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## **Continuous Glucose Monitoring at High Altitude – Effects on Glucose Homeostasis**

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**Running title:** Continuous glucose monitoring at high altitude

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**Tables:** 2

**Figures:** 4

## **Abstract**

**Purpose:** Exposure to high altitude has been shown to enhance both glucose and lipid utilization depending on experimental protocol. In addition, high and low blood glucose levels have been reported at high altitude. We hypothesized that gradual ascent to high altitude results in changes in glucose levels in healthy young adults.

**Methods:** 25 adult volunteers, split into two teams, took part in the British Services Dhaulagiri Medical Research Expedition completing 14 days of trekking around the Dhaulagiri circuit in Nepal reaching a peak altitude of 5300m on Day 11 of the trek. Participants wore blinded continuous glucose monitors (CGM) throughout. Blood samples for c-peptide, pro-insulin and triacylglycerides were taken at sea level (UK) and in acclimatisation camps at 3600m, 4650m and 5120m. Energy intake was determined from food diaries.

**Results:** There was no difference in time spent in hypoglycemia stratified by altitude. Nocturnal CGM readings (22.00-06.00 hrs) were chosen to reduce the short-term impact of physical activity and food intake and showed a significant ( $p < 0.0001$ ) increase at 3600m ( $5.53 \pm 0.22$  mmol/L), 4650m ( $4.77 \pm 0.30$  mmol/L) and 5120m ( $4.78 \pm 0.24$  mmol/L) compared to baseline altitude 1100m (vs  $4.61 \pm 0.25$  mmol/L). Energy intake did not differ by altitude. Insulin resistance and B-cell function, calculated by homeostatic model assessment, was reduced at 3600m compared to sea level.

**Conclusions:** We observed a significant increase in nocturnal CGM glucose at 3600m and above despite gradual ascent from 1100m. Taken with the changes in insulin resistance and B-cell function, it is possible that the stress response to high altitude dominates exercise enhanced insulin sensitivity, resulting in relative hyperglycemia.

**Key words:** Glycemic variability, exercise, trekking, insulin resistance, hypoglycaemia

ACCEPTED VERSION

1 **Introduction**

2

3 Ascent to high altitude (HA) is associated with significant risks but despite this mountaineering  
4 and HA trekking remain popular. As well as environmental factors, such as temperature and  
5 wind, low barometric pressure combined with physical activity induces physiological changes  
6 that can result in impaired exercise capacity, a spectrum of altitude-related illnesses and even  
7 death (1).

8

9 To evaluate how harsh and inhospitable conditions affect people operating at HA the Defence  
10 Medical Services have conducted a wide-ranging programme of research investigating the  
11 effects of HA exposure (2-13). One area that remains relatively unexplored is how glucose  
12 homeostasis is affected by prolonged HA exposure. Exercise-induced hypoglycemia in non-  
13 diabetic subjects is recognised (14). At altitude even mild neuroglycopenia could have serious  
14 repercussions for example, loss of concentration or delayed recognition of imminent danger, and  
15 may exacerbate the effects of acute mountain sickness (AMS). A greater understanding of  
16 glucose flux at altitude may allow appropriate prevention and management of both hypo- and  
17 hyperglycemia, especially in conjunction with other life-threatening conditions such as high  
18 altitude pulmonary oedema and high altitude cerebral oedema.

19

20 Glucose is the most efficient fuel that the body can utilise, consuming less oxygen per unit of  
21 energy produced than either fat or protein (15). This is of relevance in hypoxic situations, such as  
22 those at HA. Sudden exposure to HA (4300m) has been shown to lower blood glucose levels in  
23 the first 40 hours (16). It has previously been postulated that hypoxaemia may enhance  
24 utilization of glucose by mechanisms that are yet to be fully elucidated (17-19) and reduce

25 reliance on fat as a substrate (20). However, we have recently shown that acute exposure to HA  
26 *reduces* carbohydrate oxidation and increases fat oxidation during walking (21) and prolonged  
27 cycling exercise (22). These contrasting results may be due to differences in energy consumption  
28 because the degree to which blood glucose increases on rapid ascent to 4300m is higher if energy  
29 intake is adequate (23).

30

31 Loss of appetite is a near universal consequence of rapid ascent to HA and has a significant  
32 effect on the ability to maintain energy balance and, theoretically, glycaemia. Anorexia may be  
33 mediated by hypothalamic mechanisms but gastrointestinal signals causing nausea as part of the  
34 syndrome of AMS are a common exacerbating factor. It has been reported that soldiers  
35 participating in field exercises in mountainous terrain have consistently high rates of daily  
36 energy expenditure, but limited dietary energy intake (24). Increased energy requirements,  
37 reduced food intake and factors driving muscle glucose uptake may therefore cause hypoglcemia  
38 which has the potential to adversely affect performance at HA and even exacerbate AMS.

39

40 We hypothesised that ascent to HA results in a reduction in glucose levels and prolonged periods  
41 of hypoglcemia in healthy young adults. To investigate this, we undertook a novel observational  
42 study utilizing continuous glucose monitoring (CGM) in volunteers undertaking a high-altitude  
43 expedition to the Himalayas in 2016.

44 **Methods**

45

46 **Subjects**

47 Participants (n=25) were recruited from those taking part in the British Services Dhaulagiri  
48 Medical Research Expedition (BSDMRE) (25). The volunteers were divided between two teams  
49 (Team 1 and Team 2) and completed 14 days of trekking around the Dhaulagiri circuit in Nepal.  
50 Team 1 comprised 13 participants (10 male, 3 female) and Team 2 had 12 volunteers (11 male,  
51 one female). Team 1 departed 14 days before Team 2. Weather conditions and average  
52 temperatures were similar for both groups; at the time blood samples were collected (~08.00am),  
53 ambient temperatures in the research tents were 4.9, 1.2, and -6.4 °C at 3600, 4650, and 5120 m  
54 respectively. Both teams ascended to a peak altitude of 5300m, with acclimatization days on  
55 Days 7 and 10. In addition, Team 1 had a further acclimatization day at 5120m (details of  
56 altitudes and locations are in Table 1) whereas Team 2 only stayed at this altitude for one night  
57 (due to several participants suffering with AMS who needed to descend on medical advice).  
58 Food (3 meals a day and afternoon tea) were provided by a support team of porters and chefs,  
59 accompanying each team separately. Thus, individuals within each team were offered the same  
60 type (and similar quantities) of food; but the food provision was not the same between each  
61 team. In general, the trekkers woke at 06.00; after breakfast trekking began at 08.00 and  
62 continued until ~15.00 (although this was variable depending on the distance and altitude  
63 covered). During the trek, regular breaks took place and lunch was taken at around noon. On  
64 arrival at the next camp, tea and biscuits were provided and little physical activity undertaken.  
65 Supper was served at 19.00 and most people retired to their tents by 21.00.

66

67 This study was conducted according to the guidelines laid down in the Declaration of Helsinki  
68 and all procedures were approved by the Ethics Advisory Committee at Leeds Beckett  
69 University and the Ministry of Defence Research Ethics Committee (624/MODREC/14). All  
70 participants gave written informed consent.

71

## 72 **Study design**

73 All participants wore blinded continuous glucose monitors throughout (Dexcom G4, San Diego,  
74 CA). CGM monitors were placed on the triceps area (participants were given the choice of  
75 triceps or abdominal wall) and replaced every 7 days. One CGM receiver stopped working after  
76 5 days and no further data was collected from that participant (male, Team 1) and their results  
77 were excluded. Measurements of capillary blood glucose were also recorded twice each day  
78 using a Bayer Contour (Parsippany, NJ) glucometer utilizing glucose dehydrogenase testing  
79 strips.

80

81 A priori, it was decided to focus on nocturnal (22.00pm-06.00am) glucose measurements as the  
82 main outcome measurement, to minimize the effects of food intake and physical activity on the  
83 glucose levels thus hopefully allowing clearer determination of the effects of altitude. CGM data  
84 were analyzed to identify the mean blood glucose (BG) during night-time at Dharbang (1110m)  
85 and on the night of arrival at each acclimatization camp (3600m, Italian Base Camp; 4650m,  
86 Dhaulagiri Base Camp; 5120m, Hidden Valley), and each night of trekking. The overnight  
87 glycemic variability (measured by standard deviation (SD) and coefficient of variation (CV))  
88 was also assessed using EasyGV (Oxford, UK) software. Time spent in hypoglycemia (all  
89 readings) was determined at pre-specified altitudes (<2000m, 2000-3000m, 3000-4000m, and



90 >4000m). Three definitions of hypoglycemia were used; <3.9mmol/L (which correlates with the  
91 release of counter-regulatory hormones), <3.3mmol/L (associated with the onset of  
92 neuroglycopenic and adrenergic symptoms) and <2.8mmol/L (the point at which cognitive  
93 dysfunction can occur) (26). All participants were asked to complete a standardized food intake  
94 diary and daily energy intake was calculated using Nutritics dietary analysis software (v1.8 for  
95 Windows; Nutritics, Dublin). One day of food recording for one participant was excluded due to  
96 mis-recording and data was subsequently analyzed to include all remaining data (143 results) and  
97 also excluding days when participants had gastrointestinal illness affecting food intake (137  
98 results).

#### 100 **Blood sampling and assays**

101 Venous blood samples were collected at sea-level (in the United Kingdom) and at all research  
102 camps with participants in a fasted state. To prevent any extraneous influences from postural  
103 changes, all blood samples were collected after the participant had been seated for at least 5 min.  
104 One 5 mL pre-cooled EDTA tube (Sarstedt, Leicester, UK) was used to obtain samples for the  
105 determination of c-peptide and pro-insulin to investigate beta cell function and insulin  
106 sensitivity. Immediately after filling, the tube was spun at 1500 x g for 10 minutes in a centrifuge  
107 (CompactStar CS4, VWR) and then immediately frozen at either -20°C in a freezer (for UK  
108 measurements) or within a dry shipper containing liquid nitrogen (at each fixed camp) before  
109 being transferred to -80°C and stored until analysis. C-peptide was measured on plasma samples  
110 using an automated chemiluminescent immunoassay (Abbott Architect, Illinois, United States)  
111 and pro-insulin using a manual solid-phase two-site enzyme immunoassay (Merckodia  
112 Diagnostics, Upsalla, Sweden). To further understand the changes in overnight glucose observed

113 at different altitudes, we calculated insulin resistance and beta cell function using Homeostatic  
114 Model Assessment (HOMA, <http://www.dtu.ox.ac.uk/homacalculator/>). We did not collect  
115 fasting plasma glucose and therefore used the mean CGM glucose between 5am-6am on the day  
116 samples were taken. CGM glucose levels were used from the first morning of trekking (Day 1)  
117 for the sea-level HOMA calculations. Plasma triacylglycerol (TAG) concentration was  
118 determined spectrophotometrically using colorimetric analysis from a commercially available kit  
119 (Instrumentation Laboratory Company, Lexington, MA, USA).

120

## 121 **Statistics**

122 GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla California USA,  
123 [www.graphpad.com](http://www.graphpad.com)) was used for statistical analysis and graph creation. Data were checked for  
124 normality using the Shapiro-Wilk test. For unpaired data, one-way ANOVA was used with post  
125 hoc Dunnett's multiple comparison test for parametric data, and the Kruskal-Wallis test with  
126 Dunn's post hoc analysis for non-parametric data. Non-parametric repeated measures data was  
127 analyzed using the Friedman test with Dunn's post hoc analysis. To investigate differences  
128 between adjacent Trek and Rest days data were analyzed by 2-way ANOVA with Sidak's  
129 multiple comparison test. Statistical significance was set at  $p < 0.05$ .

130

131 **Results**

132

133 **Demographics**

134 The mean age of the participants was 27.7 (range 18-41). Due to severe acute mountain sickness,  
135 meaning that CGM sensors could not be replaced, data was not available for 3 participants at  
136 Hidden Valley (5120m).

137

138 **Effects of altitude on hypoglycaemia**

139 There were no differences in percent time spent in hypoglycemia overnight ( $<3.9$ ,  $<3.3$  and  $<2.8$   
140 mmol/L) when the trekkers were at altitudes of less than 2000m, between 2000-3000m and  
141 3000-4000m or at more than 4000m (Table 2).

142

143 **Effects of altitude on mean glucose levels and energy intake**

144 There was a significant increase in mean nocturnal CGM glucose at Italian Base Camp (3600m),  
145 Dhaulagiri Base Camp (4650m) and Hidden Valley Camp (5120m), compared to Dharbang  
146 (1110m) ( $5.53\pm0.22$  vs  $4.77\pm0.30$  vs  $4.78\pm0.24$  vs  $4.61\pm0.25$  mmol/L respectively;  $p<0.0001$ )  
147 (Figure 1). The mean nocturnal CGM glucose climbed steadily from Dharbang ( $4.61\pm0.25$   
148 mmol/) during the first week of the trek (Figure 2A) peaking on the second night at Italian Base  
149 Camp ( $5.64\pm0.25$  mmol/L), then falling immediately to around 5 mmol/L for the last 4 days.  
150 These results were largely replicated in both teams (Figure 2B and 2C) despite them trekking at  
151 different times. The changes in CGM glucose were not obviously a reflection of the daily energy  
152 intake values. The mean daily energy intake immediately preceding the nocturnal glucose  
153 measurements did not differ between Dharbang and the three acclimatisation Camps ( $1968\pm360$

154 vs 2220±558 vs 2354±690 vs 2363±434 Kcal, p=0.39) even when CGM glucose was most  
155 elevated (at the Italian Base Camp, 3600m). Energy intake was lowest on the first two days of  
156 the trek when several participants were suffering from gastrointestinal illness (diarrhoea and  
157 vomiting) resulting in reduced appetite independent of altitude (Supplementary Figure 1A).  
158 When results the effects of gastrointestinal disease were excluded there were no changes in  
159 energy intake at any altitude (Supplementary Figure 1B).

160

### 161 **Comparison of glucose levels on trekking and non-trekking (rest) days**

162 There was a significantly higher mean nocturnal (22.00-06.00 hrs) CGM glucose at the Italian  
163 and Dhaulagiri Base Camps on rest days compared with the day before (when participants were  
164 trekking) but lower readings were recorded at Hidden Valley Camp on the rest day (Figure 3A).  
165 Similarly, the mean daytime (06.00-22.00 hrs) CGM glucose levels were higher after a rest day  
166 at 3600m (Italian Base Camp) and Hidden Valley Camp (5120m), but not at Dhaulagiri (Figure  
167 3B). Energy intake was not different between trekking and rest days at any altitude (Figure 3C).

168

### 169 **Effects of altitude on glycaemic variability**

170 Measures of glycaemic variability were also examined. Nocturnal standard deviation and mean  
171 amplitude of glycaemia of CGM readings were not different significantly between Dharbang  
172 (1110m), Italian Base Camp (3600m), Dhaulagiri Base Camp (4650m) and Hidden Valley  
173 (5120m), however there was a statistical difference in nocturnal percent coefficient of variation  
174 (%CV) (p=0.02 by Kruksal-Wallis test). The difference between the median calibration capillary  
175 blood glucose and the temporally nearest CGM glucose reading did not change with altitude (-  
176 0.28mmol/L at <2000m; -0.42mmol/L at 2-3000m; -0.33mmol/L at 3-4000m; -0.31mmol/L at 4-

177 5000m; -0.25mmol/L at >5000m, p=0.79).

178

### 179 **Effects of altitude on beta cell function and insulin resistance**

180 There were significant reductions in C-peptide ( $p<0.05$ ) and Pro-insulin ( $p<0.0001$ ) levels  
181 between sea-level (UK) and Italian Base Camp (3600m) but no difference between sea-level and  
182 Dhaulagiri Base Camp or Hidden Valley (Figure 4A and 4B). Insulin resistance significantly  
183 differed with altitude ( $p=0.04$ ) and Holm-Sidak's multiple comparisons showed a significant  
184 ( $p<0.05$ ) reduction in insulin resistance between sea-level and Italian Base Camp, Dhaulagiri  
185 Base Camp and Hidden Valley (Figure 4C). Beta-cell function was also significantly different  
186 with altitude ( $p=0.02$ ) and Dunn's multiple comparisons showed a significant ( $p<0.05$ ) reduction  
187 in beta cell function between sea-level and Italian Base Camp (Figure 4D). The Pro-insulin:C-  
188 peptide ratio was not significantly altered by changes in altitude ( $p=0.33$ ) (Figure 4E).  
189 Triacylglycerol significantly increased with altitude ( $p<0.0006$ ) (Figure 4F).

190

191

192 **Discussion**

193

194 This is the first study to report the effects of gradual ascent to very high altitude on glucose  
195 levels measured by CGM in healthy volunteers. The participants, split into two groups, made it  
196 possible to compare whether the changes observed were reproducible in an environment where  
197 undertaking a controlled trial is not feasible. It is important to note that the ascent profile was  
198 carefully designed to minimise the risk of the participants developing AMS, thus the daily ascent  
199 was rarely more than 500m and the pace of walking set at that of the slowest team member. We  
200 believe that this means the observed results reflect changes of acclimatization, rather than sudden  
201 exposure to HA.

202

203 The lack of differences in percentage time spent in hypoglycemia as the trekkers gained altitude is  
204 likely to reflect the gradual ascent profile and adaptation to HA. Strikingly however, nocturnal  
205 glucose was significantly elevated, by around 0.8mmol/L, at 3600m compared to Dharbang  
206 (1100m) and the higher camps (at 4650m and 5120m). This was replicated in both Team 1 and  
207 Team 2. We interpret the hyperglycemia and improved insulin sensitivity demonstrated at 3600m  
208 to reflect parallel streams of adaptive physiology related to altitude (i.e. hypobaric hypoxia) and  
209 physical activity. A possible explanation is that physical activity pathway improves peripheral  
210 insulin sensitivity but the stress response to hypoxia dominates, raising blood glucose at the same  
211 time.

212

213 It has previously been shown (23) that acute (same day) ascent from sea level to 4300m increases  
214 blood glucose on Day 3 by 9.1%. Likewise, healthy volunteers exposed acutely to 3500m

215 altitude significantly increased plasma glucose from 4.59 mmol/L at sea-level to 5.53 mmol/L  
216 (28). Interestingly, a study in which individuals were flown from Kathmandu (1300m) to  
217 Namche (3500m) then trekked to Everest Base Camp (5300m) over 9 days, showed no change in  
218 fasting glucose (or insulin sensitivity) until they had been at Base Camp for 6 weeks (29). It is  
219 noteworthy that all these studies differ from ours because of their sudden exposure to HA. A  
220 reduction in the partial pressure of inspired oxygen is known to induce a stress response which  
221 includes activation of the sympathetic nervous system and increased resting levels of  
222 normetanephrine at 3375m (30). Increased catecholamines and sympathetic tone are associated  
223 with reduced insulin sensitivity at altitude (23, 28, 31, 32) which would explain the observed  
224 hyperglycaemia however our results show increased insulin sensitivity after gradual ascent to  
225 altitude. Others have shown no change during gradual acclimatization up to 5000m (29) or  
226 increases in glucose utilization on acute exposure to 4300m due to apparent increases in insulin  
227 action (19). The reasons for these divergent results are likely to related to different study  
228 protocols, including rate of ascent and the complex mechanisms that underlie variations in  
229 glucose concentration at altitude which include changes in beta cell insulin secretion, hepatic  
230 glucose production and tissue glucose uptake.

231

232 We further hypothesised that hypobaric hypoxia would result in beta cell stress resulting in an  
233 increase in the Pro-insulin:C-peptide ratio at altitude (33). We observed no change in the Pro-  
234 insulin:C-peptide ratio with altitude, however the reduced C-peptide and Pro-insulin levels at  
235 Italian Base Camp may indicate beta cell stress and relative insulin deficiency. This provides a  
236 potential mechanism whereby reduced insulin secretion occurs in response to hypoxia. Increased  
237 insulin sensitivity, as seen at all altitudes above sea-level in our study, which may be related to

238 exercise (and possibly altitude-induced) is, at least in part, due to upregulation of skeletal muscle  
239 GLUT4 receptor translocation. In adult rats exposed to 9% inspired oxygen for 30 days GLUT4  
240 protein increased by 15-20% compared to controls (34). Furthermore, in immature (aged 21  
241 days) and adult (aged 6 months) rats exposed to a simulated altitude of 4878m there was  
242 increased leg muscle GLUT4 and reduced insulin receptor density after 7 days but these changes  
243 disappeared by 28 days (35). These results could explain our observed increase in peripheral  
244 insulin sensitivity. Reduced insulin secretion also leads to increased hormone sensitive lipase  
245 activity with subsequent increased lipolysis and greater levels of circulating triglycerides, as we  
246 have shown and has been observed in response to simulated ascent to HA (36).

247

248 These results do not fit into a neat paradigm and the cellular mechanisms driving these findings  
249 are not known; thus, our proposed explanation (represented in Supplementary Figure 2) is  
250 deliberately simplified to include the components that we measured. It should be noted that this  
251 description does not account for changes in multiple factors including the distribution and  
252 number of GLUT1 receptors, alterations in hypoxia-inducible factors (e.g. HIF1 $\alpha$ ), modulation  
253 of insulin receptor density, variations in rate-limiting enzymes such as glucokinase or glucose-6-  
254 phosphate, or the response of other hormones such as growth hormone, glucagon and thyroxine  
255 to altitude exposure. Furthermore, we recognise that plasma levels of glucose and TAG do not  
256 reflect tissue uptake nor oxidation, thus reduced clearance, insulin resistance and increased  
257 lipolysis may be important.

258

259 The absence of consistent changes in markers of GV imply that the increase in mean nocturnal  
260 glucose seen at Italian Base Camp (3600m) was not due to greater glucose flux. The overall CV



261 at all altitudes are all considered to reflect low levels of GV which has previously been defined  
262 as CV of <36% (37).

263  
264 We suspected that increased food intake may play a role in the higher glucose readings seen on  
265 rest days, however the results do not bear this out – there was no greater energy intake on rest  
266 days. Although food diaries are recognised to have limited reproducibility and accuracy (27), the  
267 energy intake in our study is within the levels expected for adults at altitude. The increased CGM  
268 glucose observed on some trekking days preceding rest days may reflect an exercise-mediated  
269 increase in insulin sensitivity and increases in non-insulin mediated glucose uptake occurring on  
270 trekking days, and reduced physical activity on rest days, however the lack of consistency in  
271 these findings warrant further investigation.

272  
273 There were notable limitations to this study. The volunteers were nearly all white European  
274 young adults with reasonable levels of cardiovascular fitness and therefore the results may not be  
275 applicable to other populations. There was no standardised measurement of blood glucose (e.g. a  
276 YSI glucose meter) thus the CGM calibration by fingerprick glucose meter may be subject to  
277 error (indeed this has been noted before) (38). In addition, only two calibration readings were  
278 taken each day (the minimum recommended). The altitude and cold temperatures may also have  
279 affected the CGM readings. Continuous glucose monitoring has been investigated *in vitro* in a  
280 hypobaric chamber using solutions containing 2.9, 4.9 and 11.3 mmol/L glucose; under  
281 conditions mimicking altitude of 2500m and 5500m, continuous readings were obtained however  
282 there was a significant difference in the CGM at the lower and higher glucose concentration  
283 compared to normobaric CGM (39,40). To mitigate against cold, the participants were

284 encouraged to keep their CGM receivers inside their inner pockets. Reassuringly, the difference  
285 between CGM and calibration glucose measurements did not change significantly with  
286 increasing altitude indicating that the CGM readings were at least consistent with those obtained  
287 from the fingerprick glucometers. Our sample size was too small to detect gender-based  
288 differences in glucose homeostasis in particular whether the phase of menstrual cycle (greater  
289 insulin resistance typically occurs during the luteal phase) in female trekkers; this could be  
290 investigated in a larger group. Although this study lacks a control arm of people trekking under  
291 similar conditions at sea-level, one of the strengths is that it was done in two different teams and  
292 thus the observed changes are independent of the time of trekking and other factors that might  
293 have affected a single group of people. Nevertheless, these results provide an insight into the  
294 changes in glucose homeostasis that that occur as acclimatization to HA takes place.

295  
296 In summary, we have shown a significant increase in nocturnal CGM glucose at 3600m and  
297 above following gradual ascent from 1100m. Taken with reduced insulin resistance and evidence  
298 of B-cell dysfunction, it is possible that the stress response to high altitude leads to relative  
299 insulin deficiency and this effect is greater than exercise-induced increase in insulin sensitivity,  
300 resulting in relative hyperglycemia. Future studies could measure catecholamines, cortisol and  
301 other stress markers as well as undertaking muscle biopsies to look at GLUT expression.

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306 NEH conceived the study, collected data, analyzed data, wrote the manuscript. KD collected data  
307 and reviewed/edited manuscript. SM analyzed samples, contributed to discussion,

308 reviewed/edited manuscript. NSO conceived the study, wrote the manuscript. CN collected data.

309 AM conceived the study and reviewed/edited manuscript. JOH conceived the study and

310 reviewed/edited manuscript. DW conceived the study, wrote the manuscript.

311

312 **Conflict of interest**

313 NEH is the guarantor of this work and, as such, had full access to all the data in the study and

314 takes responsibility for the integrity of the data and the accuracy of the data analysis. The results

315 of the present study do not constitute endorsement by ACSM.

316

317

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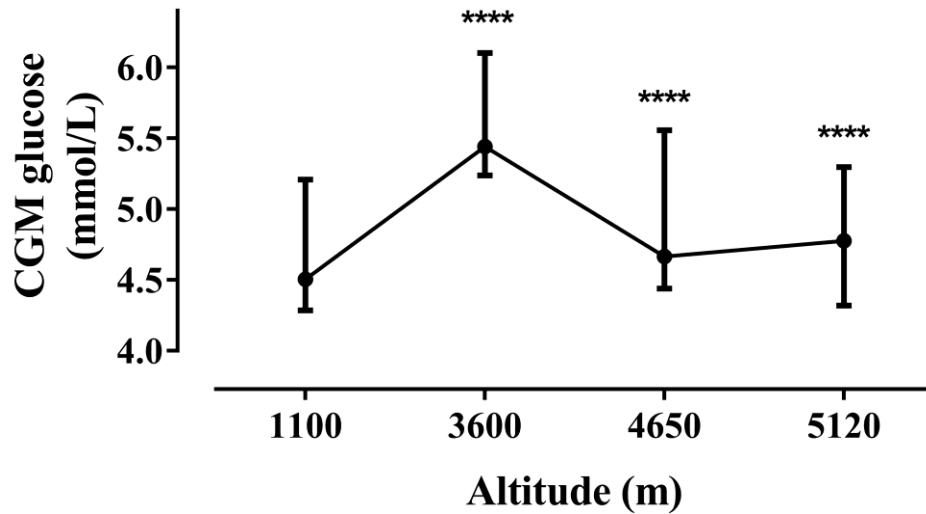
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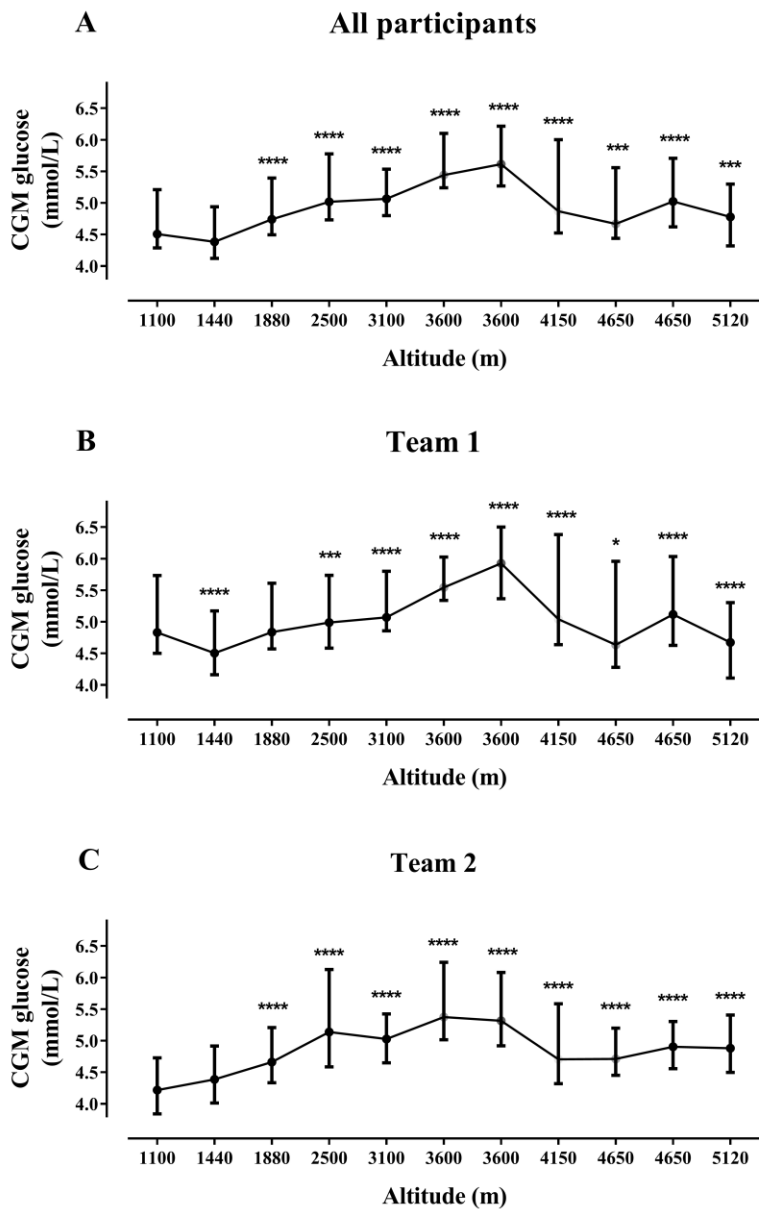
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430 **Figure 1. Nocturnal glucose levels at baseline and acclimatization camps.** Continuous  
431 glucose monitoring data collected between 22.00pm and 06.00am (n=24 at 1110, 3600 and  
432 4650m and n=21 at 5120m). Data are expressed as median and range and were analyzed by  
433 Friedman repeated measures ANOVA and Dunn's multiple comparison test for post-hoc testing.  
434 \*\*\*\* p<0.0001 vs 1100m



435

436 **Figure 2. Nocturnal glucose levels during the BSDMRE trek.** Continuous glucose monitoring

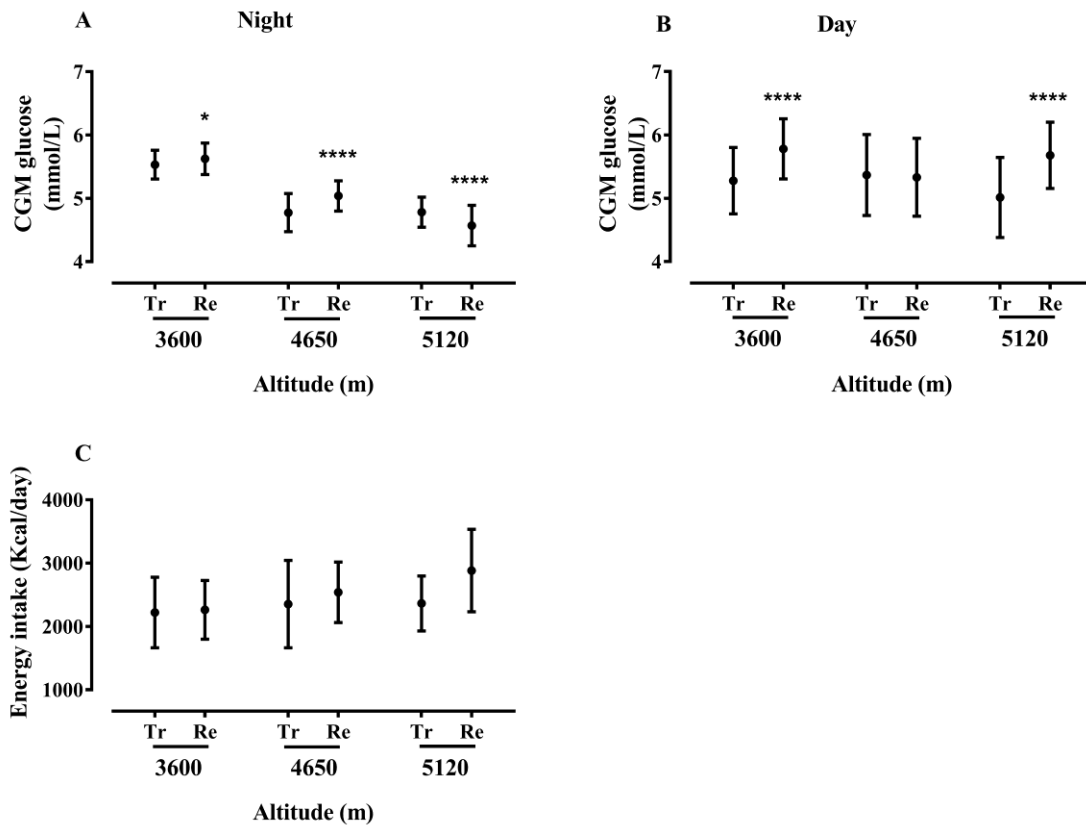
437 data collected between 22.00pm and 06.00am (n=24; n=21 at 5120m). Data are expressed as

438 median and range and were analyzed by Friedman repeated measures ANOVA and Dunn's

439 multiple comparison test for post-hoc testing. (A) all participants, n=24; (B) Team 1, n=12; (C)

440 Team 2, n=12. \*\*\*\* p<0.0001 vs 1100m

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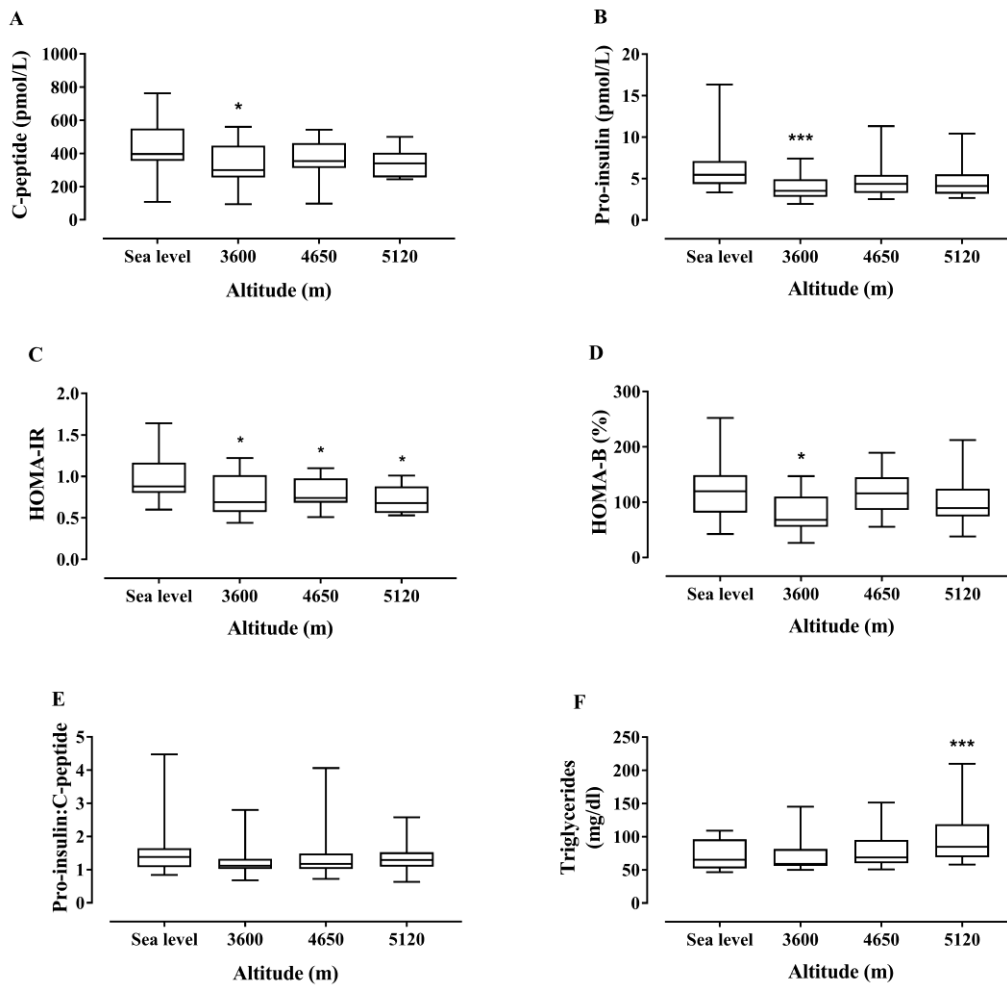
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443 **Figure 3.** Differences between (A) nocturnal (22.00-06.00 hrs) and (B) daytime (06.00-22.00  
 444 hrs) CGM glucose, and (C) Energy Intake (EI) on Trekking and subsequent Rest days. Mean ( $\pm$   
 445 SD) glucose and EI on Trekking (Tr) days and the following Rest (Re) days at 3600 (n=24  
 446 CGM, n=12 EI), 4650 (n=24 CGM, n=12 EI) and 5120m (n=12 CGM and EI, due to no rest day  
 447 for Team 2) are shown. Data were analyzed by 2-way ANOVA with Sidak's multiple  
 448 comparison test between adjacent Trek and Rest days; \* p<0.05 and \*\*\*\* p<0.0001 vs Trekking

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454 **Figure 4. Markers of beta cell secretory function and insulin resistance at baseline and**

455 **acclimatization camp.** Changes in (A) fasting C-peptide, (B) fasting Pro-insulin, (C) HOMA-

456 IR, (D) HOMA-B (E) pro-insulin:C-peptide and, (F) fasting plasma triglycerides (n=16-21 at

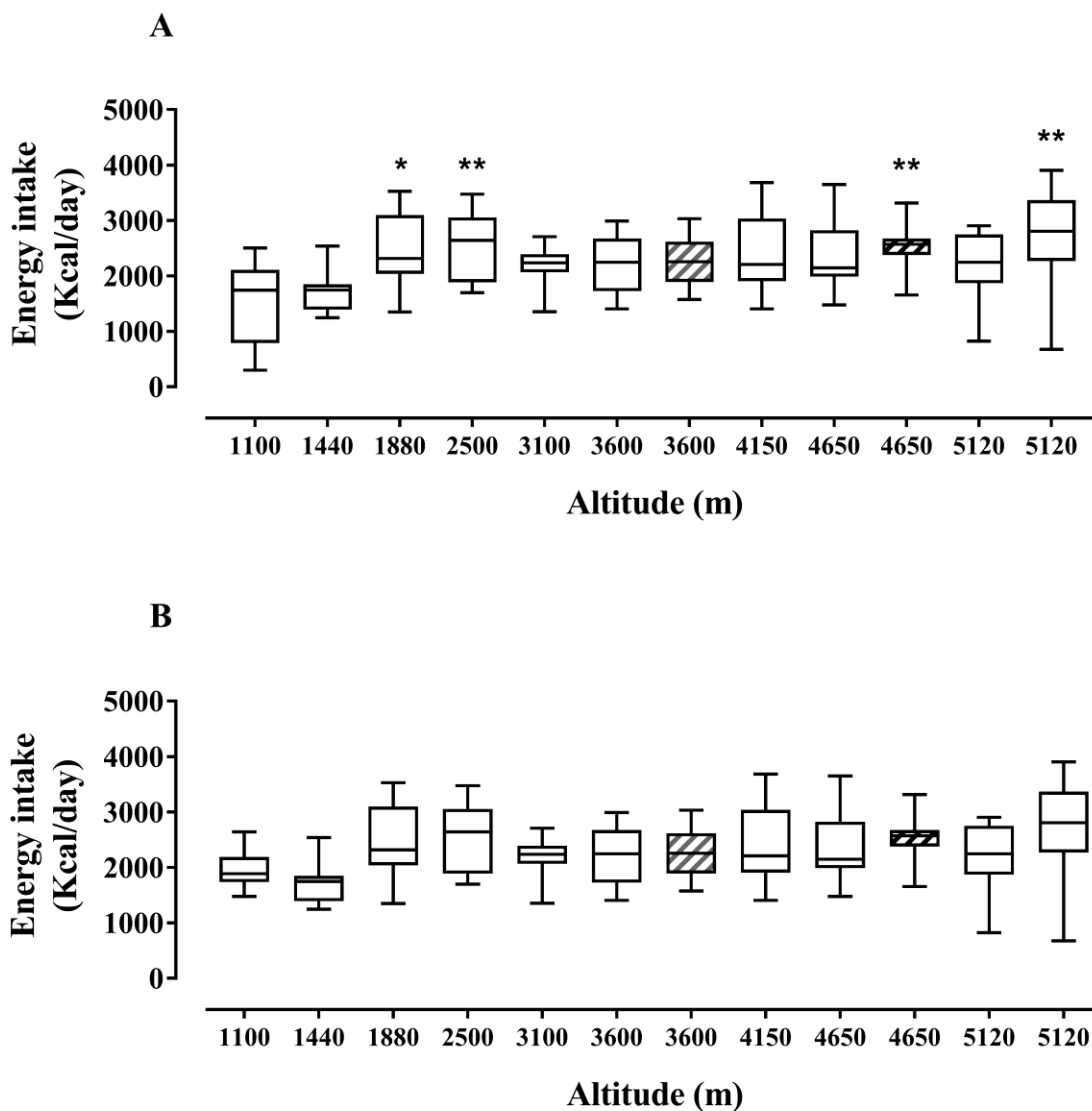
457 1110m; 18-21 at 3600m and 4650m; and n=9-11 at 5120m). Data are expressed as median  $\pm$  IQR

458 (box) and range (whiskers) and analyzed by one-way ANOVA or Kruskal-Wallis test and post-

459 hoc with Holm-Sidak's or Dunn's multiple comparison test, respectively. \*  $p < 0.05$  and \*\*\*

460  $p < 0.0001$  vs Sea level

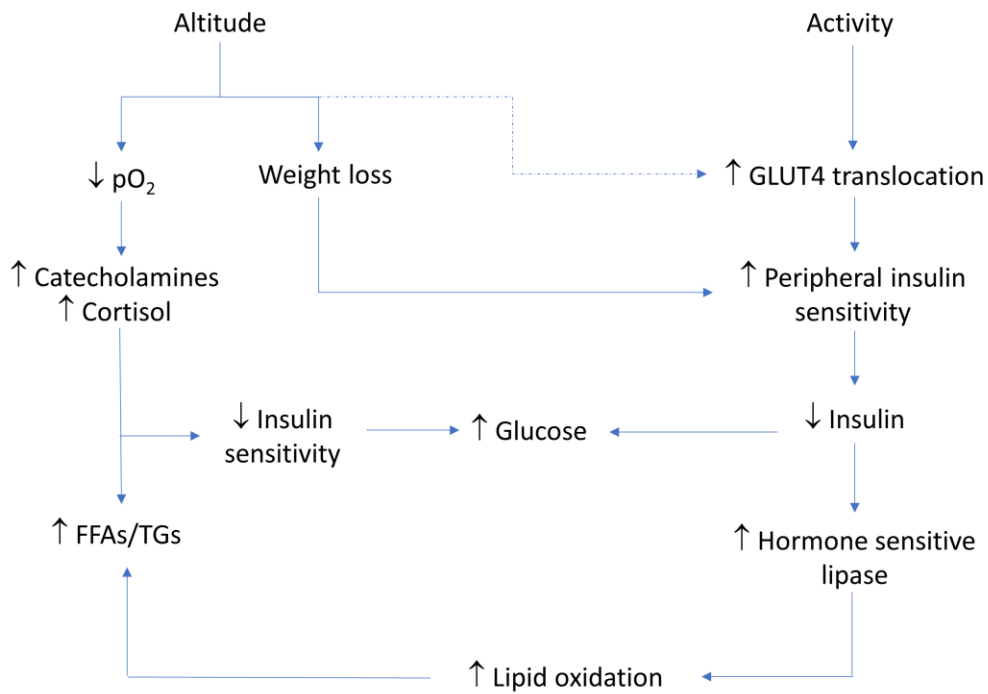
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464 **Supplementary Figure 1. Energy intake during the BSDMRE trek.** Changes in (A) energy  
 465 intake – all results, (B) energy intake - excluding results when the participant had gastro-  
 466 intestinal disease (6 results out of a total of 144). Hatched boxes show rest days. Data are  
 467 expressed as median  $\pm$  IQR (box) and range (whiskers) and analyzed by one-way ANOVA with  
 468 Dunn's multiple comparison test. n=12 (from Team 1); \* p<0.05 and \*\* p<0.01 vs 1100m

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472 **Supplementary Figure 2. Proposed pathways influencing glucose levels during**

473 **acclimatization to high altitude.** FFA – free fatty acid; TGs – triglycerides; GLUT4 – glucose

474 transporter 4.

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Day	Route	Altitude (m) reached at end of day
1	Beni to Dharbang	1110
2	Dharbang to Naura	1440
3	Naura to Bogara	1880
4	Bogara to Dobhan	2500
5	Dobhan to Sallaghiri	3100
6	Sallaghiri to Italian Base Camp	3600
7	Acclimatisation day	3600
8	Italian Base Camp to Japanese Base Camp	4150
9	Japanese Base Camp to Dhaulagiri Base Camp	4650
10	Acclimatisation day	4650
11	Dhaulagiri Base Camp to Hidden Valley	5120
12	Acclimatisation day*	5120
13	Hidden Valley to Yak Kartha	4270
14	Yak Kartha to Marpha	2500

483

484 **Table 1: Routes and altitude during the BSDMRE.** \* Team 2 did not have an acclimatization

485 day at Hidden Valley (Day 12) due to a number of team members having Acute Mountain

486 Sickness and needing to descend

487

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Altitude (m)	Percent time spent in hypoglycemia between 22.00pm – 06.00am (%)		
	<3.9 mmol/L	<3.3 mmol/L	<2.8 mmol/L
<2000	12.8 (10.6)	3.60 (4.10)	1.08 (2.35)
2000-3000	11.2 (10.1)	2.06 (2.71)	0.35 (0.81)
3000-4000	10.2 (12.1)	3.31 (5.75)	1.42 (3.69)
>4000	15.2 (12.5)	4.15 (5.87)	1.35 (2.83)

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490 **Table 2. Percent time spent in hypoglycemia at different altitudes.** Data shown are mean

491 (SD), n=24, no significant differences noted between altitudes.

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