

## DIFFERENCES IN METABOLIC POTENTIAL OF SKELETAL MUSCLE FIBRES AND THEIR SIGNIFICANCE FOR METABOLIC CONTROL

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### SUMMARY

The role of an increase in oxidative potential of skeletal muscle in the enhanced work capacity and greater use of fat as a fuel after endurance training is discussed. Evidence is presented to illustrate that this adaptive response is probably expressed at the cellular level by a more rapid translocation into the mitochondria of the ADP generated during contractile activity. The consequence of this is a tighter control over the glycolytic process thereby creating more favourable conditions for the entry of acetyl units derived from  $\beta$ -oxidation of fatty acids into the citric acid cycle.

When exposed to chronic overloads skeletal muscle is remodelled, thereby equipping it to better meet the stresses it is encountering. Examples abound in nature of the adaptive response of muscle exposed to different tasks (i.e. Armstrong, Ianuzzo & Kutz, 1977; Newsholme & Start, 1973). The principles that are outlined in this paper for the relationship between metabolic potential and control of metabolism apply to all situations where differences in metabolic potential exist. The basic model discussed is that of physical training, particularly endurance training, on skeletal muscle and metabolic control. Training was selected as the model as it is a convenient method for inducing changes in skeletal muscle and for illustrating the consequences of these adaptations on the local and total body metabolism. Thus, heavy resistance exercise taxes the tension development capacity but not prolonged energy production so the fibres enlarge without a significant modification in the metabolic systems (Gollnick *et al.* 1972). In contrast, endurance training stresses energy production, particularly aerobic energy production, but not the tension developing capacity, with the end result being augmentation of the aerobic energy-producing systems. Corollaries of these exist throughout the animal kingdom.

Key words: Metabolic control, mitochondria, training, adaptation.

## METABOLIC ADAPTATION TO ENDURANCE TRAINING

With endurance training there are increases in the mitochondrial enzymes for  $\beta$ -oxidation, the citric acid cycle and the respiratory chain as well as increases in capillary density (Saltin & Gollnick, 1983). The total body maximal oxygen uptake ( $\dot{V}_{O_2\max}$ ) is increased by training (Rowell, 1974) but the increase (15–20 %) is less than that (two-fold or more) in metabolic potential that can occur in skeletal muscle with endurance training (Saltin & Gollnick, 1983). The increased  $\dot{V}_{O_2\max}$  after endurance training is attributable to the combination of a greater cardiac output and greater extraction of oxygen from the blood (Rowell, 1974). The widening of the arteriovenous oxygen difference may be due to the elevated concentrations of mitochondrial enzymes or to an increased capillary density of the muscles (Andersen & Henriksson, 1977; Hermansen & Wachtlova, 1971). Evidence for the dissociation of the changes in  $\dot{V}_{O_2\max}$  from alterations in the enzymes for terminal oxidation of skeletal muscle comes from the observations that although changes in  $\dot{V}_{O_2\max}$  and mitochondrial enzymes of muscle occur in parallel early in training, with continued training  $\dot{V}_{O_2\max}$  levels off while the concentration of oxidative enzymes continues to rise (Henriksson & Reitman, 1977). Upon termination of training the concentration of mitochondrial enzymes in muscle declines rapidly while the  $\dot{V}_{O_2\max}$  remains unchanged (Henriksson & Reitman, 1977). In the rat, the  $\dot{V}_{O_2\max}$  increased both with endurance and sprint training whereas oxidative potential of muscle and exercise capacity were elevated only with endurance training (Fig. 1) (Davies, Packer & Brooks, 1981, 1982).

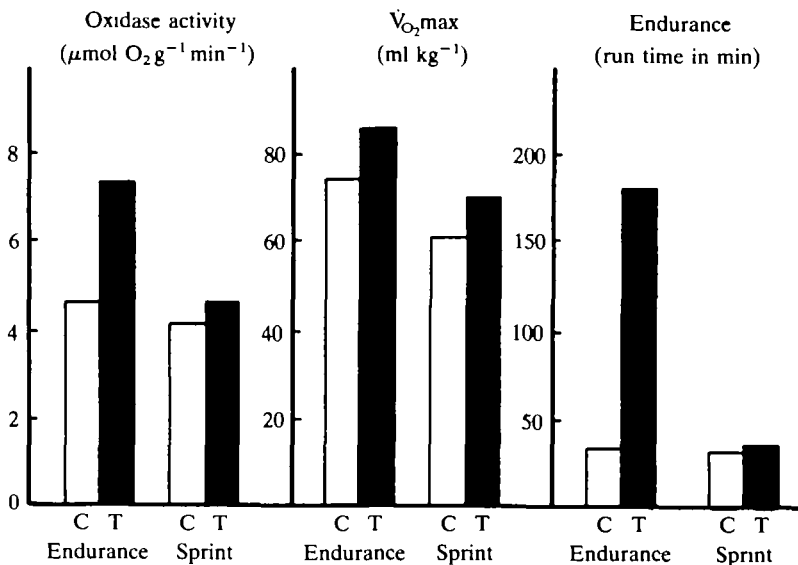


Fig. 1.  $\dot{V}_{O_2\max}$ , oxidative potential of muscle (pyruvate-succinate as substrate) and endurance capacity of rats after endurance or sprint training. (From Davies, Packer & Brooks, 1981, 1982.) C, control rats; T, trained rats.

## INTERPLAY OF ADAPTATIONS TO ENDURANCE TRAINING

The above relationships raise the question of how the disproportionate increase in mitochondrial concentration of skeletal muscle induced by endurance training is important during exercise. To answer this question it is necessary to compare the physiological and metabolic responses to exercise both before and after endurance training. Here it is recognized that most of the activity of endurance-trained individuals is at an intensity below  $\dot{V}_{O_2\max}$  and that increases in mitochondrial enzymes in skeletal muscle are evoked by training programmes with intensities below  $\dot{V}_{O_2\max}$ . For exercise at the same power output ( $\dot{V}_{O_2}$ ), the major differences between the endurance-trained and non-trained state are: (1) a three- to four-fold increase in work capacity (time to exhaustion) (Fig. 1); (2) a greater use of fat as a fuel during the exercise (Saltin *et al.* 1978; Henriksson, 1977); (3) a slower rate of glycogen utilization (Karlsson, Nordesjo & Saltin, 1974; Baldwin *et al.* 1975); (4) lower concentrations of lactate in blood and muscle (Karlsson *et al.* 1974; Saltin *et al.* 1978); and (5) an uptake rather than release of lactate from the exercising muscle (Saltin *et al.* 1978). These differences exist while the oxygen uptake and cardiac output are the same before and after training (Freedman *et al.* 1955; Saltin *et al.* 1968) and blood flow to the exercising muscle at the same oxygen consumption may not be altered by training. Since the  $\dot{V}_{O_2}$  at a given power output is constant, ATP utilization and ADP production must be the same for such exercise.

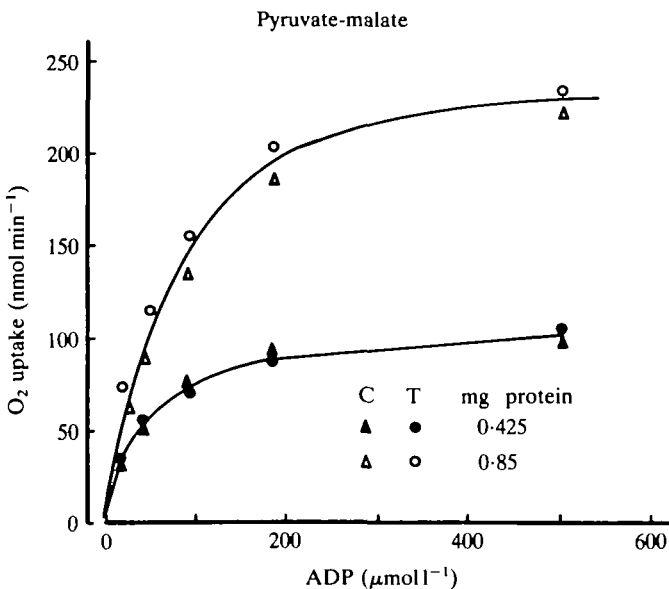


Fig. 2. The relationship between ADP concentration and reaction velocity of mitochondria prepared from the skeletal muscle of endurance trained and sedentary rats with pyruvate-malate as substrate. Responses with two concentrations of mitochondrial protein are presented to illustrate the effect that this would have *in vivo*. C, control rats; T, trained rats.

The close relationship between the oxidative potential of muscle and exercise capacity following endurance training led Gollnick & Saltin (1982) to hypothesize that the increased mitochondrial concentration of muscle exerts itself *via* a tighter regulation of the choice of fuel being oxidized. This postulation was based on a consideration of enzyme kinetics and how enzyme concentration influences the sensitivity for substrate control of individually catalysed steps. The influence of an elevated mitochondrial concentration was related to a tighter control of the Embden-Meyerhof pathway for glycogen degradation resulting in a greater use of fat. Since depletion of the intramuscular glycogen store is an event associated with the onset of fatigue, the reduced rate of glycogen depletion and the enhanced use of fat explain the ability of endurance training to extend work time at submaximal workloads. The principles of metabolic control and changes in the sensitivity of this control important for this topic have been reviewed by Kacser & Burns (1979). In addition, some data relating enzyme concentration and enzyme regulation have been published (Gollnick, 1983).

#### SUBSTRATE CONTROL OF REACTION VELOCITY FOR INDIVIDUAL ENZYME SYSTEMS

Some elements of metabolic control are presented in Fig. 2. It illustrates the relationship between the response of isolated mitochondria to changes in ADP concentration when the pyruvate-malate concentration was saturating. A similar situation existed for mitochondria when the substrate was palmitoyl-carnitine and malate (Fig. 3). These data confirm the observation that mitochondria follow Henri-Michaelis-Menten kinetics in response to increasing substrate concentrations (Chance & Williams, 1955). These figures demonstrate that the response of mitochondria to substrate depends on the amount of protein in the system but is independent of whether the mitochondria were prepared from the muscle of trained or sedentary rats. This illustrates that the response of skeletal muscle to endurance training is more closely related to the quantity than the quality of mitochondria. The similar  $K_m$  values and  $V_m$  values per unit of protein of mitochondria from skeletal muscle of endurance-trained and sedentary rats for the different experimental conditions (Table 1) further confirm that the basic property of mitochondria from muscle is not altered by endurance training. Davies *et al.* (1981) reported a similar finding for muscle from trained and sedentary rats. Green, Reichemann & Pette (1983) also demonstrated a constant proportionality of the enzymes of the citric acid cycle in the muscles of trained and control rats. An exception to this generality is a smaller proportional change in the activity of  $\alpha$ -glycerophosphate dehydrogenase with endurance training (Holloszy & Oscai, 1969; Davies *et al.* 1981) and perhaps greater enhancement of 3-hydroxyacyl-CoA dehydrogenase activity (Green *et al.* 1983). Examination of mitochondria isolated from different types of skeletal muscle, both slow and fast (red or white), revealed no differences in mitochondria as determined from  $K_m$  or  $V_m$  (data not included).

Since the  $K_m$  values of mitochondria from the muscle of sedentary and trained rats are the same, the importance of the adaptation to physical training can be related to

metabolic control and how it is influenced by the enzyme concentration. Figs 2 and 3 illustrate that at the same substrate concentration  $\dot{V}_{O_2}$  varies as a function of mitochondrial concentration. This is predictable from standard enzyme kinetic considerations. It is also clear from the substrate- $\dot{V}_{O_2}$  relationship that mitochondria behave as if they are individual enzyme units.

#### IN VIVO REGULATION OF METABOLIC RATE DURING EXERCISE

Although the responses of different mitochondrial concentrations to substrate concentrations, as demonstrated in Figs 2 and 3, illustrate an important point of enzyme control, they do not represent the situation in skeletal muscle during exercise. The kinetic relationship illustrated by changing mitochondrial protein or substrate concentration applies only to the initial velocities produced under *in vitro* conditions. To establish initial velocities for estimating  $V_m$  and  $K_m$  the decline in substrate in the reaction medium cannot exceed about 5 % of the total (Segel, 1975). During steady-state exercise there is a continuous (smoothed over time) rather than pulsed ADP production. In the steady state at the same  $\dot{V}_{O_2}$  value ADP flux through mitochondria is the same before and after training. Therefore the greater use of fat as a fuel during such exercise after endurance training is not attributable to a difference in the ATP-ADP turnover. However, it is under such circumstances that the effects of endurance training are manifest and that fatty acid use is augmented, glycogen use suppressed and lactate concentrations of muscle and blood are lower.

Regulation of mitochondrial respiration is *via* the amount of ADP translocated into the mitochondria (Tager *et al.* 1983). The limiting factor for ADP translocation into mitochondria is its interaction with transporter sites as a result of random collisions of

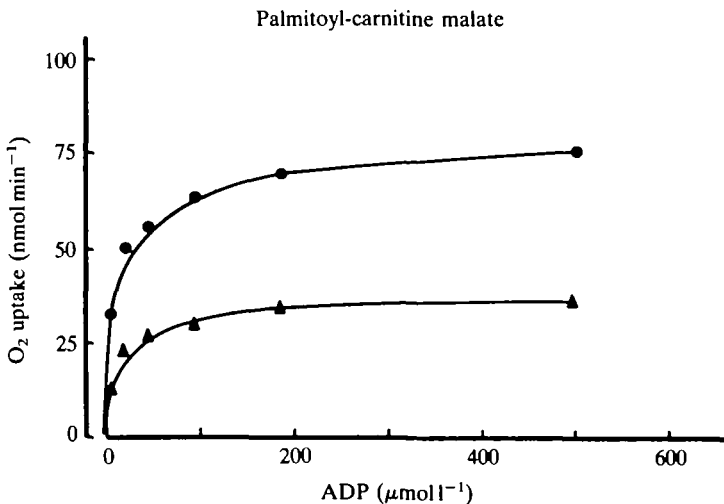


Fig. 3. The relationships between ADP concentration and reaction velocity of mitochondria prepared from the skeletal muscle of endurance trained and sedentary rats with palmitoyl-carnitine and malate as substrate.  $\blacktriangle$ , 0.325 mg protein;  $\bullet$ , 0.65 mg protein.

substrate with the adenine nucleotide translocase enzyme system. The potential for such collisions increases as a function of enzyme concentration, which depends on either the number or total surface area of mitochondria in the reaction medium (cytosol). It is unlikely in the non-trained condition, where mitochondrial concentration is low, that all of the respiratory units of the mitochondria are fully activated, in fact they may never be fully activated even during maximal power production by the muscle. It is generally agreed that no more than about 50 % of the  $V_m$  of any enzyme is used *in vivo*. It is simply that the probability for such collisions is low in this condition. If a reserve capacity exists, how does a change in mitochondrial concentration induced by endurance training serve the needs of muscle? We hypothesize that with an increase in mitochondria there is a more rapid response of the oxidative system as a result of the availability of more adenine nucleotide translocator sites. This situation would prevail whether produced by more or larger mitochondria since in both instances the end result would be an enhanced probability for random collision of ADP with the membrane-bound adenine nucleotide translocase. The effect of differences in mitochondrial concentration on the time of onset of oxygen uptake during constant ADP production is illustrated in Fig. 4. In these experiments a constant ADP production resulted from the phosphorylation of glucose from ATP by hexokinase. These data illustrate that at the same ADP production a shorter time elapsed between initiation of ADP production and the establishment of a steady-state  $\dot{V}O_2$  by the mitochondrial system as the protein concentration increased. (This phenomenon exists for isolated enzymes where a substrate is generated by another enzyme system.) The shorter time required to increase  $\dot{V}O_2$  after the onset of exercise would reduce the build-up of ADP in the cytosol. Since the ATP/ADP ratio exerts a powerful control over flux through the Embden-Meyerhof pathway, this process

Table 1.  $K_m$  and  $V_m$  values of mitochondria isolated from the muscle of trained and non-trained rats

	Non-trained	Trained
$V_m$ (nmol $O_2$ mg <sup>-1</sup> protein min <sup>-1</sup> )		
ADP*		
Pyruvate-malate	227±7	229±13
Palmitoyl	134±10	137±11
Pyruvate-malate†	172±13	187±16
Palmitoyl†	104±3	88±16
$K_m$ (μmol l <sup>-1</sup> )		
ADP*		
Pyruvate-malate	47±2	50±5
Palmitoyl	25±5	25±3
Pyruvate-malate†	5±1	9±2
Palmitoyl†	14±1	9±2

Means ± S.E.

\* Determined with saturating concentrations of pyruvate-malate or palmitoyl-carnitine and malate as substrates.

† Determined with saturating concentrations of ADP.

would be slowed resulting in lower pyruvate production. With less pyruvate available a favourable environment would be created for acetyl-CoA units derived from  $\beta$ -oxidation to enter the citric acid cycle. The lower rate of pyruvate formation coupled with a greater activity in the  $\beta$ -oxidation of fatty acids will favour the entry of fatty acid to provide the acetyl-CoA units for citrate formation within the mitochondria. It will also result in a greater percentage of the pyruvate that is produced being translocated into the mitochondria. Since lactate formation under most submaximal exercise conditions does not result from oxygen lack but is due to a mass action effect of pyruvate and lactate dehydrogenase (Jobsis & Stainsby, 1968; Connett, Gayeski & Honig, 1984; Kobayashi & Neely, 1979), the maintenance of a low cytosolic pyruvate concentration means less lactate is formed. This could account for the lower lactate concentrations observed in both the muscle and the venous outflow of trained *versus* untrained muscles during submaximal exercise (Saltin *et al.* 1978; Henriksson, 1977).

As stated above,  $\dot{V}_{O_2}$  (ADP-ATP turnover) is identical, although established later (Whipp & Wasserman, 1972; Hagberg, Hickson, Ashani & Holloszy, 1980), during exercise at the same power output before and after endurance training. In the lag period between the onset of contractile activity and establishment of the steady state  $\dot{V}_{O_2}$  level, ADP will accumulate in the cytosol and lower the  $[ATP]/[ADP]$  ratio. At some cytosolic ADP concentration, or  $[ATP]/[ADP]$  ratio, an equilibrium will be established between its production and flux through mitochondria *via* an interaction with the adenine nucleotide transporter sites. In the non-trained state this is akin to a shift to the right on the substrate-reaction velocity curve (Fig. 2). At the point of equilibrium the  $\dot{V}_{O_2}$  will be equal for both the trained and non-trained states. This is

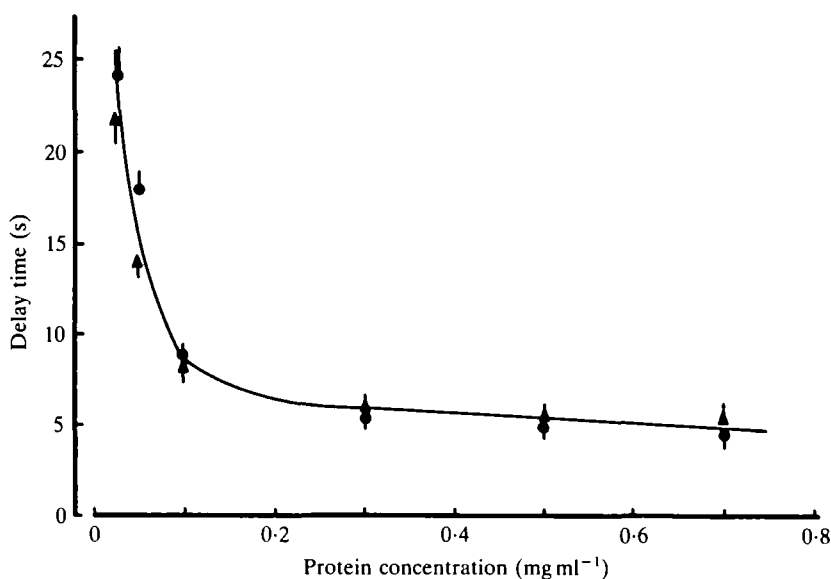


Fig. 4. The effect of increasing concentrations of mitochondrial protein on the time course of the delay in onset of  $\dot{V}_{O_2}$  when ADP was produced by phosphorylation of glucose by ATP *via* the hexokinase reaction. ●, control rats; ▲, trained rats.

consistent with the observation that three times as many twitches are required for skeletal as for heart muscle to attain half maximal oxidation-reduction of the respiratory pigments (Chance & Connelly, 1957; Ramfrez, 1959). The requirement for a higher ADP concentration (lower  $[ATP]/[ADP]$ ) to produce a similar  $\dot{V}O_2$  value, that is a similar ADP flux, has been demonstrated in isolated mitochondria by a partial blockade of the adenine nucleotide translocase with carboxyatractyloside (Kunz *et al.* 1981). The elevated ADP concentration in the cytosol needed to drive the  $\dot{V}O_2$  at the same rate as that in muscle with a high density will lower the cytosolic  $[ATP]/[ADP]$  ratio, thereby relieving the inhibition of the Embden-Meyerhof pathway and resulting in a greater glucose degradation. This can account for the greater reliance upon carbohydrate as a fuel source in the non-endurance trained state.

#### ANAEROBIC POTENTIAL

There is little or no change in the potential of most muscle fibres with altered patterns of physical activity when considered from the standpoint of the activities of the enzymes of the Embden-Meyerhof pathway. Green *et al.* (1983) have reported a reduction in this system as a result of a heavy ( $210 \text{ min day}^{-1}$ ) programme of running in the rat. However, those muscle whose fibres normally possess a high or low potential for the anaerobic degradation of glycogen or glucose generally retain this characteristic when exposed to a variety of training programmes or during inactivity. With endurance type training their relative importance is diminished by the change in aerobic potential and the control that is exerted by this change. In those fibres with high concentrations of enzymes for the Embden-Meyerhof pathway, the general principles outlined for a high aerobic potential prevail when this system is required for high metabolic activity in the absence of oxygen. Thus, the high activities of these enzymes provide a mechanism for a rapid flux of glucose through the system to produce lactate. With high concentrations of these enzymes there is a smaller chance of an accumulation of intermediates. In some situations this system can be very important in meeting the metabolic demands placed upon muscle.

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