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CONDITIONS FOR OXYGEN AND SUBSTRATE TRANSPORT IN MUSCLES IN EXERCISING MAMMALS

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Summary

The structural conditions relevant for metabolite exchange in anaerobic and aerobic work conditions in muscle tissue are reviewed. High-intensity non-steady-state exercise is supported by the phosphocreatine pool, which serves as a shuttle for high-energy phosphates produced by glycolysis and by aerobic metabolism. This is achieved through the intermediary of a topologically organized creatine kinase isozyme system. The muscle capillary network supplies substrate and environmental oxygen to the mitochondria. The network is quantitatively matched to the muscle oxidative capacity, determined structurally by mitochondrial volume. Capillary hematocrit, erythrocyte spacing and oxygen saturation of myoglobin are critical variables for oxygen release from microvessels. Myoglobin greatly helps intracellular oxygen transfer as, under aerobic work conditions, it keeps intracellular oxygen tension low and uniform in the muscle fibers. During sustained submaximal work, muscle cells are fueled by both endogenous (triglycerides and glycogen) and circulatory (lactate, glucose and fatty acids) substrates. A lactate shuttle in which lactate may move through the circulation, as well as directly from fiber to fiber, provides many of the carbohydrate-derived carbon skeletons for terminal oxidation. Glucose is taken up from the interstitial space by facilitated diffusion, mostly mediated by a glucose transporter (GLUT4) that is translocated from an intracellular location to the sarcolemma by activity and insulin. Extramyocellular transport of fatty acids is mediated by albumin, while fatty-acid-binding proteins are held responsible for intracellular fatty acid transport.

Introduction

Skeletal muscle tissue represents close to 50% of the body mass in most mammals, regardless of size. At rest, the musculature is responsible for only a small fraction of the total energy expenditure. Unlike most other tissues, muscle demonstrates a tremendous range of possible states of energy turnover. For periods of up to 10 s, humans are capable of producing well over 1000 W of mechanical power. For a few minutes, power outputs of up to 500 W can be maintained. If exercise is sustained for up to 1 h, an untrained young man is able to

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deliver around 200 W on a bicycle ergometer. Why can very high rates of energy turnover only be maintained for a short time? The primary problem, one of providing myofibrils with ATP for muscle contraction and fueling the myosin ATPase of exercising muscle, can be viewed as a transport problem. Very high intensity work is anaerobic and supported entirely by endogenous (intramuscular) substrates, whereas sustained submaximal work requires oxygen and substrates to be supplied by the circulation. The present short review will focus on topological conditions relevant for the supply of oxygen and substrate to and within muscle fibers. Both short-term *anaerobic* and long-term *aerobic* work conditions will be considered. Issues specifically related to the causes of muscle fatigue are beyond the scope of this paper. There are several excellent reviews that can be consulted (Hultmann *et al.* 1986; Wilkie, 1986; Sjögaard, 1990).

The phosphocreatine circuit

Skeletal muscle fibers contain instantaneously available energy in the form of a phosphocreatine (PCr) pool (Fig. 1). This intracellular energy store (to a lesser degree also ATP) is capable of fueling the myofibrillar ATPases for the first few seconds of vigorous activity. The PCr pool has the important primary function of an immediately available potent energy back-up. However, it is more than just the

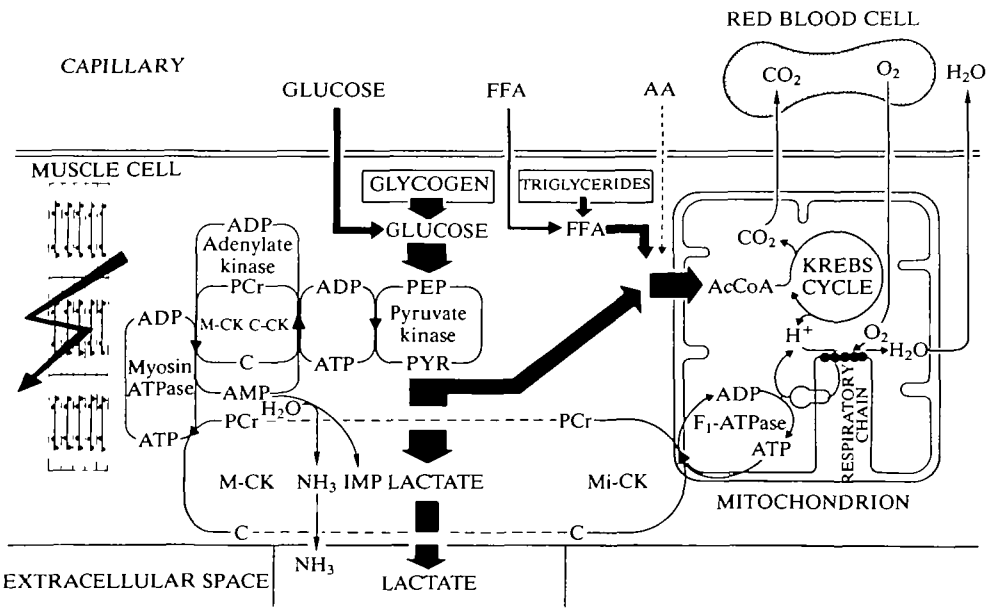


Fig. 1. Schematic drawing of pathways for energy and substrate supply in muscle cells. Mi-CK, mitochondrial form of creatine kinase; M-CK, M-line bound form of creatine kinase; C-CK, cytosolic (soluble) creatine kinase. The width of lines is an approximation of the relative capacities for flux rates. AcCoA, acetylcoenzyme A; PCr, phosphocreatine; C, creatine; FFA, free fatty acid; PYR, pyruvate; PEP, phosphoenol pyruvate; AA, amino acid.

booster rocket of muscular activity. The PCr pool is linked with most aspects of muscle cellular energy turnover through a number of compartmented creatine kinase (CK) isozymes. These isozymes are capable of channelling the intracellular energy flux and regulating local adenine nucleotide levels. The phosphocreatine system thus becomes a complex PCr circuit (Wallimann and Eppenberger, 1990). In skeletal muscle cells, the major creatine kinase isozymes are mitochondrial CK (Mi-CK), a number of cytosolic or soluble CKs (C-CK) and muscle-specific M-line-bound M-CK. The mitochondrial CK is located on the inner mitochondrial membrane at the contact sites with the outer membrane. Mi-CK serves the key role of feeding the PCr pool with energy derived from oxidative phosphorylation (see below).

For sprint-type work we first have to consider the M-line-bound CK, which has been shown to be a structural protein forming M-lines M4 and M4′ (Wallimann *et al.* 1983). These authors have also provided evidence that the activity of M-CK is sufficient for the regeneration of ATP hydrolyzed by the myosin ATPase from PCr (Wallimann *et al.* 1984). It appears, then, that the maximal short-term rate of energy turnover is not set by the creatine kinase system but rather by the activity of the myosin ATPase. The activity of the myosin ATPase has been shown to be related to the speed of muscle fiber contraction (Barany, 1967). ATPase activities are higher in fast than in slow muscle fibers of a given species. When body size is considered, higher ATPase activities are found in the same fiber types of smaller species (Hoppeler *et al.* 1980; Kram and Taylor, 1990). Scaling aspects of the CK system in muscle tissue have not, to our knowledge, been systematically analyzed. Exercise of maximal intensity lasting a few seconds can, therefore, be characterized in a simplified manner as depleting the intracellular PCr pool at a rate at which the myofibrillar ATPase can operate, with the M-CK being responsible for maintaining adequate myofibrillar ATP levels.

Glycolysis

If motor activity continues for up to a few minutes, glycolysis becomes the dominant metabolic pathway funnelling energy into the PCr pool. Glycolytic enzymes are restricted to the cytoplasm of skeletal muscle fibers. However, their distribution within fibers does not seem to be uniform (see Wallimann and Eppenberger, 1990). These authors suggest that glycolysis and the replenishment of the cellular PCr pool through a fraction of the soluble CK would occur preferentially in the I-band region of the sarcomere, where these two enzyme systems are thought to form a multienzyme complex (Maughan and Lord, 1988). CK is almost completely excluded from the actin–myosin overlap region. It appears that some of the remaining cytosolic CK is associated with the sarcolemma, where it supports transmembrane cation exchange (Sharov *et al.* 1977). Another fraction of the cytosolic CK seems to be anchored to the sarcoplasmic reticulum, functionally coupled to Ca^{2+} pumping (Rossi *et al.* 1988).

A second pathway, involving an adenylate kinase isozyme reaction, has also

been reported to be responsible for making glycolysis-derived ATP available myofibrillar ATPases (Fig. 1; Zeleznikar *et al.* 1990). The relative contributions of these two proposed pathways for ATP regeneration through glycolysis in a given situation are difficult to appreciate at present. This issue may have to be analyzed in the context of the regulation of adenine nucleotide degradation, which has been shown to occur in a manner that is specific to the type of fiber (Tullson and Terjung, 1990).

Oxygen delivery to muscle mitochondria

The high-energy turnover states of muscle are anaerobic and non-steady state. An 'oxygen debt' is incurred, which must be repaid at a later stage, during lower-intensity exercise or after cessation of the exercise (Bangsbo *et al.* 1990). Under steady-state exercise conditions, mitochondrial phosphorylation matches the total energy expenditure of the contracting muscle cell. It is generally accepted that the myofibrillar ATPase is responsible for some 70 % of the energy consumed. The remainder is used by the sarcoplasmic reticulum for Ca^{2+} sequestering and for the maintenance of ion gradients across the sarcolemma (Rall, 1985). As previously mentioned, maximal *aerobic* exercise can proceed only at a fraction of the maximal possible *contractile* activity. Therefore, under aerobic conditions, it is the respiratory and not the contractile machinery that imposes a limit to sustained muscular activity. At the whole-animal level, the state of maximal aerobic metabolism ($\dot{V}_{O_2max}$) is characterized by the fact that well over 90 % of the energy flux is directed towards the working muscle tissue. Under these conditions, a primary problem is related to oxygen transfer to muscle tissue. Because the exercise intensity eliciting $\dot{V}_{O_2max}$ can be maintained for only a few minutes, glycolysis-derived substrate for mitochondrial respiration is in abundance (Fig. 1). At the level of the muscle tissue, the analysis of this type of high-intensity aerobic work is complicated by the fact that only a fraction of the muscle fibers, those well-equipped for aerobic metabolism, are recruited (Hennig and Lomo, 1987; Armstrong and Laughlin, 1985). In any case, skeletal muscle capillaries must deliver oxygen at the rate at which it is processed by mitochondria in activated muscle fibers.

Mitochondrial oxygen consumption

Over the last decade we have analyzed the conditions for oxygen transfer in the mammalian respiratory system by combining functional measurements of maximal oxygen flow rates at the whole-animal level with the structural conditions that prevail at each step of the respiratory cascade from lungs to muscle mitochondria (Weibel and Taylor, 1981; Taylor *et al.* 1987). It was found that both the structure and function of mitochondria are relatively invariant among mammalian species. An analysis of running $\dot{V}_{O_2max}$ and an estimation of the total mitochondrial volume of the skeletal musculature carried out on eight mammalian species (ranging in size from woodmouse to horse) showed that, at $\dot{V}_{O_2max}$, skeletal muscle mitoch

a consumed 4.56 ± 0.61 ml O_2 min^{-1} ml^{-1} of mitochondria (mean±S.D.) (Hoppeler, 1990). The volume fraction of skeletal muscle fiber occupied by mitochondria can therefore be used to describe skeletal muscle oxidative capacity. The rate at which mitochondria operate at $\dot{V}_{O_2max}$ seems to be constant among species. The available evidence indicates that the oxidative capacity of muscle tissue is linearly dependent on the absolute volume of mitochondria it contains, whether in whole animals (Hoppeler, 1990), single muscles (Hoppeler *et al.* 1987) or single muscle fibers (van der Laarse *et al.* 1989).

Relating capillaries to mitochondria

It is methodologically unsophisticated to determine the oxidative capacity of muscle by measuring the volume density of mitochondria, because volume density estimates are insensitive to the highly anisotropical conformation of skeletal muscle tissue. This is not the case for the estimation of muscle capillary supply. Muscle capillaries mostly run parallel to the muscle fibers; however, they exhibit some tortuosity and branching. Several methods have been developed which allow for estimation of capillary length density in anisotropic tissue, such as skeletal and heart muscle (Mathieu *et al.* 1983; Mattfeldt, 1987). It is important to stress the point that the capillary length density is a key structural parameter of any capillary network, provided that the mean capillary diameter is known, or known to be constant, among the capillary beds to be compared. The capillary length density, $Jv(c,f)$, allows us to calculate two structural parameters that are critical for capillary exchange functions of either oxygen or substrates. These are the capillary volume, $Vv(c,f)$, and the capillary surface area, $Sv(c,f)$, per unit volume of muscle fiber. These structural parameters are related to $Jv(c,f)$ by:

$$Vv(c,f) = a(c) \times Jv(c,f)\,,$$

$$Sv(c,f) = b(c) \times Jv(c,f)\,,$$

where $a(c)$ is the mean capillary cross-sectional area and $b(c)$ is the mean capillary circumference. Capillary length density is also a useful parameter for describing diffusional properties of muscle tissue, because it allows for an approximation of the radius (R) of the 'Krogh cylinder' by:

$$R = [\pi \times Jv(c,f)]^{-1/2}\,.$$

$Jv(c,f)$ can be determined efficiently from muscle cross sections and longitudinal sections, using models (Mathieu *et al.* 1983). A number of studies have shown that the degree of capillary tortuosity is primarily dependent on the state of contraction at muscle fixation, reflected in sarcomere length (Mathieu-Costello, 1987). Neither animal size or aerobic capacity nor hypoxia or exercise seems to have a measurable influence on the capillary orientation distribution in space (Mathieu-Costello *et al.* 1989; Poole and Mathieu-Costello, 1989). Measurements of capillary circumference in muscle specimens from many mammalian species prepared under standard conditions for electron microscopy indicate that mean capillary cross-sectional area and perimeter are quite similar in mammalian

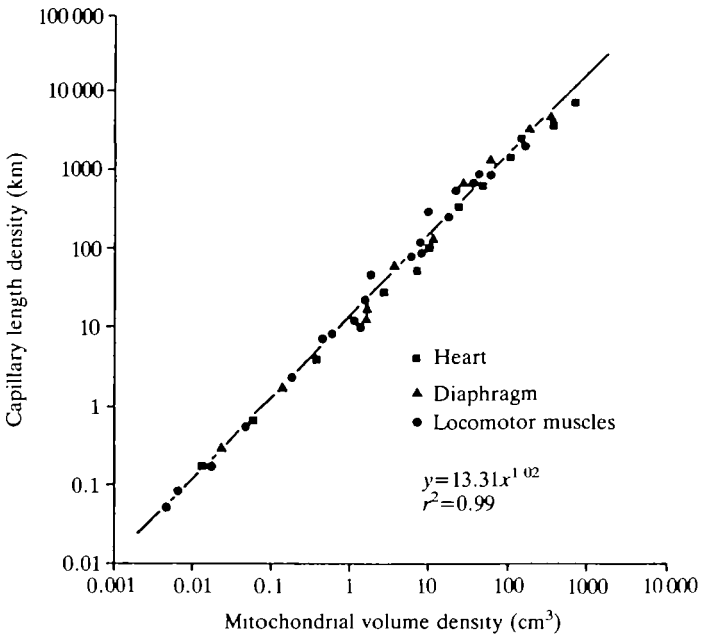


Fig. 2. Species means (13 mammalian species) of capillary length density *vs* mitochondrial volume density. Locomotor muscles include vastus medialis and semitendinosus (Adapted from Hoppeler and Kayar, 1988.)

muscles (Conley *et al.* 1987). These critical capillary dimensions have been shown to remain constant when capillary density is increased by 50% upon chronic electrical stimulation (Dawson and Hudlicka, 1989). For the purpose of the following discussion, circular capillaries of diameter 4.5 μm are assumed.

Compiling data from heart, diaphragm, semitendinosus and vastus medialis muscle from 13 mammalian species, we have plotted capillary length density in relation to mitochondrial volume density (Hoppeler and Kayar, 1988; Fig. 2). The slope of this relationship is not significantly different from 1, which indicates for this broad comparison of species that a doubling of mitochondrial volume is matched by a doubling of capillary length. It can be calculated that for each cubic centimeter of mitochondria (containing approximately 30 m^2 of inner mitochondria membrane; Schwerzmann *et al.* 1989) there is some 10 km of capillary length, 1400 cm^3 of capillary surface area and 0.16 cm^3 of capillary volume. Thus, there is roughly a ratio of 1:200 between the surface area of the capillary wall and that of the inner mitochondrial membrane, with corresponding rates of oxygen fluxes at these two sites.

Capillary transit times

The results of morphometric analyses of muscle capillary beds can yield important information on the time available for gas and substrate exchange in muscle capillary beds when combined with functional data on muscle blood flow

assumption is made that all capillaries are perfused during maximal aerobic exercise, we can calculate the average capillary transit time (tc) by:

$$tc = Vc/BF,$$

where BF is blood flow (units, $ml\,s^{-1}$; measured with the radioactive microsphere technique) in muscles of animals running at $\dot{V}_{O_2max}$ and Vc is the capillary volume (units, ml). Capillary transit times at $\dot{V}_{O_2max}$ are remarkably similar in many mammalian species and muscles recruited during running, ranging only from 0.4 s in foxes to 0.8 s in horses (unpublished observations).

It is recognized that this crude type of analysis, that is the use of bulk data for both flow and capillary structure, is capable only of unraveling some of the design principles governing the structural constraints operating on transport phenomena in muscle tissue. A closer analysis of the species mean data represented in Fig. 2 uncovers considerable variability. For any value of mitochondrial volume density, the capillary length density may vary by as much as twofold. It is obvious that the extent of the capillary network is but one, albeit rather important, determinant of muscle tissue gas and substrate exchange.

Hemoglobin concentration

In a study on adaptive variation comparing 'athletic' species such as dogs and ponies to 'sedentary' species such as goats and calves, it was found that the athletic animals had both a 2.5-fold larger maximal oxygen consumption and a 2.5-fold greater skeletal muscle mitochondrial volume (Conley *et al.* 1987). In contrast, the capillary length density differed by only a factor of 1.7. It was found that this difference was complemented by a 1.5-fold higher hematocrit at $\dot{V}_{O_2max}$ in the active species. Thus, a 2.5-fold greater oxygen delivery in athletic species was achieved in about equal parts by increasing capillary volume and the oxygen transport capacity of the blood and, consequently, by the arteriovenous oxygen concentration difference.

Erythrocyte spacing

The adaptation of the capillary bed to the needs of the muscle fibers it supplies may be achieved by any combination of structural and functional modifications. There are a number of functional factors that have been identified as playing a role in oxygen transfer from capillaries to mitochondria. In an elegant theoretical analysis, Federspiel and Popel (1986) have shown that each erythrocyte can effectively release oxygen only over a 'zone of influence' extending about half the capillary channel width in front of and behind it. Calculated 'optimal' red cell spacing is one red cell length during exercise and up to four times as much at rest. Erythrocyte spacing close to these theoretical expectations has been observed directly (Federspiel and Sarelius, 1984). The contribution of the particulate nature of blood to oxygen transport resistance from blood to mitochondria has been estimated to be between 30 and 70 % of the total (Federspiel and Popel, 1986). If higher of the proposed values is correct, one would expect a rather large drop

of oxygen tension (P_{O_2}) between the red blood cells and the muscle cell interior as well as a relatively flat P_{O_2} distribution within muscle fibers (Federspiel, 1986). This type of P_{O_2} distribution with a large drop (2.0→2.7 kPa) at the capillary wall and a rather shallow (0.27→0.40 kPa) distribution within muscle fibers is also considered to be related to the presence of myoglobin in muscle fibers (Gayeski and Honig, 1986), as well as to the heterogeneous distribution of mitochondria in muscle fibers.

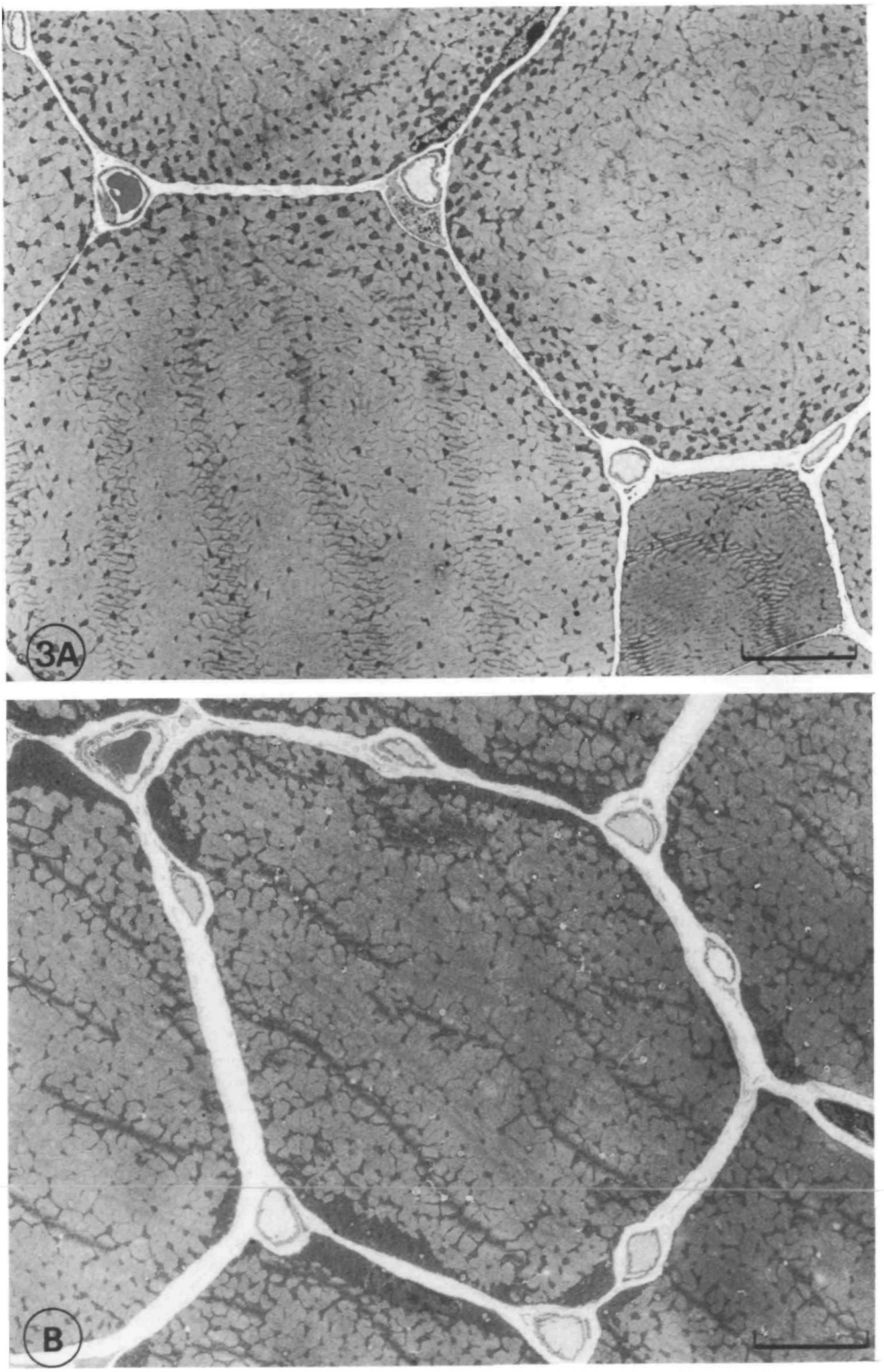
Myoglobin

Intracellular oxygen transport in muscle fibers is greatly helped by myoglobin. According to Wittenberg and Wittenberg (1989), three conditions must be met for the contribution of myoglobin-facilitated diffusion to oxygen flux to exceed that of free dissolved oxygen: (i) myoglobin must be present in significant concentrations, (ii) myoglobin must be partially desaturated, and (iii) myoglobin must be free to undergo translational diffusion in the sarcoplasma. The first two assumptions apparently hold for 'red' skeletal muscle and the heart. Direct observation demonstrates that, in heavily exercising skeletal muscle, myoglobin saturation is mostly below 50 % (Gayeski and Honig, 1986), indicating that most of the oxygen consumed in the muscle cells is transported by myoglobin-facilitated oxygen diffusion. Myoglobin spectra obtained from dog muscles rapidly frozen during near maximal work indicate a median P_{O_2} of between 0.08 and 0.8 kPa (corresponding to myoglobin saturations of 5–50 %) with almost no anoxic loci (Gayeski *et al.* 1987). It was found that $\dot{V}_{O_2max}$ of muscles was supported down to an intracellular P_{O_2} of 0.07 kPa, but dropped off precipitously below that. It may be worth noting that, like the distribution of glycolytic enzymes, the concentration of myoglobin seems to be higher in the less crowded I-band than in the A-band (Kawai *et al.* 1987). This region of the sarcomere also contains a larger volume density of mitochondria (see below). From this one might speculate that I-band-located mitochondria might benefit from direct oxymyoglobin-mediated oxygen delivery to the respiratory chain, which has been shown to be important in cardiac myocytes (see Wittenberg and Wittenberg, 1989).

Distribution of mitochondria in muscle cells

Mitochondria are not scattered randomly in muscle fibers. In muscle fiber cross sections it is common to find clusters of mitochondrial profiles directly beneath the fiber sarcolemma, particularly in regions adjacent to capillaries (Fig. 3). Also, interfibrillar mitochondria are more numerous in the peripheral than in the central portions of a muscle fiber. In a variety of muscles, mitochondria have been demonstrated to be distributed in a gradient that is highest near capillaries and decreases with distance towards the fiber center (James and Meek, 1979; Rakusan and Tomanek, 1986; Kayar *et al.* 1988*a*). These observations on intracellular

Fig. 3. Cross sections of mammalian skeletal muscle fibers showing that volume density of mitochondria increases towards the fiber border (A). Distinct accumulations of subsarcolemmal mitochondria can be observed (B). Scale bars, 10 μm.


3A
B

mitochondrial distribution have been made on many types of muscles and beg th question of whether mitochondrial location is or is not related to mitochondrial function. If the diffusion distance for oxygen or any other metabolic substrate from capillaries in mitochondria were a critical factor in mitochondrial metabolism, then all mitochondria should be located near capillaries. But, if the distance for exchanging high-energy phosphate compounds between mitochondria and myofibrils were critical to muscle work rate, then mitochondria should be distributed uniformly between myofibrils. The actual distribution of mitochondria in most muscles is intermediate between these two cases. This suggests a balance between opposing demands (Kayar and Banchero, 1987; Hoppeler *et al.* 1991). Mathematical modelling of tissue oxygen tension gradients suggests that the actual location of mitochondria in muscle fibers is compatible with a nearly constant tissue P_{O_2} across the fiber's width (Mainwood and Rakusan, 1982; Tonellato *et al.* 1989), lending support to the myoglobin saturation spectra data mentioned in the previous paragraph.

The accumulation of clusters of subsarcolemmal mitochondria is particularly noticeable in highly oxidative muscle fibers in human muscle and muscles of laboratory rodents (Fig. 3). In muscles trained for endurance exercise, there is an increase in total mitochondrial content of muscle fibers, with a greater relative increase in the subsarcolemmal than in the interfibrillar population of mitochondria (Hoppeler *et al.* 1973, 1985). This has led to the speculation that subsarcolemmal and interfibrillar mitochondria might be two biochemically distinct populations. However, the results of a number of biochemical analyses have remained equivocal (Matlib *et al.* 1981). It is currently open to debate whether subsarcolemmal mitochondria can achieve higher respiration rates than their interfibrillar counterparts or whether they have different enzymatic equipment that allows for a distinct substrate selection.

Mitochondrial connectivity

Cross sections of muscle show profiles of mitochondria that are roughly circular and of variable diameter (Figs 3 and 4). One intuitively imagines that these profiles represent sections of objects that, in three dimensions, are more or less spherical. Three-dimensional reconstructions and scanning electron microscopy have shown that muscle mitochondria may actually be quite complex in shape and variable in size (Fig. 4; Bakeeva *et al.* 1978; Ogata and Yamasaki, 1985; Kirkwood *et al.* 1986; Kayar *et al.* 1988*b*). It has been proposed that possibly all mitochondrial matter in a muscle fiber is contiguous, forming a mitochondrial reticulum (Bakeeva *et al.* 1978). The highly interconnected transverse portions of this mitochondrial network could, by virtue of the high solubility of oxygen in their lipid-rich membranes, facilitate oxygen diffusion within muscle fibers (Longmuir, 1981). However, reconstructions of mitochondria using ultrathin sections suggest that the greater axis of mitochondrial connectivity is longitudinal to the myofibrils (Kayar *et al.* 1988*b*). There is clear evidence of isolated spherical or cylindrical mitochondria in muscles with relatively low mitochondrial volume densiti

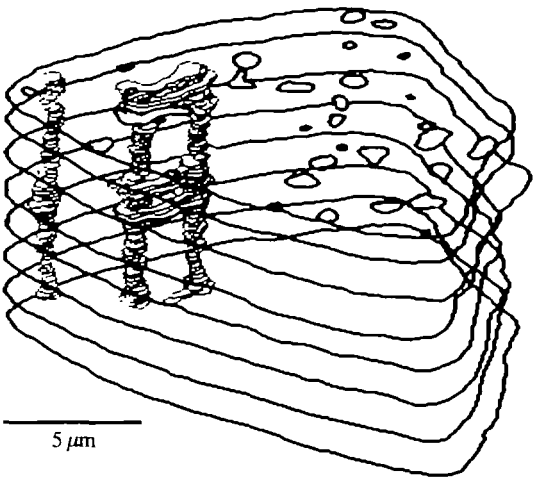


Fig. 4. Reconstruction of two mitochondria in a serially sectioned equine skeletal muscle fiber. Mitochondria are oriented mainly parallel with the main fiber axis, showing transversely oriented portions in the I-band regions. Profiles of additional mitochondria are indicated in the top section. (Adapted from Kayar *et al.* 1988*b*.)

(3–5 % of the fiber volume). In muscle fibers of around 10 % mitochondrial density, these cylindrical mitochondria are often interlinked by transverse portions in the I-band region of the sarcomere (Fig. 4). Only in muscles with over 20 % mitochondrial density (rat diaphragm) does all the mitochondrial material appear to be interconnected into a single reticulum (Bakeeva *et al.* 1978). However, even in these highly aerobic muscles, the contention of a single mitochondrial reticulum remains speculative (Hoppeler *et al.* 1991).

Substrate stores in muscle fibers

In endurance locomotion, mitochondria are capable of consuming substrates at a faster rate than they can be supplied from exogenous sources and transported into muscle cells. For continued activity, muscles therefore depend on both exogenous and endogenous substrate pools. Substrate depots, in the form of glycogen granules and lipid droplets, are stored in muscle cells and are readily visible under the electron microscope (Fig. 5). Glycogen is best measured by biochemical methods either in whole muscle homogenate or in single fibers. In untrained human vastus lateralis muscle, the glycogen concentration is around 100 mmol l^{-1} glucose kg^{-1} wet mass. These glycogen stores can be depleted to 50 % in only 6 min of intermittent supramaximal bicycling (112 % of $\dot{V}_{O_2max}$; Nordheim and Vollestad, 1990). Assuming 13 kg of active muscle mass for bicycling this would require some 90 l of oxygen, if this quantity of glycogen were burned aerobically.

Lipid droplets are usually found adjacent to mitochondria. Morphometry is the

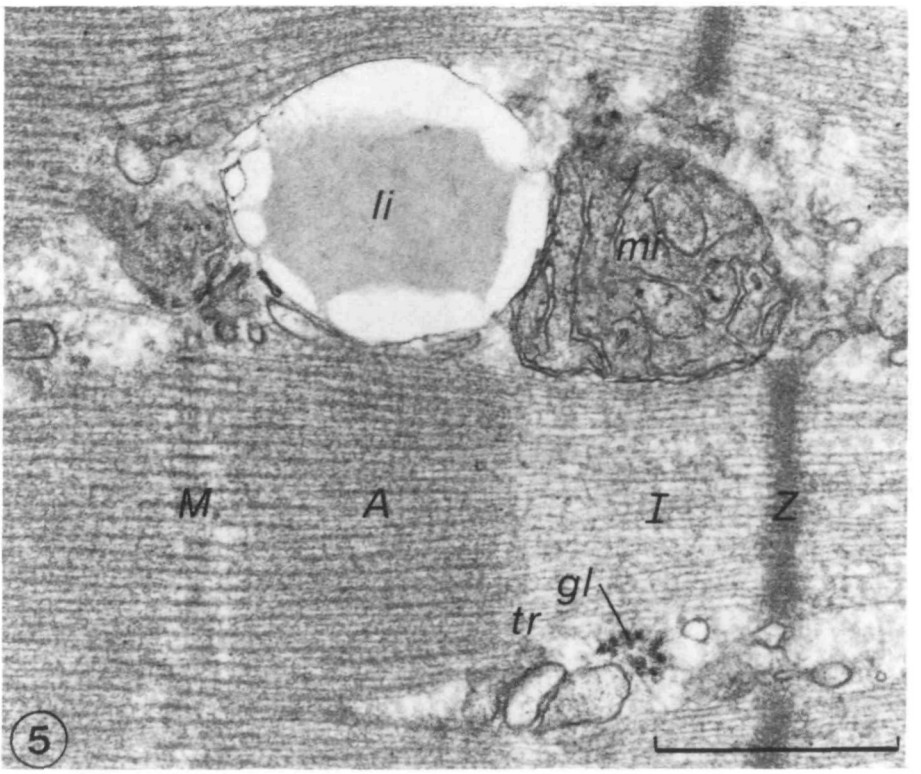


Fig. 5. Longitudinal section through a portion of a skeletal muscle fiber. *M*, M-band; *I*, I-band; *A*, A-band; *z*, Z-band; *tr*, triad; *gl*, glycogen granules; *mi*, mitochondrion; *li*, lipid droplet. Scale bar, 0.5 μm.

method of choice for quantifying these lipid stores, because their intracellular location can be positively ascertained (Fig. 5). In untrained human muscle, about 0.5 % of the fiber volume consists of intracellular lipids. These stores are doubled in endurance exercise lasting up to 2 months (Hoppeler *et al.* 1985). In extreme endurance athletes, such as professional cyclists or marathon runners, intracellular lipid stores can make up more than 2 % of the fiber volume (Hoppeler, 1986; Staron *et al.* 1989). Running an ultramarathon (100 km) results in an almost complete disappearance of intracellular glycogen and lipid stores (Oberholzer *et al.* 1976; Kayar *et al.* 1986). Such a severe depletion of intramuscular glycogen and lipid stores requires 7 days or more to restore pre-exercise levels, the time course of repletion being similar for glycogen and lipids (Staron *et al.* 1989).

Glycogen depletion studies have been instrumental in probing the recruitment pattern of muscle fiber types in activities of varying intensities and durations (see Armstrong and Laughlin, 1985). This type of study has led to the notion that the ability to mobilize and utilize fat as a fuel is an important mechanism in endurance exercise training which postpones glycogen depletion and fatigue (Holloszy at al 1986). During continuous aerobic exercise, the choice of substrate depends on the

tensity and duration of exercise. The highest aerobically sustainable workloads are supported by carbohydrate catabolism (Jones *et al.* 1980). The contribution of triglycerides is highest at intermediate work intensities (around 50 % of $\dot{V}_{O_2max}$; Hurley *et al.* 1986). Subjects trained for running ultramarathon events were capable of running for 4 h at around 70 % of their $\dot{V}_{O_2max}$ (Davies and Thompson, 1986). After the first 10 min of exercise, carbohydrates and triglycerides contributed about equally to the energy requirements. However, for the second half of the run the contribution of fat to the total energy flux increased to 70 % in these endurance athletes. Over the whole period of this 4-h run, lactate levels never rose substantially above resting levels.

Lactate, glucose and fatty acid transport in exercising muscles

The sustained performance in submaximal exercise depends critically on delivery of exogenous fuel to working muscle cells in order to delay the depletion of endogenous substrate stores. Conventionally, two major categories of blood-borne substrates are recognized: plasma glucose derived from hepatic glycolysis, gluconeogenesis or glucose uptake and plasma free fatty acids (FFA) supplied by adipose tissue triglycerides (Sahlin, 1986). There is considerable, though still somewhat controversial, evidence that lactate can also play an important role as a substrate for mitochondrial respiration in long-term exercise (Brooks, 1986*a*). The contribution of amino acids to aerobic energy production is considered to be negligible in mammals under normal circumstances (Garber *et al.* 1976).

Lactate

The quantitative contribution of lactate as a fuel in working skeletal muscle fibers is debatable (Hermansen and Vaage, 1977; Brooks and Gaesser, 1980; Brooks, 1986*b*; Sahlin, 1987). Recent experimentation by Nordheim and Vollestad (1990) provided direct evidence that, during low-intensity exercise (40 % of $\dot{V}_{O_2max}$, immediately following high-intensity exercise), circulating lactate can be used as a substrate for resynthesis of ATP in type I fibers and for glycogen resynthesis in type II fibers, as long as the blood lactate concentration is significantly elevated. Glycogen in glycolytic muscle fibers is assumed to be the main source of plasma lactate during exercise (Brooks, 1985). The transport of lactate into active muscle fibers is proposed to take place both through the capillary bed and directly from glycolytic muscle fibers to adjacent lactate-oxidizing muscle cells. Most of the lactate transport through the cell membrane is believed to be carrier-mediated (Moon *et al.* 1987), showing saturation characteristics at high lactate concentrations (Mazzeo *et al.* 1986). Around 20 % of the total lactate transport may be achieved by direct diffusion of undissociated lactic acid (Juel and Wibrand, 1989). These authors found that maximal lactate transport capacity was 12 mmol l^{-1} kg^{-1} min^{-1} at a lactate gradient of 30 mmol l^{-1} in mouse muscle.

Glucose

In contrast to oxygen, where over 80 % of the arterial content may be extracted during a single passage through the capillary bed in working muscle, the oxidizable fuels lactate, glucose and free fatty acids are poorly removed from capillaries (Walker *et al.* 1984). A possible explanation for this phenomenon is that the transport from the capillary lumen into the muscle cell may limit the maximal rate of exogenous fuel supply for these substrates. In muscle tissue, the water-soluble glucose molecule seems to be readily diffusible into the interstitial space through the relatively leaky muscle capillary endothelium. This is not the case in brain and retinal microvessels, which have 'occluding' junctions, forming a glucose impermeable blood–tissue barrier. To provide the brain with glucose, brain capillaries are endowed with a high concentration of glucose transporters absent in skeletal muscle capillaries (Harik *et al.* 1990). In muscle, the transport problem for glucose usually occurs at the sarcolemma. Across the sarcolemma, glucose transport is accomplished by facilitated diffusion *via* a glucose transporter (GLUT4) belonging to a family of structurally and functionally related glucose transport proteins (GLUT1–GLUT5; see Gould and Bell, 1990). GLUT4 is responsible in adipocytes and skeletal muscle tissue for most of the insulin-dependent glucose transport. The insulin-stimulated increase in cellular glucose uptake is mediated primarily by the translocation to the cell membrane of a latent intracellular pool of transporter molecules. In skeletal muscle tissue, contractile activity is capable of increasing the number of glucose transporters on the sarcolemma in the absence of an insulin stimulus (Goodyear *et al.* 1990). There is a large difference among skeletal muscle fiber types with regard to their insulin- and contraction-stimulated glucose uptake capacity (Henriksen *et al.* 1990). This difference is found to be directly related to the content of GLUT4 protein as well as GLUT4 mRNA. It is possible that both the number of transporters and their intrinsic activity could be modulated by contractile activity (Goodyear *et al.* 1990). Exercise training has been shown to increase the expression of skeletal muscle GLUT4 protein (Friedman *et al.* 1990).

Fatty acids

Similar to the situation with glucose transport into muscle cells, recent research has shed much light on key aspects of fatty acid transport to and within muscle cells (Fig. 6). The net mobilization rate of fatty acids in adipose tissue does not limit the maximal flux rate of this fuel into the muscle cell. Even under conditions of maximal fat utilization, the rate of re-esterification in adipocytes has been shown to be still of the order of 25 % of the total rate of fatty acid mobilization (Wolfe *et al.* 1990). Intracellular and circulating triglycerides are believed to contribute about equally to the total fat oxidized during sustained exercise (Carlson *et al.* 1971). Possible mechanisms of the cellular control of the two major identified lipases in heart and red skeletal muscle have recently been reviewed (Fig. 6; Oscai *et al.* 1990). It is proposed that fatty acids originating from circulatory chylomicrons and very-low-density lipoproteins are hydrolyzed by a lipoprotein lipas

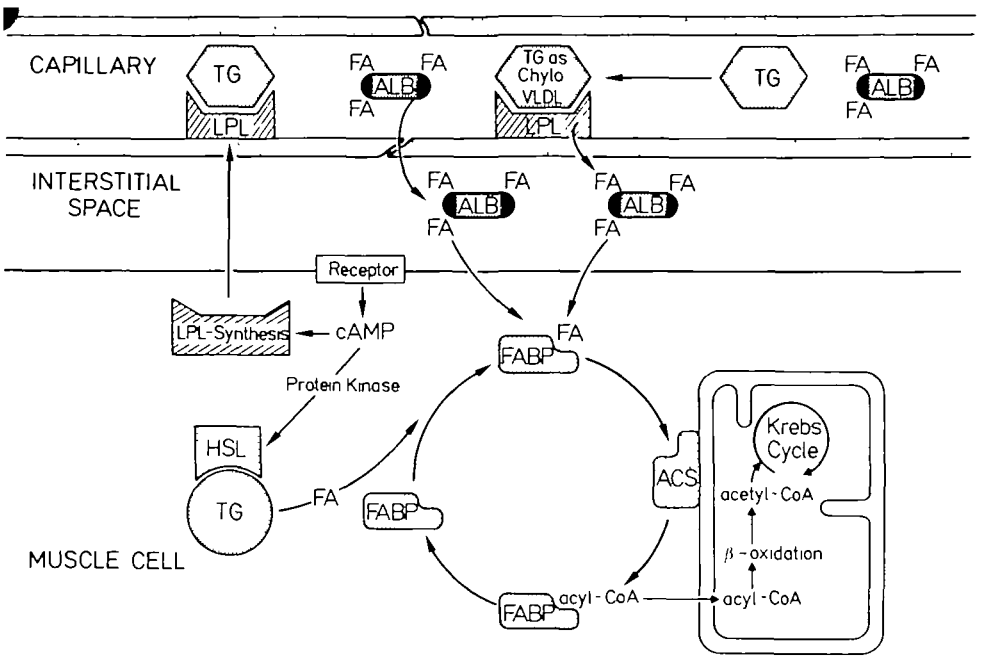


Fig. 6. Schematic drawing of the pathway for and regulation of fatty acid supply to muscle mitochondria. (Adapted from Oscai *et al.* 1990, and Glatz *et al.* 1988.) ACS, acylcoenzyme A synthetase; ALB, albumin; Chylo, chylomicrons; FA, fatty acid; FABP, fatty-acid-binding protein; LPL, lipoprotein lipase; HSL, hormone-sensitive lipase; TG, triglycerides; VLDL, very-low-density lipoproteins.

(LPL), which is synthesized in muscle cells and exported to the vascular bed. In contrast, a hormone-sensitive lipase (HSL) is held to be responsible for intracellular lysis of triglycerides. The two lipases are suggested to be activated at the same time to provide fatty acids as fuel for muscle respiration. An additional proposed role for the circulatory LPL would entail the replenishment of decreased intracellular lipid stores after exercise. Consistent with this view is the finding that LPL level is increased as a consequence of endurance exercise training (Terjung *et al.* 1983).

The method of transfer of fatty acids from muscle capillaries to respiring mitochondria is still somewhat controversial. This transfer is greatly hindered by the poor aqueous solubility of these compounds, both in the extracellular space and in the cytosol. Therefore, various transporter molecules are implicated in fatty acid movement. Transport through the interstitial space is thought to be albumin-mediated (Fig. 6; Rose *et al.* 1989). In support of this view, it has been shown that the interstitial concentration of albumin is greatly increased after chronic electrical stimulation of rabbit skeletal muscles (Heilig and Pette, 1988). The transport across the sarcolemma is likely to be determined by a physicochemical equilibrium between the extracellular fatty acid–albumin complex, the intracellular fatty-acid-binding proteins and the membrane lipid phase (Rose *et al.* 1989). Previous

reports on an energy-dependent fatty acid translocator (Stremmel, 1988) are not supported by these findings. Intracellular fatty acid transfer has recently been reviewed comprehensively (Glatz *et al.* 1988; Matarese *et al.* 1990; Zachowski and Devaux, 1990). It is recognized that a class of small (M_r 14×10^3–15×10^3) fatty-acid-binding proteins (FABPs) plays a key role in intracellular transport of lipids. FABPs are abundant proteins accounting for 3–6 % of the total cytosolic protein content in heart and red skeletal muscle. Consistent with this view, FABP level has been shown to increase after chronic electrical stimulation in a mixed skeletal muscle (Kaufmann *et al.* 1989). There exist several types of FABP in liver, intestine, heart, kidney etc., of which more than one kind may be present in each tissue. A major role of FABPs seems to be the transcytosolic transport of fatty acids from the sarcolemma to the mitochondria. FABPs are thought to act by increasing the effective solubility of fatty acids. The high concentration of FABPs and their relatively low affinity for fatty acids suggests that fatty acids are passed from one FABP molecule to the next (see Glatz *et al.* 1988). However, the various FABP types may serve many other functions. They have been implicated in enzyme modulation, prevention of the deleterious effects of fatty acids on metabolism (i.e. in ischemia), and even growth regulation (see Glatz *et al.* 1988).

Conclusions

This short report has attempted to review steady-state and non-steady-state energy metabolism of skeletal muscle in the context of the topological conditions prevailing around and within muscle fibers. From this analysis it becomes apparent that all energy or substrate supply systems share certain characteristics. (a) To allow for a wide dynamic range, fast response times of metabolic regulation and buffering of metabolic transients, there are pools of all the major metabolic intermediates pervading the muscle fibers: the phosphocreatine pool for high-energy phosphates, the 'myoglobin' pool for oxygen and the FABP pool for fatty acids. Carbohydrates are pooled as glycogen, from where rapid mobilization of carbohydrates for mitochondrial combustion and for the lactate shuttle is made possible by high activities of glycolytic enzymes (Hochachka *et al.* 1988). (b) Except for glycogen, these pools are also held to be responsible for generating a major fraction of the cellular energy flux and are believed to function as important regulators in their respective metabolic pathways.

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