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METABOLIC MEANING OF ELEVATED LEVELS OF OXIDATIVE ENZYMES IN HIGH ALTITUDE ADAPTED ANIMALS: AN INTERPRETIVE HYPOTHESIS

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Abstract. It is commonly observed that during acclimatization to altitude oxidative enzyme activities increase per g wet weight of tissue. To examine this problem in long-term adapted animals we measured citrate synthase (CS), hydroxyacylCoA dehydrogenase (HOAD), pyruvate kinase (PK), and lactate dehydrogenase (LDH) activities/g of myocardium in two domestic species (llama and alpaca) and a high altitude deer, the taruca. In all these species, we found an upward scaling of oxidative capacity (indicated by absolute activities of CS and HOAD) but a downward scaling of anaerobic/aerobic metabolic potentials of the heart (indicated by low ratios of LDH/CS, and LDH/HOAD, but high ratios of PK/LDH).

As the direction and magnitude of these long-term adaptations are the same as in shorter-term acclimatizations, we wondered why a similar pattern at the enzyme level correlates with the right shift of the O_2 dissociation curve (ODC) in the latter case, but with a left shifted ODC in the former. We hypothesize that in the long term, increased oxidative enzyme activities allow increased maximum flux capacity of aerobic metabolism. This in turn calls for physiological adjustments in O_2 transfer systems; flux limits of the former must be matched by flux limits of the latter. Only then can an acceptably high scope for aerobic activity be achieved despite reduced O_2 availability in inspired air. Such long-term match-up invariably calls for a left-shifted ODC plus other well known adjustments in O_2 transport. In the short term, right shifting the ODC may increase the total amount of aerobic work possible (by favoring O_2 unloading and thus raising tissue O_2 concentration), yet maximum flux capacity cannot be changed much because mitochondrial metabolism is designed for maintaining stable rates of ATP synthesis even at widely varying O_2 tensions. That is why even in short-term acclimatization, in order to increase flux capacity, the activities of oxidative enzymes also must be increased.

Acclimatization	Oxidative enzymes
Alpaca	Oxygen dissociation curve
High altitude	Oxygen flux
Llama	Taruca

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Although chronic hypoxia at high altitude may have severely debilitating effects on activated metabolism and performance of lowland species, it is well known that such effects can be circumvented by high altitude animals. Whereas physiological compensatory mechanisms, harnessed either during long-term adaptation or shorter-term acclimatization are now reasonably well described (see Hochachka and Somero, 1983, for discussion of interplay between time and adaptive strategies), underlying biochemical adjustments are poorly understood. Nevertheless, most recent reviews (e.g. Lahiri, 1979; Myhre, 1980) conclude that during acclimatization to high altitude hypoxia compensatory mechanisms are activated that lead to enhancement of aerobic metabolic capacities of the organism. This effect is well illustrated by increases in mitochondrial mass/g myocardium induced in ox during high altitude acclimatization (Ou and Tenney, 1970). The increase in mitochondrial mass is thought to be associated with increased activities of succinate dehydrogenase and other Krebs cycle enzymes, with increased activities or turnover rates of electron transfer components, with modified ADP dependence of respiration of isolated mitochondria, and presumably with increased maximum metabolic capacities of integrated organs and tissues (Reynafarje, 1971; Mela *et al.*, 1977). These cellular level adjustments correlate with a right-shift of the O_2 dissociation curve (ODC), which is thought to favor O_2 unloading at working tissues (Wood and Lenfant, 1979; Moore and Brewer, 1981).

The time course of such enzyme-based acclimatization is usually considered to be in the order of days or weeks (Lahiri, 1979), similar to the time-constants for synthesis and degradation of metabolic enzymes (Pette and Dölken, 1975), although some respiratory adjustments of mitochondria have been reported to occur within 30 min of the hypoxic insult (Mela *et al.*, 1977). The latter very rapid adjustments almost certainly are not accompanied by changes in enzyme amount/g tissue and their basis must be assumed, for the time being at least, to involve direct effector action at the cellular level (Mela *et al.*, 1977). However, in neither these rapid adjustments (requiring minutes) nor in the above enzyme-based acclimatizations (requiring days to weeks) are the metabolic effectors mediating the response known.

Similar studies of enzyme levels in animals living through phylogenetic time at high altitude are not as common (Barrie *et al.*, 1975; Harris *et al.*, 1971; Reynafarje, 1962). We consider this to be an unfortunate oversight, since in these organisms the ODC is almost invariably *left-shifted* (Banchero *et al.*, 1971; Lenfant *et al.*, 1971). So we took advantage of accessibility to two high altitude adapted domestic camelids (the llama and the alpaca) as well as a high altitude adapted deer, the taruca, and examined the activities of several cardiac enzymes functioning in anaerobic and aerobic phases of metabolism. We found that oxidative capacities of the heart were noticeably elevated when compared to several other mammalian species, whereas the ratios of anaerobic/aerobic metabolic capacities were relatively depressed. Aside from reporting that the changes in heart oxidative capacities during long-term adaptation to high altitude appear to be in the same direction as during shorter-term acclimatization, this paper ponders the metabolic meaning of such adjustments.

Methods and materials

Experimental animals. Specimens of adult llama *(Lama glama)*, 44–64 kg total body weight, and adult alpacas *(Lama pacos)*, 30–41 kg body weight, were obtained from the experimental herds maintained by the La Raya Veterinary Research Station (situated at 4200 m in the high Andes of southern Peru). Only males were utilized for these studies. In addition, with the permission of the Peruvian authorities five specimens of the high altitude taruca *(Hippocamelus antisensis)*, were collected between 4600 and 4800 m. Enzyme data from male and female tarucas (30–48 kg in body weight) were grouped as no trend was evident between the sexes and because the sample size was small. Unlike the camelids, which were restricted to 4200 m altitude, the taruca range widely in this area, sometimes to altitudes of over 5200 m (Merkt, unpubl. observations).

Tissue manipulations. As quickly as possible after sacrifice of the domestic animals, 50–100 g samples of the heart were removed by transecting the wall of the left ventricle and immediately frozen at −20 °C. For the taruca, the same procedure was utilized, but in this case, the myocardial samples were held at ice temperature until return to the laboratory, where they were then kept at −20 °C. The frozen tissues were subsequently returned to the University of British Columbia. Portions of each were dissected away, weighed, thawed, and homogenized for four 15-sec periods in a Polytron homogenizer (Brinkman Instruments) in 50 mM imidazole buffer, pH 7.0, containing 50 mM KCl, 7 mM $MgCl_2$ and 5 mM EDTA. The homogenate was then centrifuged at 3000 × g for 20 min and the supernatant was used as the enzyme source. The delay between sampling time and assay (3–4 weeks) is recognized as not being entirely satisfactory, as gradual enzyme degradation may lead to artifactually low estimates of true enzyme activity levels. However, the enzymes chosen for assay are reasonably stable in the frozen state (Hintz *et al.*, 1980); as it will be evident that correction of any such methodological artifacts would strengthen, not weaken, the interpretations given below, we do not consider this a serious problem.

Enzyme assays. All enzymes were assayed in a SP1800 Unicam spectrophotometer with cuvette temperature maintained at 37 °C with a constant temperature bath and circulator. All assays were at pH 7.0 in 50 mM imidazole buffer with saturating levels of all substrates and cofactors; other conditions of assay were the same as used previously (Murphy *et al.*, 1980; Emmett and Hochachka, 1981).

Basis for interpreting enzyme activity levels. It was first clearly documented by Pette *et al.* (1962) that glycolytic enzyme activities were elevated in tissues adapted for anaerobic function, while enzymes of the Krebs cycle and β-oxidation, in particular citrate synthase (CS) and hydroxyacylCoA dehydrogenase (HOAD) (Staudte and Pette, 1972), correlate with oxidative capacities. These studies form the basis for the concept of 'constant proportion groups of enzymes' and have received refinement in later studies. Thus, although CS activities/g supplies a good index of a tissue's maximum oxidative capacity both in inter-tissue and in inter-

species comparisons (Srere, 1969; Baldwin *et al.*, 1972; Marsh, 1981), HOAD does not always coadapt with CS. This is strikingly evident in systems like bee flight muscle, where capacities for fat oxidation are extremely low but vigorous glucose oxidative capacities are expressed, and where HOAD/CS ratios are consequently vanishingly small (Pette and Dölken, 1975). On the other hand, in tissues with a strong fat dependence, HOAD may be more noticeably elevated than CS activity (Hintz *et al.*, 1980), an example of which in fact will be discussed below. Because of such obvious needs for adjustment in mitochondrial enzymatic composition, CS activity is now more commonly related to oxidative capacity (Srere, 1969; Baldwin *et al.*, 1972) while HOAD activity is used more specifically as an index of relative capacity for oxidation of fatty acids. To facilitate comparisons of tissues displaying widely differing absolute enzyme activities, lactate dehydrogenase (LDH)/CS activity ratios are utilized to assess relative capacities for anaerobic *vs* aerobic metabolism (Pette and Dölken, 1975), while HOAD/CS ratios supply an index of the relative potentials for fat oxidation *vs* overall aerobic catabolism. An indication of dependence upon aerobic glycolysis *vs* anaerobic glycolysis is sometimes difficult to assess because all the enzymes in the pathway, except LDH, are utilized in both processes. However, LDH activity/g has been found to correlate well with anaerobic capacities of homologous tissues in interspecies comparisons (see Castellini and Somero, 1981). On the other hand, in comparing systems where the LDH/CS ratios are the same, the activity ratios of pyruvate kinase (PK)/LDH, or indeed the activity of any preceding glycolytic enzyme/LDH, may supply a relative index of aerobic glycolytic *vs* anaerobic glycolytic capacity (Hochachka, 1980). Thus, to obtain a qualitative impression of metabolic organization and capacity in heart and skeletal muscle of the three high altitude species chosen for study, we made the following assumptions:

(a) that CS activity/g yields an approximate measure of relative oxidative capacities of homologous tissues,
(b) that LDH/CS activity ratios yield a measure of relative anaerobic *vs* aerobic metabolic capacities,
(c) that, where LDH/CS ratios are similar, PK/LDH activity ratios yield a measure of the relative capacities for aerobic glycolysis,
(d) that HOAD activity/g yields a measure of relative capacities for fatty acid oxidation, and
(e) that HOAD/CS activity ratios yield a measure of how closely β-oxidation and Krebs cycle maximum capacities are coadapting.

Although for qualitative interspecies comparisons of homologous tissues these assumptions are not controversial, the reader may find further experimental and theoretical bases for them elsewhere (Srere, 1969; Pette and Dölken, 1975; Baldwin *et al.*, 1972; Lowry *et al.*, 1980; Hintz *et al.*, 1980; Castellini and Somero, 1981; Marsh, 1981).

Results and Discussion

Blood parameters

The O_2 transport properties and other blood biochemical parameters of camelids have been previously described (Banchero *et al.*, 1971; Banchero and Grover, 1972; Reynafarje *et al.*, 1975). In agreement with previous data, our particular specimens displayed similar features: for llama, the hematocrit was 29–37% with an average of about 31%; for alpaca, the hematocrit varied from 27 to 33% (8 individuals sampled in each case). In the taruca, the hematocrit ranged between 31 and 42%, with an average value of 34% (5 individuals sampled), while hemoglobin concentration was about 14 g/100 ml blood, a value similar to that observed for lamas and alpacas. These species then, appear to display a standard long-term adaptation of blood oxygen transport functions: low viscosity, low oxygen carrying capacity, and *left-shifted* O_2 dissociation curves (see Schmidt-Nielsen, 1979; Wood and Lenfant, 1979).

Heart enzyme activities

The activities of citrate synthase and HOAD/g of left ventricle in the three high altitude species (table 1) indicate a number of interesting differences between each other and between the high altitude group compared with other species. Firstly, and most notably, oxidative capacity as indexed by CS activity/g is by far the highest in taruca, which species, of the group studied, ranges through highest (over 5000 m) altitudes. Secondly, HOAD activity in taruca heart, occurs at only about 35% of the activity level of CS. Thirdly, CS activities/g in llama and alpaca hearts are also on the high side compared with other mammals for which data by similar techniques are available (Table 1), but what is most interesting about the two camelids is that heart HOAD is strikingly active, occurring at about 2-fold higher activities than in taruca heart, 7–10 times higher than in ox or seal hearts. This emphasis on a fat-based metabolic organization is also indicated by heart LDH/HOAD ratios, which are lowest for the two camelids, but even in taruca heart, this ratio is only 1/4 the value in ox heart, about 1/10 the value for the Weddell seal (table 1).

Another indication of a relatively high oxidative capacity in hearts of these high altitude animals is obtained from LDH/CS activity ratios, which, as mentioned above, are often utilized for comparing relative capacities for anaerobic *vs* aerobic metabolism in homologous tissues. The LDH/CS ratios are similar (about 3) for all three high altitude species studied, but are substantially lower than for other mammals: the values are about 1/10th those for seal heart and about 1/3 the values for LDH/CS in hearts of several other species (cows, dogs, rabbits, and guinea pigs) for which comparable data are available (table 1). These data lead us to the conclusion that heart metabolism in the three high altitude species displays a reduced dependence upon anaerobic glycolysis, but an elevated dependence upon oxidative metabolism.

Although anaerobic glycolytic capacity of the heart appears to be de-emphasized,

TABLE 1

Heart enzyme activities in U/g of left ventrile from taruca, llama and alpaca compared to other species. (a) data for CS and HOAD; (b) data for PK and LDH

(a) Species	μmol substrate converted $\cdot g^{-1} \cdot min^{-1}$				
	CS	HOAD	HOAD/CS	LDH/CS	LDH/HOAD
Taruca	217 ± 34	78 ± 9.7	0.36	2.5	6.9
Llama	88 ± 3.8	134 ± 4.7	1.52	3.3	2.1
Alpaca	93 ± 3.9	156 ± 4.5	1.68	3.3	2.0
Ox[1]	62 ± 7	22 ± 2	0.35	9.0	25.3
Weddell seal[1]	29 ± 7	16 ± 4	0.55	35.8	64.5
Others[2]	31 ± 2	27 ± 5	0.87	8.0	11.6
Sheep[3]	69 ± 14				
Pig[3]	59 ± 4				
Ox[3]	64 ± 4				
(b) Species	PK	LDH	PK/LDH	PK/HOAD	PK/CS
Taruca	731 ± 46	536 ± 33	1.4	9.4	3.4
Llama	223 ± 6.7	287 ± 9.8	0.8	1.7	2.5
Alpaca	212 ± 6.9	308 ± 8.3	0.7	1.4	2.3
Ox[1]	133 ± 4.5	556 ± 48	0.2	6.0	2.1
Weddell seal[1]	217 ± 51	1032 ± 93	0.2	13.6	7.5

Activities are means ± SEM; sample numbers for taruca, llama and alpaca were 4, 8 and 8, respectively.
References:

[1] Murphy et al. (1980), enzyme activities at 37 °C.

[2] Pette & Dölken (1975), enzyme activities at 25 °C; pooled data for hearts of dog, rabbit, guinea pig.

[3] Alp et al. (1975), enzyme activities at 37 °C.

the capacity for aerobic glycolysis in contrast seems enhanced in all three species, especially in taruca. As indicated in Methods and Materials, this interpretation relies upon the fact that PK may participate in both aerobic and anaerobic glycolysis, and a relative indication of the two processes can be obtained from PK/LDH activity ratios particularly since the homologous tissues in this case display similar LDH/CS ratios. This analysis shows that the PK/LDH ratios are highest for taruca (about 1.4) and are almost 2-fold higher than in the camelids (table 1). Such high PK/LDH ratios (greater than 1) seem rather rare in nature and we were able to find no other species displaying this in heart or skeletal muscle; the PK/LDH ratio closest to this value amongst mammals which we could find is for shrew leg muscle, a highly oxidative tissue, where the value is 0.92 (Emmett and Hochachka, 1981). In other mammals, such as the ten terrestrial and marine species studied by Castellini *et al.* (1981), in the ox and Weddell seal (table 1), as well as in lower vertebrates (Hochachka, 1980), heart PK/LDH activity ratios are about 0.2, or 1/4 the values for the alpaca and llama, about 1/7 the value for taruca heart (table 1). That is why we tentatively conclude that in all three high altitude species

heart metabolism displays a higher capacity for aerobic glycolysis than in other vertebrate species, and this seems particularly true for the taruca. The emphasis on aerobic glycolysis in the metabolic organization of taruca heart is also evident in the high PK/HOAD activity ratio (of about 10), which is over 5 times higher than in the camelids. This large difference is not due to a simple increase in mitochondrial mass (PK/CS ratios for example are fairly similar in them all). Rather, as is evident from absolute activities (table 1), the difference is due in part to high HOAD activities in camelid hearts and in part to their lower PK activities. (The values of PK/HOAD for other mammals analyzed in table 1 are difficult to compare because in these species the LDH/CS and indeed the LDH/HOAD activity ratios differ so much from the values for the high altitude species.)

In summary, we take the data on heart enzyme activities to quite unequivocally indicate two kinds of adjustments in the high altitude animals compared to other mammals: *firstly, an upward scaling of absolute oxidative capacity (preferentially for carbohydrate-based oxidative metabolism in taruca heart; preferentially for fat-based oxidative metabolism in the camelid hearts), and secondly, a downward scaling of ratios of anaerobic/aerobic metabolic capacities of the heart in all three species.* The capacity adjustments are indicated by absolute activities of CS and HOAD, by the ratios of HOAD/CS, PK/LDH, and PK/HOAD; the adjustments of the anaerobic/aerobic metabolic potentials are clearly indicated by the low ratios of LDH/CS and LDH/HOAD in the hearts of all three high altitude species. Metabolic signals mediating these complex adjustments, however, are not known.

These results are in general agreement with a small number of earlier direct enzyme measurements (Harris *et al.*, 1971; Barrie *et al.*, 1975) showing increased activities of enzymes in oxidative metabolism in species born and raised at high altitude, as well as with indirect evidence of increased mitochondrial mass, capillarity, and myoglobin concentrations implying increased oxidative capacity (Raynafarje, 1962; Gimenez *et al.*, 1975). What is equally interesting is that the observed enzyme adaptations in these high altitude animals, which we assume to be of a long-term nature and probably genetically based (Hochachka and Somero, 1983), are in the same direction and of similar magnitude as observed during shorter-term acclimatizations. In fact, in the context of this paper, this is the principal if simple conclusion we wish to emphasize. Even though the ODC is left-shifted in high altitude animals, right-shifted during acclimatization of lowland animals to high altitude, *under both conditions, the activities/g myocardium of enzymes in aerobic metabolism are scaled upwards.* Why should this be so?

The central metabolic problem of high altitude animals

To put the matter into proper perspective, it is important firstly to clarify that the impact of high altitude hypoxia is greatest on activity metabolism. We can illustrate this by reviewing, as West (1982) has done, the metabolic requirements of man on Mt. Everest. Interestingly, even here enough oxygen should be available

to supply basal metabolic needs; however, there is literally nowhere to go for activated aerobic metabolism and the *scope for activity (the difference between maximum rate and basal rate of oxidative metabolism) becomes vanishingly small* (West and Wagner, 1980). At near-sea level most mammals display a scope for activity of about 10 fold (Taylor *et al.*, 1981), but some species like the horse achieve a scope as high as 40 fold (Thomas and Fregin, 1981). For lowland mammals in the size range of man, the scope for activity at 5000 m altitude is decreased by about half, and on Mt. Everest aerobic scope for activity is so low it is probably sensitive to moment-by-moment changes in barometric pressure (West, 1982). This situation is undoubtedly unsatisfactory for species permanently resident at high altitudes; the central metabolic problem for these organisms, then, is *how to maintain an acceptably high scope for aerobic metabolism in the face of the reduced oxygen availability of the atmosphere.* Recognition of this issue, which applies to the heart as well as to aerobically working skeletal muscles, is necessary for understanding the metabolic meaning of upward scaling of oxidative enzyme activities.

Interpretive model of high altitude adaptation: short and long term

The centerpiece of our interpretation is that the biologically most relevant function being adjusted *in both short- and long-term adaptation to high altitude is the flux of substrates* (metabolites, oxygen, protons, and electrons) through the metabolic machinery of working heart muscle: in effect it must remain possible to accelerate flux enough to maintain an acceptably high rate of ATP turnover for an acceptably high scope. One biologically feasible alternative is to right-shift the ODC, thus favoring O_2 unloading at the working muscle, an effect further amplified by increased hematocrit and increased hemoglobin concentrations. Assuming these adjustments do not compromise O_2 loading at the lungs by too great a factor, they may on their own serve to increase the total amount of aerobic work possible (by increasing the O_2 content of the working tissues). The chief limitation of this metabolic strategy is that it leaves maximum flux capacity of oxidative metabolism largely unaffected; mitochondrial metabolism cannot be much influenced by increasing O_2 concentrations because its apparent K_m for O_2 is simply too low (in the μM range). As a result maximum flux capacity of mitochondrial metabolism, as required during maximum aerobic heart work, is relatively insensitive to very wide oxygen concentration ranges (see Wilson *et al.*, 1979a). This does not mean that the electron transfer system *per se* is insensitive to O_2 concentration. According to Wilson *et al.* (1979b), as O_2 concentrations fall, cytochrome *c* becomes more and more reduced and the ATP/ADP concentration ratios fall, but *respiration rates remain remarkably stable.* The system is obviously designed to maintain ATP synthesis rates in the face of changing O_2 availability down to very low O_2 tensions. That is why in principle it is a better solution to increase the rate at which O_2 and substrate can be fluxed through the system (*i.e., to increase enzyme activities/g of tissue*) rather than to simply increase O_2 concentration. This achieves the requisite

increase in maximum flux capacity without requiring drastic modifications in other (standard) control features (Hochachka *et al.*, 1983; Wilson *et al.*, 1979a,b). Furthermore, it in effect relieves the need for elevating O_2 concentrations in muscle, *i.e.*, the need for a right-shifted ODC. Instead, what is needed is a *physiological O_2 transport system in turn adjusted for high O_2 flux rates, high enough to match the elevated flux limits of mitochondrial metabolism.* Optimally, flux limits of the latter should be matched to flux limits of the former. In our view, it is the advantage of this condition that puts selective advantage upon a left-shifted ODC (Hlastala, 1982).

We further postulate that increasing the rate at which O_2 and substrates can be fluxed through the system is an invariable result of long-term adaptation to high altitude (providing the organism does not involve anaerobic mechanisms of adaptation in a major way). It is achieved by 'tuning up' two parts of the system, the O_2 transporting part and the cell metabolism part. Flux capacity of the latter is elevated by increasing enzyme activities and mitochondrial abundance; flux capacity of O_2 transport is elevated by left-shifting the ODC, reducing blood viscocity (low hematocrit, low hemoglobin levels), increasing capillarity, decreasing diffusion distance, and increasing facilitated diffusion capacity of muscles by increasing myoglobin levels. Because of the way the electron transfer system interacts with O_2, the most important of these mechanisms may be increased enzyme activities/g of aerobically working tissues. We are indeed tempted to suggest that the observed metabolic strategy of long-term adaptation to high altitude is imposed on organisms because of the relative insensitivity of mitochondrial metabolic rates to O_2 concentration, leaving adjustments in enzyme content/g of tissue as a necessary, if not sufficient, requirement for sustaining elevated flux capacities.

Interpretive models are useful not only as a framework for further experimentation, but also in helping explain data that are otherwise perplexing and poorly understood. We believe our model displays both these features. In particular, it helps to explain what to date has been somewhat of a paradox in the literature; namely, why short-term responses to high altitude lead to a right-shift of the ODC, while long-term responses typically lead to a left-shift. In our view, it is an expression of different metabolic strategies. In the short-term, the adaptation of enzyme levels may not be complete (see Pette and Dölken (1975) for the half life of some metabolic enzymes) so the cycling of ATP cannot be elevated enough to compensate for reduced O_2 availability; this situation may be somewhat alleviated by improved O_2 unloading at the tissues, so a right-shift of the ODC is appropriate. In the long run, however, activities of oxidative enzymes are adjusted so that a flux of O_2 through the system can be high enough to compensate for reduced atmospheric availability. Under these conditions the need is for higher O_2 flux rates through the physiological processes linking mitochondrial metabolism with atmospheric O_2. This need is invariably better satisfied with a low-viscosity blood transport system showing a left-shifted ODC suited for O_2 loading, a situation previously pondered by Hebbel *et al.* (1978) in their analysis of human llamas.

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