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Metabolic insight into mechanisms of high-altitude adaptation in Tibetans

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ABSTRACT

Recent studies have identified genes involved in high-altitude adaptation in Tibetans. Genetic variants/haplotypes within regions containing three of these genes (*EPAS1*, *EGLN1*, and *PPARA*) are associated with relatively decreased hemoglobin levels observed in Tibetans at high altitude, providing corroborative evidence for genetic adaptation to this extreme environment. The mechanisms that afford adaptation to high-altitude hypoxia, however, remain unclear. Considering the strong metabolic demands imposed by hypoxia, we hypothesized that a shift in fuel preference to glucose oxidation and glycolysis at the expense of fatty acid oxidation would improve adaptation to decreased oxygen availability. Correlations between serum free fatty acid and lactate concentrations in Tibetan groups living at high altitude and putatively selected haplotypes provide insight into this hypothesis. An *EPAS1* haplotype that exhibits a signal of positive selection is significantly associated with increased lactate concentration, the product of anaerobic glycolysis. Furthermore, the putatively advantageous *PPARA* haplotype is correlated with serum free fatty acid concentrations, suggesting a possible decrease in the activity of fatty acid oxidation. Although further studies are required to assess the molecular mechanisms underlying these patterns, these associations suggest that genetic adaptation to high altitude involves alteration in energy utilization pathways.

1. Introduction

Native high-altitude populations have persisted for hundreds of generations in oxygen-deprived (hypoxic) environments. Recent genome-wide scans of positive selection in Tibetans have identified hypoxia-sensing and -regulated genes as candidates for high-altitude adaptation [1–10]. A few of the candidate regions thought to underlie Tibetans' adaptation were previously shown to be associated with their hemoglobin concentrations [Hb] [1,7,10], which are lower than those of native highland groups from the Andes or visitors to high altitude [1,7,10,11]. Positively selected regions containing the *EPAS1* gene, which encodes the HIF-2α subunit of the hypoxia inducible factor (HIF) complex, were associated with [Hb] in Tibetan populations examined in two separate studies [1,12]. Two additional genomic regions were associated with Hb concentration in a Qinghai Tibetan population. One contains *EGLN1*, which encodes the proline hydroxylase PHD2 that negatively regulates HIFs' α subunits in an oxygen-dependent manner [12]. The second is the genomic region containing *PPARA*, which encodes the nuclear peroxisome proliferator activated receptor alpha (PPARα) that regulates fatty acid metabolism and is in turn regulated by HIF [7].

It is unclear whether these previously identified associations reflect the action of selection directly on [Hb] or are consequences of natural selection acting on other advantageous traits [13,14]. Members of the hypoxia inducible factor (HIF) pathway orchestrate molecular responses during hypoxic stress through a complex series of cellular metabolites [15]. In the absence of adequate oxygen, energy production from oxidative metabolism may be diminished. At the same time, if oxidative metabolism proceeds under hypoxia, reactive oxidative intermediates will accumulate in mitochondria. Either of these conditions can result in cell death. Recent work on the whole-organism level has revealed that HIF plays a major role in regulating metabolism, highlighting a strong relationship between HIF and metabolic demands in humans [16].

The hypoxia signaling system triggers a pleiotropic response that increases tissue oxygenation by increasing vascularization and oxygen carrying capacity of the blood [1,2]. Simultaneously, HIF signaling triggers alterations in metabolism to decrease tissue oxygen demand [17,18]. Oxidation of fatty acids yields less ATP per molecule of oxygen consumed than oxidation of carbohydrates, suggesting that decreased fatty acid oxidation could also be a favorable adaptation to hypoxia [19]. Several studies have demonstrated decreased reliance

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on fat metabolism at high altitude, both in people living habitually at high altitude [19] and in those not dwelling at high altitude but acclimatized [20,21]. Another metabolic change induced by hypoxia is a conversion from oxidative glucose metabolism to glycolysis in order to maintain energy production. This occurs through up-regulation of glucose uptake and glycolysis and down-regulation of mitochondrial glucose oxidation [22,23].

These observations suggest that natural selection on HIF-related genetic variants in a hypoxic environment could be associated with significant metabolic changes in high-altitude Tibetans. If natural selection on genes in the HIF pathway has reduced fatty acid oxidation and increased glycolysis, then we predict that individuals who have the favorable HIF-related haplotypes should exhibit metabolic profiles consistent with these adaptations. Specifically, they should manifest increased concentrations of lactate and serum free fatty acids and/or triglycerides.

Here we examine relationships that provide insight into this hypothesis by comparing genetic variation in a Tibetan population with concentrations of several serum metabolites. We show that the putatively advantageous *EPAS1* haplotype previously associated with [Hb] [1,10] is also highly associated with changes in serum lactate concentration. In addition, the putatively adaptive *PPARA* haplotype exhibit an association with serum free fatty acid concentration in this population.

2. Results and discussion

We employed the analytic strategies (iHS [24] and XP-EHH [25] statistics) used in our previous study of Tibetan high-altitude adaptation [7] to identify targets of natural selection in a different Tibetan population from the Tuo Tuo River region in the Qinghai–Tibetan Plateau (Simonson et al. unpublished data). Both *EPAS1* and *EGLN1* gene regions exhibit significant signals of selection for the iHS test [24] ($p<0.004$ and $p<0.01$, respectively), although the genomic region containing *PPARA* did not reach significance ($p<0.14$) in this population for tests of selection performed (see Simonson et al. 2011 for discussion of differences among selection signals in Tibetan populations). Interestingly, a recent study of adaptation in Ethiopian highlanders shows that variants within the *PPARA* genomic region exhibit a signal of selection in Ethiopian highlanders; furthermore, this putatively selected haplotype is associated with decreased [Hb] in this population [26]. Considering variants/haplotypes in these three regions have been associated with [Hb] in one or more populations from the Qinghai–Tibetan Plateau [3,5,6], we examined whether these regions were associated with variation in metabolites.

We measured serum concentration of triglycerides, free fatty acids, β-hydroxybutyrate, and lactate in non-fasted serum samples from 36 individuals from the Tuo Tuo River region (Simonson et al. unpublished data). We performed Spearman rank-order correlation analysis between the serum concentration and the selected haplotypes (0, 1, or 2 copies; see Table 1). Lactate concentration was positively associated with the putatively adaptive *EPAS1* haplotype ($p<0.003$; Fig. 1, Table 1). Additionally, the previously identified *PPARA* haplotype of interest [7] exhibited a significant positive relationship with serum free fatty acids ($p<0.01$; Fig. 1, Table 1). The putatively advantageous *EGLN1* gene region was not associated with any of the serum metabolite levels measured.

Table 1
Haplotype–phenotype significance values for Spearman rank-order correlation analysis of metabolites measured in Tibetans living at 4500 m. Significant differences are indicated in bold text.

	EGLN1		*EPAS1*		*PPARA*	
	P value	Rho	P value	Rho	P value	Rho
Triglycerides	0.150	0.245	0.307	0.175	0.973	-0.006
Free fatty acids	0.505	−0.115	0.860	−0.031	**0.014**	0.406
Three hydroxybutyrate	0.230	−0.205	0.590	0.093	0.182	0.227
Lactate	0.070	0.305	**0.003**	0.482	0.424	0.137

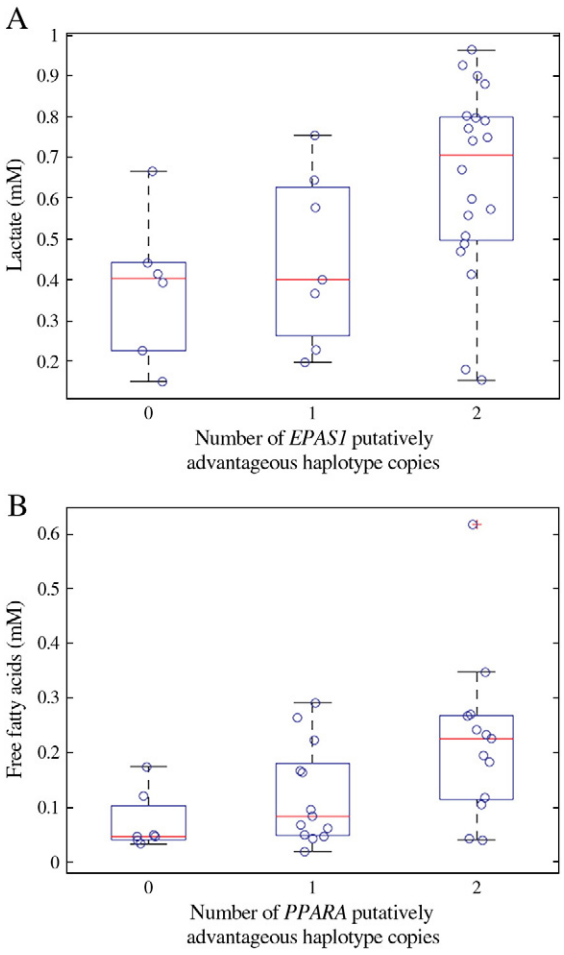

Fig. 1. Association of previously identified adaptive haplotypes and metabolites. A) Lactate concentration is plotted against the group of putatively advantageous haplotypes (0, 1, or 2) at the *EPAS1* locus. B) Serum free fatty acid concentration (FFA) is plotted against the number of putatively advantageous *PPARA* haplotype copies (0–2). A box-and-whisker plot overlying the data points shows the median, upper and lower quartiles, and extreme measurements as a red line, boxed ends, and dashed lines, respectively.

Both *EPAS1* and *PPARA* are involved in hypoxia signaling [27]. We observed that the putatively adaptive *EPAS1* haplotypes were associated with increased lactate concentration, which is consistent with decreased glucose oxidation. Humans acutely exposed to hypoxia consequently exhibit increased anaerobic glucose metabolism [28], and HIF-2α's metabolic activity has been shown to be required for the shift to anaerobic metabolism that facilitates adaptation to hypoxia in skeletal muscle of mice [15]. Additionally, individuals with Chuvash polycythemia, an autosomal recessive disorder in which HIF degradation is impaired, exhibit higher lactate levels during exercise than do normal individuals, illustrating a metabolic regulatory role of HIF under exercise conditions [16]. Mice lacking *EPAS1*, however, exhibit lactic acidosis [29], implying that the adaptive Tibetan polymorphism could be associated with either increased or decreased HIF-2α activity.

Because fatty acid oxidation consumes more oxygen than glycolysis for energy production, it is conceivable that a decrease in fatty acid oxidation activity is preferred and adaptive to the hypoxia environment. Indeed, a previous report demonstrates a high prevalence of hypertriglyceridemia in Tibetan highlanders [30]. We measured fasting triglyceride concentration in a separate cohort of Tibetans and Han Chinese living in an urban environment near sea level, and confirmed this observation of significantly higher triglyceride concentration in Tibetans

(298 ± 23 compared to 139 ± 8 mg/dl in the Han, p = 0.006; data not shown). We therefore examined the relationship of the putatively adapted haplotypes with serum free fatty acid and triglyceride concentrations in the high-altitude Tibetan samples. Free fatty acid concentration was positively associated with the *PPARA* putatively adapted haplotype, although serum triglyceride concentration was not. It should be pointed out, however, that the sera were not collected in the fasting state, and serum triglyceride concentration varies acutely with fasting, feeding, and composition of the diet.

PPARA encodes the nuclear receptor protein PPARα, a major regulator of fatty acid oxidation [31,32]. Down regulation of several genes involved in fatty acid oxidation, including *PPARA*, has been observed in rats exposed to hypoxia [33]. Previous studies have shown that PPARα activation is associated with lower serum free fatty acid and triglyceride concentration [34]; hence the putatively adaptive haplotype is consistent with decreased expression or activity of PPARα. We did not, however, observe decreased β-hydroxybutyrate, which is indicative of fatty acid breakdown, with the *PPARA* haplotype (Table 1), so there is no direct evidence of decreased fatty acid oxidation. Another explanation for increased lipid concentration would be increased lipid synthesis. Fat anabolic pathways are up regulated in hypoxia, mediated at least in part by up regulation of PPARγ [32,35] and SREBP-1 [36], but we have no data from our studies to determine the degree to which increased synthesis, increased lipolysis, or decreased metabolism of fatty acids contribute to the observed phenotype.

Recent studies suggest that some of the PPARα-dependent effects of hypoxia on fat metabolism may be mediated through HIF-2α [27]. Paradoxically, mice with either deletion of *Epas1* or liver-specific over expression of *Epas1* exhibit hepatic steatosis and both models show evidence of decreased fatty acid oxidation [29,37]. Thus, the interrelationships among the status of the primary hypoxia signaling pathways, their downstream metabolic effectors, and the final metabolic phenotype of the organism are highly complex. In addition, environmental factors are crucial in determining metabolic status. Thus, determining the specific roles of changes in *EPAS1*, and *PPARA* genomic regions on the observed changes in metabolites will require further study.

Hypoxia-induced regulation of metabolism and its alteration in adapted populations may carry implications for the risks of diabetes and obesity. The putatively adaptive haplotypes may result in a relative inability to shift between fat and glucose oxidation, so-called metabolic inflexibility [38]. Such inflexibility and fatty acid oxidation capacities [39,40] are both implicated in the pathogenesis of type 2 diabetes mellitus. Tibetan highlanders have a relatively low prevalence of diabetes [41], but the diet is also relatively low-calorie [42] and high altitudes are associated with lower body weights among Tibetans [43]. As populations move to lower altitudes and encounter a more industrialized lifestyle and higher calorie diets, however, the metabolic adaptations to altitude could have health implications. For example, increasing total fat and calorie consumption with a metabolic profile that will not support fat oxidation could result in accumulation of lipid intermediates thought to play a role in diabetes pathogenesis [39,40]. Further study of the metabolic implications of high altitude adaptation may allow interventions to ameliorate this risk and also identify potential new targets to treat obesity and diabetes.

Our results demonstrate increased lactate and free fatty acid concentrations in Tibetans with putatively advantageous *EPAS1* and *PPARA* haplotypes. This pattern provides insight consistent with the hypothesis that anaerobic glucose metabolism is increased and fatty acid oxidation may be decreased in the Tibetans with the putatively adaptive haplotypes compared to Tibetans without the adapted haplotypes living at the same altitude. Controlled studies including more dynamic metabolic analyses and studies at different altitudes will be required to better understand the physiological significance of these patterns.

3. Materials and methods

3.1. DNA sample collection

DNA was extracted from whole blood samples for individuals (non-smokers, no chronic diseases). This population, who speak the Kham dialect, is from the Tuo Tuo River area in the Qinghai–Tibetan Plateau (~4500 m), People's Republic of China. Informed consent was obtained for all participants according to guidelines approved by the Institutional Review Boards at the High Altitude Medical Research Institute (Xining, Qinghai, People's Republic of China).

3.2. SNP genotyping

DNA samples were genotyped using Affymetrix 6.0 SNP Array technology (>900,000 SNPs) at Capital Bio Corporation (Beijing, China). We used default parameters for the Birdseed algorithm (version 2) to determine genotypes for all samples (Affymetrix, Santa Clara, CA, USA). Genotypic data were analyzed using the Affymetrix Genotyping Console 3.1 (Affymetrix).

3.3. Estimates of relatedness

We used unrelated samples as described in Simonson et al. [7] and the program ERSA [44] to exclude related individuals in the second set of Tibetan samples, excluding one member of the pair if their relationship exceeded that expected for first cousins. Based on these criteria, a total of 36 unrelated individuals were included in the genotype–phenotype analyses.

3.4. Phenotype collection

We measured metabolites in a cohort of Tibetans, excluding related individuals (N = 36) using kit protocols for lactate (Point Scientific, Inc., Lincoln Park, MI), β-hydroxybutyrate (WAKO Diagnostics, Richmond, VA), free fatty acids (Half Micro Test, Roche, Penzberg Germany) and triglycerides (Sigma Chemical, St. Louis, MI).

3.5. Genotype–phenotype association

We determined whether the selection candidate regions that were previously associated with [Hb] (Table 1) are also associated with metabolites. We performed selection scans using the iHS (Voight et al., 2006) and XP-EHH (Sabeti et al., 2007) test statistics, and defined three-SNP haplotypes as previously described [7]: SNPs within 200 kb genomic region with the most extreme iHS scores comprise each of the putatively advantageous haplotypes (Hg 18 build haplotype-defining SNPs: chromosome 1 positions 229601735, 229604075, and 229793717 for *EGLN1*; chromosome 2 positions 46490868, 46590298, and 46592661 for *EPAS1*; chromosome 22 positions 44807657, 44827140, and 44866192 for *PPARA*).

We tested for an association between these haplotypes and free fatty acid, lactate, triglyceride, and β-hydroxybutyrate concentrations in 36 Tibetan individuals from the Tuo Tuo River area on the Qinghai–Tibetan Plateau (Simonson et al., unpublished data) using a nonparametric Spearman rank test. The number of putatively advantageous haplotype copies (0, 1, or 2) was determined using SNP haplotypes described above for *EPAS1*, *EGLN1*, and *PPARA* regions and compared to metabolite concentrations.

Conflict of interest

None of the authors have a conflict of interest in the materials presented.

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