Metabolic basis to Sherpa altitude adaptation

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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 to 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 known, however. 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). 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 adaptation appeared to be related, in part, to a putatively advantageous allele for the peroxisome proliferator-activated receptor A (*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 have an impact upon our understanding of human diseases in which hypoxia is a feature.

metabolism | altitude | skeletal muscle | hypoxia | mitochondria

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 and 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, for example, encoding erythropoietin (*EPO*) and vascular endothelial growth factor A (*VEGFA*) (3).

The Tibetan Plateau has an average altitude of some 4,500 m. Humans were first present on the plateau ∼30,000 y ago, with the earliest permanent settlements appearing 6,000–9,000 y ago (4), a period sufficient to drive the natural selection of genetic variants (and associated features) favoring survival and performance in sustained hypoxia (5, 6). Evidence supports the selection of genetic variants encoding components of the 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 y ago and exhibits remarkable physical performance at extreme altitude (9).

Although 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, as well as 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), whereas exposure to more moderate high altitude is associated with a reprogramming of muscle metabolism (19) even without altered mitochondrial density (20), including down-regulation 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 (OXPHOS) coupling efficiency (20). Sherpas have lower muscle mitochondrial densities than unacclimatized Lowlanders (22), but little is known

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Significance

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, that 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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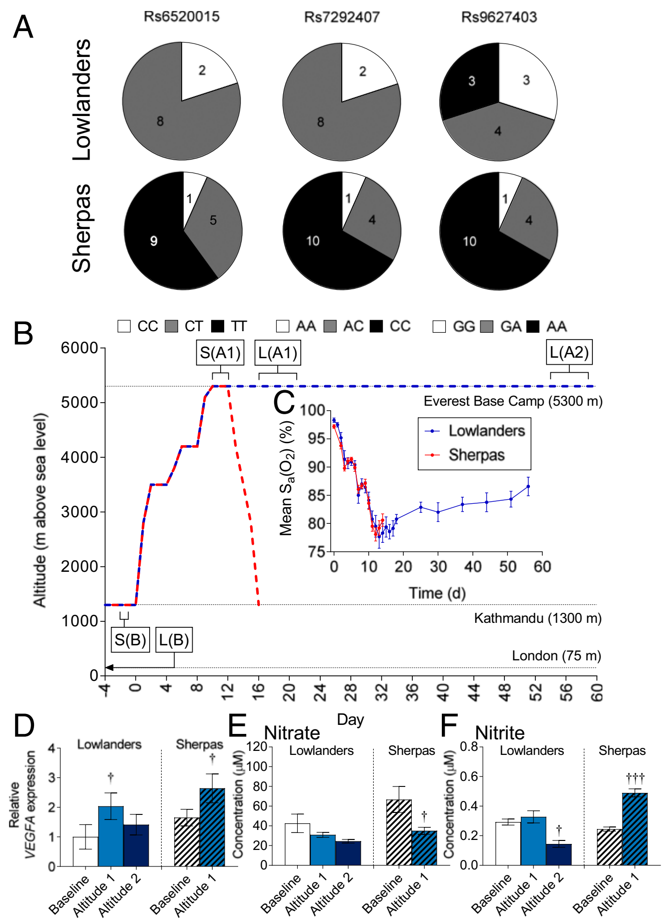

Fig. 1. Subject genetics, ascent profile, arterial blood O_2 saturation, muscle hypoxia, and circulating NO metabolites. (*A*) Genotypes of Lowlanders and Sherpas at three *PPARA* SNPs. Subjects homozygous for the putatively advantageous allele are shown in black, heterozygous subjects are shown in gray,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

of their metabolic adaptation to hypoxia, or any genetic selection that might underpin it. A role has been suggested for peroxisome proliferator-activated receptor α (PPARα), a transcriptional regulator of FAO in liver, heart, and muscle. HIF downregulates PPARα in some tissues (23), although 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 Mount Everest Base Camp (EBC) laboratory. Sherpas (n = 15) were a gender-matched (73% male, compared with 70% in Lowlanders) and age-matched (26.8 ± 1.2 y, compared with 28.0 ± 1.6 y in Lowlanders) group living in Kathmandu and the Solukhumbu and Rolwaling valleys. No subject ascended higher than 4,200 m in the 3 mo preceding the trek, or above 2,500 m in the preceding 3 wk. In addition, Sherpas presented evidence of sole Sherpa ancestry for two generations (i.e., four Sherpa grandparents). The frequency of putatively advantageous *PPARA* alleles (8) was higher in Sherpas than Lowlanders (Fig. 1*A* and Table S1), with genotype frequencies of the cohorts being significantly different at two single-nucleotide polymorphisms (SNPs), rs6520015 and rs7292407 (P = 0.0091), although not at rs9627403. This finding 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 (OGTTs), took place in London (35 m) for Lowlanders and in Kathmandu (1,300 m) for Sherpas (25). All subjects then followed an identical ascent (Fig. 1*B*) from Kathmandu to EBC (5,300 m), whereupon further testing took place at an early time point (A1: 15–20 d postdeparture for Lowlanders, 11–12 d for Sherpas) and a late time point (A2: 54–59 d postdeparture) 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. 1*C*), whereas muscle expression of the HIF-target *VEGFA* increased in all subjects (Fig. 1*D*), indicating a molecular response to hypoxia. Following measurements at the early time point, the Lowlanders remained at EBC for 2 mo 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 time point, suggesting further acclimatization had occurred.

To our surprise, there were no differences in circulating *N*-nitrosamine (RNNO), S-nitrosothiol, nitrate, or nitrite concentrations between Lowlanders and Sherpas at baseline (Fig. 1 *E* and *F* and Fig. S1). In Lowlanders, a transient increase in plasma RNNO levels occurred upon arrival at EBC ($P < 0.05$) but disappeared by the later time point (Fig. S1*A*). In Sherpas, plasma nitrate levels fell at altitude ($P < 0.05$; Fig. 1*E*) and nitrite levels increased ($P < 0.05$; Fig. 1*F*), whereas nitrite levels fell by the later time point ($P < 0.05$) in Lowlanders. 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 FAO 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. 2*A*); 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. 2*B*). The *PPARA* gene contains 139 SNPs. One of the tagging SNPs reported by Simonson et al. (8) is rs6520015; however, it appears to be a noncoding variant. It is thus uncertain whether the SNP itself affects transcriptional regulation or whether it tags a functional variant elsewhere, modifying expression or mRNA stability. Ascent to EBC did not alter *PPARA* expression in either group; however, despite this finding, *CPT1B* expression decreased by 44% in Lowlanders ($P < 0.05$) but did not decrease further in Sherpas. This result suggests that the Lowlander response to hypoxia involves decreased PPARα transcriptional activity without changes in *PPARA* expression, similar to hypoxic rat skeletal muscle (27).

Gene expression changes do not necessarily reflect protein levels or activity; therefore, we measured activity of the β-oxidation enzyme 3-hydroxyacyl–CoA dehydrogenase, finding it to be 27% lower in Sherpas than Lowlanders at baseline ($P < 0.05$), and not changing in either group following ascent (Fig. 2*C*). Moreover, fatty acid oxidative phosphorylation capacity (FAO_P) was measured as the oxygen flux in saponin-permeabilized muscle fibers with octanoyl carnitine, malate, and ADP, using high-resolution respirometry (28). FAO_P was 34% lower in Sherpas than

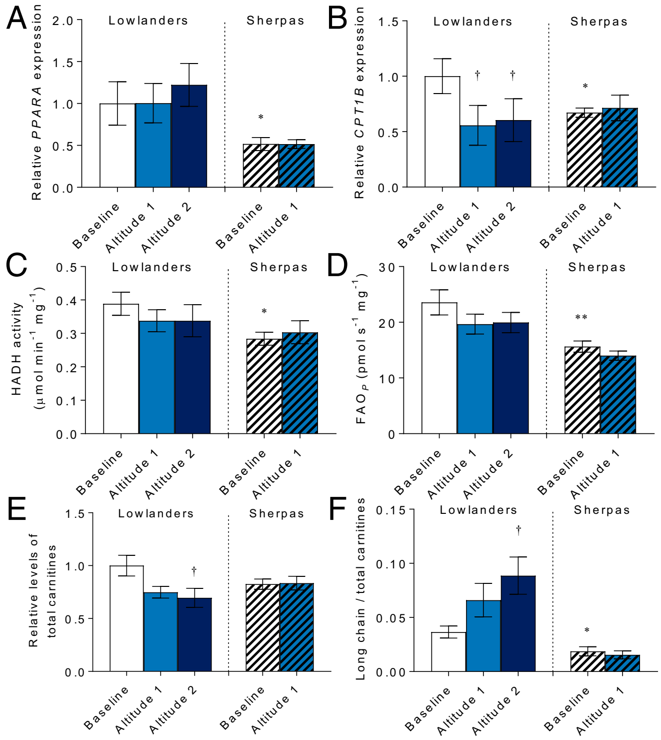

Fig. 2. FAO and regulation in muscle. *PPARA* expression (*A*), *CPT1B* expression (*B*), 3-hydroxyacyl-CoA dehydrogenase (HADH) activity (*C*), oxidative phosphorylation with octanoyl carnitine and malate (FAO_P) (*D*), total carnitine (*E*), and long chain/total carnitine ratio (*F*) in Lowlanders and Sherpas are shown. Gene expression and carnitine levels are expressed relative to Lowlanders at baseline. Mean ± SEM (n = 6–13). $^{*}P \le 0.05$, $^{**}P \le 0.01$ in Lowlanders vs. Sherpas at baseline. $^{\dagger}P \le 0.05$ at baseline vs. altitude within cohort.

Lowlanders at baseline ($P < 0.01$), and did not change in either group following ascent (Fig. 2*D* and Fig. S2). Ex vivo measurements may be particular to assay conditions used; therefore, we also measured muscle metabolite levels to indicate changes in metabolism in vivo. Total carnitine concentrations decreased in Lowlanders with time spent at EBC ($P < 0.05$), although they were not significantly different from total carnitine concentrations in Sherpas at baseline (Fig. 2*E*). The ratio of long-chain acylcarnitines to total carnitines, however, increased in Lowlanders with time at altitude ($P < 0.05$; Fig. 2*F*), suggesting incomplete FAO results in accumulation of potentially harmful lipid intermediates (29). In Sherpa muscle, however, the ratio of long-chain acylcarnitines to total carnitines was lower than in Lowlanders at baseline ($P < 0.05$), perhaps resulting from lower expression of CPT-1. In further contrast to Lowlanders, the ratio of long-chain acylcarnitines to total carnitines remained low in Sherpa muscle at altitude.

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

Upon ascent to altitude, 6- and 5-carbon TCA cycle intermediates increased in Sherpa muscle ($P < 0.05$; Fig. 3 *B*–*E*), suggesting improved coupling of intermediary metabolism, TCA

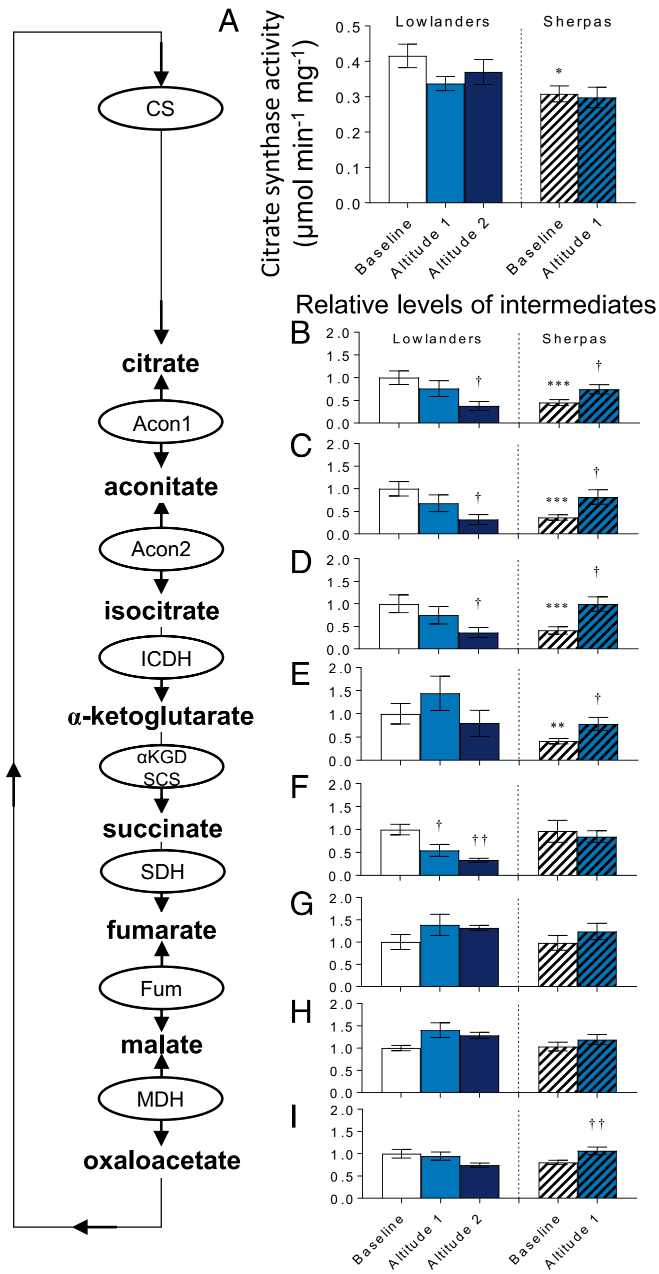

Fig. 3. TCA intermediates and activity in muscle. CS activity (*A*) and TCA cycle intermediates (*B*–*I*) in Lowlanders and Sherpas are shown. Metabolite levels are expressed relative to Lowlanders at baseline. Mean ± SEM (n = 7–14). $^{*}P \le 0.05$, $^{**}P \le 0.01$, $^{***}P \le 0.001$ in Lowlanders vs. Sherpas at baseline. $^{\dagger}P \le 0.05$, $^{\dagger\dagger}P \le 0.01$ at baseline vs. altitude within cohort.

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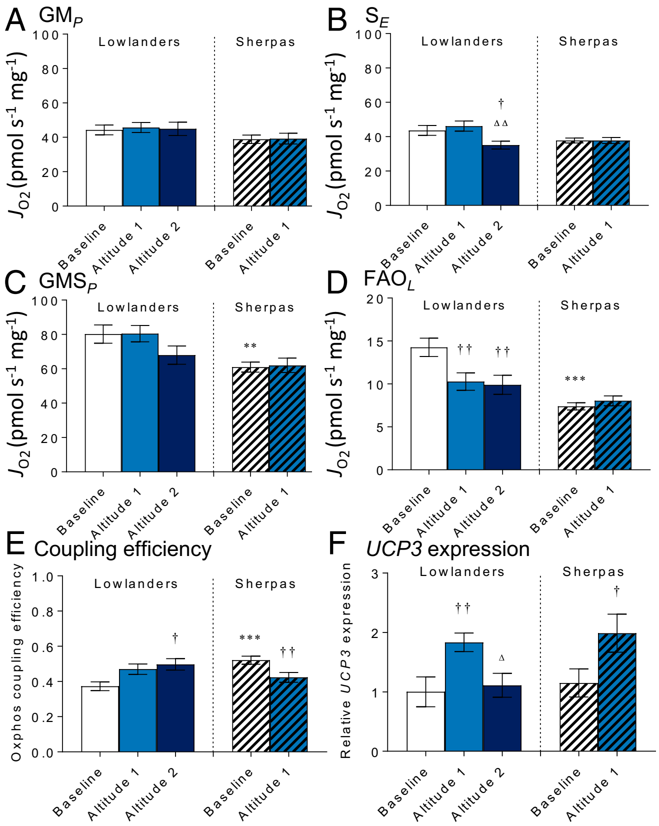

Fig. 4. Mitochondrial oxygen consumption, efficiency, and uncoupling protein expression. N-OXPHOS (GM$_P$) (*A*), S-ETS capacity (S$_E$) (*B*), and NS-OXPHOS capacity (GMS$_P$) (*C*) in permeabilized muscle fibers from Lowlanders and Sherpas are shown. Octanoyl carnitine and malate-supported LEAK (FAO$_L$) (*D*) and OXPHOS coupling efficiency (*E*) are shown. (*F*) Muscle *UCP3* expression relative to Lowlanders at baseline. Mean ± SEM (n = 7–11). $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ in Lowlander vs. Sherpas at baseline. $^{\dagger}P \leq 0.05$, $^{\dagger\dagger}P \leq 0.01$ at baseline vs. altitude within cohort. $^{\triangle}P \leq 0.05$, $^{\triangle\triangle}P \leq 0.01$ at altitude 1 vs. altitude 2 within cohort. G, glutamate; J_{O2}, oxygen flux; M, malate, S, succinate.

cycle, and oxidative phosphorylation. In Lowlanders, however, citrate, aconitate, and isocitrate decreased at altitude ($P < 0.05$; Fig. 3 *B*–*D*), despite no significant change in CS activity, perhaps reflecting impairments upstream. Interestingly, α-ketoglutarate concentrations were maintained in Lowlanders at altitude (Fig. 3*E*), despite decreased succinate downstream, which could be explained by the fall in both α-ketoglutarate dehydrogenase and isocitrate dehydrogenase, as reported previously in Lowlanders following an identical ascent to EBC (21). α-Ketoglutarate plays regulatory roles in hypoxia, including 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 its distinct intermediary substrate metabolism.

Greater Mitochondrial Coupling Efficiency in Sherpas. To understand further 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 (S-pathway through complex II; Fig. 4 *A* and *B* and Fig. S3) as a substrate, but Sherpas had a lower OXPHOS capacity with malate, glutamate, and succinate combined to reconstitute TCA cycle function (NS pathway; $P < 0.01$; Fig. 4*C*). There were no early changes in either group upon ascent. By the later time point, 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 at >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. 4 *D* and *E*), indicating more efficient use of O_2. Upon ascent to EBC and with sustained time at altitude, LEAK decreased in Lowlanders ($P < 0.01$), although it remained higher than in Sherpas (Fig. 4*D*), and coupling efficiency improved ($P < 0.05$; Fig. 4*E*). 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

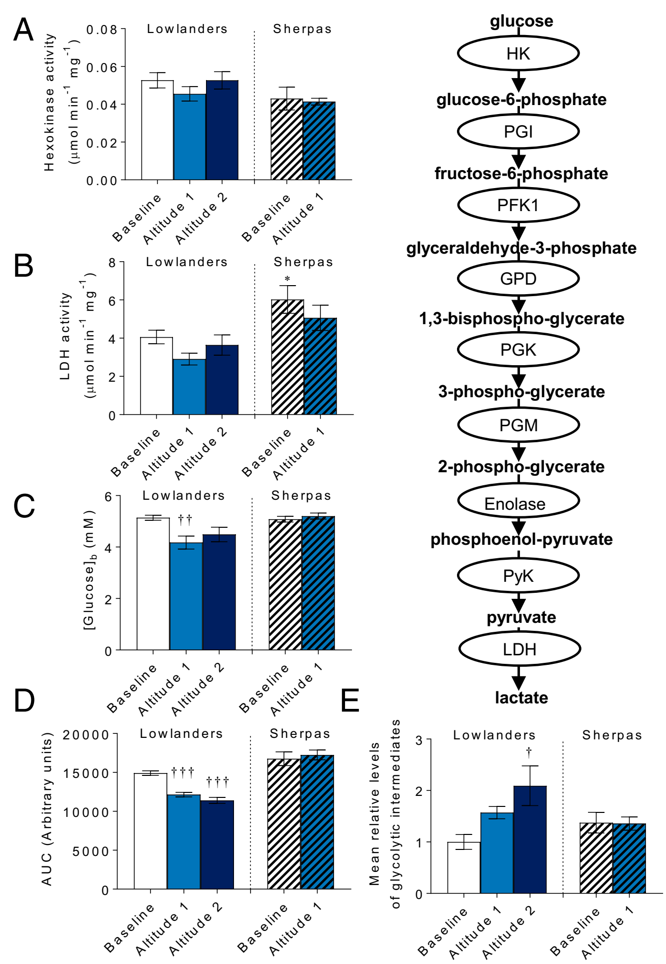

Fig. 5. Muscle glycolysis and blood glucose homeostasis. Hexokinase (*A*) and lactate dehydrogenase (LDH) (*B*) activity are shown. Fasting blood glucose (*C*) and glucose clearance during OGTT (*D*) are shown. (*E*) Total muscle glycolytic intermediates relative to Lowlanders at baseline. Mean ± SEM (n = 5–14). AUC, area under the curve. $^{*}P \leq 0.05$ in Lowlanders vs. Sherpas at baseline. $^{\dagger}P \leq 0.05$, $^{\dagger\dagger}P \leq 0.01$, $^{\dagger\dagger\dagger}P \leq 0.001$ at baseline vs. altitude within cohort.

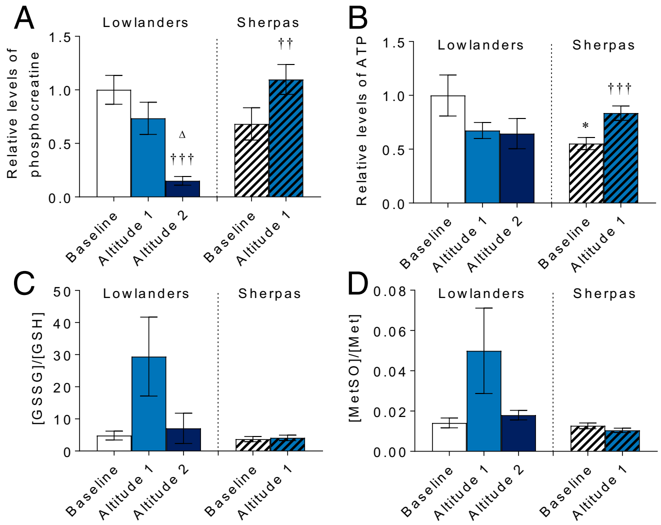

Fig. 6. Muscle energetics and oxidative stress. PCr (*A*), ATP (*B*), oxidized/reduced glutathione (GSSG/GSH) (*C*), and sulfoxide/total methionine (MetSO/Met) (*D*), all expressed relative to Lowlanders at baseline, are shown. Mean ± SEM (n = 8–14). $^{††}P \le 0.01$, $^{†††}P \le 0.001$ at baseline vs. altitude within cohort. $^{\triangle}P \le 0.05$ at altitude 1 vs. altitude 2 within cohort.

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, however, 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. 4*F*). 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, whereas the OXPHOS efficiency of Lowlanders 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), because glycolysis 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. 5*A*); however, lactate dehydrogenase activity was 48% higher in Sherpa muscle than in Lowlanders ($P < 0.05$), indicating greater capacity for anaerobic lactate production (Fig. 5*B*). Fasting blood glucose was the same in Sherpas and Lowlanders at baseline, and decreased upon ascent in Lowlanders ($P < 0.01$; Fig. 5*C*), who also showed faster clearance of glucose during an OGTT ($P < 0.001$; Fig. 5*D*) 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. 5*E*), 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 be explained to some extent by altered HIF activities. Many genes encoding glycolytic enzymes are upregulated by HIF-1 (42), whereas hypoglycemia is seen in Chuvash polycythemia (CP), 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. At altitude, Lowlanders showed progressive loss of muscle phosphocreatine (PCr; $P < 0.001$; Fig. 6*A*), indicating a loss of energetic reserve, which may relate to down-regulation of muscle creatine kinase, as reported previously (21). By contrast, in Sherpa muscle, PCr increased at altitude ($P < 0.01$). Similarly, Sherpa muscle ATP levels, which were lower than in Lowlanders at baseline ($P < 0.05$), increased at altitude ($P < 0.001$; Fig. 6*B*), illustrating that Sherpa metabolism is better suited to maintaining muscle energetics at altitude than Lowlander metabolism either in the short term or following acclimatization. Moreover, with short-term exposure, markers of oxidative stress (reduced/oxidized glutathione and methionine sulfoxide) increased in Lowlander muscle but not Sherpa muscle (Fig. 6 *C* and *D*), indicating superior redox homeostasis in the Sherpas. Antioxidant protection may represent another outcome of convergent evolution, having been reported in Andean subjects in association with protection of fetal growth (44), whereas glutathione levels are raised in CP, suggesting a possible role for HIF activation (45).

Conclusions

It has long been suspected that Sherpa people are better adapted to life at high altitude than Lowlanders (46). Recent findings have suggested a genetic basis to adaptation in populations around the world (6), and we show here that Sherpas have a metabolic adaptation associated with improved muscle energetics and protection against oxidative stress. Genetic selection on the *PPARA* gene is associated with decreased expression, and thus lower fatty acid β-oxidation and improved mitochondrial coupling compared with Lowlanders, with a possible compensatory increase in fatty acid ω-oxidation. Sherpas also have a greater capacity for lactate production. With acclimatization to altitude, Lowlanders accumulate potentially harmful lipid intermediates in muscle as a result of incomplete β-oxidation, alongside depletion of TCA cycle intermediates, accumulation of glycolytic intermediates, a loss of PCr despite improved mitochondrial coupling, and a transient increase in oxidative stress markers. In Sherpas, however, there are remarkably few changes in intermediary metabolism at altitude but increased TCA cycle intermediates and PCr and ATP levels, with no sign of oxidative stress.

Genetic selection, by definition, requires an increased likelihood of advantageous gene variants being passed on to offspring. Hence selection might occur if the disadvantageous variant is associated with poorer survival to reproductive age and beyond, including greater fetal/neonatal mortality. Evidence supports precisely such effects, with fetal growth at altitude being poorer in Lowlander populations than in many native highlanders (47), including Tibetans (48) and Sherpas (49). Likewise, gene variants may affect survival through childhood or fecundity/fertility in the hypoxic environment. We cannot speculate on the mechanism by which *PPARA* variants prove advantageous; however, PPAR isoforms are expressed in the placenta (50) and influence female reproductive function (51). It would be of interest to seek association of the *PPARA* variants with birth weight and measures of placentation in high-altitude natives and Lowlanders exposed to hypoxia.

Our findings suggest a metabolic basis to Sherpa adaptation that may permit the population to survive and perform at high altitude. Such adaptations may also underpin the superior performance of elite climbing Sherpas at extreme high altitude.

Materials and Methods

Subjects were selected from the participants of Xtreme Everest 2 (25). All Lowlanders were born and lived below 1,000 m, were not descended from a high-altitude–dwelling population, and were of European (Caucasian) origin. Subjects gave written consent, and underwent medical screening. All protocols were approved by the University College London Research Ethics Committee and Nepal Health Research Council. Vastus lateralis biopsies were taken from the midthigh, muscle fibers were prepared for respirometry (28), and respiration was measured using substrate-uncoupler-inhibitor titrations (Tables S3 and S4). Enzyme activities were assayed as described elsewhere (27). RNA was extracted, and Taqman assays were used to analyze gene expression (Table S5). For metabolite analysis, a methanol/chloroform extraction (52) was followed by liquid chromatography mass spectrometry. 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 were genotyped using TaqMan assays for allelic discrimination (Applied Biosystems; 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 χ^2 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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