Defining the resistance to oxygen transfer in tissue hypoxia

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Summary. Studies of O_2 supply in freshly isolated adult mammalian cells provide new insight into the factors that limit mitochondrial oxygenation in vivo. Of particular importance, mitochondria are present at high densities and often in apparent clusters, both of which contribute to local O_2 gradients under hypoxic conditions. Current evidence indicates that the mitochondrial distribution is a component of the differentiated phenotype of adult mammalian cells and that specific motors and anchoring mechanisms are present to allow redistribution in response to developmental, physiological and pathological challenges. To compare the importance of resistance to O_2 transfer under different conditions and at different sites along the supply path in vivo, a simple mathematical expression of relative resistance to O_2 supply is introduced. Under various pathophysiological conditions, this resistance increases in specific regions of the pulmonary, circulatory or cellular supply path and results in O_2 deficiency in the mitochondria. Regardless of cause, the relative resistance increases dramatically in the vicinity of mitochondrial clusters during hypoxia.
Key words. Oxygen transfer; tissue hypoxia; mitochondria; respiration, cellular; diffusion, intracellular; adaptation to hypoxia.

The supply of energy for most mammalian functions depends on the delivery of O_2 from the atmosphere to the mitochondria with cells. Insufficient O_2 supply, termed hypoxia, limits cell functions. The factors that define the resistance to O_2 transfer are therefore important in many aspects of physiology and pathology.

Barcroft[3] recognized that there are three general causes of hypoxia. These are deficient ventilatory supply of O_2 to blood (hypoxic hypoxia), deficient O_2 carrying capacity of blood (anemic hypoxia) and deficient circulatory function (stagnant hypoxia). A critical site of resistance to O_2 transport is implicit in each of these classifications. However, Barcroft's classification is not based on quantification of the resistances to O_2 transfer but upon the recognition that pathologies affecting specific sites impede O_2 transfer and have the common characteristic that the O_2-consuming components of cells are insufficiently supplied.

After 70 years of investigation, it is perhaps surprising that the locations and importance of resistances to O_2 transfer remain a central issue. However, the quantification of relative resistances at specific sites has been difficult because the path is highly heterogenous and adaptive to physiological and pathological challenges. Moreover, it is experimentally difficult to dissect the path, especially within organs and tissues, without changing the sites and magnitudes of resistance.

During the past several years we have focussed on O_2 supply at the cellular level because this approach provides the useful simplification that respiratory and circulatory heterogeneities and adaptive changes are eliminated[11]. Thus, an understanding of cellular oxygenation allows better interpretation of the sites of resistance to O_2 supply in vivo. In the current presentation we review these studies of O_2 supply at the cellular level and present a conceptual framework for discussing the quantitative importance of resistance to O_2 transport at sites within the cell and at other sites in the O_2 delivery path.

Potential sites of resistance to O_2 transport in cells

Crossover theorem in steady-state analysis. There is considerable utility in application of the crossover theorem[21] to understand metabolite flux and regulation in enzymology. One aspect of this theorem is that the site in a pathway responsible for a change in flux can be identified by comparison of the intermediate metabolite concentrations under the steady states before and after change in flux (fig. 1). For example, the site of effect of an inhibitor can be ascertained because the metabolite concentrations D–E are decreased while A–C are increased after the inhibitor is added (fig. 1).

A similar approach can be applied to O_2 supply from the lung to the mitochondria under different conditions to determine the important site(s) of resistance (fig. 2). A site of change in resistance to O_2 supply can be identified by the effects on O_2 concentration at preceding and subsequent sites. In this case, however, only the O_2 concentration in the vicinity of mitochondria is important for maintaining the O_2 consumption rate because the rate is determined by the kinetics of cytochrome oxidase. The other sites along the path also contribute to supply limitation because they affect the overall ability to supply O_2 to the terminal oxidase. Flux at these sites generally occurs by diffusion, which is proportional to the difference in concentration (ΔC) across the diffusion path rather than to the concentration (C) at any given site (see below). Thus, expression in terms of the concentration gradient (ΔC) provides a convenient means to identify sites of resistance, and comparison of two steady states provides a means to determine the sites of change in resistance to supply.

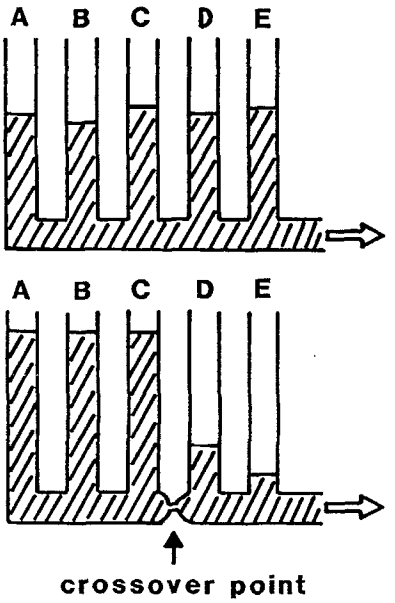

Figure 1. Illustration of crossover analysis to identify sites of rate limitation in a metabolic pathway. Under steady-state conditions, metabolite concentrations, A–E, are maintained at steady-state concentrations by the balance of production and removal, dictated by the activities and kinetic properties of the relevant enzymes of the pathway (upper panel). Following addition of an inhibitor or other manipulation to alter the flux, the site of effect (crossover point) can be identified by the changes in preceding and subsequent metabolite concentrations (lower panel).

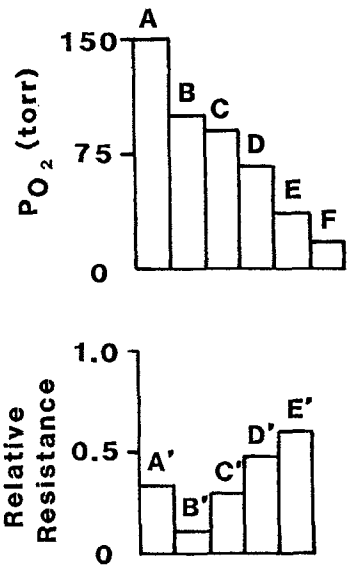

Figure 2. O_2 values and relative resistance at different sites in the path of O_2 supply. Top: O_2, given in partial pressure, decreases along the path from the atmosphere to the mitochondria. Following a change in resistance at a specific site, the change can be identified because the subsequent sites will have lower partial pressures. A: air; B: mixed alveolar air; C: mixed arterial blood; D: mean capillary blood; E: cytoplasm; F: mitochondria. Bottom: The actual contribution to resistance may be considerably larger than indicated by the partial pressure (concentration) changes because the significance of a gradient increases as the absolute value decreases[15]. Thus, a normalized expression, relative resistance, provides a better indicator of the importance of specific sites in limiting O_2 supply. A': flux between air and alveoli; B': flux between alveoli and arterial blood; C': flux between arterial and capillary blood; D': flux between capillary and cytoplasm; E': flux between cytoplasm and mitochondria.

A difficulty with this approach is that the significance of a concentration gradient (ΔC) increases as the average concentration ($\bar{C}$) of the species decreases[15]. From the Fick equation, the likelihood that a significant concentration gradient occurs with a given biological solute can be expressed as the ratio of flux (J) of the solute to its average concentration, $J/\bar{C}$[15]. As discussed below, a similar expression can be derived to express the relative resistance to O_2 supply at different sites within the supply path.

Relative resistance to O_2 supply. Diffusion of solutes in various geometries has been defined mathematically[4]. A general form of the Fick equation that is useful for discussion of resistance to transport is:

$$\Delta C = \frac{J}{D} \times G \qquad (1)$$

where ΔC is the concentration gradient of a solute, J is the flux, D is the diffusion coefficient of the specified solute, and G is a term or function appropriate for the geometry of the system.

To provide a normalized expression of the resistance to O_2 supply at given sites, the ΔC values can be divided by the maximal concentration (C_{max}) for the region of interest:

$$\frac{\Delta C}{C_{max}} = \frac{J}{C_{max}} \times \frac{G}{D} \qquad (2)$$

The resulting expression ($\Delta C/C_{max}$) is dimensionless and provides a general index of the magnitude of resistance at sites along the supply path. We term this expression the *relative resistance* (RR); it can be a value from 0 to 1 with the higher value indicating a high contribution of resistance to O_2 supply. For a gas such as O_2, the expression can also be given in partial pressure (P) where $RR = \Delta P/P_{max}$.

Each of the right-hand terms affects the RR. The geometry term is important in the actual definition of RR at the cellular level. However, if one assumes that the structural determinants of O_2 supply are unchanged by an acute transition from normoxia to hypoxia, we can consider this term constant.

Similarly, the effective diffusion coefficient for solutes in cells (D_c) is important in the actual definition of the resistance ratio, but it is also likely to be relatively constant during hypoxic transitions. The precise value of D_c for O_2 is not known, but several factors contribute to the D_c being lower than the corresponding value in water: a) the diffusion volume is less than the cell volume because only about 65% of the cell water is free[6] and some of the volume is occupied by high concentrations of macromolecules, b) the diffusion path may be greater than the linear distance[9], c) the viscosity is greater than that in water[22], d) multiple aqueous phases can occur and have boundaries that impede diffusion[27], and e) solutes may bind to relatively immobile macro-

molecules [1]. From the analyses described below and from several other techniques, the D_c for O_2 as well as many other small solutes are in the range of 0.2–0.4 times the corresponding value in water. This low D_c, in effect, raises the C_{max} at which significant RR occurs by a factor of 2.5- to 5-fold.

The two most important variables in the definition of RR during hypoxia are J and C_{max}. A significant resistance to transport occurs only as ΔC becomes large relative to the maximal concentration of the solute, C_{max}, occurring within G. This will occur if J is large or C_{max} is small. In cells, the respiration rate (K) determines J, and K is highly variable. K is a function of both the mitochondrial density and the work load placed upon the cell. As cells become O_2-limited by any cause, RR at the cellular level will become large if K is not dramatically decreased. Because cytochrome oxidase does not become limited until very low O_2 concentrations are obtained, the relative resistance at the cellular level increases during hypoxia. Thus, when compared to the RR values at different sites in vivo, cellular resistance becomes more important.

Use of intracellular enzymes and transport systems as in situ probes of solute concentrations

In isolated cells, resistance to diffusion can occur at an extracellular unstirred layer, the plasma membrane, the aqueous cytoplasm and the vicinity of mitochondria (fig. 3). Definition of the relative contributions of resistance at individual sites requires means to measure O_2 at the respective regions within the cells. For this purpose, we have utilized enzymes with different intracellular localizations as endogenous sensors for O_2 concentration [15].

Intracellular proteins provide sensitive probes for the concentrations of specific biomolecules and ions with the spatial resolution of the subcellular organelles [15]. Experimentally, calibration problems limit the application of this approach, but useful data have been obtained for O_2, ATP, H^+ and other solutes [12]. The principle is that any enzyme or transport system that is dependent mostly upon a single component can be used to estimate the local concentration of that component if a suitable means to calibrate the system is available. This approach was used in pioneering studies by Millikan et al. [23] to measure oxygenation of myoglobin. However, the measurement was limited by optical problems and heterogeneity associated with bulky tissues. Only recently has the quantitation of tissue spectroscopy been clarified by the recognition that the internal pathlength of light is greater than the linear distance [7].

The application of optical spectroscopy to cells has been easier to calibrate and verify. This can be illustrated by considering the hepatic cytochrome P-450, an O_2-dependent enzyme that is present in the endoplasmic reticulum. This enzyme detoxifies lipophilic foreign compounds,

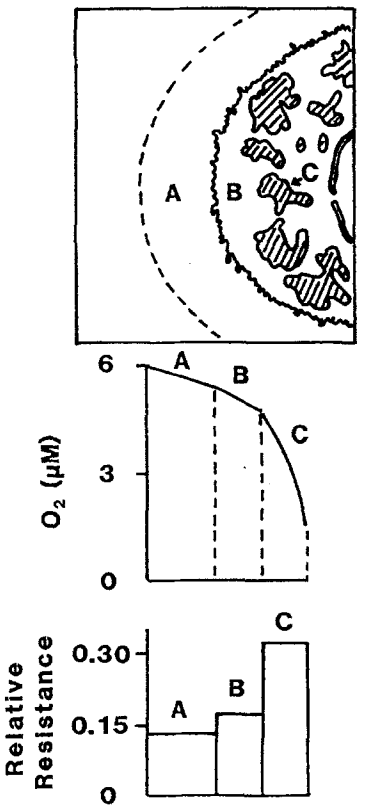

Figure 3. Schematic drawing of sites of resistance to O_2 supply at the cellular level. Top: Region of hepatocyte illustrating the unstirred layer (A), the plasma membrane and aqueous cytoplasm (B) and the clusters of mitochondria (C). In the middle panel, estimates of the O_2 gradients in these regions are presented based upon data obtained with studies of isolated hepatocytes [10]. Bottom: calculation of the relative resistance to O_2 supply for the different regions.

which can be supplied to cells at concentrations that give maximal activity. The enzyme is not under allosteric regulation, and conditions can be established in which the activity of the enzyme is dependent only on the concentration of O_2. Measurement of the O_2 dependence of alprenolol metabolism in hepatocytes showed that the rate of reaction was half-maximal (P_{50} value) with a suspending medium O_2 concentration of 9.8 μM [13]. Measurement of the O_2 dependence of the same reaction in isolated liver microsomes showed that the P_{50} value was 8.7 μM. An $[O_2]$ of 10 μM corresponds to a P_{O_2} of about 6 Torr. Thus, at the P_{50} value, the gradient in O_2 concentration between the suspending medium of cells and the endoplasmic reticulum averages only about 1.2 μM. Under this condition, RR due to the unstirred layer, plasma membrane and intervening cytoplasm is about 0.12. Similar values are obtained with other substrates for cytochrome P-450 [13]. This means that at an extracellular O_2 concentration in the range of 10 μM, RR due to the unstirred layer and plasma membrane is small. This is schematically visualized in figure 3 as region A and part of region B.

Resistance to O_2 transport into mitochondria in cells

Early studies by Krogh[20] showed that bulk phase diffusion of O_2 through tissue sections occurs at about half of the rate of O_2 diffusion in water. Based on this and known cellular O_2 consumption rates, mathematical modeling of O_2 diffusion into cells (assuming uniform O_2 consumption rate within the cells) and into mitochondria (assuming single mitochondria of 1 μm diameter or less) suggested that significant O_2 gradients are not likely to occur either within cells or in the vicinity of a single mitochondrion[4,5]. However, comparison of the O_2 dependence of mitochondrial functions in isolated mitochondria and in freshly isolated cells from adult rat liver, heart and kidney showed that cells required approximately 10-fold higher O_2 concentrations for the same extent of mitochondrial cytochrome oxidation and O_2 consumption[2,13,14,19]. The difference in O_2 dependence in these two systems is not due to respiratory control since the difference remains after elimination of respiratory control by protonophores. Rather, the difference in O_2 dependence can be explained by the existence of intracellular O_2 concentration gradients. With hepatocytes exposed to 10 μM O_2, intracellular mitochondria respond as though they are exposed to only about 1.4 μM[10]. Thus, RR is about 0.86 for the space from the suspending medium to the mitochondria (regions A, B, and C in fig. 3). Compared to the RR given above for O_2 supply into the endoplasmic reticulum (0.12), these results show that the resistance to O_2 transport is greater into mitochondria than into endoplasmic reticulum of cells. This suggests that the greatest resistance to O_2 transport within these cells is associated with the mitochondria.

The resistance to O_2 transport could be due principally to a relatively low D_c or to the spatial distribution of mitochondria within the cytoskeleton. To distinguish between these possibilities, we determined the O_2 dependence of mitochondrial function in cells permeabilized with digitonin and reconstituted with ADP and respiratory substrates[10]. The results showed that mitochondria retained within the intact cytoskeleton required about 4-fold higher O_2 concentrations to produce the same extent of cytochrome oxidation as isolated mitochondria. At the P_{50} value in cells, the corresponding extent of oxidation of cytochrome *c* in digitonin-treated cells and in isolated mitochondria provide estimates of RR of 0.32 for diffusion into mitochondria (regions B–C, fig. 3) and 0.3 for transport through the unstirred layer, plasma membrane and aqueous cytoplasm (regions A–B, fig. 3). Thus, approximately half of the resistance to O_2 transport into the mitochondria under these conditions is a consequence of the spatial distribution of mitochondria.

The heterogenous distribution of mitochondria within cells determines the resistance to O_2 transport in the vicinity of the mitochondria. Mathematical analyses show that the cellular O_2 concentration dependence of mitochondria can be explained by the occurrence of clusters of mitochondria 3–4 across. The two-dimensional depictions from transmission electron microscopy suggest that three-dimensional aggregates also occur and can be relatively large in some cell types. Methodologies are now available for three-dimensional reconstructions of mitochondria[18] and need to be applied to quantify the extent of clustering of mitochondria in three dimensions for the cell preparations used in these studies. This is of critical importance because our recent results show that the relative resistance to oxygenation of mitochondria in cells changes in association with phenotypic changes[16].

Relative resistance to O_2 supply during acute and chronic hypoxia

Because of the very low Km_{O_2} for cytochrome oxidase, the O_2 consumption rate is preserved until very low O_2 concentrations occur within the vicinity of the mitochondria. Thus, as discussed above, substantial O_2 concentration gradients occur within the vicinity of the mitochondria and the relative resistance to O_2 supply is increased. This effect is readily apparent if one plots the RR for the O_2 supply path under normoxic and hypoxic conditions (fig. 4b). The RR is much larger in the vicinity of mitochondria under hypoxic conditions.

We have recently studied the O_2 dependence of mitochondrial function in hepatocytes from chronically hypoxic rats. The results (fig. 4b, right panel) show that the relative resistance to O_2 supply in the vicinity of the mitochondria is decreased in these cells (A. H. Sillau, T. Y. Aw and D. P. Jones, manuscript submitted). Thus, cells are adaptive during chronic hypoxia to reduce the RR to O_2 supply to mitochondria.

This effect may be due to a change in mitochondrial clustering (fig. 4a) which decreases the relative resistance in the vicinity of mitochondria. Costa et al.[8] found that liver mitochondria are not as extensively clustered in hypoxic liver as under normal conditions. Apparently, the cellular O_2 concentration dependence is changed by redistribution of mitochondria[16]. This could occur by either disruption of clustering or reduction of cluster size (fig. 4a), but morphometric data are not available on this point.

A 'control strength' description of O_2 supply

Unlike most metabolic pathways where a single rate-determining step can control flux, all steps in the supply path for O_2 can ultimately affect the O_2 supply to the mitochondria. An analogous situation occurs under normal conditions with mitochondria because mitochondrial respiratory rate is affected by many factors such that no single component is rate determining[26]. To quantify the role of the different sites in rate determination of this process, *control strength* analyses have been introduced which define the relative importance of different

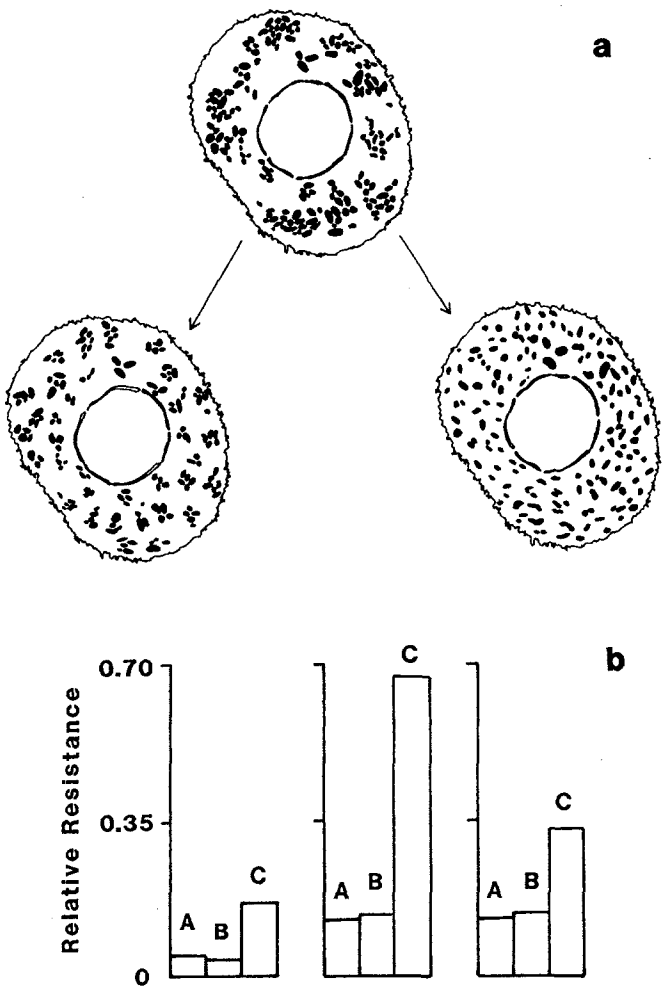

Figure 4. Possible patterns of mitochondrial redistribution in hepatocytes from chronically hypoxic rats (a). Data of Costa et al.[8] show that mitochondria are less clustered in the cells from hypoxic animals. Sillau et al.[25] found that the cellular O_2 dependence was consistent with a decrease in clustering of mitochondria. In this figure, the two possible modes of redistribution are illustrated, namely, a retention of clustering with a reduction in cluster size (left) and a loss of clustering (right); *b* The relative resistance to O_2 supply in cells from normoxic animals exposed to normoxia (left) and hypoxia (middle) and the compensatory effect on relative resistance during hypoxia due to redistribution of mitochondria following chronic hypoxia (right). The relative resistances were calculated from data of Jones[10] and Sillau et al.[25] for the unstirred layer (A), the plasma membrane and cytoplasm (B) and the mitochondria (C). The results show that the relative resistance in the region of mitochondria is increased in cells from normoxic animals due to exposure to hypoxia in vitro. In the right panel, exposure of cells from chronically hypoxic animals to the same extent of hypoxia in vitro resulted in a substantially smaller relative resistance to supply to the mitochondria.

sites[17, 24]. In this analysis, large changes in inhibition at specific sites result in a relatively small change in flux. The implication of this type of regulation is that by sharing rate determination among many sites, the system has maximal stability. A large inhibition at one site can be compensated by adjustments at other sites to maintain near-normal flux.

An underlying principle of O_2 supply may also involve shared rate determination such that many sites contribute to flux limitation. This would minimize the deleterious consequences of rate limitation at any individual site because compensations at other sites could stabilize flux. Thus, both in adaptation and acclimatization, the general rule may be that multiple sites of the path of O_2 supply contribute to supply limitation. This would minimize the impact of impaired function at one site because it would provide compensation by a relative improvement in function at other sites.

Summary and perspectives

Studies of O_2 supply to mitochondria in intact cells show that the cellular O_2 dependence is modulated by the density and clustering of mitochondria. Of particular importance, supply of O_2 to mitochondria becomes diffusion-limited under hypoxic conditions. To compare the importance of resistance at different sites along the supply path, the *relative resistance* for a given region is defined as the ratio of the concentration gradient across that region to the maximal concentration in that region. Under normoxic conditions, the relative resistance is similar in different regions of the supply path. Under various pathophysiological conditions, this resistance increases in specific regions of the pulmonary, circulatory or cellular supply path and results in O_2 deficiency to the mitochondria. Regardless of cause, the relative resistance increases dramatically in the vicinity of mitochondrial clusters during hypoxia. Acclimatization to chronic hypoxia results in decreased mitochondrial clustering and a decrease in the relative resistance to O_2 supply in mitochondria. Thus, supply of O_2 to mitochondria within cells is important in the overall resistance to O_2 supply in mammalian systems. Future studies are needed to quantitatively define the relative resistances throughout the supply path under relevant physiological and pathological conditions.

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Human muscle structure after exposure to extreme altitude

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Summary. Muscle structural changes during typical mountaineering expeditions to the Himalayas were assessed on muscle biopsies. A significant reduction in muscle fiber size (−20%) and a loss of muscle oxidative capacity (−25%) were observed. The capillary network was not affected by catabolism. It is concluded that the oxygen supply to muscle mitochondria after high altitude exposure is thus improved.
Key words. High altitude; hypoxia; muscle; computed tomography; capillaries; fiber size; human; exercise.

Introduction

Participants in mountaineering expeditions to the Himalayas are exposed to chronic hypoxia at about 5000 m altitude (base camp) for several weeks, with occasional excursions (with or without supplementary oxygen) to altitudes in excess of 8000 m. The adaptational consequences of this type of exposure to high altitude for the cardiovascular system have been well studied (see reviews by Sutton et al.[15], Cerretelli and di Prampero[4] and Banchero[1]). However, little is known in humans about structural adaptations of skeletal muscle tissue as a consequence of high altitude exposure. From animal experimentation it would appear that chronic exposure to hypoxia leads to an increase in muscle tissue capillarity[9] and oxidative capacity[7]. These generally held assertions have been questioned, mainly on the grounds that there were technical limitations, and confounding variables such as simultaneous exposition to hypoxia and cold, in some of the older studies[1, 13]. Banchero[1] comes to the conclusion that chronic normothermic hypoxia has no influence on skeletal muscle capillarity in sedentary laboratory animals, and that exercise or cold exposure must further challenge the oxygen delivery system before measurable structural adaptations can take place in muscle tissue.

With regard to human muscle adaptations to high altitude, experimental data are rather scarce. Short exposure to 4300 m (18 days) has no effect on oxidative and glycolytic enzyme activities measured in biopsies of M. vastus lateralis[16]. However, there is an almost complete lack of structural data on adaptations of human skeletal muscle tissue to continued exposure to the stresses of high altitude.

Methods

$\dot{V}_{O_2}$max tests, computed tomographies and muscle biopsies were obtained before and 10–15 days after the return of expeditions to the Himalayas, one in 1981 and one in