# Skeletal muscle: microcirculatory adaptation to metabolic demand

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**ABSTRACT**

HEPPLE, R. T. Skeletal muscle: microcirculatory adaptation to metabolic demand. *Med. Sci. Sports Exerc.*, Vol. 32, No. 1, pp. 117–123, 2000. The issue of whether skeletal muscle is master or slave of the cardiovascular system depends on frame of reference. Acute manipulations of convective $O_2$ delivery clearly show that $O_2$ supply sets the upper limit of muscle $\dot{V}O_{2max}$. However, studies of adaptation to chronic conditions such as training and hypoxia show that skeletal muscle has a remarkable capacity to meet changes in metabolic demand. Moreover, there are several lines of evidence that these adaptations are essential to changes in $\dot{V}O_{2max}$. Studies show that with training, electrical stimulation, and chronic hypoxia, the ratio of capillary surface per fiber surface and fiber mitochondrial volume/fiber length is preserved, suggesting a primary regulated feature in skeletal muscle is matching the structural capacity for $O_2$ flux to mitochondrial metabolic demand. Adaptations in both capillarity and mitochondrial respiratory capacity have also been shown to be important components in the adaptive increase in $\dot{V}O_{2max}$ with training. Collectively, this evidence argues against skeletal muscle being simply a slave to the cardiovascular system. **Key Words:** CAPILLARIZATION, MITOCHONDRIA, $\dot{V}O_{2max}$, PLASTICITY, HYPOXIA, TRAINING

The view one takes on whether skeletal muscle is the slave of the cardiovascular system depends on frame of reference. For example, the presence of a muscle diffusing capacity limitation to muscle $\dot{V}O_{2peak}$ has often been considered evidence of $O_2$-supply limitation of the mitochondria (e.g., 65). The contrary view has also been presented that because after training within a species, and across species representing a wide aerobic scope, the variable resistors from lung to skeletal muscle mitochondria increase in proportion to $\dot{V}O_{2max}$, there is a symmorphosis rather than a single-step limitation (e.g., 44,72). Whereas studies employing acutely altered $O_2$ delivery clearly demonstrate the fundamental role of $O_2$ in determining skeletal muscle aerobic function (2,39,75), the danger in thinking that skeletal muscle is, therefore, a slave to the cardiovascular system is that it undermines the importance of skeletal muscle plasticity in the adaptive variability of $\dot{V}O_{2max}$ both within and between species. In this respect, the highly adaptable nature of skeletal muscle in response to altering metabolic demand argues against its often inferred role as slave to the cardiovascular system. Moreover, as will be shown, there is considerable evidence that these adaptations are vital to the adaptation in $\dot{V}O_{2max}$.

Before considering how muscle adapts to metabolic demand, a brief description of some of the design features of skeletal muscle will be provided, focusing on the structure of the capillary bed and fiber mitochondria.

**Microvascular design.** In skeletal muscle of mammals, the capillaries run largely parallel to the longitudinal axis of muscle fibers, exhibiting a degree of tortuosity and branching that contributes to their overall length (49,63). The degree of tortuosity varies as a function of muscle fiber sarcomere length (49) and is an important determinant of both erythrocyte hemodynamics (62) and total capillary surface area (50). Although some early investigations suggested the contrary, recent evidence indicates that neither endurance training (61), nor more intense muscle activation using chronic electrical stimulation (52), alters the degree of tortuosity. Thus, adaptation in the size of the capillary bed occurs by increasing capillary number rather than the degree of capillary tortuosity (52,61). Similarly, the degree of tortuosity is not a function of muscle aerobic capacity *per se* because, for example, the very aerobic hummingbird flight muscle has a lower capillary tortuosity than mammalian limb muscle (e.g., rat soleus muscle) (54).

**Mitochondrial design.** Skeletal muscle mitochondria have often been described as spheroid organelles that increase both in size and number with adaptation to training. However, this view is evolving due to recent investigations showing that mitochondrial structure can be quite complex, and varies considerably among fiber types and even between species (58,59). For example, there is a progressive increase in the complexity of mitochondrial structure with increasing muscle fiber mitochondrial content, with mitochondria in the most aerobic muscle fibers having considerable interconnections between what would appear on a transverse section of muscle to be distinct mitochondria

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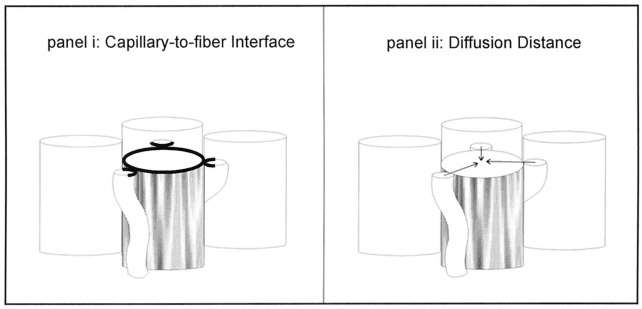


**Figure 1—Muscle capillary-to-fiber interface and diffusion distance. Left panel shows that the size of the capillary-to-fiber interface is determined by the relative proportion of capillary surface to fiber surface (represented in bold on transverse section). A larger capillary-to-fiber interface can be achieved by increasing capillary per fiber number, reducing fiber size, or both. Right panel highlights the radial diffusion distance for $O_2$ in moving from capillaries to the most distant mitochondria. Intercapillary diffusion distance can be reduced by increasing capillary per fiber number; however, this has little effect on maximal diffusion distance since the latter is determined primarily by fiber cross-sectional area.**

(37,38,58,59). This interconnection has also been found between subsarcolemmal and interfibrillar mitochondria, although the extent appears quite variable (37,38). Moreover, muscles having a high mitochondrial volume density, such as rat diaphragm (mitochondrial volume density $\geq$ 20%), demonstrate evidence suggesting a single mitochondrial reticulum (4). It is also noteworthy that the highly interconnected network between the mitochondria and endoplasmic reticulum in HeLa cells has been found to conduct intracellular $Ca^{2+}$ fluxes (67), suggesting that these complex structural arrangements are important to mitochondrial function.

Although there may be interconnection between subsarcolemmal and intermyofibrillar mitochondria, the biochemical features of these mitochondrial subpopulations appear to be different (9,13,71). There have also been some data to suggest biochemical differences between mitochondria isolated from fast twitch glycolytic muscle fibers and those isolated from slow twitch muscle fibers (36). Despite our growing appreciation for mitochondrial specialization between and within muscle fibers, the functional consequences remain relatively unknown.

**$O_2$ diffusion from capillary to muscle fiber mitochondria.** The steep $O_2$ gradient between red cell and muscle fiber interior suggests a resistance to the diffusion of $O_2$ in the short distance between red cell and the first few micrometers inside the muscle fiber sarcolemma (30,66). Consistent with this notion, the size of the capillary-to-fiber interface (Fig. 1), rather than capillary density (which incorporates diffusion distance), has been shown to be of primary importance when considering the structural capacity for $O_2$ flux (54). Increasing the number of capillaries around a fiber increases the size of the capillary-to-fiber interface (assuming no change in fiber size), which is thought to enhance $O_2$ conductance into muscle fibers (21,60). Structural data obtained by Mathieu-Costello and colleagues in the very aerobic hummingbird and bat flight muscles supported the notion that the size of the capillary-to-fiber interface plays an important role in determining $O_2$ flux rates in aerobic muscles (50). They also pointed to the role of a small fiber size (as seen in the very aerobic flight muscle) in maximizing the size of the capillary-to-fiber interface for the volume of mitochondria in the muscle fibers, besides reducing diffusion distances (54). The concept that the capillary-to-fiber interface is a major site of resistance for $O_2$ flux is also consistent with evidence supporting a diffusion limitation to $\dot{V}O_{2max}$ (69,74,75), described in this symposium by Dr. Richardson.

## MATCHING MICROCIRCULATORY STRUCTURE TO METABOLIC DEMAND

If mitochondria are important in determining muscle $\dot{V}O_{2max}$, we would expect that mitochondria should demonstrate a close relationship to the structural capacity for $O_2$ flux into muscle fibers. Whereas the early work of Ranvier (64) and Krogh (40) showed more oxidative muscles have a greater capillary supply, not all studies have found this to be the case (56). Part of this discrepancy was due to the use of indices of capillarity that did not account for the interaction between sarcomere length and the degree of capillary tortuosity, which is well-known to introduce considerable variability into measurements such as capillary density (e.g., 49). In this respect, there have been substantial advancements in the methods available to describe capillary geometry in recent yr that have dramatically improved our understanding of the relationships between capillarity and fiber mitochondrial volume (50). For example, as first reported by Hoppeler and Kayar across locomotory muscles, diaphragm, and heart (32), there is a robust relationship observed between mitochondrial volume per fiber volume and capillary length per fiber volume (Fig. 2) across a broad range of skeletal muscle aerobic capacity (55). Capillary length per fiber volume takes into account the length added to capillaries by tortuosity and branching, and is an important determinant of both capillary surface area and red cell path length (50). That a relationship between capillary

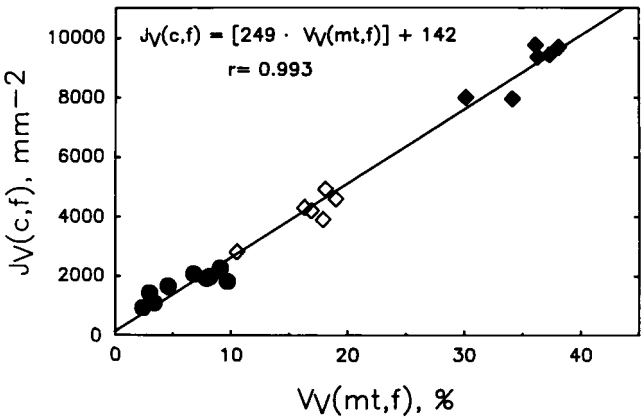


**Figure 2—Capillary length per fiber volume, $J_V(c,f)$, as a function of mitochondrial volume per fiber volume, $V_V(m,f)$, across a broad scope of muscle aerobic capacity. Reproduced with permission from Mathieu-Costello, O., J. M. Szewczak, R. B. Logemann, and P. J. Agey. Geometry of blood-tissue exchange in bat flight muscle compared with bat hindlimb and rat soleus muscle. *Am. J. Physiol.* 262*(Regul. Integr. Compar. Physiol.)*:R955–R965, 1992.**

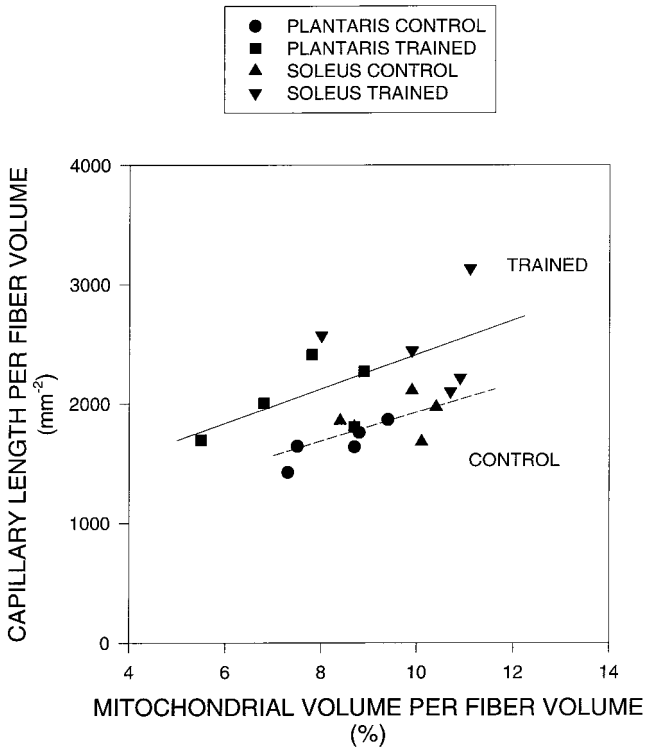


**Figure 3—Capillary length per fiber volume as a function of mitochondrial volume per fiber volume before and after endurance training in rat soleus and plantaris muscles. Reproduced from Poole, D. C., and O. Mathieu-Costello. Relationship between fiber capillarization and mitochondrial volume density in control and trained rat soleus and plantaris muscles. *Microcirculation* 3:175–186, 1996, by permission of Stockton Press for The Microcirculatory Society Inc.**

length per fiber volume and mitochondrial volume per fiber volume should exist is, therefore, intuitively reasonable. However, note that the intercept of the relationship changes with adaptation to exercise training, such that there is a greater capillary length per fiber volume for a given mitochondrial volume per fiber volume compared to untrained muscles (60) (Fig. 3). Thus, although this is a strong relationship, it is apparently not one that is preserved in adapting to increased $O_2$ demand within a species.

The importance of the capillary-to-fiber interface to $O_2$ flux was mentioned in the previous section, and there have been two recent investigations considering changes in mitochondria and capillaries from this perspective. Poole and Mathieu-Costello (60) showed that although capillary length per fiber volume was increased for a given mitochondrial volume per fiber volume after endurance training in rats, the relationship between the size of the capillary-to-fiber interface and mitochondrial volume/fiber length was maintained (Fig. 4) and not different in muscles of different fiber type composition (e.g., plantaris vs soleus), suggesting this may be the primary regulated feature between capillaries and mitochondria. Mathieu-Costello et al. (52) showed that this was also true after more intense muscle activation through chronic electrical stimulation, yielding a two- to three-fold increase in capillary length per fiber volume and mitochondrial volume per fiber volume in rat fast-twitch muscles (extensor digitorum longus and tibialis anterior muscles). Thus, although capillaries serve numerous functions, including delivery of metabolic substrates (e.g., glucose, free-fatty acids, and $O_2$) and removal of metabolic by-products (e.g., $CO_2$ and lactic acid), the degree of capillarity is regulated foremost as function of $O_2$ demand irrespective of fiber type composition in skeletal muscle (52,73).

## PLASTICITY OF SKELETAL MUSCLE WITH CHANGES IN METABOLIC DEMAND

Skeletal muscle is an extremely adaptable organ, demonstrating impressive structural and functional plasticity in response to alterations in metabolic demand. The diversity of muscle adaptation will be explored through examination of skeletal muscle structure and function with adaptation to exercise training and chronic hypoxia.

**Microcirculatory changes with training in older humans.** The microcirculation, capillaries in particular, play a key role in $O_2$ delivery to muscle fibers. As mentioned above, a significant resistance to the diffusion of $O_2$ into muscle fibers was shown to occur at the capillary-to-fiber interface. As such, increased capillarization has been argued to be of fundamental importance to increasing $O_2$ conductance after training (5,60). This notion is consistent with the results of many studies that have shown increased muscle capillarity after training in young adults (1,31,35) and the results of Ingjer (34), who showed previously that the number of capillaries around a fiber is proportional to differences in $\dot{V}O_{2max}$ across a spectrum of training levels in young adults.

Aging causes a progressive decline in $\dot{V}O_{2max}$ that is associated with decrements in function of virtually all components of the respiratory system, from lung to skeletal

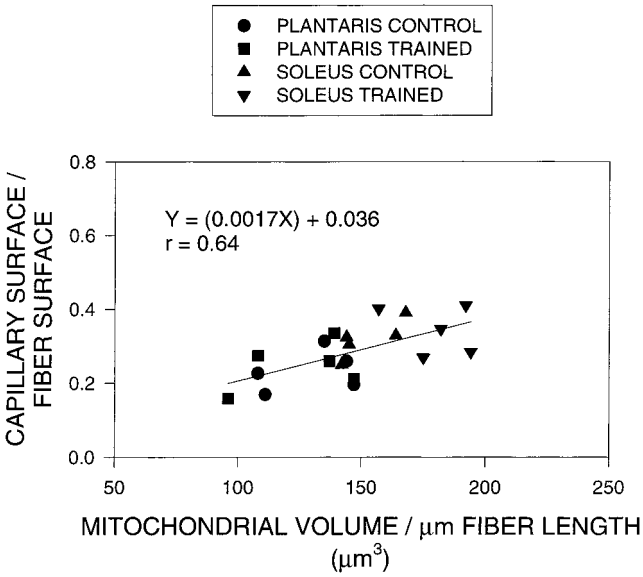


**Figure 4—Capillary surface per fiber surface as a function of mitochondrial volume/fiber length before and after endurance training. Reproduced from Poole, D. C., and O. Mathieu-Costello. Relationship between fiber capillarization and mitochondrial volume density in control and trained rat soleus and plantaris muscles. *Microcirculation* 3:175–186, 1996, by permission of Stockton Press for The Microcirculatory Society Inc.**

muscle mitochondria. These changes are due to both aging processes *per se* and a significant decline in habitual physical activity with aging (28). Among the changes found in skeletal muscle are a reduction in muscle fiber number and cross-sectional area (27,42) [note: some animal models deviate in this respect, e.g., the Fisher 344 rat (12,43)], and impaired mitochondrial function (16,45,76).

Interestingly, whereas $\dot{V}O_{2max}$ is only increased after endurance training in young adults (24,33), both endurance (22,47,70) and resistance training (15,23) increase $\dot{V}O_{2max}$ in older humans. Despite the well-known changes in skeletal muscle with aging, training studies have demonstrated that skeletal muscle retains considerable plasticity in aging humans and other animals (8,48,79). Recently, we examined the role plasticity in muscle fiber capillarity played in the adaptive response to training in older men (22,23). We assigned 18 older men (68 ± 1 y) to 1 of 2 training groups, taking biopsies from the vastus lateralis muscle and conducting maximal cycle exercise tests on each subject before and after training. One group underwent 9 wk of high-intensity resistance training of the legs and then 9 wk of aerobic training on a cycle ergometer. The other group underwent two consecutive 9-wk periods of aerobic training on a cycle ergometer. We found that after 18 wk of training, both groups increased their $\dot{V}O_{2max}$ to a similar extent (≈17%), suggesting there was no synergistic effect of resistance and aerobic training on changes in $\dot{V}O_{2max}$ in this population. Both groups also demonstrated significant increases in capillary-to-fiber ratio; however, given the importance of the capillary-to-fiber interface to $O_2$ flux (54), we wanted to examine how changes in $\dot{V}O_{2max}$ related to changes in capillarity using a method for estimating the size of the capillary-to-fiber interface on biopsy material (19), and compare this to the relationship of $\dot{V}O_{2max}$ and capillary density (a determinant of intercapillary diffusion distance). This analysis revealed that whereas only endurance training increased capillary density, both resistance and endurance training increased the size of the capillary-to-fiber interface, suggesting an increased structural capacity for $O_2$ flux after

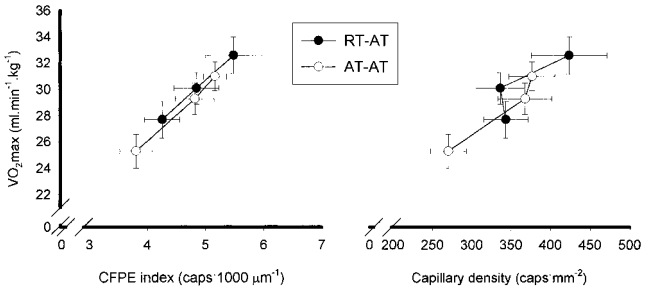


**Figure 5—Changes in $\dot{V}O_{2max}$ as a function of changes in the CFPE index (a measure of the size of the capillary-to-fiber interface for biopsy material) and capillary density in older men after resistance and/or aerobic training. RT-AT: group that underwent 9 wk of high-intensity resistance training of the legs followed by 9 wk of aerobic training on a cycle; AT-AT: group that underwent two consecutive 9 wk periods of aerobic training on a cycle. Adapted from ref. 22. Hepple, R. T., S. L. M. MacKinnon, J. M. Goodman, S. G. Thomas, and M. J. Plyley. Resistance and aerobic training in older men: effects on $VO_2$peak and the capillary supply to skeletal muscle. *J. Appl. Physiol.* 82:1305–1310, 1997.**

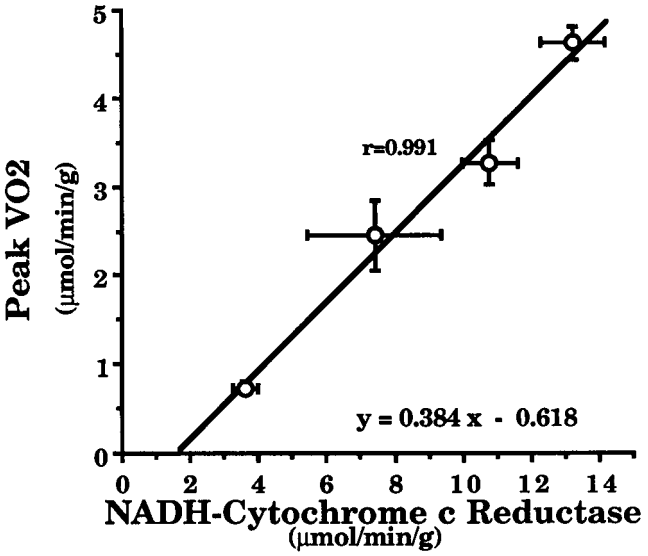


**Figure 6—Peak muscle $\dot{V}O_2$ as a function of mitochondrial complex III (NADH-cytochrome c reductase) activity in the perfused rat hindlimb. Reproduced with permission from McAllister, R. M and R. L. Terjung. Acute inhibition of respiratory capacity of muscle reduces peak oxygen consumption. *Am. J. Physiol. (Cell Physiol. 28)* 259:C889–C896, 1990.**

both types of training (22). Consistent with this interpretation, changes in $\dot{V}O_{2max}$ were found to parallel changes in the size of the capillary-to-fiber interface (but not capillary density), regardless of training modality, suggesting a common feature in the adaptive response to resistance and aerobic training in this population (Fig. 5). Furthermore, these data provided functional evidence that changes in the structural capacity for $O_2$ flux are related to the size of the capillary-to-fiber interface rather than changes in diffusion distance *per se* in older humans.

**Mitochondrial electron transport chain activity and $\dot{V}O_{2max}$.** One of the conclusions often drawn from investigations showing increased $\dot{V}O_{2max}$ with increased $O_2$ delivery is that mitochondrial oxidative capacity is excessive. However, this conclusion is not consistent with results obtained when mitochondrial electron transport chain (ETC) capacity is experimentally reduced. McAllister and Terjung (57) found that in untrained rats peak muscle $\dot{V}O_2$ decreased proportionally with reductions in complex III (NADH-cytochrome c reductase) enzyme activity (Fig. 6) and that there was no excessive ETC capacity that could be inhibited without reducing peak muscle $\dot{V}O_2$. Furthermore, when complex III activity was reduced to pretraining levels in endurance trained rats, the training-induced increase in peak muscle $\dot{V}O_2$ also returned to pretraining levels, suggesting that an increased mitochondrial ETC activity is essential to realize an increase in $\dot{V}O_{2max}$ after training (68). It has also been pointed out previously (17) that the mass-specific blood flows (and thus, $O_2$ delivery) achieved using this model (the isolated perfused rat hind limb) are well below those seen with a less invasive *in situ* rat preparation (46) or *in vivo* during maximal treadmill running in rats (41). Taken together, these results suggest that mitochondrial ETC capacity is not excessive even when blood flow and $O_2$

delivery are less than maximal (68), and, thus, the nature of the interaction between mitochondrial oxidative capacity and $O_2$ delivery in determining $\dot{V}O_{2max}$ warrants further study.

One explanation for these apparently disparate findings concerns similarities in the effect of $O_2$ and mitochondrial content on respiratory control in muscle (e.g., 25). It is known that a given absolute level of submaximal exercise (and thus, $\dot{V}O_2$) in hypoxia causes a greater perturbation of intracellular metabolites such as phosphocreatine (PCr) and the ADP/ATP ratio (3,18,77,78). In contrast, the same absolute level of exercise in hyperoxia causes a smaller perturbation of these metabolites than in normoxia or hypoxia (18,26). As pointed out by Hogan et al. (25), the effect of altering $O_2$ supply on muscle metabolism is the same as that seen when comparing muscles of a different mitochondrial content, i.e., muscles having greater mitochondrial content exhibit a smaller metabolic perturbation for a given $\dot{V}O_2$ (11). Similarly, the experiments of McAlister and Terjung (57) showed that poisoning complex III of the ETC also results in a greater metabolic perturbation (based on a greater muscle lactate efflux) for a given submaximal $\dot{V}O_2$. If the failure to maintain intracellular homeostasis plays a role in determining muscle $\dot{V}O_{2max}$ (29), then the tighter respiratory control obtained by an elevated mitochondrial content after training may be a possible mechanism by which mitochondria contribute to an increased $\dot{V}O_{2max}$ after training (68), independent of $O_2$ availability (e.g., hyperoxia, hypoxia, or normoxia). Similarly, the increased $\dot{V}O_{2max}$ with increases in $O_2$ delivery may be due to the tighter mitochondrial respiratory control observed when mitochondria are better oxygenated. In this paradigm, $O_2$ availability modifies rather than limits muscle $\dot{V}O_{2max}$, because mitochondria exert an effect on $\dot{V}O_{2max}$ independent of $O_2$ supply *per se*.

**Adaptation to hypoxia.** Despite considerable evidence that adaptation to hypoxia does not increase capillary per fiber number or fiber mitochondrial content in mammals (7), intermittent hypoxia coupled with exercise does stimulate capillary proliferation and mitochondrial adaptation. For example, endurance training with hypoxia (10) or ischemia (14) in humans induces greater increases in capillary-to-fiber ratio and mitochondrial volume and enzyme activity than the same exercise stimulus under normoxic or nonischemic conditions. Similarly, endurance training in rats chronically exposed to hypoxia (barometric pressure = 463 torr) induces a greater increase in capillary-to-fiber ratio than the same training at sea level (6).

Recent findings in birds have shown an increased capillary-to-fiber ratio, altered capillary geometry, and increased fiber mitochondrial volume in flight muscle of pigeons kept at altitude (3800 m, inspired $PO_2$ = 91 torr) for 5 months (51), and in finches living and flying at 4000 m compared with sea-level finches of the same subfamily (Cardulinae) (53). Capillary geometry in flight muscle of birds demonstrates an interesting branching pattern where venular capillaries run perpendicular to the longitudinal axis of the muscle fibers, forming capillary manifolds (63). The change in capillary geometry seen in flight muscle of pigeons kept in hypoxia for 5 months and in finches living and flying at 4000 m included a greater increase in venular than arterial capillaries, consistent with a greater stimulus for adaptation at the lower $PO_2$ of venular capillaries (53). To determine the role of muscle aerobic capacity in the adaptation of both capillary per fiber number and capillary geometry in altitude finches, the less aerobic leg muscle was recently compared with the flight muscle in the altitude finches (20). Interestingly, whereas capillary-to-fiber ratio was also greater in leg muscle of altitude finches (as in flight muscle), there was no change in capillary geometry in leg muscle. Thus, although the relative metabolic demands of both flying and hopping are greater in the hypoxia of altitude than at sea level, the higher absolute metabolic demand of flight muscle appears to cause a concomitant change in capillary geometry (i.e., an increased proportion of venular capillary branches). This illustrates a remarkable specificity of adaptation between muscles within a given organism to meet the particular metabolic demands encountered while flying and hopping in this hypoxic environment (20). In addition, these results in birds, in conjunction with those of Bigard et al. in rats (6), suggest that the level of activity (i.e., muscle use) plays an important role in determining skeletal muscle adaptations to chronic hypoxia (53).

Another interesting feature of the adaptation to hypoxia in birds is that, similar to what is seen with endurance training (60) and electrical stimulation (52) in rat muscle, the ratio of capillary surface per fiber surface and mitochondrial volume/fiber length is maintained in pigeons kept in hypoxia for 5 months (51) and in both the flight (53) and leg (20) muscle of altitude finches, compared with their sea-level counterparts. If mitochondria were solely $O_2$-supply dependent, we might expect a relatively greater adaptation in the capillary surface per fiber surface to compensate for the lower $PO_2$ in hypoxia. This shows that capillarity is regulated as a function of fiber metabolic demand, irrespective of the $O_2$ supply conditions (e.g., normoxia or hypoxia) (53), and supports the idea that adaptations in mitochondria are important to the adaptive response independent of $O_2$ supply *per se*.

## CONCLUSIONS

Our understanding of the limitations to $\dot{V}O_{2max}$ has gradually evolved to appreciate the complex interplay of convective $O_2$ delivery, $O_2$ diffusional conductance, and muscle metabolic factors. In this respect, the central role of $O_2$ delivery has to be acknowledged, for it is obvious a muscle can use no more $O_2$ than it receives. Furthermore, the increase in $\dot{V}O_{2max}$ and, thus, mitochondrial respiratory rate, with acutely increased $O_2$ delivery clearly demonstrates that $O_2$ availability to the mitochondria certainly sets the upper limit of $\dot{V}O_{2max}$. However, these results do not exclude independent effects of mitochondria, which by setting the $O_2$ demand, and capillaries, which by determining $O_2$ conductance, contribute to determining the maximal $O_2$ extraction possible for a given level of $O_2$ delivery. In this respect, there is complementary adaptation in capillaries and mitochondria, demonstrated by a constant ratio of the size of the

capillary-to-fiber interface and mitochondrial volume/fiber length with adaptation to training, electrical stimulation, and chronic hypoxia.

The above examples illustrate only a sampling of the scope of skeletal muscle plasticity. This plasticity is clearly an important and powerful part of an organism's adaptive reserve/response. Whereas acute studies of altered convective $O_2$ delivery might suggest a skeletal muscle that is at the mercy of the cardiovascular system, examination of skeletal muscle adaptation to chronic processes, such as exercise training and hypoxia, shows a structure with a remarkable capacity to adapt to the demands imposed upon it. Given this perspective, skeletal muscle, although certainly dependent on the cardiovascular system for $O_2$ supply, is master of its own domain.

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