Continuous Glucose Monitoring at High Altitude – Effects on Glucose Homeostasis

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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±0.22mmol/L), 4650m (4.77±0.30mmol/L) and 5120m (4.78±0.24mmol/L) compared to baseline altitude 1100m (vs 4.61±0.25mmol/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.

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

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Introduction

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

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

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

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

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

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

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Methods

Subjects

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

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

Study design

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

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

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

Blood sampling and assays

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

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

Statistics

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

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Results

Demographics

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

Effects of altitude on hypoglycaemia

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

Effects of altitude on mean glucose levels and energy intake

There was a significant increase in mean nocturnal CGM glucose at Italian Base Camp (3600m), Dhaulagiri Base Camp (4650m) and Hidden Valley Camp (5120m), compared to Dharbang (1110m) (5.53±0.22 vs 4.77±0.30 vs 4.78±0.24 vs 4.61±0.25 mmol/L respectively; $p<0.0001$) (Figure 1). The mean nocturnal CGM glucose climbed steadily from Dharbang (4.61±0.25 mmol/) during the first week of the trek (Figure 2A) peaking on the second night at Italian Base Camp (5.64±0.25 mmol/L), then falling immediately to around 5 mmol/L for the last 4 days. These results were largely replicated in both teams (Figure 2B and 2C) despite them trekking at different times. The changes in CGM glucose were not obviously a reflection of the daily energy intake values. The mean daily energy intake immediately preceding the nocturnal glucose measurements did not differ between Dharbang and the three acclimatisation Camps (1968±360

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

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

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

Effects of altitude on glycaemic variability

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

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

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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).

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Discussion

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

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

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

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

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

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

These results do not fit into a neat paradigm and the cellular mechanisms driving these findings are not known; thus, our proposed explanation (represented in Supplementary Figure 2) is deliberately simplified to include the components that we measured. It should be noted that this description does not account for changes in multiple factors including the distribution and number of GLUT1 receptors, alterations in hypoxia-inducible factors (e.g. HIF1α), modulation of insulin receptor density, variations in rate-limiting enzymes such as glucokinase or glucose-6-phosphate, or the response of other hormones such as growth hormone, glucagon and thyroxine to altitude exposure. Furthermore, we recognise that plasma levels of glucose and TAG do not reflect tissue uptake nor oxidation, thus reduced clearance, insulin resistance and increased lipolysis may be important.

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

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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

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

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

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NEH conceived the study, collected data, analyzed data, wrote the manuscript. KD collected data and reviewed/edited manuscript. SM analyzed samples, contributed to discussion, reviewed/edited manuscript. NSO conceived the study, wrote the manuscript. CN collected data. AM conceived the study and reviewed/edited manuscript. JOH conceived the study and reviewed/edited manuscript. DW conceived the study, wrote the manuscript.

Conflict of interest

NEH is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. The results of the present study do not constitute endorsement by ACSM.

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Figure Legends

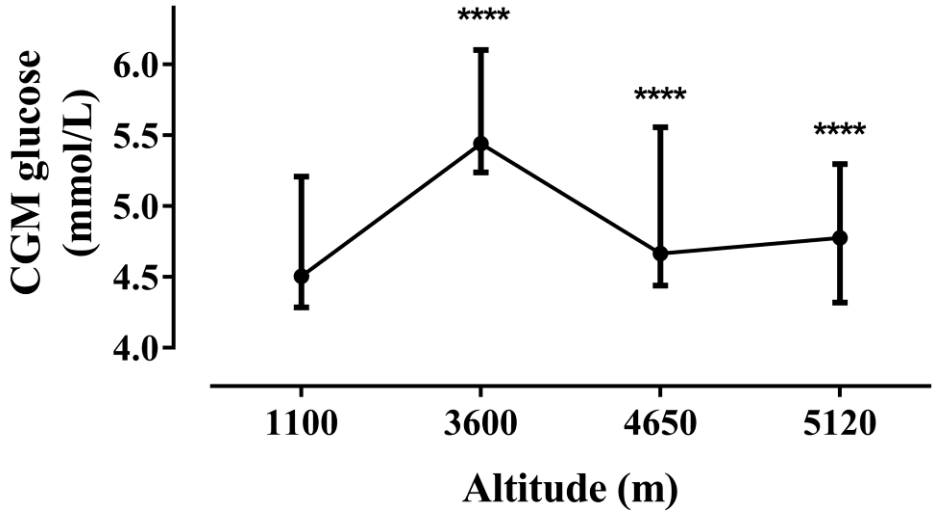

Figure 1. Nocturnal glucose levels at baseline and acclimatization camps. Continuous glucose monitoring data collected between 22.00pm and 06.00am (n=24 at 1110, 3600 and 4650m and n=21 at 5120m). Data are expressed as median and range and were analyzed by Friedman repeated measures ANOVA and Dunn's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

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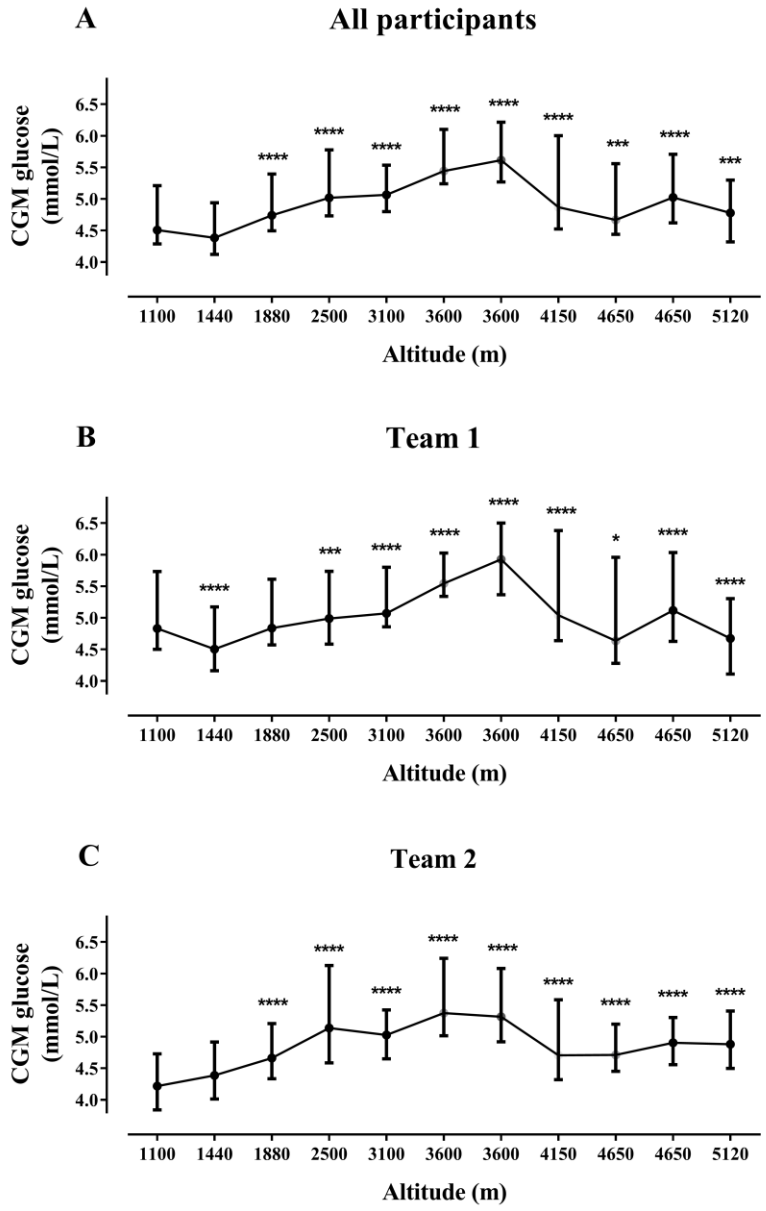

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436 **Figure 2. Nocturnal glucose levels during the BSDMRE trek.** Continuous glucose monitoring

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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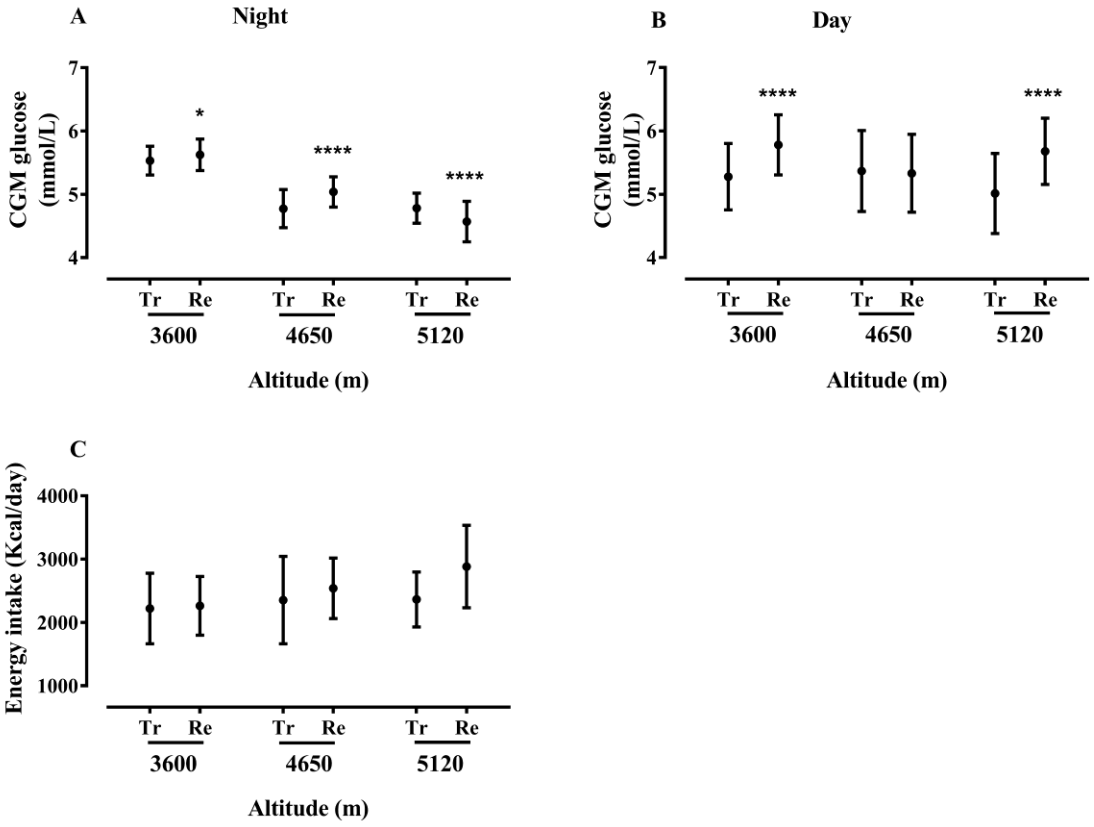

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443 **Figure 3.** Differences between (A) nocturnal (22.00-06.00 hrs) and (B) daytime (06.00-22.00
444 hrs) CGM glucose, and (C) Energy Intake (EI) on Trekking and subsequent Rest days. Mean (±
445 SD) glucose and EI on Trekking (Tr) days and the following 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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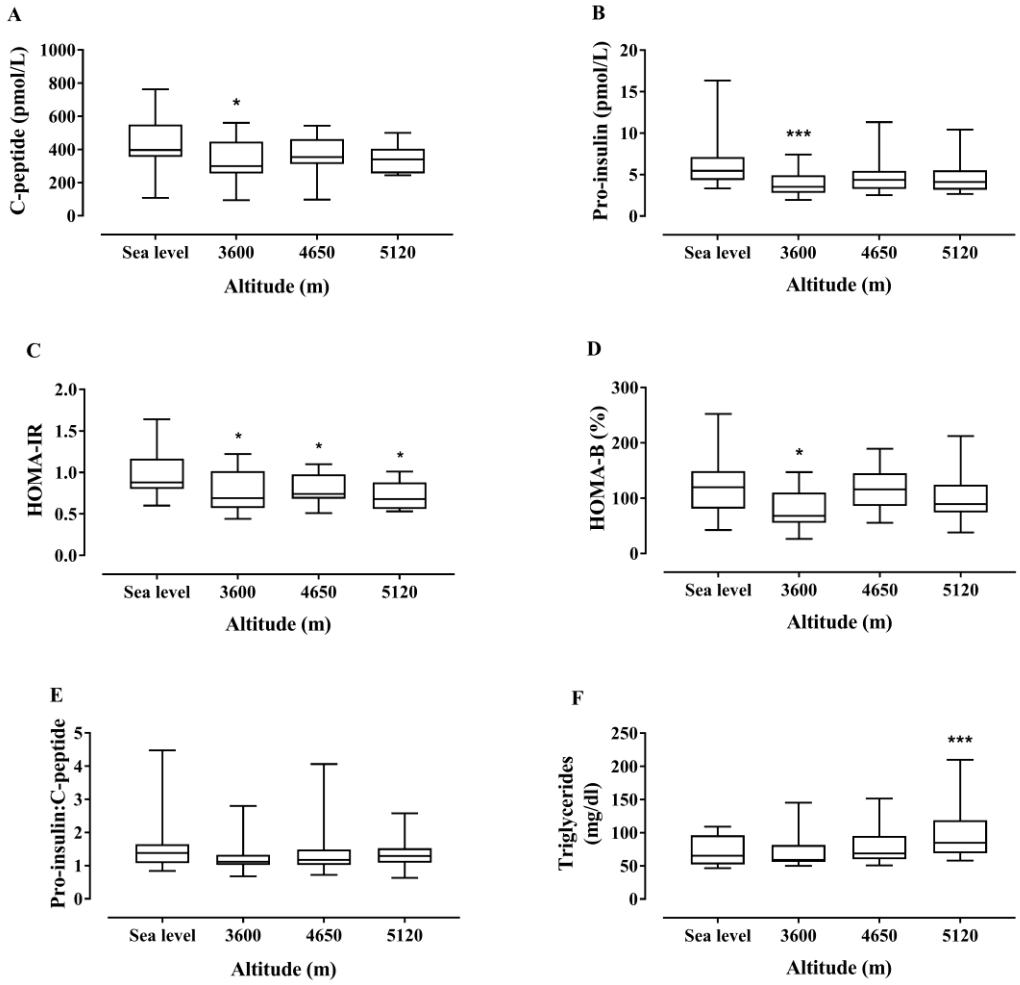
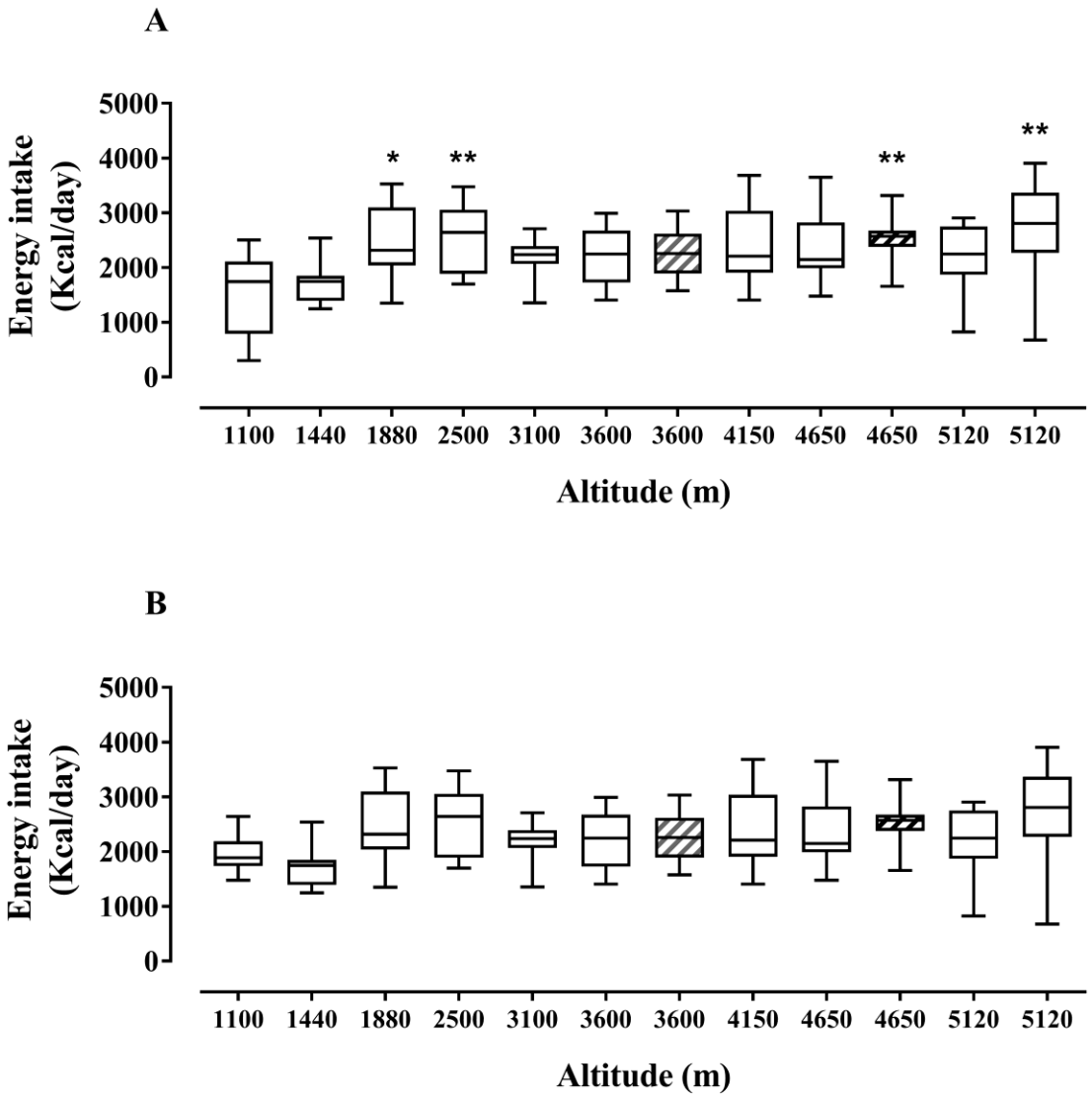
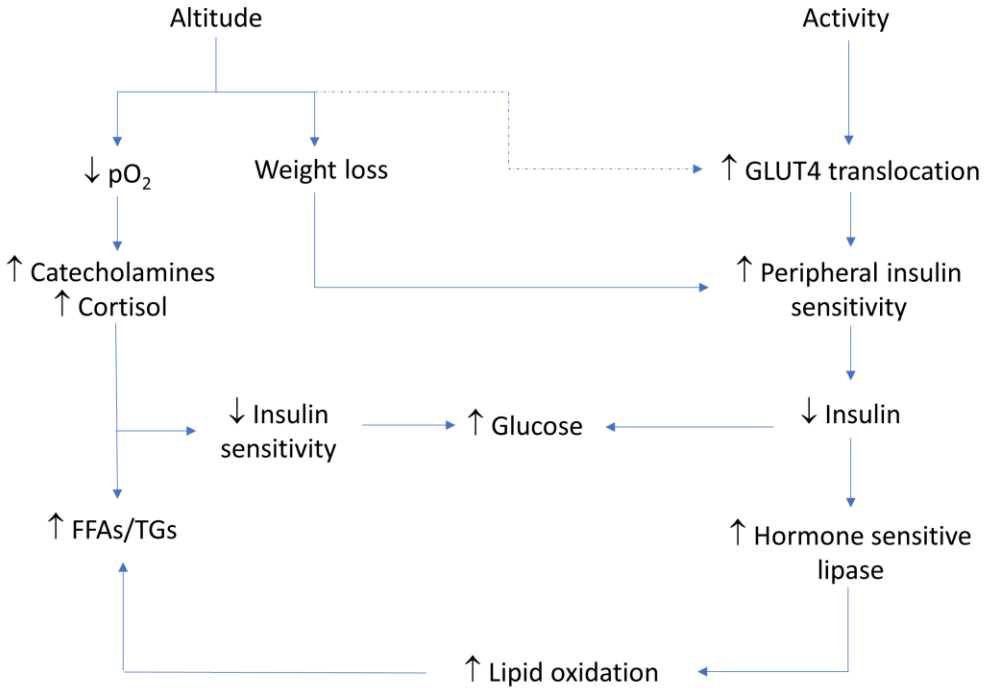


Figure 4. Markers of beta cell secretory function and insulin resistance at baseline and acclimatization camp. Changes in (A) fasting C-peptide, (B) fasting Pro-insulin, (C) HOMA-IR, (D) HOMA-B (E) pro-insulin:C-peptide and, (F) fasting plasma triglycerides (n=16-21 at 1110m; 18-21 at 3600m and 4650m; and n=9-11 at 5120m). Data are expressed as median ± IQR (box) and range (whiskers) and analyzed by one-way ANOVA or Kruskal-Wallis test and post-hoc with Holm-Sidak's or Dunn's multiple comparison test, respectively. * $p<0.05$ and *** $p<0.0001$ vs Sea level



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



Supplementary Figure 2. Proposed pathways influencing glucose levels during acclimatization to high altitude. FFA – free fatty acid; TGs – triglycerides; GLUT4 – glucose 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

Table 1: Routes and altitude during the BSDMRE. * Team 2 did not have an acclimatization day at Hidden Valley (Day 12) due to a number of team members having Acute Mountain Sickness and needing to descend

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Altitude (m)	Percent time spent in hypoglcemia 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)

Table 2. Percent time spent in hypoglycemia at different altitudes. Data shown are mean (SD), n=24, no significant differences noted between altitudes.

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