Supporting Information

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SI Materials and Methods

Study Design. The design and conduct of Xtreme Everest 2 have been described previously (25). Healthy Lowlanders (n = 10, seven male) and healthy age- and gender-matched Sherpas (n = 15, 11 male) were selected from recruited participants. All Lowlander subjects were born and lived below 1,000 m, were not descended from a high-altitude–dwelling population (e.g., Tibetan, Andean, Ethiopian), and were of European (Caucasian) origin. These subjects were Xtreme Everest 2 investigators selected to be resident at the EBC laboratory throughout the expedition for the purpose of conducting research. The researchers were selected on the basis of their availability and ability to contribute to the scientific program of the expedition, but not due to any proven ability to perform at high altitude; indeed, a number of these subjects were altitude-naive at the time of departure. Sherpa subjects were drawn from communities in the Solokhumbu and Rolwaling valleys and were required to provide evidence that all parents and grandparents were Nepali Sherpas. Two of the Sherpa subjects were first cousins, but no other subjects (Sherpa or Lowlander) were related.

Subjects gave written consent for participation, and were subjected to medical screening before the expedition, which involved completion of a health questionnaire and a check-up with the chief medical officer. Potential participants with serious cardiac or respiratory disease were excluded. A local, medically qualified translator was present at all times to ensure effective communication between scientific investigators and Sherpa subjects. All protocols were approved by the University College London Research Ethics Committee and the Nepal Health Research Council. All subjects from both cohorts were free from altitude exposure for at least 3 mo before the expedition and were physically active, but neither particularly sedentary nor highly trained. Subjects were flown from Kathmandu, Nepal (1,300 m) to Lukla in the Solokhumbu region (2,800 m), before ascending on foot to EBC (5,300 m) by a matched 10-d ascent profile. Diet was not strictly controlled; however, all subjects were presented with similar communal fare at tea houses, lodges, and camps throughout the expedition, and this diet did not include large quantities of foods known to be rich in nitrogen oxides, such as green leafy vegetables or cured meats.

Muscle Sample Collection and Preparation. Biopsies of the vastus lateralis muscle were taken from the midthigh using Tilley–Henckel forceps under local anesthesia (2% lignocaine, 1:80,000 adrenaline) of the skin and superficial muscle fascia. A 5-mm incision was made, and 150 mg of wet-weight tissue was collected, with repeat biopsies taken adjacent to previous biopsies. Sherpa biopsies were taken in Kathmandu and again 11–12 d after departure (1–2 d at 5,300 m). Lowlander biopsies were taken in London (35 m) before the expedition, 15–20 d after departure (5–10 d at 5,300 m), and again 54–59 d after departure (44–49 d at 5,300 m). Atmospheric parameters from the three laboratories have been reported elsewhere (25). The London and Kathmandu biopsies were taken to assess baseline metabolic profile. Thereafter, biopsies were taken at EBC within 21 d of the start of the ascent to assess the effects of shorter term high-altitude exposure on metabolism, whereas biopsies taken after 55 d indicated the effects of more sustained high-altitude exposure.

Ideally, biopsies would have been carried out on the subjects at the same times following the onset of exposure; however, Sherpas and Lowlanders were studied on different days after arrival at EBC for logistical reasons. The Lowlander subjects, being Xtreme Everest 2 investigators, needed to establish camp; construct the laboratory; and unpack, calibrate, and validate equipment for high-resolution respirometry upon arrival. Because these measurements cannot be made on frozen samples, biopsy sampling only occurred once respirometry could be carried out. The Sherpa subjects, however, arrived and departed within guided trekking groups after the laboratory had been established. These treks followed preordained ascent/descent schedules to and from Kathmandu, with the subjects spending three nights at EBC. There was therefore a narrow window of opportunity during which Sherpas could be studied. Following measurements at the high-altitude exposure time point, the Lowlander subjects were scheduled to remain at EBC for a further 2 mo for the purpose of carrying out research, presenting us with the additional opportunity of collecting further valuable data pertaining to longer term metabolic acclimatization to hypobaric hypoxia in a group resident at 5,300 m. It was not, however, possible for us to collect comparable longer term data for the Sherpa subjects on this expedition, although we acknowledge that such data would have been of interest.

No food or caffeine was allowed within the 12 h preceding each biopsy. The muscle sample was immediately placed in ice-cold biopsy preservation medium (BIOPS) [CaK_2EGTA (2.77 mM), K_2EGTA (7.23 mM), $MgCl_2{\cdot}6H_2O$ (6.56 mM), taurine (20 mM), PCr (15 mM), imidazole (20 mM), DTT (0.5 mM), MES (50 mM), and Na_2ATP (5.77 mM) at pH 7.10], which was filtered and stored at −40 °C or lower until use to prevent bacterial growth. Following this procedure, the muscle sample was cleared of any fat or connective tissue and divided into sections as follows: 15 mg was snap-frozen in liquid nitrogen for metabolomics, 20 mg was snap-frozen in liquid nitrogen for gene expression and enzyme activity assays, and 50 mg was retained in ice-cold biopsy preservation medium for high-resolution respirometry. Frozen samples were flown back to the United Kingdom on liquid nitrogen and stored at −80 °C until use.

Measurement of NO Metabolites. Venesection was performed for the measurement of circulating biomarkers. Plasma was separated from blood cells by centrifugation of whole blood at 800 × g for 15 min and immediately frozen in 1-mL aliquots in liquid nitrogen. Samples were stored under liquid nitrogen for the duration of the expedition, transported back to the United Kingdom on dry ice, and kept at −80 °C until analysis.

NO metabolite concentrations were quantified immediately after thawing of frozen plasma aliquots in the presence of an excess of *N*-ethylmaleimide (NEM; in PBS, 10 mM final concentration). For the analysis of circulating total nitroso species, aliquots of NEM-treated EDTA plasma were directly injected into a triiodide-containing reaction chamber, and the NO produced from the reduction of protein nitroso species was quantified by gas phase chemiluminescence (CLD 77sp; EcoMedics), as described elsewhere (52). The concentration of nitroso species in these samples was estimated from the difference in quantification of the NO signal after sample pretreatment with mercuric chloride with sulfanilamide vs. sulfanilamide alone. For nitrite/nitrate analysis, NEM-treated samples were deproteinized with ice-cold methanol (1:1 vol/vol), separated by centrifugation, and subjected to analysis by high-pressure liquid chromatography using a dedicated nitrite/nitrate analyzer (ENO20; Eicom). Sample processing was performed in a staggered fashion to ensure reproducible processing times, and reported values are corrected for background contaminant levels.

Genetics. Total genomic DNA was isolated from whole-blood samples using LGC Genomics' DNA extraction service (www.lgcgroup.com/services/extraction/dna-extraction/). In brief, samples were extracted using detergent-driven cell lysis, followed by guanidinium isothiocyanate-mediated DNA binding to silica. Contaminants were removed by washing, and DNA was subsequently eluted into a low-salt buffer (10 mM Tris, 1 mM EDTA). Three SNPs on the *PPARA* gene (rs9627403, rs7292407, and rs6520015) were genotyped using the TaqMan platform for allelic discrimination (Applied Biosystems). PCR amplification was performed on 384-well plates using TaqMan Predesigned SNP Genotyping Assays (Applied Biosystems) and conditions recommended by the manufacturer. Reactions were analyzed by individuals blinded to subject/racial status and phenotypic data using the Applied Biosystems TaqMan 7900HT system and the sequence detection system software v2.4. All samples were genotyped twice, with 100% concordance. The TaqMan SNP Genotyping Assay ID numbers for each *PPARA* SNP are shown in Table S2.

OGTT. OGTTs were carried out to assess whole-body insulin sensitivity. After an overnight fast, subjects were challenged with an oral dose of 75 g of glucose dissolved in water. Blood was collected at 0, 15, 30, 60, 90, and 120 min after ingestion, and the blood-glucose concentration was measured using a standard AccuChek Glucometer (Roche Applied Science). The area under the curve was then calculated using the trapezoidal rule. An OGTT was performed on Lowlanders in London before altitude exposure, 16–21 d after departure (6–11 d at 5,300 m), and 55–60 d after departure (45–50 d at 5,300 m). An OGTT was performed on Sherpa subjects in Kathmandu before altitude exposure, and 12–13 d after departure (2–3 d at 5,300 m). For all subjects, an OGTT was performed the day following biopsy collection to avoid confounding experiments on muscle metabolism.

High-Resolution Respirometry. Skeletal muscle fiber bundles were prepared from the respirometry-designated sample according to previously described methods (28). After permeabilization, fiber bundles were blotted on filter paper and weighed using a microbalance (Mettler–Toledo). Respiration of fiber bundles was then measured in a mitochondrial respiration medium (MiR05) containing EGTA (0.5 mM), $MgCl_2{\cdot}6H_2O$ (3 mM), K-lactobionate (60 mM), taurine (20 mM), KH_2PO_4 (10 mM), Hepes (20 mM), sucrose (110 mM), and defatted BSA (1 $g{\cdot}L^{-1}$) at pH 7.4, using two substrate-uncoupler-inhibitor titration (SUIT) protocols, detailed in Tables S3 and S4. Respirometry was performed such that there was crossover of personnel between the three laboratories.

Malate (M; 5 mM) and octanoyl carnitine (Oct; 0.2 mM) were added initially to stimulate LEAK respiration (FAO_L; Fig. 4*D*, Fig. S2, and Table S3). ADP (saturating concentration ≥ 10 mM) activated phosphorylation of ADP to ATP, resulting in OXPHOS limited by the capacity of β-oxidation (FAO_P, F-OXPHOS; Fig. 2*D*). Addition of glutamate (G; 10 mM), followed by succinate (S; 10 mM), saturated convergent electron entry to the Q-junction in the FN-pathway ($OctGM_P$) and the FNS-pathway ($OctGMS_P$), respectively. Cytochrome *c* (10 μM) addition was used as a quality control to confirm outer mitochondrial membrane integrity; all assays with an increase in O_2 consumption of >15% following cytochrome *c* addition were excluded from further analysis. Carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP) was used (stepwise titration of 0.25 μM) to uncouple oxidative phosphorylation and investigate ETS capacity ($OctGMS_E$). Finally, rotenone was added (0.5 μM) to inhibit complex I [and thus FAO (38)] and isolate succinate-linked ETS capacity (S_E). The OXPHOS coupling efficiency (Fig. 4*E*) was calculated as follows to give an indication of mitochondrial coupling (37):

$$j \approx P = \frac{P - L}{P},$$

where $j \approx P$ = OXPHOS coupling efficiency, P = OXPHOS capacity following ADP addition, and L = LEAK respiration before ADP addition.

A second SUIT protocol was used to interrogate ETS function in the absence of fatty acid substrates (Fig. S3 and Table S4). Malate (5 mM) was added initially, followed by glutamate (10 mM), to measure LEAK respiration. ADP (saturating concentration ≥ 10 mM) activated phosphorylation of ADP to ATP, resulting in N-pathway OXPHOS capacity (Fig. 4*A*). Addition of succinate (10 mM) stimulated convergent electron entry to the Q-junction through complexes I and II (NS-pathway; Fig 4*C*). Cytochrome *c* (10 μM) addition was used as a quality control to confirm outer mitochondrial membrane integrity; all assays with an increase in O_2 consumption of >15% following cytochrome *c* addition were excluded from further analysis. FCCP was used (stepwise titration of 0.25 μM) to uncouple oxidative phosphorylation and investigate ETS capacity. Finally, rotenone was added (0.5 μM) to inhibit complex I and isolate succinate-linked ETS capacity (Fig. 4*B*).

Enzyme Activity Assays. Enzyme activity assays were performed as described previously (19). Briefly, ∼10 mg of vastus lateralis from each individual was homogenized with an Eppendorf pestle in an Eppendorf tube containing 300 μL of homogenization buffer containing Hepes (20 mM), EDTA (1 mM), and Triton X-100 (0.1% vol/vol). The samples were then centrifuged (380 × *g*, 30 s, 4 °C), and the supernatant was collected. This supernatant was centrifuged again (380 × *g*, 30 s, 4 °C), and the resulting supernatant was collected to obtain a homogeneous suspension. The protein concentration of chamber and tissue homogenates was measured using the Quick Start Bradford protein assay (Bio-Rad). All assays were performed using a spectrophotometer (Evolution 220; Thermo Scientific) at 37 °C in a reaction volume of 1 mL. CS activity was quantified with homogenate diluted to 10 μg of protein per milliliter in an assay buffer containing Tris (20 mM), 5,5′-dithiobis-2-nitrobenzoic acid (0.1 mM), and acetyl-CoA (0.3 mM) at pH 8.0. The reaction was initiated by the addition of oxaloacetate (0.5 mM), and the absorbance change at 412 nm was measured. The 3-hydroxy acyl dehydrogenase activity was assayed with homogenate diluted to 20 μg of protein per milliliter in an assay buffer containing imidazole (50 mM), NADH (0.15 mM), and Triton X-100 (0.1% vol/vol) at pH 7.4. The reaction was initiated by the addition of 0.1 mM acetoacetyl-CoA (0.1 mM), and the absorbance change at 340 nm was measured. Hexokinase activity was quantified with homogenate diluted to 60 μg of protein per milliliter in an assay buffer containing imidazole (20 mM), ATP (1 mM), $7H_2O{\cdot}MgCl_2$ (5 mM), DTT (5 mM), NAD^+ (2 mM), and glucose-6-phosphate-dehydrogenase (3.125 U) at pH 7.4. Glucose (5 mM) was added to trigger the reaction, and the absorbance change at 340 nm was measured. Activity of lactate dehydrogenase was quantified with homogenate diluted to 2 μg of protein per milliliter with an assay buffer containing Hepes (50 mM) and NADH (0.3 mM) at pH 7.0, and the reaction was triggered by the addition of pyruvate (0.5 mM). The reaction was monitored by measuring absorbance at a wavelength of 340 nm.

Reverse Transcription Quantitative PCR. RNA was extracted from frozen skeletal muscle samples using a Qiagen RNeasy Fibrous Tissue Mini kit as per the manufacturer's instructions, except that the incubation step with DNase I was excluded because this step was found to lower RNA yields. The Taqman assays used are detailed in Table S5.

Mass Spectrometry. A methanol/chloroform extraction protocol was used, as described previously (51). First, 600 μL of

chloroform/methanol (2:1 mixture) was added to cryovials containing ~20 mg of frozen skeletal muscle and a metallic bead. Samples were lysed in a tissue lyser (Qiagen, 3 × 2 min, 22 s^{-1}) and sonicated for 15 min. Metallic beads were then removed before 200 μL of chloroform and 200 μL of distilled water were added. Samples were thoroughly vortexed before centrifugation (~20,000 × *g*, 15 min), which resulted in clear separation of an aqueous phase (upper), protein pellet (middle), and organic phase (lower). The aqueous and organic fractions were carefully extracted using a positive displacement pipette and transferred to separate Eppendorf tubes. A further 600 μL of chloroform/methanol (2:1 mixture) was added to the protein pellet, and the lysis, mixing, and centrifugation steps were repeated to maximize metabolite recovery. Both the aqueous and organic fractions were dried under nitrogen and stored at −80 °C until further analysis.

Due to their high polarity, compounds that contain phosphate were measured using hydrophilic interaction liquid chromatography, where an aqueous layer is formed on the surface of the stationary phase and this layer allows retention of the analytes. Samples were reconstituted in 200 μL of acetonitrile/water (7:3 mixture), vortexed, and analyzed. The instrumentation comprised an Acquity Ultra Performance Liquid Chromatography unit (Waters Ltd.) interfaced with an AB Sciex 5500 triple quadrupole (AB Sciex). Mobile phases were run at 0.6 mL·min^{-1}, where mobile phase A consisted of 10 mM ammonium acetate adjusted to pH 9.5 with ammonia and mobile phase B was acetonitrile. Mobile phase B was held for 1 min at 70%, decreased to 40% over 2.5 min, returned to 70% by 3.6 min, and maintained for 2.4 min. The total run time was 6 min. Data were acquired in both positive and negative ionization modes using capillary spray voltages of 3.5 kV and 2.5 kV, respectively. The ion transfer tube was set to operate at 356 °C, and the vaporizer temperature was set to 420 °C. Sheath, auxiliary, and sweep gases were set to 52, 16, and 2 arbitrary units, respectively.

Other aqueous metabolites were measured using the same instrumentation, but the chromatographic separation was performed using an ACE C18-PFP 3-μm column (2.1 × 150 mm; Advanced Chromatography Technologies Ltd.). The mobile phase gradient was run at 0.5 mL·min^{-1} using water (mobile phase A) and acetonitrile (mobile phase B). The gradient started at 0% B and increased to 60% B from 1.6 to 4.5 min, followed by reequilibration for 2 min. The total run time was 6.5 min. Data were acquired in both positive and negative ionization modes using capillary spray voltages of 3.5 kV and 2.5 kV, respectively. The ion transfer tube was set to operate at 350 °C, whereas the vaporizer temperature was set to 400 °C. Sheath, auxiliary, and sweep gases were set to 50, 15, and 2 arbitrary units, respectively.

Half of the organic fraction and half of the aqueous fraction were combined with 200 μL of acetonitrile containing an internal standard mix of eight deuterated carnitines [1.63 μM (d9) free carnitine, 0.3 μM (d3) acetyl carnitine, 0.06 μM (d3) propionyl carnitine, 0.06 μM (d3) butyryl carnitine, 0.06 μM (d9) isovaleryl carnitine, 0.06 μM (d3) Oct, 0.06 μM (d9) myristoyl carnitine, and 0.12 μM (d3) palmitoyl carnitine; Cambridge Isotope Laboratories, Inc.] and dried under nitrogen. Samples were derivatized with 100 μL of 3 M HCl in butanol for 15 min at 65 °C. The resulting mixture was dried under nitrogen and finally reconstituted in 4:1 acetonitrile/0.1% formic acid in water, vortexed, and placed into autosampler vials. The strong mobile phase used for analysis was acetonitrile with 0.1% formic acid (B), and the weak mobile phase was 0.1% formic acid in water (A). The analytical ultra performance liquid chromatography (UPLC) gradient used a Synergi Polar RP phenyl ether column (50 × 2.1 mm, 2.5 μm; Phenomenex) starting with 30% B in 0.1% formic acid, followed by a linear gradient to 100% B for 3 min, and held at 100% B for the next 5 min with a further 2 min reequilibration. The total run time was 10 min, and the flow rate was 0.5 mL·min^{-1} with an injection volume of 2 μL. Analytes were measured using a multiple reaction monitoring (MRM) method, with the daughter ion being set to 85.0 Da for each compound.

Protein pellets were dissolved in 1 mL of 1 M NaOH solution and heated for 10 min at 80 °C. Samples were then centrifuged (16,000 × *g*, 10 min). Sample protein concentration was quantified using a bicinchoninic acid (BCA) assay kit (BCA1-1KT; Sigma), and absorbance at a wavelength of 562 nm was then quantified using a spectrophotometer (Evolution 220; Thermo Scientific).

Data were processed using the vendor software and normalized to total protein content and to the intensity of the internal standards.

It was not feasible in a field study such as this one to avoid autooxidation during sample processing, which is known to affect redox; thus, the redox ratios reported do not correspond to true physiological levels, which would have been obtainable with direct addition of thiol-alkylating agents. Nevertheless, we demonstrate that ratios change substantially, reflecting redox differences in relation to oxidative load. Addition of a thiol-alkylating agent would have compromised the analysis of other metabolites.

Statistics. To compare Sherpa and Lowlander cohorts at baseline, an unpaired, two-tailed Student's *t* test was performed (considering significance at $P \leq 0.05$). Genotype frequencies were compared between Sherpas and Lowlanders using a χ^2 test. To assess the effects of an ascent to high altitude on both cohorts, a one-way ANOVA with repeated measures was performed. If a significant difference was reported, post hoc pairwise comparisons were carried out with a Tukey correction.

Data Sharing. All data are available from the University of Cambridge data repository, which is available at https://www.repository.cam.ac.uk/handle/1810/263797.

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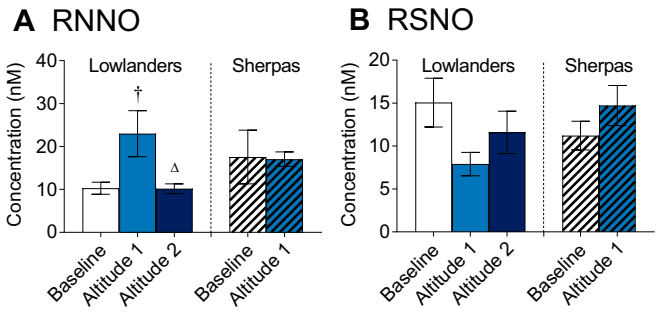

Fig. S1. Circulating nitrogen oxide levels. (*A*) N-nitrosamine (RNNO) and (*B*) S-nitrosothiol (RSNO) concentrations in blood plasma of Lowlanders and Sherpas at baseline (B) and at early (A1) and late (A2) altitudes are shown. Mean ± SEM (n = 10–12). $^{\dagger}P \leq 0.05$ at B vs. A1 within cohort. $^{\triangle}P < 0.001$ at A1 vs. A2 within cohort.

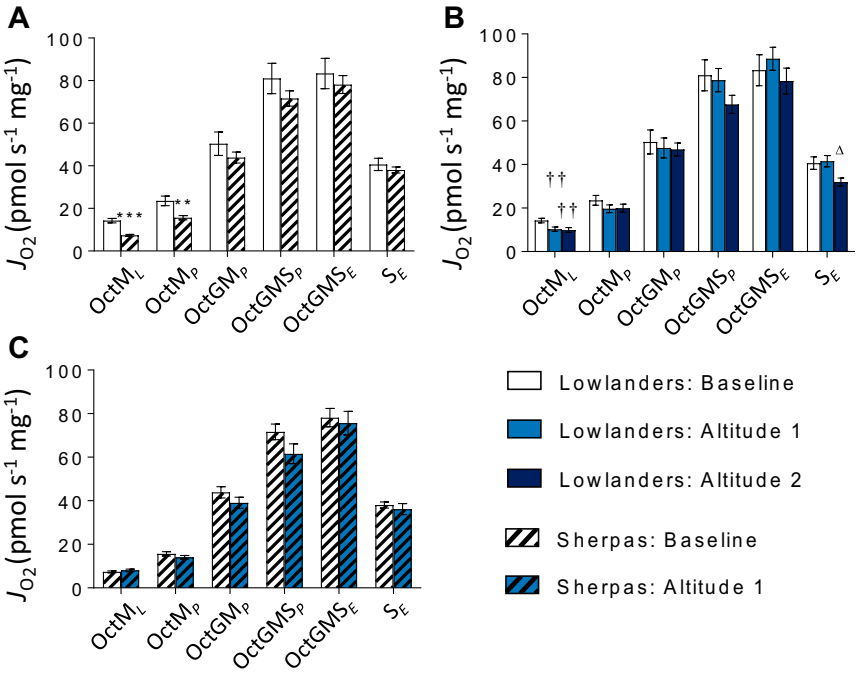

Fig. S2. Mitochondrial respiratory function by SUIT protocol 1, in the presence of Oct. FAO-LEAK (OctM$_L$), FAO-OXPHOS (OctM$_P$), N-OXPHOS (OctGM$_P$), NS-OXPHOS (OctGMS$_P$), NS-ETS capacity (OctGMS$_E$), and S-ETS capacity (S$_E$) are illustrated. Lowlanders vs. Sherpas at baseline (B) (*A*), Lowlanders at B and at early-altitude (A1) and late-altitude (A2) time points (*B*), and Sherpas at B and A1 time points (*C*). Mean ± SEM (n = 10–11). $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ for Lowlanders vs. Sherpas at B. $^{\dagger\dagger}P \leq 0.01$ at B vs. A1 within cohort. $^{\triangle}P \leq 0.05$ at A1 vs. A2 within cohort. J_{O2}, oxygen flux.

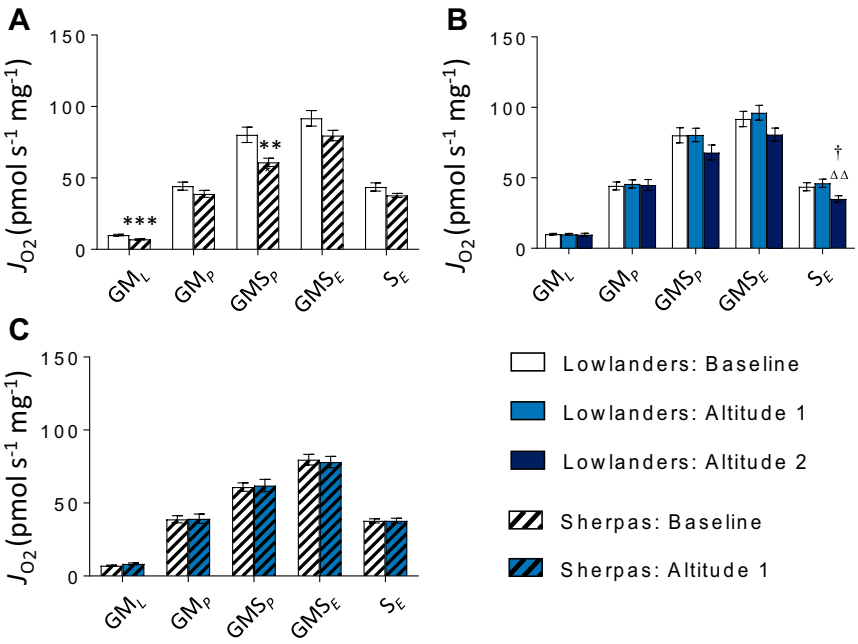

Fig. S3. Mitochondrial respiratory function by SUIT protocol 2, in the absence of Oct. N-LEAK (GM_L), N-OXPHOS (GM_P), NS-OXPHOS (GMS_P), NS-ETS capacity (GMS_E), and S_E are illustrated in Lowlanders vs. Sherpas at B (*A*), Lowlanders at B at A1 and A2 time points (*B*), and Sherpas at B and A1 time points (*C*). Mean ± SEM (n = 10–11). $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ for Lowlanders vs. Sherpas at B. $^{\dagger}P \leq 0.05$ at B vs. altitude within cohort. $^{\Delta\Delta}P \leq 0.01$ at A1 vs. A2 within cohort.

Table S1. *PPARA* SNP positions and putatively advantageous alleles

PPARA SNP	HG 18 position*	Selected allele	Alternate allele	TaqMan SNP Genotyping Assay ID
rs9627403	Chr22: 44827140	A	G	C_30661738_10
rs7292407	Chr22: 44832376	C	A	C_189279291_10
rs6520015	Chr22: 44842095	T	C	C_26019862_10

SNP positions and alleles are shown as identified by Simonson et al. (8) and by TaqMan SNP Genotyping Assay ID information.
*Based on University of California Santa Cruz (UCSC) Genome Browser Human Reference Build 18 (HG 18). Chr, chromosome.

Table S2. Relative levels of glycolytic intermediates

Intermediate	L(B)	L(A1)	L(A2)	S(B)	S(A1)
Glucose-6-phosphate and fructose-6-phosphate	1.00 ± 0.17	1.44 ± 0.25	3.29 ± 0.95†	1.33 ± 0.43	1.76 ± 0.35
Dihydroxyacetone phosphate	1.00 ± 0.18	1.20 ± 0.25	0.86 ± 0.13	1.23 ± 0.26	1.40 ± 0.12
2-Phosphoglycerate and 3-phosphoglycerate	1.00 ± 0.16	2.07 ± 0.28†	2.13 ± 0.22†	1.56 ± 0.22	0.92 ± 0.08††

Two pairs of metabolites, glucose- and fructose-6-phosphate and 2- and 3-phosphoglycerate, could not be distinguished from each other, so combined levels are shown. Levels of all intermediates are shown relative to Lowlanders at baseline as mean ± SEM (n = 7–14 per group). A1, early-altitude exposure; A2, late-altitude exposure; B, baseline; L, Lowlanders; S, Sherpas.
$^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ B vs. A1 within cohort.

Table S3. SUIT protocol 1

No.	SUIT	State	Figs.
1	Malate, 5 mM		Fig. 4*D* and Fig. S2
	Octanoyl carnitine, 0.2 mM	$OctM_L$	
2	ADP, 10 mM*	$OctM_P$	Fig. 2*D* and Fig. S2
3	Glutamate, 10 mM	$OctGM_P$	Fig. S2
4	Succinate, 10 mM	$OctGMS_P$	Fig. S2
5	Cytochrome *c*, 10 μM	$OctGMSc_P$	
6	FCCP, 0.25–1.5 μM†	$OctGMS_E$	Fig. S2
7	Rotenone, 0.5 μM	S_E	Fig. S2

Reagents were added in the order 1–7 (stub column) to give the final concentrations shown. *c*, cytochrome c; $_L$, LEAK state respiration; $_P$, OXPHOS state respiration; S_E, S-ETS capacity.
*The minimum amount of ADP added was 10 mM. Higher concentrations were required to reach saturation in some cases.
†FCCP was titrated in 0.25 μM steps until an inhibitory effect was observed.

Table S4. SUIT protocol 2

No.	SUIT	State	Figs.
1	Malate, 5 mM		
	Glutamate, 10 mM	GM_L	Fig. S3
2	ADP, 10 mM*	GM_P	Fig. 4*A* and Fig. S3
3	Cytochrome *c*, 10 μM	GMc_P	
4	Succinate, 10 mM	GMS_P	Fig. 4*C* and Fig. S3
5	FCCP, 0.25–1.5 μM†	GMS_E	Fig. S3
6	Rotenone, 0.5 μM	S_E	Fig. 4*B* and Fig. S3

Reagents were added in the order 1–6 (stub column) to give the final concentrations shown.
*The minimum amount of ADP added was 10 mM. Higher concentrations were required to reach saturation in some cases.
†FCCP was titrated in 0.25 μM steps until an inhibitory effect was observed.

Table S5. Details of Taqman assays selected to assess gene expression

Gene	Amplicon size	Assay no.
*ACTB**	63	Hs01060665_g1
*HPRT1**	82	Hs02800695_m1
*PPIA**	97	Hs04194521_s1
*RNA18S**	90	Hs03928985_g1
VEGFA	59	Hs00900055_m1
PPARA	62	Hs00947536_m1
UCP3	74	Hs01106052_m1
CPT1B	133	Hs03046298_s1

*Housekeeping genes used as controls for normalization of target genes.