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**Reduced insulin sensitivity as a marker for acute mountain
sickness?**

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Stellt eine reduzierte Insulinsensitivität einen Marker für akute Höhenkrankheit dar?

Dexamethason (Dex) verringert bei Bergsteigern die Symptome der Höhenkrankheit. Neben den gewünschten Effekten hat Dex viele Nebenwirkungen, wie z.B. eine Verschlechterung des Glukosemetabolismus, Euphorie und Schlaflosigkeit.

Ziel der Studie war es, durch die Analyse von Parametern von Bergsteigern, die 5 Tage auf 4559m Höhe verbrachten, Informationen über den Effekt von Dex auf den Metabolismus in grosser Höhe zu erhalten.

Blutproben wurden in Zürich (ZH, 490m) und an den Tagen 2 und 4 auf der Capanna Regina Margherita (MG2, MG4; 4559m) genommen. Das Blutplasma wurde auf verschiedene Hormone wie Insulin, Cholecystokinin (CCK) sowie auf verschiedene Metaboliten wie Glukose untersucht. Eine Probandengruppe bestand aus Bergsteigern, die aufgrund von akuter Höhenkrankheit am Abend von MG2 mit Dex behandelt wurden, eine weitere Gruppe blieb unbehandelt.

Neben den erwarteten Effekten von Dex auf den Glukosemetabolismus (Glukose, Insulin und Laktat am Tag 4 erhöht) hatte die mit Dex behandelte Gruppe bereits in Zürich, d.h. vor jeglicher Behandlung und Höhenexposition, eine geringere Insulinsensitivität. CCK war in der mit Dex behandelten Gruppe im Vergleich zur unbehandelten Gruppe an MG4 niedriger.

Wir vermuten, dass Individuen mit niedriger Insulinsensitivität empfänglicher für akute Höhenkrankheit sind. CCK könnte am positiven Effekt von Dex auf höhenbedingte Appetitlosigkeit beteiligt sein.

Insulin Sensitivität, Höhenkrankheit, Dexamethason

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Reduced insulin sensitivity as a marker for acute mountain sickness?

Dexamethasone (dex) alleviates the symptoms of mountaineers suffering from acute mountain sickness (AMS). However, besides its benefits, dex has a wide range of side effects on mountaineers such as a diabetogenic impact on glucose metabolism, euphoria and insomnia.

To study the effect of dex on metabolism at high altitude, we analyzed parameters obtained from mountaineers who spent 5 days at 4559m of altitude.

Blood samples were taken in Zurich (ZH, 490m) and 2 and 4 days after a fast ascent to the Capanna Regina Margherita (MG2 and MG4, 4559m). Plasma was analyzed before and after ingestion of a 430kcal solid meal for several hormones including insulin, cholecystokinin (CCK) and erythropoietin (EPO) and several metabolites including glucose. One group consisted of mountaineers that required dex in the evening of day 2 due to the occurrence of AMS and the other remained untreated.

Besides expected effects of dex on glucose metabolism (increased glucose, insulin and lactate levels on day 4), the dex-treated group had a lower insulin sensitivity and lower levels of EPO already at baseline in Zurich i.e. before any treatment and exposure to hypoxia. CCK was lower on MG4 in the dex-treated group compared to the untreated group.

We speculate that individuals with low insulin sensitivity are more susceptible to AMS. CCK may be involved in the improving effect of dex on high altitude anorexia.

Insulin sensitivity, acute mountain sickness, dexamethasone

Reduced insulin sensitivity as a marker for acute mountain sickness?

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Running head: Insulin sensitivity and AMS

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Abstract

Background Dexamethasone alleviates the symptoms of mountaineers suffering from acute mountain sickness (AMS). However, besides its benefits, dexamethasone has a wide range of side effects on mountaineers such as a diabetogenic impact on glucose metabolism, euphoria and insomnia.

Objective To study the effect of dexamethasone on metabolism at high altitude, we analyzed parameters obtained from mountaineers who spent 5 days at 4559m of altitude.

Design Blood samples were taken in Zurich (ZH, 490m) and 2 and 4 days after a fast ascent to the Capanna Regina Margherita (MG2 and MG4, 4559m). Plasma was analyzed before and after ingestion of a 430kcal solid meal for several hormones including insulin, cholecystokinin (CCK) and erythropoietin (EPO) and several metabolites including glucose. One group consisted of mountaineers that required dexamethasone in the evening of day 2 due to the occurrence of AMS and the other remained untreated.

Results Besides expected effects of dexamethasone on glucose metabolism (increased glucose, insulin and lactate levels on day 4), the dexamethasone-treated group had a lower insulin sensitivity and lower levels of EPO already at baseline in Zurich i.e. before any treatment and exposure to hypoxia. CCK was lower on MG4 in the dexamethasone-treated group compared to the untreated group.

Conclusion We speculate that individuals with low insulin sensitivity are more susceptible to AMS. CCK may be involved in the improving effect of dexamethasone on high altitude anorexia.

Key words: High altitude, insulin resistance, dexamethasone, erythropoietin

Introduction

Increasing numbers of people living at or near sea level spend their spare time with activities such as hiking, skiing or climbing at high altitude. Mountaineering is often accompanied by acute mountain sickness (AMS), with an incidence of 15-80% depending on individual susceptibility, acclimatization, speed of ascent and height of summit higher than 2500m (13, 20, 33, 34). Most common symptoms of AMS are headache, insomnia, fatigue, dizziness, breathlessness, anorexia and nausea (13, 19, 46, 53). High altitude illness often manifests as high altitude pulmonary edema (HAPE) and high altitude cerebral edema (HACE) that, without effective treatment, might lead to death (27). Therefore, a long list of possible prophylactic or therapeutic medications exists to make high altitude traveling safer and more comfortable (30-32). Apart from standard medications such as non-steroidal anti-inflammatory drugs (NSAID; ibuprofen, paracetamol) against headache or acetazolamide that accelerates acclimatization by inducing a metabolic acidosis, the corticosteroid dexamethasone is commonly used by mountaineers to prevent or cure themselves from AMS (14, 21, 28). It is well known that dexamethasone taken prophylactically improves exercise capacity in HAPE-susceptibles (9). The underlying molecular mechanisms for dexamethasone-mediated improvement of mountaineers suffering from AMS are complex and only partly understood. This is due to the fact that the primary cause of AMS is not well investigated. There is evidence that hypoxia-induced systemic inflammation plays a role in the development of AMS (15, 22, 23). Thus, the anti-inflammatory and immunosuppressant action of dexamethasone could at least partly be responsible for dexamethasone's beneficial effect in AMS. Another possible effect might be the modulation of the release and action of hormones, especially gastrointestinal hormones that control food intake. Cholecystokinin (CCK) has been shown to be upregulated in plasma at high altitude and thus may play a role in the development of the anorexia of AMS (3). CCK is also known to be decreased by dexamethasone in cell cultures and rats (42).

Of note dexamethasone has a wide range of undesired side effects. Besides the immunosuppressant effect that potentially leads to a higher risk of

infection and impaired wound healing, glucocorticoids influence fat and glucose metabolism in a catabolic and diabetogenic manner (26, 48, 50). The change in insulin sensitivity and beta cell activity of the pancreas can be assessed with methods like the homeostasis assessment model (HOMA), the glucose to insulin ratio or the quantitative insulin sensitivity check index (QUICKI) (18, 37). Due to increasing numbers of patients with metabolic syndrome, decreased insulin sensitivity or manifest diabetes worldwide and the improvements in the efficiency of antidiabetic treatment, a greater number of patients with metabolic problems might be exposed nowadays to hypoxic conditions. Unfortunately the risk for diabetic dexamethasone-treated patients is even higher as dexamethasone and high altitude hypoxia itself lead to changes in glucose homeostasis (17, 25) and therefore result in changed insulin requirement and eventually a deterioration of their metabolic disorders. As a primary endpoint we investigated potential metabolic differences in patients with AMS in particular concerning metabolites, glucose metabolism and gastrointestinal hormones. This part of the data was analyzed retrospectively depending on whether individuals required dexamethasone or not. Second we investigated the effect of dexamethasone treatment in a subgroup of participants. We analyzed blood plasma samples of a group of healthy mountaineers who took part in a large-scale study; the study also included analysis of food intake, gastrointestinal mucosal injury, pulmonary high altitude illness and sleep. These results will be reported elsewhere.

Methods

Subjects

Twenty-five healthy and experienced mountaineers (15 male; 22-60 years) were recruited by adverts in mountain journals. As we were interested in AMS and therefore avoided acclimatization, volunteers were not allowed to stay more than 3 nights above 2500m one month prior to the fast ascent. Exclusion criteria were chronic diseases, regular medication, history of transplantation, clinically significant heart valve diseases and congenital heart or lung disease. Also, volunteers were expected to have normal body weight ($>18 \text{ kg/m}^2$, $< 25 \text{ kg/m}^2$) but two persons with a BMI slightly above 25 and

one with a BMI of 31.38 were included. Participants had to show normal eating behavior and were not allowed to require special diets.

The Ethics Committee of the Canton of Zurich approved the study (EK-1677) and conformed to the declaration of Helsinki.

Study procedure

Subjects underwent baseline physical examinations to ensure they matched the inclusion criteria. First blood samplings were done in Zurich (ZH, 490m above sea level, pO₂ 140-150 mmHg) to generate baseline values. Groups of 4-5 subjects each were then exposed to hypoxia using a fixed schedule; all experiments were performed in the summer of 2009 over a period of 5 weeks. Each group started at day 0 in Alagna Valsesia (Italy, 1205m), was transported by cable car to 3000m, then walked to the Capanna Gnifetti at 3600m (pO₂ 94-103 mmHg) in the Monte Rosa region and stayed there overnight. On day 1 the groups ascended to arrive at the Capanna Regina Margherita (4559m, pO₂ 81-91 mmHg) in the late morning, where the tests at high altitude were done. (Fig. 1A)

AMS assessment and medication

Occurrence of AMS was evaluated using the Lake Louise Score (LLS>5) and by medical examination by experienced physicians. This study was not designed to assess the effects of dexamethasone on high altitude physiology in a randomized double blind placebo controlled fashion. Therefore for safety reasons only subjects with high HAPE susceptibility, a LLS greater 5 in the morning or evening of MG2 or necessity identified by medical examination were treated with 2x8 mg/day dexamethasone (9-fluor-16a-methylprednisolone, Dexamethasone Galepharm, 4 mg, Galepharm AG, 8700 Kuesnacht, Switzerland) starting on the evening of MG2, i.e. after the last blood sample had been taken on that day. One person had to be treated with dexamethasone already earlier on day 2 so that this subject was excluded from analysis. Due to the occurrence of AMS, 14 subjects had to be treated with dexamethasone (DEX) and 11 served as untreated controls (CON).

Study design and sample proceeding

Blood samples and gastrointestinal biopsies were taken at ground level in Zurich (ZH) and on day 2 and day 4 (MG2 and MG4) at the Capanna Regina Margherita. On the morning of each test day, a venous catheter was placed in the forearm to allow blood sampling. Part of the study was to analyze gastric emptying which included a protocol of repeated blood plasma sampling in the morning before an ad libitum breakfast, just before and 30, 60, 90, 120, 180, 240 min after a test meal of two 125 mg ^{13}C -octanoate containing labeled muffins (1.76 g dietary fiber, 12.52 g protein, 18.06 g fat, 52.91 g carbohydrates, 430 kilocalories), and between an additional muffin preload and ad libitum dinner in the evening of each test day (Fig. 1B). EDTA-tubes were centrifuged at the hut and plasma samples were kept as aliquots frozen in liquid nitrogen until they were stored in -80°C at Zurich for later analysis. Analytical results of ^{13}C octanoate metabolism are not shown in this report. For dinner, subjects were offered pasta, bolognese sauce, grated parmesan cheese as well as two sorts of biscuits. They were free to choose what and how much they ate of the different food components; all food consumed was weighed on a kitchen scale to the nearest gram and caloric food intake was calculated.

Plasma analysis

To analyze the blood plasma samples Milliplex® MAP Kit Human Endocrine (Millipore, Billerica, MA, USA) was used for amylin and glucagon. Human gastrin I (1-17) was measured with the Enzyme Immunoassay Kit (Enzo Life Sciences, Lausen, Switzerland) and protein extraction was done as recommended in the protocol. Further kits used were Human Erythropoietin Immunoassay (Quantikine, IVD, R&D Systems, Minneapolis, USA) and Ultra sensitive Human Insulin RIA Kit (Millipore).

CCK-8 (active), PYY 1-36 and PYY 3-36 (truncated form) were measured using Radioimmunoassay Kits (Eurodiagnostica, Burgdorf, Switzerland) by Prof. Christoph Beglinger, University Hospital Basel, Switzerland. Blood metabolites were measured with a Cobas Mira analyzer (Roche, Switzerland): triglycerides with an enzymatic dye test, glucose by an enzymatic UV-test, L-lactate colorimetric, BHB with a kinetic enzymatic method and FFA with an enzymatic colorimetric test. Plasma levels of IL-6, IL-8 IL-1 β and TNF- α were

measured using the Milliplex® MAP Human Cytokine/Chemokine Assay (Millipore).

Calculations

Homeostasis model assessment (insulin sensitivity: $\text{HOMA S} = (\text{glucose} \times \text{insulin}) / 22.5$); beta cell activity: $\text{HOMA B} = (20 \times \text{insulin}) / (\text{glucose} - 3.5)$), fasting glucose to insulin ratio (G:I) and quantitative insulin sensitivity check index ($\text{QUICKI} = 1 / ((\text{LOG}(\text{insulin})) + (\text{LOG}(\text{glucose})))$) were calculated by using the measured values of fasting insulin (mU/l) and fasting glucose (mmol/l). (18, 37)

Statistics

Statistical analysis was performed with Graphpad Prism (CA, USA). Part of the data was skewed and had different variances. To compare two groups at one test day, significance was determined by students t-test with Welch's correction. For comparison of different test days within the groups the Wilcoxon matched-pairs signed rank test was used. Two way ANOVA including Bonferroni posthoc test was used for comparing hormone levels and metabolites that were measured over the course of the day. Area under the curve (AUC) was calculated for various time periods for hormones with the value of the blood sample "before muffin" set as baseline. AUC therefore indicates the changes in plasma levels relative to baseline. Odds ratios were calculated to define the risk to become sick from AMS in subjects with baseline EPO plasma levels lower than 6 mU/ml and a HOMA S level higher or equal 2 at baseline in ZH. In the case of HOMA S one value equaled zero, 0.5 was added to all values to make the calculation of the odds ratio possible. Significance was assumed with a p-value smaller or equal to 0.05.

Results

Endogenous cortisol was measured as an internal treatment control standard in all individuals before and after medication with dexamethasone. (Fig. 2A) Retrospective analysis indicated that endogenous cortisol did not differ

between groups in ZH or at MG2; as expected, however, endogenous cortisol was markedly lower after treatment (MG4DEX vs. MG4CON $p<0.001$); in other words endogenous cortisol was only suppressed in treated participants. Based on similar cortisol levels on MG2 compared to ZH in both groups, the ascent on day 0 and day 1 had no influence on MG2.

Baseline characteristics

No significant differences were found between the dexamethasone-treated (DEX) and untreated groups (CON) in body mass index, body weight, age, gender and in systolic and diastolic blood pressure at all test days (table 1). Eight of 25 subjects were known to have suffered from HAPE before.

Caloric food intake ad libitum dinner

Food intake in kcal during the ad libitum dinner was lower in DEX on all three days ($p<0.05$) and decreased in both groups on MG2 compared to ZH (ZHCON vs. MG2CON $p<0.01$; ZHDEX vs. MG2DEX $p<0.05$); food intake normalized to nearly baseline levels on MG4 (MG2CON vs. MG4CON $p<0.05$; MG2DEX vs. MG4DEX $p<0.01$) (Fig 2C).

Hypoxia and clinical high altitude illness

Both groups showed a significant decrease in arterial PO₂ on MG2 and MG4 (mean of 12.4 ± 1.2 kPa in ZH to 5.2 ± 0.6 kPa on MG2 and 5.9 ± 0.6 on MG4); mean peripheral oxygen saturation also decreased compared to baseline levels. ($97.3\pm1.4\%$ in ZH to $75.3\pm8.5\%$ at MG2 and $81.5\pm7.3\%$ at MG4). As expected, EPO levels increased in all subjects on MG2; EPO decreased on MG4 compared to MG2 but remained higher on MG4 compared to ZH (MG2CON and MG4CON to ZHCON $p<0.01$; MG2DEX and MG4DEX to ZHDEX $p<0.001$). Unexpectedly, EPO levels of the dexamethasone-treated group were lower than in the untreated control group at baseline in ZH ($p<0.05$) i.e. before any prior treatment with dexamethasone. The odds ratio for the risk of suffering from AMS for subjects with EPO levels lower than 6 mU/ml at baseline in ZH was 10.5 with a confidence interval (CI) of 1.36-81.09 and a p-value of 0.03 in the Fisher's exact test. EPO levels decreased from

MG2 to MG4 in both groups but EPO was significantly lower in MG4DEX than in MG4CON ($p<0.05$) (table 1).

Lake Louise Score (LLS) increased in both groups on MG2 compared to ZH ($p<0.01$) but only tended to be higher in MG2DEX compared to MG2CON ($p=0.07$). On MG4 LLS decreased in both groups but the decrease was significant only in the dexamethasone-treated group ($p=0.16$ for MG2CON vs. MG4CON; $p<0.05$ for MG2DEX vs. MG4DEX). In both groups LLS remained higher at MG4 compared to ZH ($p<0.05$) (Fig. 2B).

Inflammation markers

IL-8 and IL-1 β levels were similar across groups but TNF- α was lower in MG4DEX than in MG4CON ($p<0.05$) (Fig. 2D). IL-6 increased in both groups on MG2 compared to ZH (CON $p<0.05$, DEX $p<0.01$). IL-6 decreased on MG4 but the difference was only significant in the control group compared to MG2 ($p<0.05$) but not in the dexamethasone treated group (MG2DEX to MG4DEX $p=0.07$) (Fig. 2E).

Metabolites

We detected no significant changes due to AMS and dexamethasone treatment in plasma levels of triglycerides, beta hydroxybutyrate and free fatty acid (data not shown). Glucose was higher in MG4DEX compared to MG4CON at most time points of the day ($p<0.001$ morning, before muffin, 60, 90 min pp; $p<0.01$ 30min pp; $p<0.05$ 120,180,240 min pp) (Fig. 3B).

Gastrointestinal and pancreatic hormones

We did not detect any difference in blood plasma levels of amylin, PYY and gastrin at any time point across groups, i.e. due to hypoxia or dexamethasone treatment. Amylin, CCK and PYY increased as expected after the muffin test meal in both groups and on all days.

CCK

Interestingly, CCK levels were lower in MG4DEX compared to MG4CON 30 ($p<0.05$) and 60 ($p<0.01$) min after the test meal (Fig. 3A). The AUC of CCK was also smaller in DEX compared to CON on MG2 30, 60 and 90 min after the test meal ($p<0.05$). The AUC was greater in MG4CON compared to ZH

30, 60 and 90 min postprandially ($p < 0.01$, < 0.01 , < 0.05); compared to MG4DEX, it remained significantly greater over all time points after the muffin test meal (before breakfast $p < 0.05$, all other time points $p < 0.01$) (data not shown).

Amylin

Amylin had a greater AUC at MG2 in CON 30 and 60 min after the muffin test meal ($p < 0.05$). Ninety to 240 min after the muffin the AUC of amylin was greater in CON at MG2 compared to ZH ($p < 0.001$) (data not shown).

Insulin

Retrospective analysis of baseline data in ZH indicated that plasma insulin was higher in DEX compared to CON at 60 min ($p < 0.01$). Comparison of the study days within groups showed no significant difference at any time point in CON but insulin was higher in DEX on MG2 compared to ZH 30 and 60 min postprandially ($p < 0.001$, < 0.01). Two-way ANOVA revealed an overall effect of dexamethasone and altitude in MG4DEX compared to ZHDEX ($p < 0.001$) but did not detect a significant difference between time points. MG4DEX had higher levels at 30 and 60 min than MG2DEX ($p < 0.001$). Insulin levels were higher in MG4DEX compared to MG4CON after the test meal and just before dinner ($p < 0.001$ 60, 90 min pp; $p < 0.01$ 30, 120 min pp; $p < 0.5$ 180 min pp, before ad lib) (Fig. 3C). Fig. 2F represents fasting insulin levels taken before breakfast and demonstrates the significantly higher levels in ZHDEX compared to ZHCON ($p < 0.01$) and in MG4DEX to MG4CON ($p < 0.001$).

The AUC of insulin was smaller in MG2DEX compared to ZHDEX at the time points 30, 60 and 90 min ($p < 0.05$, < 0.01 , < 0.05), and greater in MG4DEX compared to ZHDEX at the time points 120, 180 and 240 min ($p < 0.05$, < 0.01 , < 0.001). At 180 min MG4CON was greater than ZHCON ($p < 0.05$). At the time points 180 and 240 min AUC of insulin was greater in MG4DEX compared to MG4CON ($p < 0.05$) (data not shown).

Glucagon

Glucagon levels were higher in MG4DEX compared to MG4CON at the same points as indicated by glucose and insulin levels (Fig. 3D). The AUC was significantly smaller in MG4CON compared to MG4DEX at the time points 180 and 240 min pp ($p < 0.05$).

Assessment of insulin sensitivity

Calculation of HOMA levels indicated that insulin sensitivity (HOMA S) was lower in MG4DEX than in MG4CON ($p < 0.001$). While this effect was expected considering the known effect of dexamethasone on glucose metabolism, it is interesting that insulin sensitivity also was lower in ZHDEX compared to ZHCON ($p < 0.01$) i.e. before any treatment already at baseline condition. The odds ratio for the risk of becoming sick from AMS for subjects with HOMA S baseline levels in ZH higher or equal 2 was calculated as 22.45 with a confidence interval of 1.1-480.3 and $p = 0.014$ in the Fisher's exact test. HOMA S increased in MG2CON compared to ZH ($p < 0.05$) and in MG4DEX compared to ZH ($p < 0.01$) (Fig. 2H). Beta cell activity (HOMA B) remained unchanged. Fasting glucose to insulin ratio (Fig. 2G) was lower in ZHDEX and MG2CON compared to ZHCON ($p < 0.05$, $p < 0.01$) and also lower in MG4DEX than in MG4CON ($p < 0.05$). QUICKI values were significantly lower in ZHDEX and MG2CON than in ZHCON ($p < 0.05$) and lower in MG4DEX compared to ZHDEX and MG4CON ($p < 0.01$, $p < 0.001$) (Fig. 2I).

These findings altogether indicate that baseline insulin sensitivity was lower in individuals at risk of AMS; these individuals required dexamethasone treatment in the evening of MG2 because of the development of AMS symptoms.

Discussion

The exact causes and mechanisms of AMS remain unknown. Here we intended primarily to study specifically the effects of AMS on metabolism. Second we were interested in side effects of dexamethasone treatment on metabolism in AMS prone patients under exposure of high altitude-induced hypoxia. Our results did not only confirm the expected effect of dexamethasone on glucose homeostasis but also revealed differences between the two groups in EPO levels and insulin sensitivity already at baseline in Zurich, i.e. at low altitude and before treatment with dexamethasone.

The changes in LLS reflect the fast ascent to high altitude with an increase on MG2 and a decrease due to acclimatization on MG4 in both groups as

expected (20, 27). The prevalence of 57% of AMS in our subjects lies in the upper range of the data reported by other groups that found a prevalence of 30-60% at altitudes between 4243m and 5671m (4, 20, 33, 54). This may be due to the fast ascent in our study and to the study design that included eight subjects with a known HAPE susceptibility. The decrease in LLS on MG4 after dexamethasone clearly shows the effective improvement in well-being of the subjects who suffered from AMS but were successfully treated with dexamethasone, as it has been shown previously (49).

Endogenous cortisol was measured and analyzed to proof the efficacy of the dexamethasone medication. The suppressive effect of dexamethasone on endogenous cortisol production was as expected (8, 39), i.e. the dexamethasone treated group showed a marked decrease of endogenous cortisol on MG4. Hence the medication of the individuals suffering from AMS was in principle considered successful.

EPO

Erythropoietin is generally up-regulated by hypoxia (7). As expected EPO levels increased on MG2 after the fast ascent, subsequently slightly decreased on MG4 due to acclimatization but still remained higher than in ZH. Levels of EPO in the dexamethasone-treated group were significantly lower on MG4; surprisingly, levels were already lower in ZH in these individuals, i.e. at baseline before any treatment.

Endogenous EPO has a relatively short plasma half-life of 6-9 hours (7). Therefore our results indicate that the EPO production was still higher on MG4 compared to ZH but lower compared to MG2. The significantly lower EPO plasma level in the dexamethasone-treated group on MG4 can be explained by the effect of dexamethasone on gene transcription of cytokines via the glucocorticoid receptor (2, 40, 47). Hypoxia inducible factor (HIF) enhances EPO gene transcription in response to hypoxia (51) and Gaber et al showed in cell culture experiments that dexamethasone suppresses the expression of HIF (12). Unfortunately this effect interferes with the acclimatization to high altitude hypoxia and leads to the urgent necessity of descent in case of AMS despite medication with dexamethasone. Further

possible influences e.g. on EPO clearance from the blood are not well investigated yet.

In apparent contrast to our study Pavlicek et al. reported no difference in baseline levels but a significantly higher EPO plasma level in HAPE susceptibles at high altitude (41). Of note, one of the inclusion criteria for our study was, that the subjects were not allowed to spend more than 3 nights at 2500m or higher one month prior to the study, but we did not completely exclude activities at altitude. Altitude activities prior to the study and a better general state of physical fitness may have lead to the slightly but significantly higher EPO levels in ZH in the group that did not suffer from AMS (11). Whether there is any connection between EPO levels at near sea level and susceptibility to AMS cannot be answered by our results.

Glucose metabolism

Glucose metabolism was affected by dexamethasone on MG4 as expected. It has been shown in other studies that dexamethasone increases glucose levels (36); however, our study could not confirm increased glucose levels due to hypoxia or AMS itself as has been shown by Larsen et al. (25). Glucocorticoids affect glucose metabolism in several ways, they impair insulin dependent glucose uptake in the periphery and enhance gluconeogenesis in the liver (43, 44).

Our data show that glucose and insulin levels increased and therefore insulin sensitivity decreased after dexamethasone treatment on MG4, which was expected (1). No significant changes due to hypoxia could be seen on MG2. Most surprisingly, we found a lower insulin sensitivity in Zurich at baseline – which has to be clearly differentiated from diagnosed diabetes – in the subjects that later on suffered from AMS and were treated with dexamethasone. This may be an indication of higher susceptibility of those individuals for AMS. We did not evaluate the subjects with respect to any hereditary predisposition for diabetes. Of note Henriksen et al showed that muscle glucose metabolism of normoglycemic relatives of type 2 diabetic patients is differentially altered by dexamethasone (17). The lower EPO levels in the dexamethasone treated group already at baseline may also play a role as it has been shown that EPO improves glucose tolerance in mice by

changing metabolism in muscles (10, 22, 33), and EPO treatment of anemia in uremic patients improves insulin resistance and hyperinsulinemia (35). Insulin sensitivity is impaired in patients with metabolic syndrome but other symptoms like increased blood pressure or higher body mass index did not appear to be prevalent in our subjects (45). Although they did not yet show any obvious symptoms of diabetes or even clear changes in blood glucose levels, people predisposed for diabetes may have more difficulties to handle those side effects of dexamethasone because it aggravates their insulin resistance, which may have been undetected before. Therefore we analyzed insulin sensitivity by different models using fasting insulin and glucose levels but all indicated consistent findings. Unfortunately we did not perform hyperinsulinemic-euglycemic clamps or oral glucose tolerance tests in our study, which would make our analyses more precise. Another possible indicator for assessing the blood glucose concentration over a longer period is glycated hemoglobin (HbA1c) (5, 16). Nevertheless, we believe that the calculations of HOMA, QUICKI, glucose to insulin ratio combined with the values of fasting insulin are helpful and reliable indices to assess insulin resistance (18, 37, 38).

Hormones

Neither altitude hypoxia nor AMS or dexamethasone seemed to have an impact on PYY and gastrin. CCK levels of MG4CON tended to be higher only at one time point (30 min postprandially) compared to ZH and MG2 but this did not reach significance; further we could therefore not confirm the increase of resting plasma CCK that Bailey et al found at high altitude (3). The AUC showed a significantly higher increase of CCK in MG4CON compared to ZH and MG4DEX; this may indicate that hypoxia could still have had some influence; this finding, combined with the significantly lower levels in MG4DEX compared to MG4CON confirms an effect of dexamethasone on CCK levels similar to what had already been reported by Gatineau et al in cell culture and rat experiments (42). Therefore CCK may play a role in the improvement of AMS associated anorexia after dexamethasone treatment.

Although we found no significant difference at single time points in amylin plasma levels that would implicate an effect of hypoxia or dexamethasone, we

found significant differences in the AUC. Amylin increased more in MG2CON compared to MG2DEX at the time points 30 and 60 min and compared to ZHCON for the time points 90, 120, 180 and 240 min. Therefore the food intake induced amylin response seems to be different in subjects at high altitude compared to sea level and compared to subjects suffering from AMS. This needs to be further investigated but at least our data suggest that dexamethasone has no effect on amylin levels.

Caloric food intake

The overall effect of hypoxia on eating, in particular the significant decrease of caloric food intake during the ad libitum dinner in both groups on MG2 confirms the results of earlier studies (29, 52). The increase back to nearly baseline (ZH) levels in both groups on MG4 was probably due to acclimatization and the dexamethasone treatment in the DEX group (6). Subjects at risk for AMS (DEX) ate less than the control group on all three test days. As we did not measure hormone levels and metabolites during and after the ad libitum dinner potential underlying mechanisms need to be tested in future studies.

Cytokines

Consistent to other studies, IL-6 increased due to high altitude hypoxia; no difference could be seen in other cytokines measured (15, 23). IL-6 and TNF- α decreased significantly in the dexamethasone treated group on MG4, which was expected due to the effective anti-inflammatory effect of dexamethasone.

Finally, some limitations of our study need to be mentioned. First, medication was not blinded and we had no placebo control group included due to the primary endpoint of this large scale study. In other words, any individual who required dexamethasone treatment for their medical condition and development of AMS received the drug. The other individuals were untreated. Nonetheless, we believe that the main conclusion of our study, i.e. that AMS-prone individuals may be less insulin sensitive before exposure to hypoxia, is interesting and remains valid. The data were analyzed retrospectively, therefore we did not carry out tests like the oral glucose tolerance tests or

hyperinsulinemic-euglycemic clamps that would make the evaluation of glucose metabolism more complete. Second, due to some difficulties in blood sampling and analysis, the group size (n) of some parameters is rather small. However, our results provide evidence indicating that dexamethasone in the treatment of AMS has not only positive effects on inflammation and altitude anorexia but also a potentially dangerous influence on glucose metabolism, in particular as our data indicate that people with decreased insulin sensitivity may be more susceptible to AMS. Finally, this study emphasizes a clear need for further investigation and reminds the reader that dexamethasone is a potent drug but, due to the numerous possible side effects, also potentially dangerous, especially at high altitude.

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None of the authors has any conflict of interest with regard to this manuscript.

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Isabelle Aeberli: food intake study

Max Gassmann: study design

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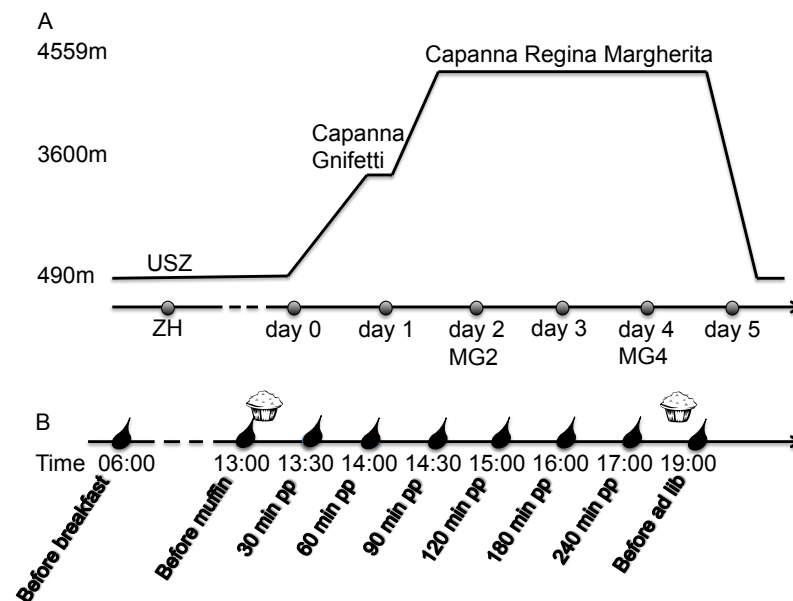


Fig. 1
Scheme of study travel itinerary (A) and blood sampling (B)

Table 1. baseline characteristics

| | ZH | | MG2 | | MG4 | |
|------|-------------|-------------|----------------------------|----------------------------|----------------------------|------------------------------|
| | CON | DEX | CON | DEX | CON | DEX |
| age | 39.8 ± 10.4 | 46.5 ± 8.8 | | | | |
| BW | 72.0 ± 7.5 | 70.0 ± 12.1 | | | | |
| BMI | 23.5 ± 1.1 | 23.9 ± 2.8 | | | | |
| SBP | 127 ± 14 | 131 ± 14 | 132 ± 12 | 125 ± 12 | 137 ± 11 | 129 ± 13 |
| DBP | 76 ± 11 | 80 ± 8 | 81 ± 7 | 78 ± 9 | 83 ± 8 | 80 ± 5 |
| EPO | 7.2 ± 1.7 | 5.4 ± 2.0 | 78.4 ± 33.8 ^{§§§} | 69.0 ± 22.2 ^{§§§} | 41.0 ± 17.3 ^{§§§} | 23.3 ± 8.5 ^{***§§§} |
| SPO2 | 97.2 ± 1.6 | 97.3 ± 1.3 | 78.8 ± 6.6 ^{§§§} | 72.5 ± 9.1 ^{§§§} | 83.4 ± 6.2 ^{§§§} | 79.9 ± 7.9 ^{§§§} |

Subjects divided in dexamethasone treated group (DEX) and control group (CON); table includes age, body weight (BW), body mass index (BMI), systolic (SBP) and diastolic blood pressure (DBP), erythropoietin (EPO), peripheral oxygen saturation (SPO2). ZH: Zurich, baseline; MG2: day 2 and MG4: day 4 at the Capanna Regina Margherita.

Values are means ± SD; * indicates a significant difference to control at the same day, § indicates a significant difference within the same group to baseline in ZH; §: p<0.05, §§: p<0.01, §§§: p<0.001

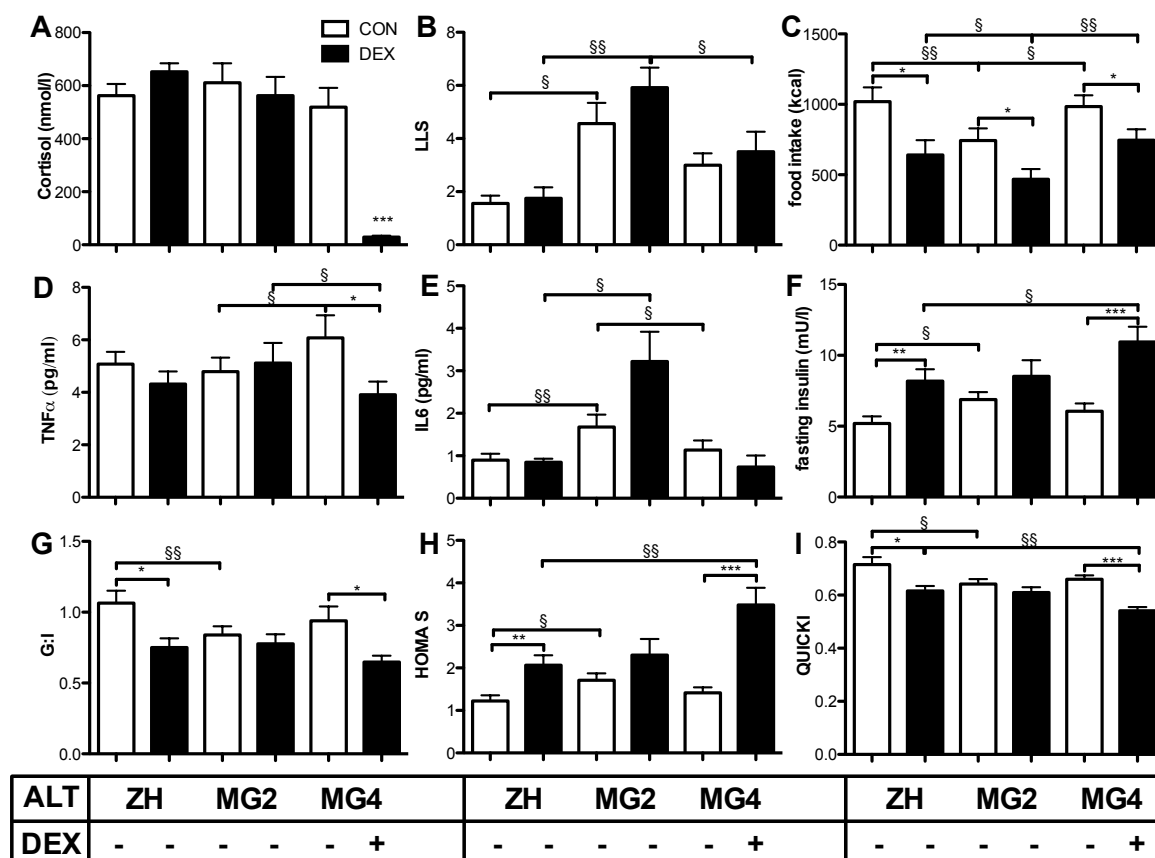


Fig. 2

Effects of hypoxia and dexamethasone on plasma concentrations of cortisol (A; n CON=9, DEX=12), tumor necrosis factor alpha (TNFα), interleukin-6 (IL-6) (D, E; n CON=9, DEX=11) and fasting insulin (F; n CON=9, DEX=12) in the first sample of each test day, on Lake Louise score (B; n CON=11, DEX=14), fasting glucose to insulin ratio (G:I) (G; n CON=9, DEX=12), quantitative insulin sensitivity check index (QUICKI) (I; n CON=9, DEX=12), insulin sensitivity by homeostasis model assessment (HOMA S) (H; n CON=9, DEX=12) and food intake at ad libitum dinner (C; CON=9, DEX=12)

§ implicates significant differences within the groups between different test days, tested with Wilcoxon matched-pairs signed rank test.

* implicates significant differences between the groups on the same test day, tested with unpaired students t test with Welch's correction

§: p<0.05, §§: p<0.01, §§§: p<0.001

Error bars represent standard error of the mean.

ZH: Zurich, baseline; MG2: day 2 and MG4: day 4 at the Capanna Regina Margherita;

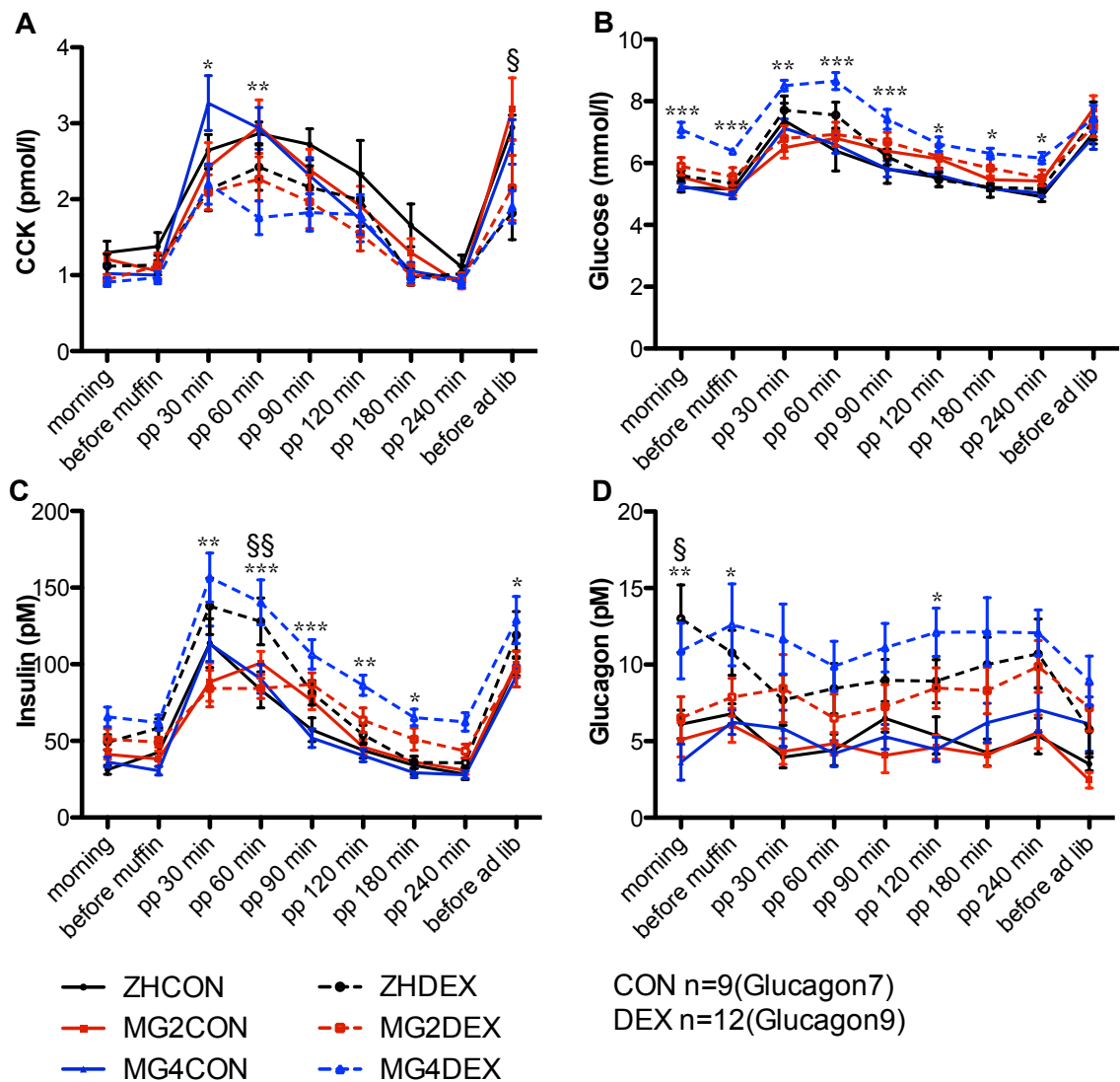


Fig. 3

Plasma levels in Zurich (ZH) and on day 2 (MG2) and day 4 (MG4) at high altitude, of CCK (A), glucose (B), insulin (C) and glucagon (D) measured nine times each test day and separated retrospectively into two groups (dexamethasone treated (DEX) and control group (CON)) *indicates significant differences between MG4CON and MG4DEX; § indicates significant differences between ZHCON and ZHDEX

Error bars represent standard error of the mean.

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