area of research (9, 29) as the high ^{13}C-

179 enrichment of exogenous glucose and fructose provides a strong signal in expired CO_2. This

180 cancels the confounding effects of relatively small fluctuations in background enrichment of

181 expired CO_2 seen from rest to exercise following a Western European diet (30) on the calculation

182 of exogenous carbohydrate oxidation. Endogenous carbohydrate oxidation was calculated by

183 subtracting exogenous carbohydrate oxidation from total carbohydrate oxidation.

184

185 Computations were made on the assumption that, in response to exercise, ^{13}C is not irreversibly

186 lost in pools of tricarboxylic acid cycle intermediates and/or bicarbonate, and that lactate produced

187 from either glucose or fructose is either oxidized in muscle or recycled through gluconeogenesis

188 to be used subsequently by complete oxidation. Essentially exogenous carbohydrate oxidation is

189 calculated irrespective of the pathway that finally produces $^{13}CO_2$ that can be measured. The

190 calculations are based on the assumption that $^{13}CO_2$ recovery in expired gases was complete or

191 almost complete during exercise (31). Such computation has been shown to underestimate

192 exogenous carbohydrate oxidation rates at the beginning of exercise because of the delay between

193 $^{13}CO_2$ production in tissues and expired $^{13}CO_2$ at the mouth (32). Based on this, exogenous

194 carbohydrate oxidation rates are presented from 60 minutes onwards during the exercise period,

195 where it is expected that there would be isotopic equilibrium in the tissues and at the mouth.

196

197 Using the isotopic compositions of plasma glucose (R_{glu}) the oxidation rate of plasma glucose was

198 computed at 60, 90 and 120 minutes during exercise (equation 2 (33)).

199

200 $$\text{Plasma glucose oxidation (g·min}^{-1}) = \dot{V}CO_2\ [(R_{exp} - R_{ref})/\ (R_{glu} - R_{ref})]\ /k \quad (2)$$

201

202 The oxidation rate of muscle glycogen (g·min^{-1}), either directly or through the lactate shuttle (34),

203 was calculated by subtracting plasma glucose oxidation from total carbohydrate oxidation. Finally,

204 the amount of glucose released from the liver was estimated as the difference between plasma

205 glucose and exogenous carbohydrate oxidation (33).

206

207

208 *Statistical Analysis*

209 GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla California USA) was

210 used for graph creation. Data were normally distributed (Shapiro-Wilk) and are presented as mean

211 ± SD. Two-way repeated measures ANOVA was used to compare differences in fuel use and blood

212 related variables over time and between conditions. Post-hoc analysis was performed for any

213 significant effects using paired samples t-tests. Paired t-tests were also used to compare mean

214 differences in relative and absolute fuel use, as well as heart rate, SpO_2 and RPE between

215 conditions. This was supported where appropriate with 95% confidence intervals. Data were

216 evaluated using SPSS for Windows version 22 (Chicago, USA) with statistical significance

217 determined as $P < 0.05$. Due to participant attrition potentially affecting the power of the study,

218 we have placed greater emphasis on effect sizes (ES) in the interpretation of these data. Cohen's *d*

219 effect sizes were calculated, and interpreted using a threshold scale, where 0-0.2 was considered

220 to be a trivial effect, 0.2-0.6 a small effect, 0.6-1.2 a moderate effect, 1.2-2.0 a large effect, and

221 >2.0 a very large effect (35).

222

223 **Results**

224 **Total Carbohydrate and Fat Oxidation**

225 There was a *small*, but non-significant difference in total energy expenditure for 2 hours of

226 continuous cycling between HA and sea level conditions (1154.0 ± 170.5 kcal vs. 1245.3 ± 160.5

227 kcal, ES=0.54; P=0.114). *Large* and *moderate* effect sizes indicated lower absolute carbohydrate

228 oxidation at HA compared with sea level during the first hour, second hour and for the entire 2

229 hours of continuous cycling, though non-significant (table 1). *Small* non-significant effects were

230 observed for the relative contribution of carbohydrate to the total energy yield during the second

231   hour of exercise when comparing HA and sea level trials ($50.2 \pm 10.2\%$ vs. $54.9 \pm 6.3\%$, ES=0.46;

232   P=0.34). Alternatively, only *small* and *trivial* effect sizes for absolute fat oxidation were observed

233   during the exercise periods (table 1). *Small* non-significant effects were also observed for the

234   relative contribution of fat oxidation to the total energy yield during the second hour of exercise

235   when comparing HA and sea level trials ($49.8 \pm 10.2\%$ vs. $45.08 \pm 6.3\%$; ES =0.46; P=0.34), figure

236   1.

237

238   **Exogenous and Endogenous Carbohydrate oxidation.**

239   Exogenous carbohydrate oxidation rates were *moderately* lower at HA compared with sea level,

240   during the second hour of continuous cycling, being significant at 60 (ES=0.99, P=0.003), 90

241   (ES=1.19, P=0.017) and 120 minutes (ES=0.99, P=0.024), figure 2a. A *small* effect was observed

242   for the relative contribution of exogenous carbohydrate oxidation to the total energy yield between

243   HA and sea level, which approached significance ($32.6 \pm 6.1\%$ vs. $36.0 \pm 6.1\%$, ES=0.56,

244   P=0.059), figure 1. Further, absolute exogenous carbohydrate oxidation during the second hour of

245   exercise, was *moderately* and significantly lower at HA compared with sea level (table 2). There

246   was a *moderate* but non-significant effect towards lower absolute endogenous carbohydrate

247   oxidation at HA compared with sea level for the second hour of exercise (table 2). Furthermore,

248   the relative contribution of endogenous carbohydrate oxidation to the total energy yield was *trivial*

249   and non-significant between HA ($17.6 \pm 13.2\%$) and sea level ($19.0 \pm 9.8\%$, ES=0.1, P=0.725).

250

251   **Oxidation of plasma glucose, liver glucose and muscle glycogen**

252   A lower rate of plasma glucose oxidation was seen at HA compared with sea level during the

253   second hour of exercise, with *moderate* (60 minutes, ES=1.07) and *large* (90 minutes, ES=1.61

254 and 120 minutes, ES=1.33) but non-significant effects (P=0.113), figure 2b. In addition, the

255 absolute contribution of plasma glucose to the total energy yield was *moderately* and significantly

256 lower at HA compared with sea level (table 2). The rate of liver glucose oxidation produced non-

257 significant (P=0.471) *moderate* (60 minutes, ES=0.75), *trivial* (90 minutes, ES=0.12) and *small*

258 effects (120 minutes, ES=0.36) between conditions, figure 2c. Further, *trivial* and non-significant

259 effects were observed for both the relative (HA: $3.2 \pm 1.2\%$ vs. sea level: $3.1 \pm 0.8\%$, ES=0.09,

260 P=0.635, figure 1) and absolute contributions of liver glucose (table 2) to the total energy yield

261 between conditions during the second hour of exercise. The rate of muscle glycogen oxidation

262 produced non-significant *moderate* (60 minutes, ES=0.92, P=0.085) and *trivial* effects (90

263 minutes, ES=0.04, P=0.841 and 120 minutes, ES=0.15, P=0.708) between conditions, figure 2d.

264 *Trivial* non-significant effect was observed for the relative contribution of muscle glycogen to the

265 total energy yield for the second hour of exercise when comparing HA and sea level ($14.4 \pm 12.2\%$

266 vs. $15.8 \pm 9.3\%$, ES=0.11, P=0.934), figure 1. Furthermore, the absolute contributions of muscle

267 glycogen showed a *small* non-significant effect between conditions (table 2).

268

269 **Blood Biochemistry**

270 Plasma glucose concentrations were *moderately* higher at HA compared with sea level at 90 and

271 120 minutes (ES=0.74 and 0.87), with the condition and time interaction approaching significance

272 (P=0.072), figure 3a. *Moderate* to *large effect* sizes indicate higher plasma lactate concentrations

273 during exercise at HA compared with sea level (ES=0.86), but failed to reach statistical

274 significance (P=0.324), figure 3b. There were mainly *small* and non-significant effects for serum

275 insulin responses during exercise between conditions, which approached significance (ES<0.60,

276 P=0.07), figure 3c. Serum free fatty acid concentrations were *moderately* higher during the initial

277 30 minutes of exercise at HA compared with sea level (ES>0.69), but there was a non-significant

278 condition and time interaction (P=0.469), figure 3d. Metanephrine concentration was *moderately*

279 higher at HA compared with sea level at 60 minutes (ES=094) and *largely* higher at 120 minutes

280 (ES= 1.40), however, these differences only approached significance (P>0.075), figure 3e. A *very*

281 *large* effect for normetanephrine concentration was observed, being significantly higher at HA

282 compared with sea level at both 60 (ES= 2.02, P=0.009,) and 120 minutes (ES=2.36, P=0.006),

283 figure 3f.

284

285 **Heart Rate, Rating of Perceived Exertion and SpO$_2$**

286 Table 3 shows heart rate, RPE and SpO$_2$ at HA and sea level during the 2 hours of cycling, as well

287 as during the initial and last hour of cycling.

288
289 **Discussion**
290
291 This study, to our knowledge, is the first to compare exogenous and endogenous (liver and muscle)

292 carbohydrate oxidation, as well as fat oxidation during matched relative intensity (55% W$_{max}$)

293 cycling at terrestrial HA and sea level in women. Exogenous carbohydrate oxidation supplied as

294 glucose and fructose made a significant contribution to the total energy yield in both conditions

295 during the second hour of exercise. However, the primary findings are that exogenous

296 carbohydrate oxidation was reduced in acute hypoxia compared with normoxia in these women

297 leading to lower absolute whole body carbohydrate oxidation at HA. The latter was not associated

298 with alterations in the use of endogenous glycogen stores.

299

300 The suppressed rate of exogenous carbohydrate oxidation at HA (0.86 g.min^{-1}) compared with sea

301 level (1.03 g.min^{-1}) is supported by literature in men when the relative (10) and absolute (29)

302 exercise intensity was matched. Our previous study in men produced similar rates of exogenous

303 carbohydrate oxidation at HA (0.92 g.min^{-1}) (10). In contrast to our earlier work in men (10), the

304 differences in the rates of exogenous carbohydrate oxidation in these women was large enough for

305 the relative and absolute contributions to the total energy yield to demonstrate the same pattern of

306 response, being lower at acute hypoxia compared with sea level. These differences in the absolute

307 and relative exogenous carbohydrate responses between men and women who took part in an

308 identical study remains to be explained. Young et al. (29) reported suppressed exogenous glucose

309 oxidation in men, *albeit* when the absolute exercise intensity was matched between HA (0.19

310 g.min^{-1}) and sea level (0.38 g.min^{-1}). However, their rates were lower compared with the present

311 study, which in part may be due to the lower workload, as well as the different mode of exercise

312 compared with the present study (walking vs. cycling). Further, their study is limited as the

313 glucose-fructose beverage was only enriched with ^{13}C- glucose, hence their data is likely

314 underestimated as based solely on exogenous glucose oxidation, not including any fructose

315 contribution. In contrast, Peronnet et al. (9) showed no differences in exogenous glucose oxidation

316 following glucose ingestion (1.75 g.min^{-1}) when matching both the relative and absolute exercise

317 intensity between HA and sea level. In comparison the rates of exogenous carbohydrate oxidation

318 were also lower at both HA (0.43 g.min^{-1}) and sea level (relative: 0.54 g.min^{-1}, absolute: 0.50

319 g.min^{-1}) compared with the present study. The greater availability of carbohydrate in the present

320 study is due to the likely higher overall carbohydrate absorption rates (36), due to glucose and

321 fructose's distinctly different intestinal transport mechanisms.

322

323 Acute hypoxic exposure is associated with reduced insulin sensitivity in both men (37) and women

324 (38) due to increased catecholamine's and cortisol levels. However, overall the hyperglycemia in

325 both conditions was similar, which is in contrast to studies that have reported elevated plasma

326 glucose concentrations following carbohydrate ingestion during acute hypoxia compared with sea

327 level (9, 29). Furthermore, the plasma glucose concentrations in the present study, stimulated

328 insulin to similar concentrations, despite at least *moderately* higher metanephrine and

329 normetanephrine concentrations at HA. Therefore, the present study does not support the idea of

330 insulin resistance being a plausible explanation for the reduced exogenous carbohydrate oxidation

331 during exercise on acute exposure to HA.

332

333 The absolute whole body carbohydrate oxidation was reduced in women at HA compared with sea

334 level. These data are consistent with the previous literature in men (9, 10), following glucose-

335 fructose or glucose ingestion during matched relative exercise intensity. When comparing these

336 data with our previous research in men (10), the reduction in whole body carbohydrate oxidation

337 during 2 hours of exercise at HA is less (~40g vs. ~129g). This may be explained by the lower

338 absolute cycling intensity for the women (105.0 ± 17.2 watts) compared with the men (114.9 ± 9.7

339 watts), with a lower total energy expenditure (~1154 kcal vs. ~1347 kcal). However, preferential

340 use of fat as a fuel source by women may also be an explanation.

341 A small difference of 7% in total energy expenditure between HA and sea level is due to the lower

342 absolute workload at HA (105.0 ± 17.2 vs. 114.7 ± 18.9 watts). This will have only marginally

343 contributed to the lower absolute oxidation of whole body carbohydrate at HA and is unlikely to

344 be the full explanation as the magnitude of difference (~24%) far outweighs any difference in total

345 energy expenditure. The primary explanation is that reduced exogenous carbohydrate oxidation

346 makes the most significantly contribution towards the lower whole body carbohydrate oxidation

347 during the second hour of exercise at HA.

348   In contrast to the literature in men (10, 39), absolute and relative fat oxidation did not significantly

349   increase during exercise at HA compared with sea level. These data are supported by the similar

350   free fatty acid concentrations, suggesting the rate of utilization is comparable despite the higher

351   normetanephrine and metanephrine concentrations. The fact that fat oxidation was not

352   significantly affected by acute exposure to HA in these women, may be due to their higher fat

353   oxidation at sea level (baseline effect) compared with men. This has been ascribed to women

354   having a greater proportion of body fat (12), a greater lipid content in muscles fibers (11), a higher

355   percentage of type 1 muscle fibers (40) and better mobilization of fatty acids from subcutaneous

356   adipose tissue linked to cyclic changes in estrogen and progesterone (13). However, it is interesting

357   to note that the magnitude of fat oxidation in women at HA, was very similar compared with our

358   previous study in men (50% vs. 51%), as was the use of pre-existing muscle glycogen (17% vs.

359   16%), exogenous carbohydrate oxidation (32% vs. 30%) and glucose release from the liver (3%).

360   This suggests that women and men fuel exercise at HA in a very similar fashion following the

361   ingestion of glucose-fructose.

362

363   The sample size in the present study is smaller than originally intended, due to participant attrition.

364   Post-hoc power analysis revealed that the rate of exogenous carbohydrate oxidation provided a

365   power of 57%, 71% and 57% for 60, 90 and 120 minutes of cycling, respectively, using the mean

366   difference and associated standard deviation at each time point and an alpha level of 0.05.

367   Therefore, this study is slightly underpower compared with the accepted 80% and should be

368   interpreted in this context.

369

370   In conclusion, acute exposure to hypoxia reduced the exogenous oxidation of glucose and fructose

371   compared with normoxia during 2 hours of cycling at the same relative exercise intensity. This

372 may have practical implications, as the conventional carbohydrate ingestion recommendations

373 alter fuel use compared with normoxia. Thus, further research is required to identify an optimal

374 dose for women on acute HA exposure, as well as establishing the mechanism for reduced

375 exogenous carbohydrate oxidation.

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386 **Conflict of Interest**

387 The authors report no conflict of interest.

388
389 **References**
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Figure Captions

Figure 1. The relative (% of energy yield) contribution of exogenous and endogenous substrate oxidation during the second hour of cycling at high altitude and sea level.

Figure 2. Oxidation rates of exogenous CHO (a), plasma glucose (b), glucose released from the liver (c) and muscle glycogen (d) during the second hour of cycling. * high altitude significantly lower compared with sea level (P<0.05).

Figure 3. Plasma glucose (a), plasma lactate (b), serum insulin (c), serum free fatty acids (d), plasma metanephrine (e) and plasma normetanephrine (f) concentrations at rest and during 2 hours of cycling. * high altitude significantly higher compared with sea level (P<0.05).