METABOLIC BASIS TO SHERPA ALTITUDE ADAPTATION

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Abstract

The Himalayan Sherpas, a human population of Tibetan descent, are highly adapted to life in the hypobaric hypoxia of high altitude. Mechanisms involving enhanced tissue oxygen *delivery* in comparison with Lowlander populations, have been postulated to play a role in such adaptation. Whether differences in tissue oxygen *utilization* (i.e. metabolic adaptation) underpin this adaptation is not however known. We sought to address this issue, applying parallel molecular, biochemical, physiological and genetic approaches to the study of Sherpas and native Lowlanders, studied before and during exposure to hypobaric hypoxia on a gradual ascent to Mount Everest Base Camp (5,300 m). When compared with Lowlanders, Sherpas demonstrated a lower capacity for fatty acid oxidation in skeletal muscle biopsies, along with enhanced efficiency of oxygen utilization, improved muscle energetics and protection against oxidative stress. This in part appeared to be related to a putatively advantageous allele for the *PPARA* gene, which was enriched in the Sherpas compared with the Lowlanders. Our findings suggest that metabolic adaptations underpin human evolution to life at high altitude, and could impact upon our understanding of human diseases in which hypoxia is a feature.

186 words (250 max)

Significance Statement

A relative fall in tissue oxygen levels (hypoxia) is a common feature of many human diseases including heart failure, lung diseases, anemia and many cancers, and can compromise normal cellular function. Hypoxia also occurs in healthy humans at high altitude due to low barometric pressures. Human populations resident at high altitude in the Himalayas have evolved mechanisms that allow them to survive and perform, including adaptations that preserve oxygen delivery to the tissues. Here we studied one such population, the Sherpas, and found metabolic adaptations, underpinned by genetic differences, which allow their tissues to use oxygen more efficiently, thereby conserving muscle energy levels at high altitude, and possibly contributing to the superior performance of elite climbing Sherpas at extreme altitudes.

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Introduction

At high altitude, low barometric pressure is accompanied by a fall in the partial pressure of inspired O_2, resulting in *hypobaric hypoxia*. The cellular response to hypoxia is orchestrated by the Hypoxia Inducible Factor (HIF) transcription factors, with HIF-1α and HIF-2α respectively mediating responses to short-term and more sustained hypoxia (1). In normoxia, prolyl-hydroxylases target HIFα subunits for destruction (2). Under low O_2 partial pressures, however, HIF-1α/HIF-2α are stabilized and dimerize with the nuclear HIF-1β subunit. This dimer interacts with hypoxia-response elements in promoter regions to increase expression of specific genes, e.g. *EPO* (encoding erythropoietin) and *VEGFA* (vascular endothelial growth factor A) (3).

The Tibetan Plateau has an average altitude of some 4,500 m. Humans were first present on the Plateau ~30,000 years ago, with the earliest permanent settlements appearing 6-9,000 years ago (4) – a period sufficient to drive the natural selection of genetic variants (and associated features) favouring survival and performance in sustained hypoxia (5, 6). Evidence supports the selection of genetic variants encoding components of the hypoxia-inducible factor (HIF) pathway, such as *EPAS1* (encoding HIF-2α) (7) and *EGLN1* (prolyl-hydroxylase-2, PHD2) (8) in Tibetan populations. One population, the Sherpas, migrated from Tibet to eastern Nepal ~500 years ago and exhibit remarkable physical performance at extreme altitude (9).

Whilst the human adaptive response to hypoxia is incompletely understood, mitigation against the fall in convective O_2 delivery plays an important role. In Lowlanders, increased ventilation and cardiac output, and the production of more O_2-carrying red blood cells help to sustain O_2 delivery

and content (10, 11). Likewise, exhaled concentrations of nitric oxide (NO), a key regulator of blood flow, are higher in Tibetans than Lowlanders (12), as are circulating NO metabolites and limb blood flow (13). The rise in red cell mass in response to hypobaric hypoxia is not as great in Tibetans as in Lowlanders, however (14, 15), suggesting that adaptation involves more than just increased O_2 delivery. In fact, acclimatization also involves alterations in O_2 *use*. In Lowlander muscle, mitochondrial density declines with sustained exposure to extreme altitude (16-18), whilst exposure to more moderate high altitude is associated with a reprogramming of muscle metabolism (19) even without altered mitochondrial density (20), including downregulation of electron transfer complexes (19) and tricarboxylic acid (TCA) cycle enzymes (21), loss of fatty acid oxidation (FAO) capacity (19, 20) and improved oxidative phosphorylation coupling efficiency (20). Sherpas have lower muscle mitochondrial densities than unacclimatized Lowlanders (22), but little is known of their metabolic adaptation to hypoxia, or any genetic selection which might underpin it. A role has been suggested for peroxisome proliferator-activated receptor alpha (PPARα), a transcriptional regulator of FAO in liver, heart and muscle. HIF downregulates PPARα in some tissues (23), whilst there is evidence for selection of variants in its encoding gene (*PPARA*) in some Tibetan subgroups (8, 24). We hypothesized that metabolic adaptation, and PPARα in particular, play a central role in the Sherpa adaptation to hypobaric hypoxia.

Results and Discussion

Selection of *PPARA* Variants in Sherpas

Lowlander and Sherpa subjects were participants of the research expedition, Xtreme Everest 2 (25). The Lowlanders comprised 10 investigators selected to operate the Everest Base Camp (EBC) laboratory. Sherpas (n = 15) were a sex-matched (73% male, *cf.* 70% in Lowlanders) and age-matched (26.8 ± 1.2 yr, *cf.* 28.0 ± 1.6 yr in Lowlanders) group living in Kathmandu and the Solukhumbu and Rolwaling valleys. No subject ascended higher than 4,200 m in the 3 months preceding the trek, nor above 2,500 m in the preceding 3 weeks. In addition, Sherpas presented evidence of sole Sherpa ancestry for 2 generations (i.e. 4 Sherpa grandparents). The frequency of putatively advantageous *PPARA* alleles (8) was higher in Sherpas than Lowlanders (Fig. 1A; Table S1), with genotype frequencies of the cohorts being significantly different at 2 single nucleotide polymorphisms (SNPs), rs6520015 and rs7292407 (P = 0.0091), though not rs9627403. This reflected patterns reported in some other Tibetan groups (26).

Muscle Hypoxia and Circulating NO Metabolites

Baseline testing, including blood sampling, muscle biopsy sampling, high-resolution respirometry of permeabilized muscle fibers and oral glucose tolerance tests (OGTT) took place in London (35 m) for Lowlanders and Kathmandu (1,300 m) for Sherpas (25). All subjects then followed an identical ascent (Fig. 1B) from Kathmandu to EBC (5,300 m) whereupon further testing took place at an early timepoint (A1; 15-20 d post-departure for Lowlanders, 11-12 d for Sherpas), and a late timepoint (A2; 54-59 d post-departure) for Lowlanders only. At the time of sampling, both groups had passed through the acute phase of hypoxic exposure (<24 h) (1) and had been sufficiently exposed to chronic hypoxia for acclimatization to have occurred. Indeed, arterial hemoglobin-O_2

saturations were similarly low in both groups (Fig. 1C), whilst muscle expression of the HIF-target *VEGFA* increased in all subjects (Fig. 1D), indicating a molecular response to hypoxia. Following measurements at A1, the Lowlanders remained at EBC for 2 months to carry out research, presenting an opportunity to collect data pertaining to longer-term metabolic acclimatization. Interestingly, *VEGFA* expression was no longer elevated by this timepoint, suggesting further acclimatization had occurred.

To our surprise, there were no differences in circulating N-nitrosamine (RNNO), S-nitrosothiol (RSNO), nitrate (NO_3^-) or nitrite (NO_2^-) concentrations between Lowlanders and Sherpas at baseline (Fig. 1E-H). In Lowlanders, a transient increase in plasma RNNO levels occurred upon arrival at EBC ($P < 0.05$) but disappeared by the later timepoint (Fig. 1E). In Sherpas, plasma nitrate levels fell at altitude ($P < 0.05$; Fig. 1G) and nitrite levels increased ($P < 0.05$; Fig. 1H), whilst in Lowlanders nitrite levels fell by the later timepoint ($P < 0.05$). The absence of large differences in NO metabolites between the groups at baseline or at altitude, suggested an adaptive phenotype in Sherpas that is distinct from other Tibetan highlanders (13).

Lower Fatty Acid Oxidation Capacity in Sherpas

Skeletal muscle biopsies revealed marked differences in gene expression and FAO capacity between Sherpas and Lowlanders. Expression of *PPARA* mRNA was 48% lower in Sherpas than Lowlanders ($P < 0.05$; Fig. 2A), thus the putatively advantageous *PPARA* allele is associated with diminished expression. Correspondingly, expression of the PPARα target *CPT1B* was 32% lower in Sherpas at baseline compared with Lowlanders ($P < 0.05$; Fig. 2B). The *PPARA* gene contains 139 SNPs. rs6520015 is one of the tagging SNPs reported by Simonson *et al* (8), however it appears to

155 be a non-coding variant. It is thus uncertain whether the SNP itself affects transcriptional
156 regulation, or whether it tags a functional variant elsewhere, modifying expression or mRNA
157 stability. Ascent to EBC did not alter *PPARA* expression in either group, yet despite this *CPT1B*
158 expression decreased by 44% in Lowlanders ($P < 0.05$) but did not decrease further in Sherpas. This
159 suggests that the Lowlander response to hypoxia involves decreased PPARα transcriptional activity
160 without changes in *PPARA* expression, similar to hypoxic rat skeletal muscle (27).

161 Gene expression changes do not necessarily reflect protein levels or activity, therefore we
162 measured activity of the β-oxidation enzyme 3-hydroxyacyl-CoA dehydrogenase (HADH), finding it
163 to be 27% lower in Sherpas than Lowlanders at baseline ($P < 0.05$), and not changing in either
164 group following ascent (Fig. 2C). Moreover, fatty acid oxidative phosphorylation capacity (FAO_P)
165 was measured as the oxygen flux in saponin-permeabilized muscle fibers with octanoylcarnitine,
166 malate and ADP, using high-resolution respirometry (28). FAO_P was 24% lower in Sherpas than
167 Lowlanders at baseline ($P < 0.01$), and did not change in either group following ascent (Fig. 2D, Fig.
168 S1). *Ex vivo* measurements may be particular to assay conditions used, therefore we also
169 measured muscle metabolite levels to indicate changes in metabolism *in vivo*. Total carnitine
170 concentrations decreased in Lowlanders with time spent at EBC ($P < 0.05$), though were not
171 significantly different to those in Sherpas at baseline (Fig. 2E). The ratio of long chain acylcarnitines
172 to total carnitines, however, increased in Lowlanders with time at altitude ($P < 0.05$; Fig. 2F),
173 suggesting incomplete FAO results in accumulation of potentially-harmful lipid intermediates (29).
174 In Sherpa muscle, however, the long chain acylcarnitine to total carnitine ratio was lower than in
175 Lowlanders at baseline ($P < 0.05$), perhaps resulting from lower expression of CPT-1. In further
176 contrast with Lowlanders, the long chain acylcarnitine to total carnitine ratio remained low in
177 Sherpa muscle at altitude.

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179 **TCA Cycle Regulation at High Altitude**

180 We therefore sought to understand whether there were differences between the populations in
181 other aspects of mitochondrial metabolism. The TCA cycle enzyme citrate synthase (CS) is a
182 candidate marker of mitochondrial content in human muscle (30). At baseline, Sherpas had a 26%
183 lower muscle CS activity than Lowlanders ($P < 0.05$; Fig. 3A), in agreement with findings of 17-33%
184 lower mitochondrial volume density in Sherpa *vastus lateralis* compared with Lowlanders (22). In
185 accordance with lower CS activity, concentrations of 6- and 5-carbon intermediates downstream
186 of CS (citrate, aconitate, isocitrate, α-ketoglutarate) were lower in Sherpas than Lowlanders ($P <$
187 0.001). However, concentrations of 4-carbon intermediates (succinate, fumarate, malate,
188 oxaloacetate) were not different (Fig 3B-I). This suggests an alternative strategy to supply the TCA
189 cycle with succinate. Intriguingly, recent analysis of a large SNP dataset from low and high altitude-
190 adapted populations in the Americas and Asia (31) aimed to identify pathways of convergent
191 evolution, and highlighted fatty acid ω-oxidation as the most significant cluster of overlapping
192 gene sets between high altitude groups (32). ω-oxidation, is normally a minor pathway in
193 vertebrates, becoming more important when β-oxidation is defective (33), and through successive
194 cycles oxidizes fatty acids to adipate and succinate in the endoplasmic reticulum, after which
195 succinate enters the mitochondria with anaplerotic regulation of the TCA cycle (34).

196

197 Upon ascent to altitude, 6- and 5-carbon TCA cycle intermediates increased in Sherpa muscle ($P <$
198 0.05; Fig. 3B-E), suggesting improved coupling of intermediary metabolism, TCA cycle and
199 oxidative phosphorylation. In Lowlanders, however, citrate, aconitate and isocitrate decreased at
200 altitude ($P < 0.05$; Fig. 3B-D), despite no significant change in CS activity, perhaps reflecting

impairments upstream. Interestingly, α-ketoglutarate concentrations were maintained in Lowlanders at altitude (Fig. 3E), despite decreased succinate downstream, which could be explained by the fall in both α-ketoglutarate dehydrogenase and isocitrate dehydrogenase, reported previously in Lowlanders following an identical ascent to EBC (21). α-ketoglutarate plays regulatory roles in hypoxia, including a suppression of HIF stabilization (35), but also supporting glutathione synthesis (36). Taken together, these results indicate different TCA cycle regulation in Sherpas and Lowlanders. The replete TCA cycle of Sherpas at altitude contrasts sharply with the depletion of TCA cycle intermediates in Lowlanders, and suggests a coupling of the TCA cycle in Sherpa muscle to their distinct intermediary substrate metabolism.

Greater Mitochondrial Coupling Efficiency in Sherpas

To further understand whether mitochondrial function differs between Sherpas and Lowlanders, we used high-resolution respirometry, to probe electron transfer system (ETS) capacity and coupling efficiency in permeabilized muscle fibers. At baseline, there was no significant difference between the two groups in OXPHOS or ETS capacities with either malate and glutamate (N-pathway through Complex I) or succinate as substrates (S-pathway through Complex II; Fig. 4A,B; Fig. S2), but Sherpas had a lower OXPHOS capacity with malate, glutamate and succinate combined to reconstitute TCA cycle function (NS-pathway; $P < 0.01$; Fig. 4C). There were no early changes in either group upon ascent. By the later timepoint however, succinate-linked respiration had fallen in Lowlanders ($P < 0.05$), consistent with previous findings of decreased succinate dehydrogenase (Complex II) levels in subjects with sustained exposure >5,300 m (21).

In addition, we measured muscle fiber respiration in the absence of ADP (LEAK), i.e. O_2 consumption without ADP phosphorylation. Expressing LEAK relative to OXPHOS capacity, it is possible to calculate OXPHOS coupling efficiency (37, 38). At baseline, Sherpa muscle mitochondria had lower LEAK respiration and greater coupling efficiency than Lowlander mitochondria ($P < 0.001$; Fig. 4D,E), indicating more efficient use of O_2. Upon ascent to EBC and with sustained time at altitude, LEAK decreased in Lowlanders ($P < 0.01$), though it remained higher than in Sherpas (Fig. 4D), and coupling efficiency improved ($P < 0.05$; Fig. 4E). In Sherpas at altitude, LEAK did not change although coupling efficiency decreased ($P < 0.01$). One possible explanation for these differences in coupling efficiency might be the altered expression of uncoupling protein 3 (UCP3). *UCP3* is a transcriptional target of PPARα and lower UCP3 levels at altitude might improve the efficiency of O_2 utilization. In previous studies, however, muscle UCP3 expression increased with acute hypoxia (17, 39), which may offer some protective benefit considering its possible role as an antioxidant (39). Notably though, UCP3 levels decreased with more sustained exposure to extreme altitude (17). Here, *UCP3* was upregulated in Sherpas at altitude in association with decreased coupling efficiency ($P < 0.05$; Fig. 4F). However, *UCP3* expression also increased in Lowlanders in the short-term ($P < 0.01$) in whom there was decreased LEAK respiration. Moreover, *UCP3* expression returned to baseline in Lowlanders with longer-term exposure with no further change in LEAK respiration. Overall, our results indicate that Sherpa muscle mitochondria are characterized by a lower OXPHOS capacity and greater, albeit declining, efficiency, whilst in Lowlanders OXPHOS efficiency improved with acclimatization.

Glycolysis and Glucose Metabolism

Next we investigated the capacity to derive cellular energy via glycolysis, which is increased in hypoxic cells (40), as this may allow ATP levels to be maintained when O_2 is limited. Hexokinase activity was the same in both groups at baseline, and did not change at altitude (Fig. 5A), however lactate dehydrogenase (LDH) activity was 48% higher in Sherpa muscle than in Lowlanders ($P < 0.05$), indicating greater capacity for anaerobic lactate production (Fig. 5B). Fasting blood glucose was the same in Sherpas and Lowlanders at baseline, and decreased upon ascent in Lowlanders ($P < 0.01$; Fig. 5C), who also showed faster clearance of glucose during an OGTT ($P < 0.001$; Fig. 5D) in agreement with previous reports (41). In Sherpas, however, there was no indication of altered glucose homeostasis. Meanwhile, over time at altitude glycolytic intermediates increased in Lowlander muscle (Fig. 5E) with increased glucose-6-phosphate/fructose-6-phosphate and 2-phosphoglycerate/3-phosphoglycerate (Table S2). In contrast, total glycolytic intermediates did not change in Sherpa muscle, although 2-phosphoglycerate/3-phosphoglycerate decreased. These findings, might to some extent be explained by altered HIF activities. Many genes encoding glycolytic enzymes are upregulated by HIF-1 (42), whilst hypoglycemia is seen in Chuvash polycythemia, an autosomal recessive disorder in which HIF degradation is impaired (43). Taken together, our findings suggest an increased reliance on glucose by Lowlanders under resting conditions at altitude compared with Sherpas, but a greater capacity for lactate production in Sherpas which may prove effective upon exertion.

Energetics and Oxidative Stress

Finally, to understand the implications of Sherpa metabolic adaptation we investigated muscle energetics and redox homeostasis. Lowlanders at altitude showed progressive loss of muscle phosphocreatine (PCr; $P < 0.001$; Fig. 6A), indicating a loss of energetic reserve, which may relate

268 to downregulation of muscle creatine kinase, as reported previously (21). By contrast, in Sherpa
269 muscle, PCr increased at altitude ($P < 0.01$). Similarly, Sherpa muscle ATP levels, which were lower
270 than in Lowlanders at baseline ($P < 0.05$), increased at altitude ($P < 0.001$; Fig. 6B), illustrating that
271 Sherpa metabolism is better suited to maintaining muscle energetics at altitude than Lowlander
272 metabolism in either the short-term or following acclimatization. Moreover, with short-term
273 exposure, markers of oxidative stress (reduced/oxidized glutathione and methionine sulfoxide)
274 increased in Lowlander muscle, but not Sherpa muscle (Fig. 6C,D), indicating superior redox
275 homeostasis in the Sherpas. Antioxidant protection may represent another outcome of convergent
276 evolution, having been reported in Andean subjects in association with protection of fetal growth
277 (44), whilst glutathione levels are raised in Chuvash polycythemia suggesting a possible role for HIF
278 activation (45).
279
280 **Conclusions**
281
282 It has long been suspected that Sherpa people are better adapted to life at high altitude than
283 Lowlanders (46). Recent findings have suggested a genetic basis to adaptation in populations
284 around the world (6), and here we show that Sherpas have a metabolic adaptation associated with
285 improved muscle energetics and protection against oxidative stress. Genetic selection on the
286 *PPARA* gene is associated with decreased expression, and thus lower fatty acid β-oxidation and
287 improved mitochondrial coupling compared with Lowlanders, with a possible compensatory
288 increase in fatty acid ω-oxidation. Sherpas also have a greater capacity for lactate production.
289 With acclimatization to altitude, Lowlanders accumulate potentially-harmful lipid intermediates in
290 muscle as a result of incomplete β-oxidation, alongside depletion of TCA cycle intermediates,
291 accumulation of glycolytic intermediates, a loss of PCr despite improved mitochondrial coupling,

292 and a transient increase in oxidative stress markers. In Sherpas, however, there are remarkably
293 few changes in intermediary metabolism at altitude, but increased TCA cycle intermediates and
294 PCr and ATP levels, with no sign of oxidative stress.
295
296 Genetic selection, by definition, requires an increased likelihood of advantageous gene variants
297 being passed on to offspring. This might occur if the disadvantageous variant is associated with
298 poorer survival to reproductive age and beyond, including greater fetal/neonatal mortality.
299 Evidence supports precisely such effects with fetal growth at altitude being poorer in Lowlander
300 populations than many native highlanders (47), including Tibetans (48) and Sherpas (49). Likewise,
301 gene variants may affect survival through childhood or fecundity/fertility in the hypoxic
302 environment. We cannot speculate on the mechanism by which *PPARA* variants prove
303 advantageous, however PPAR isoforms are expressed in the placenta (50) and influence female
304 reproductive function (51). It would be of interest to seek association of the *PPARA* variants with
305 birth weight and measures of placentation in high altitude natives and Lowlanders exposed to
306 hypoxia.
307
308 Our findings suggest a metabolic basis to Sherpa adaptation, which may permit the population to
309 survive and perform at high altitude. Such adaptations may also underpin the superior
310 performance of elite climbing Sherpas at extreme high altitude.
311

Materials and Methods

Subjects were selected from the participants of Xtreme Everest 2 (25). All Lowlanders were born and lived below 1,000 m, not descended from a high altitude-dwelling population and of European (Caucasian) origin. Subjects gave written consent, and underwent medical screening. All protocols were approved by UCL Research Ethics Committee and Nepal Health Research Council. Vastus lateralis biopsies were taken from the mid-thigh, muscle fibers prepared for respirometry (28) and respiration measured using substrate-uncoupler-inhibitor titrations (Tables S3, S4). Enzyme activities were assayed as described (27). RNA was extracted and Taqman® assays used to analyse gene expression (Table S5). For metabolite analysis, a methanol/chloroform extraction (52) was followed by liquid chromatography mass spectrometry (LC-MS). OGTTs were carried out on fasted subjects on the day after biopsies. Blood plasma NO metabolites were quantified as described (53). Genomic DNA was isolated from whole blood and PPARA SNPs genotyped using TaqMan® for allelic discrimination (Applied Biosystems, UK; Table S1). To compare cohorts at baseline, an unpaired two-tailed Student's t-test was used (significance at $P \leq 0.05$). Genotype frequencies were compared using a Chi-squared test. To assess the effects of altitude, a one-way ANOVA with repeated measures was used. Post-hoc pairwise comparisons were carried out with a Tukey correction.

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Figure Legends

Figure 1 Subject genetics, ascent profile, arterial blood O_2 saturation, muscle hypoxia and circulating NO metabolites. A) Genotypes of Lowlanders and Sherpas at 3 *PPARA* SNPs - subjects homozygous for the putatively advantageous allele in black, heterozygous subjects in gray and subjects homozygous for the non-advantageous allele in white (digits in segments refer to number of subjects with genotype); B) Ascent profile including timing of biopsies; C) Arterial hemoglobin-O_2 saturations; D) Muscle *VEGFA* expression, and E-H) plasma nitrogen oxides in Lowlanders (L) and Sherpas (S) at baseline (B) and early (A1) and late (A2) altitude. Mean ± SEM (n = 4-15). †$P \leq 0.05$; †††$P \leq 0.001$ B vs A1 within cohort. $^{\Delta}P \leq 0.05$ A1 vs A2 within cohort.

Figure 2 Fatty acid oxidation and regulation in muscle. A) *PPARA* expression; B) *CPT1B* expression; C) HADH activity; D) Oxidative phosphorylation with octanoylcarnitine&malate (FAO_P); E) Total carnitine; F) Long chain/total carnitine ratio in Lowlanders and Sherpas. Gene expression and carnitine levels are expressed relative to Lowlanders at baseline. Mean ± SEM (n = 6-13). *$P \leq 0.05$; **$P \leq 0.01$ Lowlanders vs Sherpas at baseline. †$P \leq 0.05$ baseline vs altitude within cohort.

Figure 3 TCA intermediates and activity in muscle. A) Citrate synthase activity and B-I) TCA cycle intermediates in Lowlanders and Sherpas. Metabolite levels are expressed relative to Lowlanders at baseline. Mean ± SEM (n = 7-14). *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$ Lowlanders vs Sherpas at baseline. †$P \leq 0.05$; ††$P \leq 0.01$; baseline vs altitude within cohort.

Figure 4 Mitochondrial oxygen consumption, efficiency and uncoupling protein expression. A) N-OXPHOS (GM_P), B) S-ETS capacity (S_E) and C) NS-OXPHOS capacity (GMS_P) in permeabilized muscle fibers from Lowlanders and Sherpas. D) Octanoylcarnitine&malate-supported LEAK (FAO_L) and E) OXPHOS coupling efficiency. F) Muscle *UCP3* expression relative to Lowlanders at baseline. Mean ± SEM (n = 7-11). **$P \leq 0.01$; ***$P \leq 0.001$ Lowlander vs Sherpas at baseline. †$P \leq 0.05$; ††$P \leq 0.01$ baseline vs altitude within cohort. $^{\Delta}P \leq 0.05$; $^{\Delta\Delta}P \leq 0.01$ altitude 1 vs 2 within cohort.

Figure 5 Muscle glycolysis and blood glucose homeostasis. A) Hexokinase and B) Lactate dehydrogenase activity. C) Fasting blood glucose and D) glucose clearance during OGTT. E) Total muscle glycolytic intermediates relative to Lowlanders at baseline. Mean ± SEM (n = 5-14). *$P \leq 0.05$ Lowlanders vs Sherpas at baseline. †$P \leq 0.05$; ††$P \leq 0.01$; †††$P \leq 0.001$ baseline vs altitude within cohort.

Figure 6 Muscle energetics and oxidative stress. A) Phosphocreatine, B) ATP, C) Oxidized/reduced glutathione (GSSG/GSH) and D) Sulfoxide/total methionine (MetSO/Met), all expressed relative to Lowlanders at baseline. Mean ± SEM (*n* = 8-14). ††*P* ≤ 0.01; †††*P* ≤ 0.001 baseline vs altitude within cohort. $^{\Delta}P \leq 0.05$ altitude 1 vs 2 within cohort.