CHAPTER 6

CELLULAR OXIDATION OF PYRUVATE AND LACTATE

Physical activities lasting a minute or more, such as the 1,500-m run, absolutely require the presence and use of oxygen in active muscle. Moreover, recovery from all-out, fatiguing exercise is essentially an aerobic process. Far more energy can be realized from a substrate through oxidation than through glycolytic processes. Within muscle cells are specialized structures called mitochondria that link the breakdown of foodstuffs, the consumption of oxygen, and the maintenance of ATP and CP levels. As opposed to glycolysis, which involves carbohydrate materials exclusively, cellular oxidative mechanisms allow for the continued metabolism of carbohydrates as well as for the breakdown of derivatives of fats and proteins. Even though the processes of cellular oxidation are far removed from the anatomical sites of breathing and the pumping of blood, it is the processes of cellular oxidation that breathing and beating of the heart serve. Seen in their proper perspective, the two most familiar physiological processes (breathing and the heartbeat) play a key role in energy transduction.

Figure 6-1 Hicham El Guerrouj of Morocco celebrates after setting a new world record in the mile run (3:43.13). Performances such as this one require the highest sustained metabolic power output humanly possible. Such performances depend on the ability to consume and utilize oxygen as well as the ability to access immediate and nonoxidative energy sources. SOURCE: © AP/Wide World Photos.

■ Mitochondrial Structure

Cellular oxidation takes place in cellular organelles called *mitochondria* (mitochondrion, singular). Pyruvate and lactate (products of glycolysis) as well as

products of lipid and amino acid metabolism are metabolized within mitochondria. These organelles have been called "the powerhouses of the cell." It is within mitochondria that almost all oxygen is consumed and ADP is phosphorylated to ATP. Consequently, those activities that last more than a minute are powered by mitochondria. As suggested in Figure 6-2, mitochondria appear in two places in skeletal muscle. A significant population of mitochondria is located immediately beneath the cell membrane (sarcolemma); these are subsarcolemmal *mitochondria* and are in a position to receive O_2 provided by the arterial circulation. Subsarcolemmal mitochondria are believed to provide the energy required to maintain the integrity of the sarcolemma. Energy-requiring exchanges of ions and metabolites across the sarcolemma are most probably supported by subsarcolemmal mitochondria activity. Deeper within muscle cells are the *intermyofibrillar* mitochondria. As the name implies, these mitochondria exist among the contractile elements of the muscle. Intermyofibrillar mitochondria probably have a higher activity per unit mass (specific activity) than subsarcolemmal mitochondria, and they probably play a major role in maintaining the ATP supply for energy transduction during contraction.

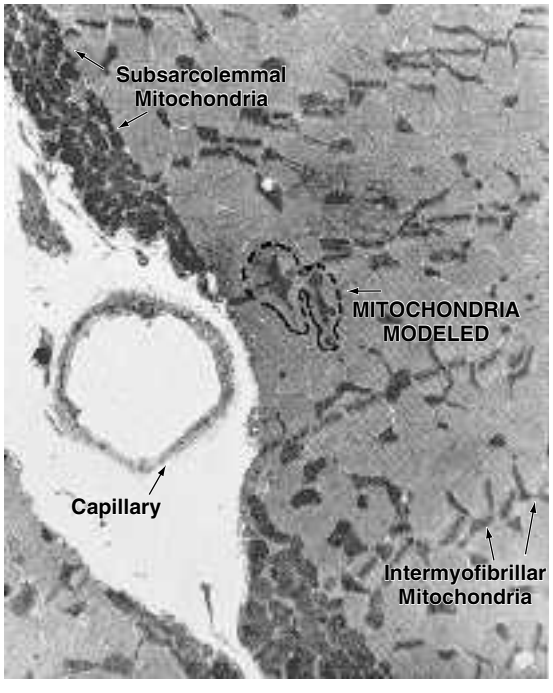
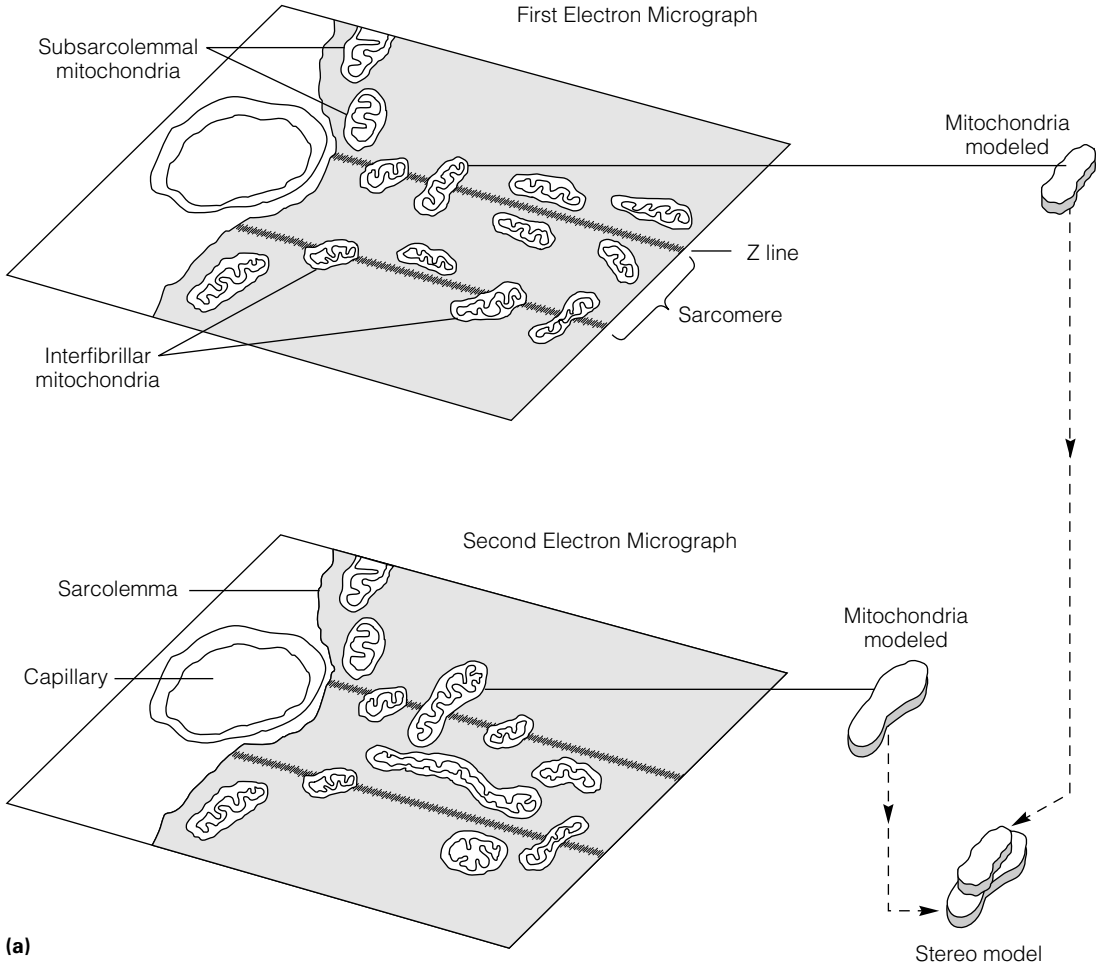


Figure 6-2 Mitochondria are found to be distributed within two areas of a muscle cell. Subsarcolemmal mitochondria are found immediately beneath the cell membrane (i.e., the sarcolemma). Intermyofibrillar mitochondria are found among the muscle cell's contractile elements. Micrograph courtesy of E. E. Munn, C. Greenwood, S. P. Kirkwood, L. Packer, and G. A. Brooks.

Because of their appearance in cross-sectional electron micrographs and in micrographs of isolated mitochondria, it has long been believed that the mitochondria exist as discrete capsule-shaped organelles. However, seminal work on diaphragm muscle by the Russian scientists Bakeeva, Chentsov, and Skulachev (1978) indicates that mitochondria are interconnected in a network (the mitochondrial reticulum), much like the sarcoplasmic reticulum. Instead of thousands of mitochondria, a muscle cell may actually contain relatively few subsarcolemmal and intermyofibrillar mitochondria, but each mitochondrion has thousands of branches.

Figure 6-3 is the result of collaborative efforts by researchers in Berkeley, California, and Babraham, England. From serial cross sections through rat soleus muscle (Figure 6-3a), a particular "mitochondrion" can be identified and traced, and a model constructed, based on knowledge of the magnification and section thickness. The same "mitochondrion" can continue to be modeled in subsequent slices through the muscle (i.e., serial cross sections). These models of individual sections can then be stacked up to reveal the appearance of a mitochondrial reticulum (Figure 6-3b).

Red-pigmented muscle fibers obtain their color, in part, from their number of mitochondria, which are red. By comparison, pale muscle fibers contain few mitochondria (see Figure 5-6). More detailed studies on animal muscle that suggest the presence of a mitochondrial reticulum are supported by cross-sectional analyses of human muscle biopsy specimens. Figure 6-4 from Hans Hoppeler (1986) in Bern, Switzerland, shows muscle ultrastructure at three magnifications and illustrates the anatomical relationship between capillary O_2 delivery and the mitochondrial reticulum.



(b)

Figure 6-3 In all probability, "mitochondria" do not exist as separate, individual entities within muscle cells but rather as parts of a network, or "reticulum." Evidence for the mitochondrial reticulum in limb skeletal muscles was obtained by modeling the mitochondria seen in electron micrographs of serial cross sections through rat soleus muscles. The process of modeling is illustrated in (a), and the resulting model is pictured in (b). (Model [part b] courtesy of E. A. Munn and G. Greenwood, Babraham, England.)

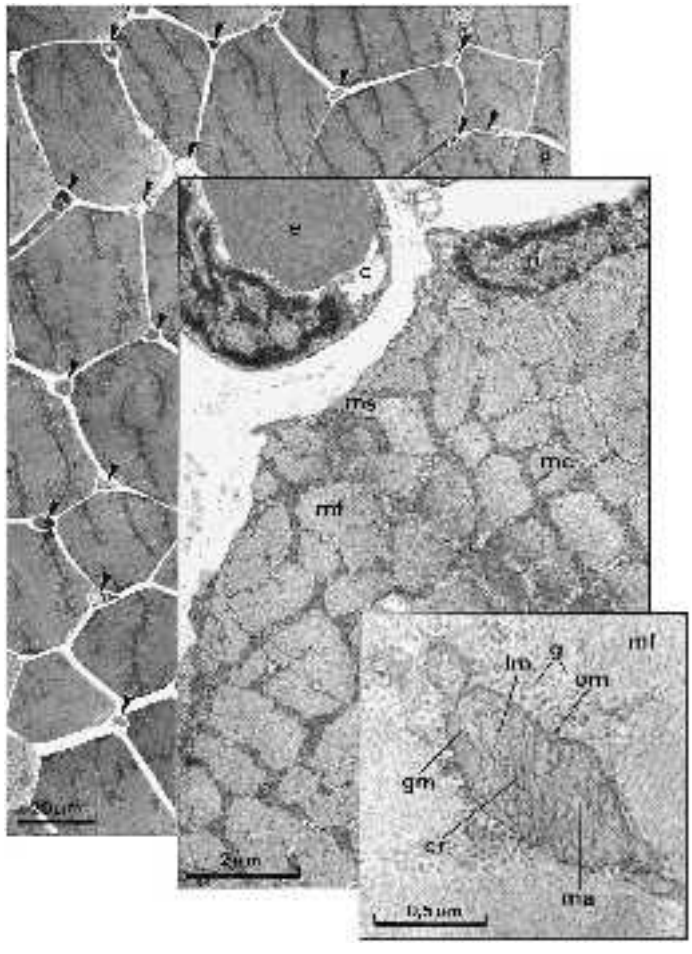


Figure 6-4 Cross sections of human skeletal muscle tissue illustrating the sampling design used for analyzing muscle respiratory structures. The low-level magnification is used for assessing capillarity and fiber size. The intermediate magnification allows for estimating the volume density of mitochondria and other sarcoplasmic components. The highest magnification allows measurement of mitochondria compartmental spaces and membrane surface areas (arrows = capillaries; c = capillary; e = erythrocyte; mc = central mitochondria; ms = subsarcolemmal mitochondria; mf = myofibrils; cr = cristae; g = glycogen; gm = mitochondrial granule; ma = matrix; im = intermembrane space; om = outer mitochondrial membrane. SOURCE: Hoppeler, 1986. Used with permission.

As noted by Hoppeler and Ewald Weibel and associates, the essentially cylindrical shape of muscle fibers means that 50% of the cell volume is in the outer 25% of cross-sectional diameter. This means that subsarcolemmal and interfibrillar components of the mitochondrial reticulum occupy approximately equal portions of muscle cell volume.

■ The Mitochondrial Reticulum: Mitochondria Are Interconnected Tubes, Not Individual Spheres

Recognition of the existence of a muscle mitochondrial reticulum has brought about the development of new concepts of the distribution of oxygen, energy metabolites, and energy. Bakeeva and Skulachev (Bakeeva et al., 1978) have pointed out that oxygen is more soluble in the phospholipid environment of mitochondrial membranes than in the cytosol. Most importantly, presence of the mitochondrial reticulum (Figures 6-4 and 6-5) creates channels for distribution of the chemiosmotic (energy) gradient whereby energy can be transmitted from the cell surface, where oxygen is in high concentration, through the mitochondrial reticulum to deep within the cell, where oxygen delivery is less and oxygen concentration is lower. The mitochondrial chemiosmotic gradient is discussed more later (see Figure 6-11), but here it is important to note that oxygen and all mitochondrial constituents are necessary to maintain the chemiosmotic gradient over

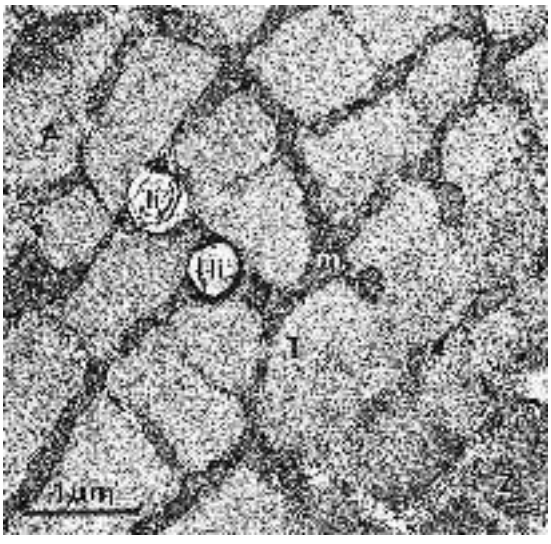


Figure 6-5 Cross section of a portion of a human muscle fiber exposing the A- and I-band and the Z-line regions. Lipid droplets (li) are seen in contact with mitochondria (m). It is evident that the mitochondria in this muscle fiber form an extensively branched tubular network, or reticulum. SOURCE: Hoppeler, 1986. Used with permission.

the distance from the sarcolemmal surface to deep within the fiber.

■ Mitochondrial Structure and Function

The lower right panel of Figure 6-4 is an electron micrograph of a muscle mitochondrion, whereas Figure 6-6 provides a schematic representation of a mitochondrion. Area 1, the outer membrane, functions as a barrier to maintain important internal constituents (e.g., NADH) and to exclude exterior factors. The outer membrane contains specific transport mechanisms to regulate the influx and efflux of various materials. Area 2, the intermembrane space, also contains enzymes for exchange and transport. Area 3, the inner membrane, can be divided into two surfaces. Area 3a functions, in part, along with the outer and inner membrane constituents in a transport capacity. For example, the enzyme carnitine transferase is found on the inner wall of fragmented mitochondria. Carnitine transferase is involved in moving lipids into mitochondria. The mitochondrial inner membrane is also relatively impermeable to protons, which helps preserve the chemiosmotic gradient. Area 3b of the inner membrane is referred to as the cristae membrane, so called because it is made up of many folds, or *cristae* (*crista*, singular). The cristae membrane is the main mitochondrial site where oxidative phosphorylation takes place. Because area 3a is confluent with area 3b, and the chemical compositions are similar,

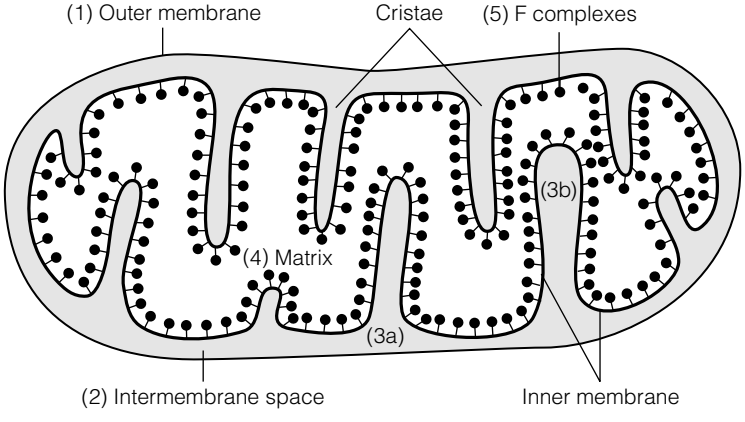


Figure 6-6 Schematic representation of a mitochondrion (reticulum fragment). Areas indicated are (1) the outer membrane, (2) the intermembrane space, (3) the inner membrane, and (4) the matrix. The inner membrane is diagrammed as existing in either of two orientations: juxtaposed to the outer membrane (3a) or protruding into the matrix (3b). F complexes (5) protrude from the inner membrane, which is a mobile phospholipid structure. Inward folds of the inner membrane form the cristae. See the text for a description of the functions of each area.

area 3a, the part of the inner membrane that is juxtaposed to the outer membrane, can phosphorylate as well.

Keep in mind that the membrane constituents of mitochondria are not rigidly locked into position the way a leg is attached to the trunk of a body. Rather, the inner membranes change in conformation (shape) as mitochondria function, and the membrane constituents themselves display a high degree of mobility such that various components can move both along and within the membranes.

The actual mitochondrial site of phosphorylation is the F complex, which looks like a ball on a stalk on the mitochondrial inner membrane. The F complex is alternatively termed the *elementary particle* and is made up of two subunits: the stalk (F_0 complex) and the ball (F_1 complex).

Area 4, the matrix, is not simply a space but contains nearly half the mitochondrial protein. In the matrix are located LDH and the Krebs cycle enzymes. We describe those in detail next.

■ The Krebs Cycle

Pyruvate (and lactate) gain entry to the mitochondrial matrix via a carrier protein (MCT) located in the inner membrane (see Figure 5-14). Additionally, there are possibilities for lactate and pyruvate transporters to exist in the outer membrane. However, the mitochondrial outer membrane is permeable to low molecular weight substances, so a transporter is unnecessary. The sequence of events of pyruvate metabolism, catalyzed first by the enzyme complex pyruvate dehydrogenase (PDH) and then by the Krebs cycle enzymes, is illustrated in Figures 6-7 and 6-8.

The series of enzymes depicted in Figures 6-7 and 6-8 is called the *Krebs cycle* after 1953 Nobel laureate Sir Hans Krebs, who did much of the work elaborating the pathway. This series of enzymes is also often referred to as the *citric acid cycle* (the first constituent is citric acid) and the *tricarboxylic acid cycle* (the initial constituents have three carboxyl groups, the TCA cycle).

Although the TCA cycle is generally referred to as a cycle, it is important to realize that it is imperfect. Various substances can leave the cycle, and others can gain entry to it. This will be discussed later in detail. Figure 6-7 is intended to show the key role of pyruvate dehydrogenase (PDH) in regulating flux into the TCA cycle. The purposes of PDH and the TCA cycle are revealed to be decarboxylation (CO_2 formation), ATP production, and most importantly, NADH production. The figure shows that there are four places where NAD^+ is reduced to NADH, and one place each where FADH and ATP are formed. Recalling that each NADH is equivalent to three ATPs and that each FADH is equivalent to two ATPs, the purpose of the TCA cycle is revealed: to continue the metabolism of pyruvate from glucose (as well as from the intermediate products of lipid and protein catabolism) and to trap part of the energy released in the forms of ATP and the high-energy reduced compounds NADH and FADH.

The enzyme pyruvate dehydrogenase is a complex that has three parts and functions in several steps to convert pyruvate to acetyl-CoA, and one additional step to reduce NAD^+ to NADH. Both thiamine pyrophosphate (TPP) and flavine adenine dinucleotide (FAD) serve as cofactors for the complex. In addition, PDH is an enzyme controlled by phosphorylation state (Figure 6-9). The PDH enzyme component of PDH complex is inhibited when phosphorylated by the action of a specific kinase that uses ATP. Thus, high ATP/ADP, acetyl-CoA/CoA, and NADH/NAD^+ act to reduce glycolytic flux to the TCA by inactivation of PDH. Conversely, dephosphorylation of PDH by a specific phosphatase serves to activate the enzyme. Dephosphorylation is promoted by high levels of pyruvate and Ca^{2+} as well as by decreases in ATP/ADP, acetyl-CoA/CoA, and NADH/NAD^+. Also important, insulin binding to the cell surface promotes dephosphorylation (activation) of PDH by some, as yet unknown, second messenger. The chemical agent dichloroacetate (DCA) serves to dephosphorylate and to activate PDH, thus increasing flux from glycolysis to mitochondria. For this reason, DCA is sometimes used to reduce lactic acidosis.

As implied by the name, pyruvate dehydrogenase, in the conversion of pyruvate to acetyl-CoA, an NAD^+ molecule is reduced to NADH. NADH is termed the "universal hydrogen carrier." In

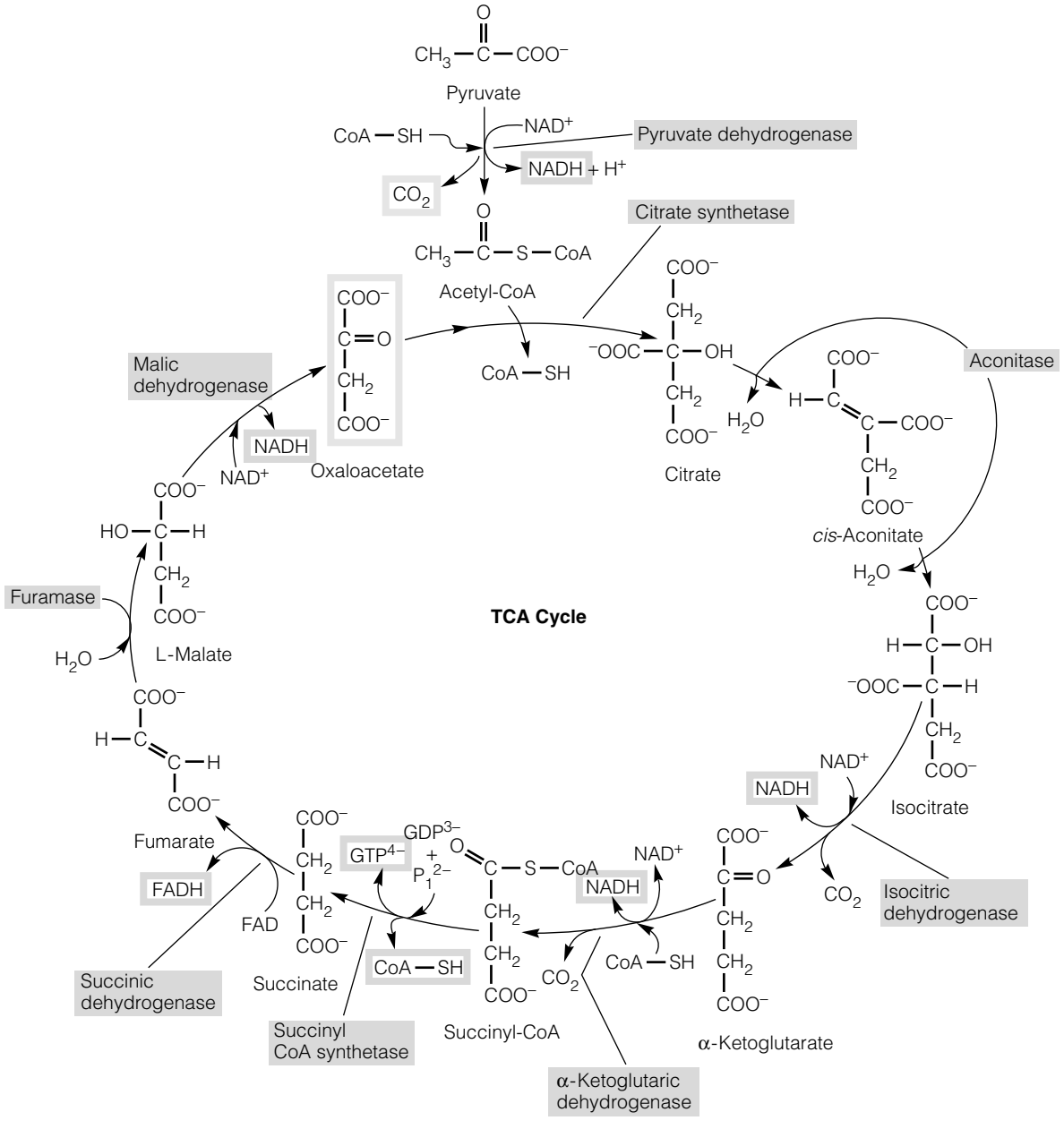


Figure 6-7 Detailed diagram of the Krebs cycle intermediates and catalyzing enzymes. As the result of one acetyl-CoA entering and traversing the cycle, one GTP (guanosine triphosphate, energetically equivalent to an ATP) and several high-energy reducing equivalent compounds (NADH and $FADH_2$) are formed. These substances result in significant mitochondrial electron transport and ATP production. Modified from McGilvery, 1975.

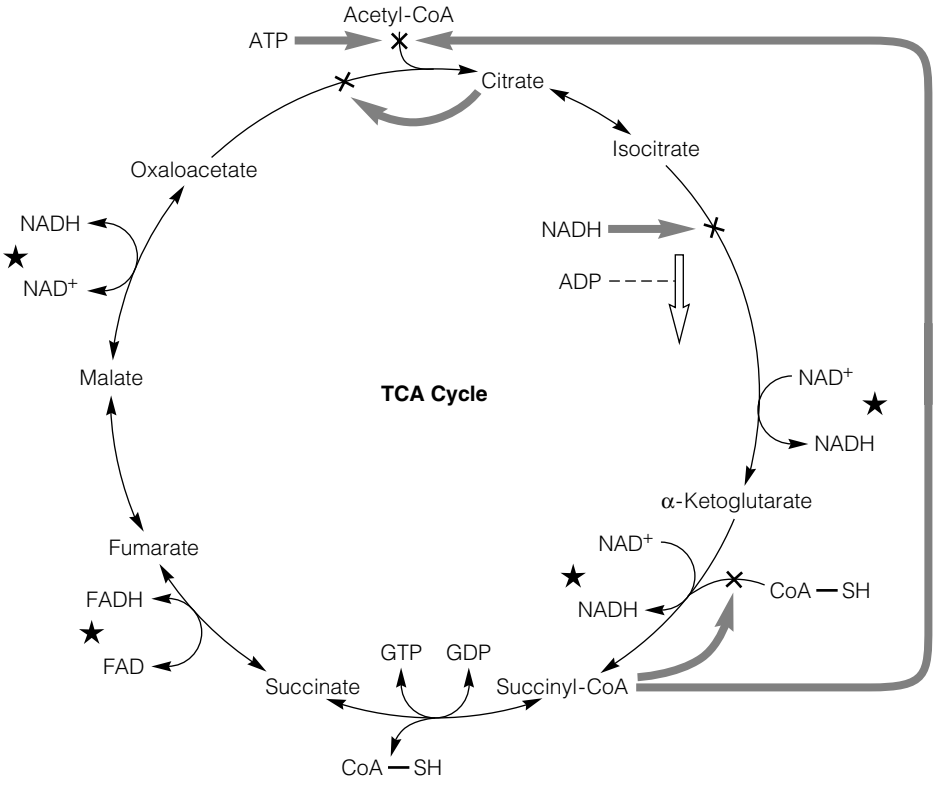


Figure 6-8 Regulation of the citric acid cycle. The starred reactions require oxidized coenzymes; the ratio of oxidized to reduced coenzymes is governed by the availability of ADP and P_i for oxidative phosphorylation, thereby regulating the cycle to demand. However, the nearly irreversible isocitric and α-ketoglutaric dehydrogenase reactions can still proceed at low NAD^+ levels. They are controlled through inhibition by NADH and succinyl-CoA, respectively. Also, isocitric dehydrogenase is activated by ADP and the dehydrogenase is activated by Ca^{2+}, which enters mitochondria during muscle contraction. Finally, when the high-energy phosphate supply is high and GTP accumulates, the accumulating succinyl-CoA inhibits the initial citrate synthetase reaction in competition with oxaloacetate. This effect also tends to balance the initial part of the cycle, which consumes oxaloacetate, against the final part, which produces oxaloacetate. With the exception of succinic dehydrogenase, which bonds to the inner membrane, TCA cycle enzymes exist in the mitochondrial matrix. SOURCE: McGilvery, 1975.

addition, pyruvate dehydrogenase functions in concert with coenzyme A (CoA), which is referred to as a coenzyme or a cofactor. A cofactor is a low-molecular-weight substance whose presence is required to allow an enzyme to work. CoA is sometimes referred to as coenzyme 1, as it was the first such factor discovered; it is also sometimes referred to as the "universal acetate carrier." In Figure 6-10, the terminal sulfur bond of CoA is shown to be the site at which acetate binds. As the result of pyruvate dehydrogenase, pyruvate is decarboxylated, releasing a CO_2 molecule, and the remaining two-carbon unit (acetate) is combined with CoA to give acetyl-CoA. In summary, pyruvate dehydrogenase is an

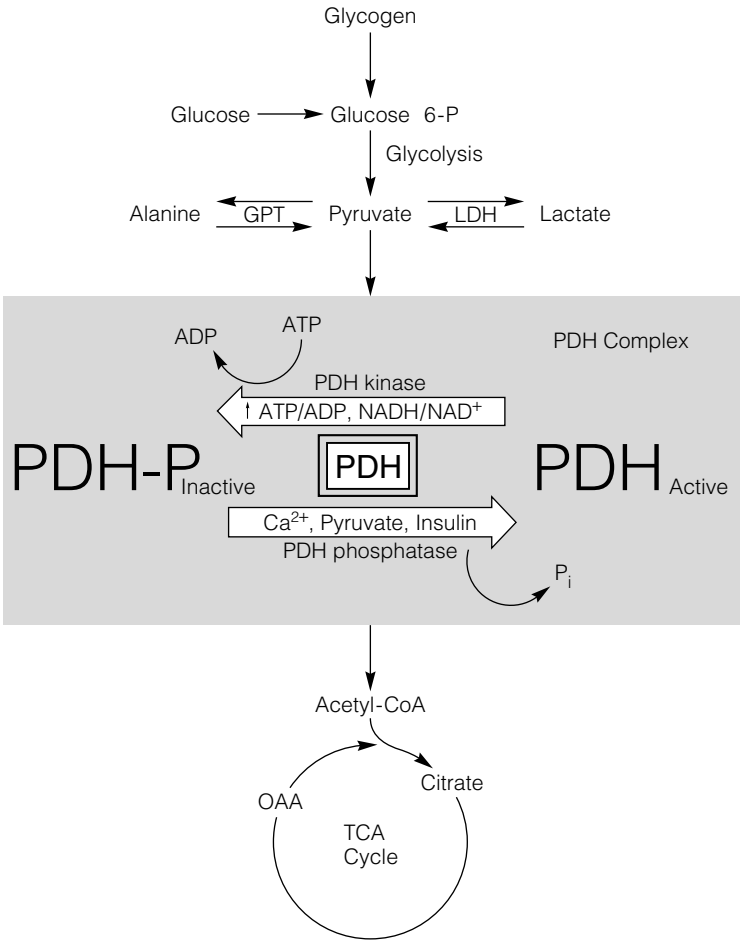


Figure 6-9 The enzyme pyruvate dehydrogenase (PDH) is one component of the "PDH complex," which regulates glycolytic flux (flow) to the TCA cycle or, alternatively, to lactate and alanine. Phosphorylation inhibits PDH, whereas dephosphorylation activates the enzyme and enzyme complex.

important enzyme, not only because of its complex function, but also because it is a rate-limiting enzyme. The activity of PDH plays a major role in determining the rates of glycolysis, lactate production, and carbohydrate supply for mitochondrial oxidation.

Acetyl-CoA is the entry substance into the TCA cycle. Acetyl-CoA can be formed from pyruvate as well as from fatty and amino acids. Under the influence of the enzyme citrate synthetase, acetyl-CoA and oxaloacetic acid (OAA) condense to give citric acid. As we shall see, the presence of OAA may be a regulating factor controlling the rate of the TCA cycle.

Several steps into the TCA cycle is the enzyme isocitric dehydrogenase (IDH). This is the rate-limiting enzyme of the TCA cycle, much as PFK is the rate-limiting enzyme in glycolysis. Like PFK, IDH is an allosteric enzyme stimulated by ADP. IDH, together with the other dehydrogenases of the TCA cycle, as well as pyruvate dehydrogenase, are sensitive to the redox (reduction-oxidation) potential and calcium ion (Ca^{++}). Simply defined, the redox potential is the NADH/NAD^+ ratio. The dehydrogenases are inhibited by a high redox potential and are stimulated by a decline in redox potential. When muscle starts working, components of the electron transport chain (ETC, below) become

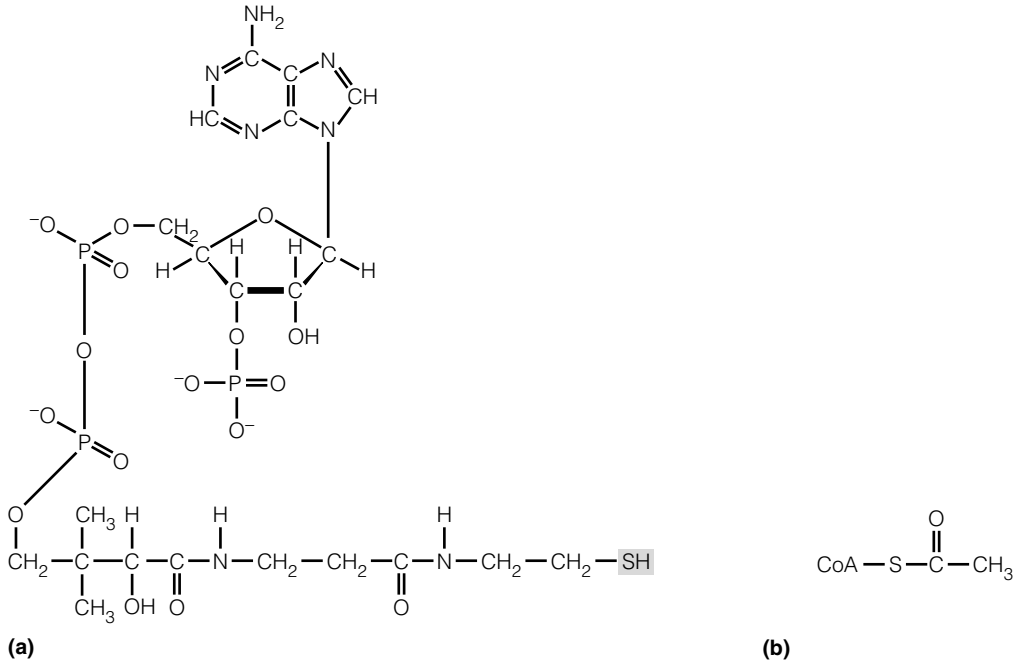


Figure 6-10 (a) Structure of coenzyme A (CoA) showing the binding site (–SH) for acetate. (b) Acetyl-CoA is formed from the union of CoA and acetate.

oxidized. Oxidation of the the ETC in turn oxidizes the NADH/NAD^+ complex, thus activating the Krebs cycle dehydrogenases.

In resting cells, the concentration of free Ca^{++} is low ($\leq 10^{-7}$ M). However, Ca^{++} release from the sarcoplasmic reticulum as part of excitation-contraction coupling causes free cytosolic [Ca^{++}] to rise (range 10^{-7} to 10^{-5} M) if only transiently (Figure 18-14). Some of the free cytosolic Ca^{++} is sequestered in the mitochondrial reticulum, thus activating the Krebs cycle dehydrogenases and, in a way, linking muscle contraction to oxidative metabolism.

■ The Electron Transport Chain

The *electron transport chain (ETC)* is located on the mitochondrial inner membrane, probably in both areas 3a and 3b (see Figure 6-6). The ETC constituents are arranged in the sequence indicated in Figure 6-11. We owe much of our present understanding of how mitochondria function to Peter Mitchell (Nobel Prize in chemistry, 1978) and his chemiosmotic theory of oxidative phosphorylation. The term *oxidative phosphorylation* refers to two separate processes that usually function together. Oxidation is a spontaneous process that is linked or coupled to the phosphorylation, the union of P_i with ADP to make ATP. Phosphorylation is an endergonic process driven by oxidation. It is important to note that although the linkage in oxidative phosphorylation is tight, certain situations—for example, heat buildup in muscles from prolonged work—can cause the linkage to be loosened or uncoupled.

The function of the ETC can be simply described as follows. Reducing equivalents containing a high-energy hydrogen and electron pair gain entry to the beginning of the chain. The hydrogen and electron then move from areas of electronegativity

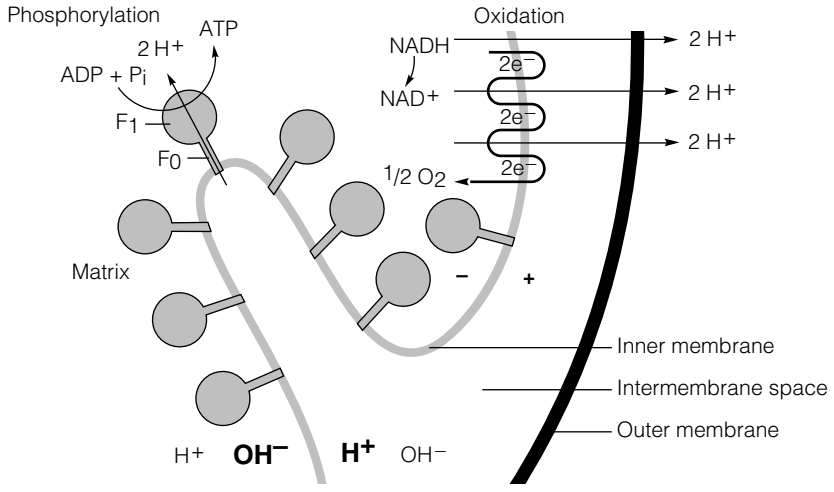


Figure 6-11 Orientation of components of the electron transport chain (ETC) within the mitochondrial inner membrane. Entry of hydride ions (H) from high-energy reduced compounds (e.g., NADH) into the ETC results in the oxidation of those compounds, electron transport, and the expulsion of protons (H^+). This creates a chemical and electrical gradient across the inner membrane. The entry of protons through specific portals into the F_0–F_1 complex (an elementary particle like a stalk and ball) provides the energy for the phosphorylation of ADP to ATP.

(NAD^+) toward areas of electropositivity (atomic oxygen). Along the ETC, the electron is stripped from the hydrogen, which continues along the chain; the resulting proton (H^+) is pumped outside the mitochondria (see Figure 6-11). For each NADH entering, three pairs, or a total of six protons, are pumped out. For each FADH entering, two pairs of protons are pumped out. Outside the mitochondrion, a region of decreased pH and positive charge is created. This chemical and osmotic potential ultimately supplies the energy to phosphorylate ADP. An inner membrane portal channels the potential energy of the external proton field to the ATPase on the matrix side of the inner membrane.

According to Mitchell, pairs of protons enter the mitochondrion and are directed to the stalk (or F_0) part of the elementary particle. These protons attack the oxygen of phosphate groups previously bound to the ball (or F_1) part of the elementary particle. The combination of phosphate and atomic oxygen results in the formation of water and an energized phosphate ion. This phosphate ion is then in position to unite with ADP, which has also previously been bound to F_1. This forms ATP. Phosphorylation may thus be written as two partial reactions:

$$2\,H^+ + PO_4^{3-} \rightarrow H_2O + PO_3^{-5} \qquad (6\text{-}1)$$

$$PO_3^{-5} + ADP^{3-} \rightarrow ATP^{4-} \qquad (6\text{-}2)$$

The formation of ATP according to Mitchell's chemiosmotic theory represents a reversal of the hydrolysis of ATP. Recalling Equation 3-2, the hydrolysis of ATP can be written as

$$ATP + HOH \underset{\text{Phosphorylation}}{\overset{\text{Hydrolysis}}{\underset{\leftarrow}{\xrightarrow{\text{ATPase}}}}} ADP + P_i \qquad (6\text{-}3)$$

In the mitochondrion, the phosphorylation of ADP is made possible by linking ATP production to the formation of water. With energy input, the mitochondrial ATPase is driven against its equilibrium, toward ATP production.

Function of the ETC

The electron transport chain functions based on three factors. First, each constituent can exist in reduced (higher energy, with electron) and oxidized (lower energy, without electron) forms. Second, the ETC constituents are sequentially arranged in close proximity on the inner membrane, forming a "respiratory assembly." Again, these respiratory assemblies are linked through the mitochondrial reticulum and run from the cell surface to deep within the fiber. Distance, therefore, does not impede electron movement. Third, and most important, the ETC constituents are arranged such that the redox potential of each constituent is greater (i.e., more positive) than that of the previous constituent. As a result, electrons can move from NADH (electronegative redox potential) to atomic oxygen (electropositive). Oxygen is called "the final electron acceptor."

Control of the ETC

We have seen that the adenylate energy charge regulates both glycolysis and the Krebs cycle. It should therefore not be surprising that ADP and ATP, respectively, stimulate and inhibit the ETC. The control of muscle metabolism is elegantly simple. As soon as muscle contracts, ATP is split and ADP is formed. The change in the relative amounts of these substances then sets in motion biochemical events to re-form the spent ATP. When exercise stops, the cellular respiratory mechanisms soon reestablish normal levels of ATP, ADP, and AMP. Consequently, whole-body O_2 consumption rapidly declines toward resting levels after exercise. The mechanism by which ATPase activity of the contractile elements is buffered by cytoplasmic creatine phosphate, with the ultimate result being phosphorylation of mitochondrial ADP to ATP, is termed the *creatine phosphate shuttle* (Figure 6-12).

Recall from Chapter 3 (Eq. 3-3) that in the muscle cytosol after a contraction, the level of ATP is buffered by the creatine kinase reaction and the degradation of creatine phosphate, which acts as a "chemical capacitor" restoring ADP produced during contractions to ATP. Recall also that the level of ADP is further reduced by the myokinase reaction (Eq. 3-4). As well, in our introduction to Atkinson's concept of an adenylate energy charge, we noted that in terms of cytosolic signals, AMP was more important than ADP, which is in turn more important than ATP, which is the least important signal. However, as emphasized by Martin Kushmerick and Kevin Conley of the University of Washington, mitochondrial ADP is a powerful regulator of mitochondrial respiration (Kushmerick and Conley, 2002).

As shown in Figure 6-12, ATP hydrolysis by the contractile elements does result in free creatine in the cytosol that is rapidly phosphorylated by the mitochondrial reticulum to CP, the result being mitochondrial ADP. It has been long known (Chance and Williams, 1956) that isolated mitochondria are highly responsive to ADP, the presence of which stimulates oxidative phosphorylation according to the strict stoichiometry as defined in Equation 6-2 and associated discussion of the electron transport chain (ETC). For each mol atom of oxygen used by the ETC, for NADH-linked substrates in mitochondria 3 ADP are phosphorylated to 3 ATP (i.e, the ADP/O = P/O = ATP/O = 3). From studies on exercising humans (Gaesser and Brooks, 1975), we know that in working human muscle each mol of O_2 results in phosphorylation of 6 mol of ADP to ATP (i.e., $ADP/O_2 = 6$). Therefore, even though levels of ATP and ADP are highly buffered in the cytosol, because of the creatine kinase reaction and relationship between cytosolic CP degradation and mitochondrial ADP supply illustrated in Figure 6-12, ADP exerts exquisite control over mitochondrial O_2 consumption. By means of the creatine kinase reaction equilibrium, the magnitude and rate of ATP demand (mostly to support processes related to muscle contraction and relaxation), the magnitude and rate of ATP supply, and the magnitude and rate of cellular oxygen consumption are unified. So, to reiterate from Chapter 5, *energy flux is the most important regulator of metabolism.*

The Number of ATP from a Glucose Molecule

The breakdown of glucose and glucose 6-phosphate in muscle is extremely important because it can oc-

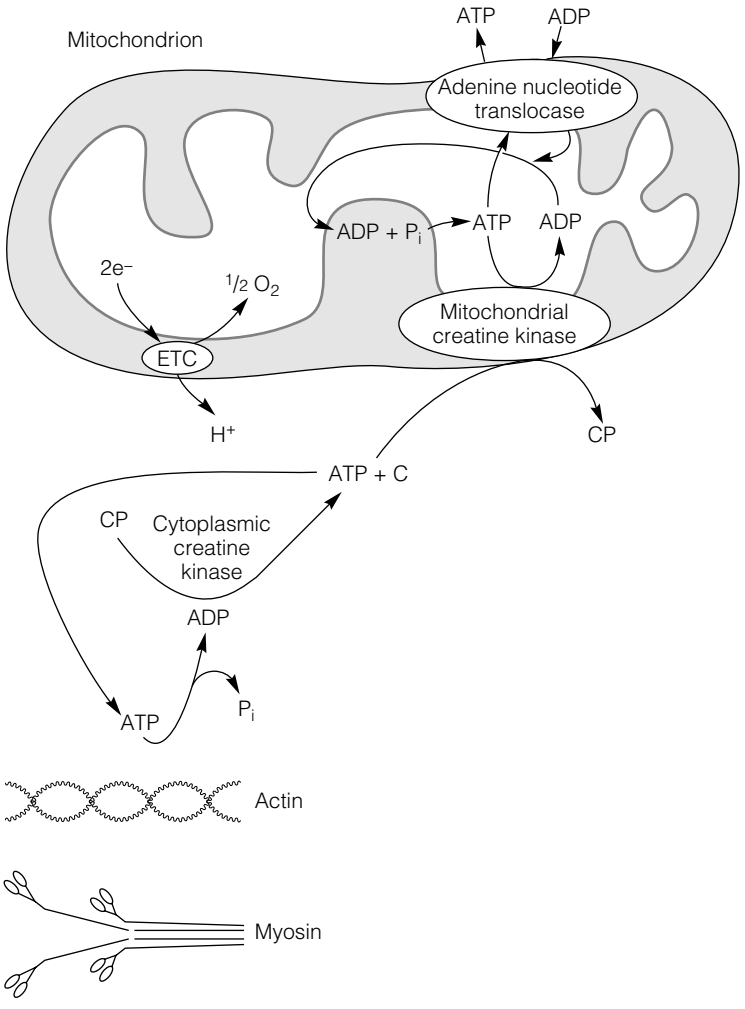


Figure 6-12 A model of the control of cellular respiration by creatine phosphate and ADP (the creatine phosphate shuttle). The model begins at the lower left, where the contractile proteins actin and myosin hydrolyze ATP. The resulting ADP is phosphorylated by cytoplasmic creatine kinase, with CP serving as the phosphate donor. The resulting cytoplasmic creatine is rephosphorylated by mitochondrial creatine kinase. Thus, ATP hydrolysis in the cytoplasm results in ADP formation in mitochondria. The rates of electron transport and O_2 consumption in mitochondria respond to the presence of ADP, phosphorylating it to ATP.

cur rapidly and therefore can supply energy rapidly. Further, glycolysis can occur in the presence or absence of oxygen. This means that energy requirements in muscle can be supplied by glycolysis for finite periods of time under anaerobic conditions.

Under aerobic conditions, for each glucose unit a net of two ATPs are formed in the cytoplasm, and the energy equivalents of two cytoplasmic NADH can be shuttled into the mitochondria. Recalling that the reducing equivalent energy of cytoplasmic NADH can give rise to either NADH or FADH within mitochondria, and that the P:O of NADH is 3 and the P:O of FADH is 2, then, under aerobic conditions, glycolysis yields four to six ATPs in mitochondria in addition to the two cytoplasmic ATPs. Together with the ATP formed in the TCA cycle and the ATP produced as the result of reducing equivalents donated from the TCA cycle to the ETC, the ATP yield per glucose is 36 to 38 molecules of ATP per molecule of glucose.

As we noted earlier, when glycolysis is anaerobic, a net of two ATPs is formed per glucose.

When the substrate for glycolysis originates from glycogen—that is, when glucose 6-phosphate is supplied by glycogenolysis—it is more difficult to estimate the ATP yield per glucosyl unit cleaved

from glycogen. When a molecule of glucose enters the glycolytic pathway through the action of the enzyme hexokinase, a molecule of ATP is required to energize glucose to the level of glucose 6-phosphate. In glycogenolysis, this energizing step is bypassed, and theoretically we could consider that the energy of one phosphorylation is saved. Thus, the ATP yield for a glucosyl unit derived from glycogen under anaerobic conditions may be 3. However, it may be incorrect to assume that glycogen yields relatively more ATP than does glucose. This is because it takes energy to synthesize glycogen from glucose or glucose 6-phosphate. Although glycogen synthesis may have preceded glycogenolysis in time by hours or days, the energy requirement for the process should be taken into account. Further, ATP was required to activate the phosphorylase, the enzyme catalyzing glycogenolysis. In starting glycolysis from glucose, each molecule has to be phosphorylated. In glycogenolysis, the enzyme rather than the substrate is phosphorylated.

The activity of phosphorylase is much higher in muscle than is the activity of hexokinase. Consequently, entry of glucosyl units into glycolysis during exercise is more rapid from glycogen than from glucose. It follows, therefore, that the ATP yield for anaerobic glycolysis during heavy exercise reflects the dominant role of glycogenolysis and is closer to 3 than to 2.

■ Effects of Training on Skeletal Muscle Mitochondria

Beginning in the late 1960s and early 1970s, John Holloszy in St. Louis, Missouri, and Philip Gollnick in Pullman, Washington, ushered in a new era of study on what has been called *exercise biochemistry.* In their initial papers and in subsequent reports with associates, Gollnick (1969) and Holloszy (1967, 1975; Baldwin et al., 1972; Holloszy and Booth, 1976; Holloszy et al., 1971) have identified a number of specific effects of endurance training on skeletal muscle mitochondria and respiratory capacity. Researchers in other laboratories have obtained similar results. Table 6-1 summarizes some of the results of these studies. In response to the endurance-training procedure utilized, several enzymes of the TCA cycle and constituents of the ETC have been observed to double in activity.

A major question in exercise biochemistry has been: How is the increase in muscle mitochondrial capacity (Table 6-1) accomplished? Do the mitochondria adapt in specific ways, such as by an in-

TABLE 6-1

Effect of Endurance Training on Respiratory Capacity of Rat Skeletal Muscle Mitochondria[a]

Muscle	Group	Muscle Citrate Synthase ($\mu mol \cdot g^{-1} \cdot min^{-1}$)	Muscle Carnitine Palmityl-transferase ($\mu mol \cdot g^{-1} \cdot min^{-1}$)	Muscle Cytochrome *c* ($nmol \cdot g^{-1}$)
Fast-twitch white	Exercised	18 ± 1	0.20 ± 0.02	6.3 ± 0.7
	Sedentary	10 ± 1	0.11 ± 0.01	3.2 ± 0.3
Fast-twitch red	Exercised	70 ± 4	1.20 ± 0.09	28.4 ± 2.1
	Sedentary	36 ± 3	0.72 ± 0.06	16.5 ± 1.6
Slow-twitch red	Exercised	41 ± 3	1.20 ± 0.05	—
	Sedentary	24 ± 2	0.63 ± 0.07	—
Heart	Exercised	160 ± 4	—	46.6 ± 0.9
	Sedentary	158 ± 1	—	47.1 ± 1.0

[a] In response to endurance training, mitochondrial components in different types of rat skeletal muscles double in concentration. The heart does not change.

SOURCE: Baldwin et al. (1972), Holloszy (1967, 1975), Holloszy and Booth (1976), Holloszy et al. (1971), Oscai et al. (1971a, 1971b).

crease in the number or density of enzymes on the mitochondrial cristae, or are there simply more mitochondria (a larger mitochondrial reticulum)? The answer to this question has been investigated by scientists at the University of California, Berkeley. Their results clearly indicate that mitochondria do not increase in specific activity—that is, in enzymatic activity per unit of mitochondrial protein (Table 6-2, top). Rather, there are more mitochondria or there is a more elaborate reticulum (Table 6-2, bottom). With a few minor exceptions, such as the labile, nonstructural enzyme α-glycerol phosphate

TABLE 6-2

Specific Activity (Top) and Mitochondrial Content (Bottom) in Skeletal Muscle from Endurance-Trained Rats and Sedentary Controls[a–c]

	Mitochondrial Protein Specific Activity ($nmol \cdot mg^{-1}$)	
Parameter	**Control Group ($n = 10$)**	**Endurance Group ($n = 10$)**
Cytochrome *a*	0.37 ± 0.02	0.35 ± 0.08
Cytochrome *b*	0.32 ± 0.02	0.36 ± 0.02
Cytochrome *c* (+ c_1)	0.91 ± 0.05	0.82 ± 0.03
Flavoprotein	1.13 ± 0.08	1.04 ± 0.05
b/*a*	0.86	1.03
c (+ c_1)/*a*	2.46	2.34
Flavoprotein/*a*	3.05	2.97

	Mitochondrial Content of Muscle ($mg \cdot g^{-1}$)		
Parameter	**Control Group ($n = 9$)**	**Endurance Group ($n = 9$)[b]**	**Percentage Difference of Control from Endurance Trained**
Pyruvate–malate oxidase	15.5 ± 0.7	25.1 ± 1.2	+ 62
Succinate oxidase	20.1 ± 1.0	43.6 ± 2.1	+ 117
Palmitoyl carnitine oxidase	21.1 ± 2.6	50.4 ± 4.7	+ 138
Cytochrome oxidase	19.2 ± 0.8	38.3 ± 1.8	+ 99
Succinate dehydrogenase	14.9 ± 0.8	30.9 ± 1.8	+ 108
NADH dehydrogenase	17.5 ± 0.8	27.9 ± 1.7	+ 59
Choline dehydrogenase	25.9 ± 1.7	55.7 ± 3.8	+ 115
Cytochrome *c* (+ c_1)	16.2 ± 0.6	31.6 ± 1.2	+ 95
Cytochrome *a*	18.2 ± 0.7	36.5 ± 1.6	+ 101
Average	18.7 ± 1.2	37.8 ± 3.7	+ 99

[a] Values are mg mitochondrial protein • g wet muscle^{-1} (means ± SE). Mitochondrial content of muscle was calculated as muscle activity/mitochondrial specific activity, for the relevant oxidases, dehydrogenases, and cytochromes.
[b] All endurance-trained values were significantly higher than controls, $P < 0.01$ (one-tailed *t* test).
[c] In terms of specific activity (units of activity or component content per unit of mitochondrial protein), no difference is seen between endurance-trained and sedentary control groups (top table), whereas the muscle mitochondrial content doubles (bottom table).

SOURCE: Davies et al. (1981, 1982).

dehydrogenase, most mitochondrial constituents increase in direct proportion to the amount of mitochondrial material. Further, the Berkeley group showed no change in protein/lipid ratio with training. Training does affect the mechanisms of mitochondrial replication and destruction, however. As a result of endurance training, skeletal muscle contains more mitochondrial material, but its activity is the same as that of untrained individuals.

Examinations of muscle from untrained and trained animals under the electron microscope (Figure 6-13) provide results consistent with the biochemical analyses (Tables 6-1 and 6-2). Mitochondria in tissues of trained rats may appear to be more numerous. We may say that the mitochondrial reticulum is more elaborate in response to training.

As dramatic as the effects of training on the respiratory capacity of muscle are, at least two problems need to be resolved. First, what specific aspects of endurance training result in these changes? Why does progressive resistance (weight) training not improve mitochondrial capacity? Obviously, endurance training presents some stimulus to the muscle that weight training does not. The identities of these training stimuli that cause mitochondrial protein to increase in response to endurance training and contractile protein to increase in response to heavy resistance training are under investigation.

A second major question concerns the significance of the doubling of mitochondrial activity. Table 6-1 indicates large increments in the respiratory capacities of mitochondria in all three types of skeletal muscle fibers. In humans and in laboratory animals, training may increase by severalfold the length of time a submaximal work load can be endured. However, in both humans and laboratory animals, maximal O_2 consumption capacity increases only 10 to 15% in response to endurance training. In results obtained by the U.C. group, muscle mitochondrial cytochrome oxidase activity correlated 0.92 with running endurance but only 0.70 with $\dot{V}_{O_2max}$ (Table 6-3). Consequently, it appears that mitochondrial capacity may be better related to endurance than $\dot{V}_{O_2max}$. The reason for this needs to be elaborated, but most likely increased mitochondrial mass increases the sensitivity of respiratory control. This training effect is illustrated in Figure 6-14. A greater mitochondrial mass means that a given rate of oxygen consumption can be achieved with a higher ATP/ADP ratio. Superior respiratory control due to increased mitochondrial mass is thought to feed back on regulatory enzymes of carbohydrate degradation, thereby allowing more lipid and less carbohydrate to be consumed at a given $\dot{V}_{O_2}$ after training. The increase in mitochondrial mass due to endurance training may also be the means for increasing fat utilization as a fuel during exercise (Chapter 7). It may be that training has a greater effect on subsarcolemmal mitochondria, thereby improving the ability to maintain the in-

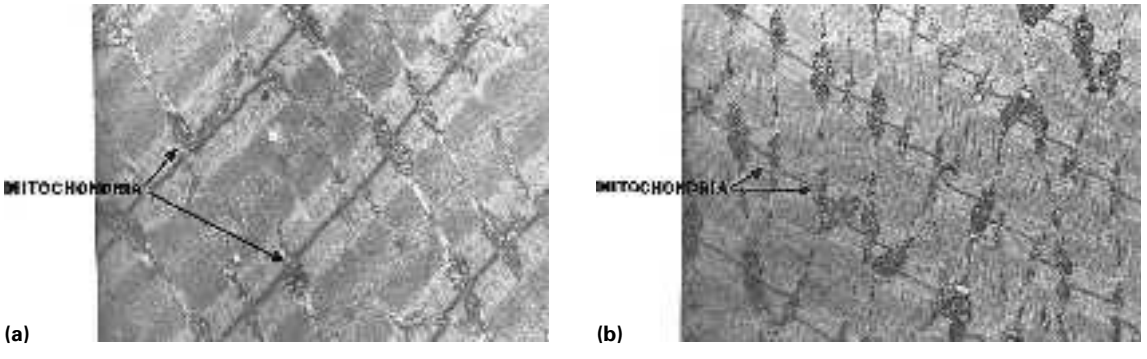


Figure 6-13 Electron micrographs of mitochondria in tissues of (a) untrained and (b) trained rats. Mitochondria in trained animals appear to be more numerous, probably because there is a more elaborate mitochondrial reticulum. Micrographs courtesy of P. D. Gollnick.

TABLE 6-3

Correlation Matrix for Muscle Oxidases, $\dot{V}_{O_2max}$, and Maximal Endurance in Rats on a Treadmill[a,b]

	Pyruvate–Malate Oxidase	Palmitoyl Carnitine Oxidase	$\dot{V}_{O_2max}$ (weight normalized)	Maximal Endurance
Cytochrome oxidase	0.95	0.93	0.74	0.92
Pyruvate–malate oxidase	—	0.89	0.68	0.89
Palmitoyl carnitine oxidase	—	—	0.71	0.91
$\dot{V}_{O_2max}$ (weight normalized)	—	—	—	0.70

[a]All correlations reported were statistically significant ($P < 0.01$).
[b]Running endurance of rats as measured on a standardized treadmill test correlates significantly better with skeletal muscle mitochondrial activity (i.e., cytochrome oxidase activity) than with maximal O_2 consumption ($\dot{V}_{O_2max}$).

SOURCE: Davies et al. (1981, 1982).

tegrity of the cell membrane and thus improving endurance during heavy exercise. Training may have a lesser effect on the interfibrillar mitochondria—those supportive of muscular contraction. Alternatively, it is likely that $\dot{V}_{O_2max}$ is limited by blood flow (cardiac output) but that endurance is dependent on mitochondrial function. From this perspective, it may be that exercise exhausts the functional capability of mitochondria and that having a large, elaborate reticulum, or many mitochondria, retards the eventual fatigue point. This question is being investigated.

Doubling the mitochondrial content in muscle due to training (as determined by biochemical procedures) is consistent with an elaboration of the mitochondrial reticulum due to training. This elaboration of the mitochondrial reticulum due to training, however, has yet to be demonstrated by appropriate morphometric studies. Similarly, it has not yet been established whether subsarcolemmal mitochondria are arranged in a reticulum, or whether the subsarcolemmal reticulum (if it exists) is continuous with the intrafibrillar reticulum. However, on the basis of electron microscopic information now available

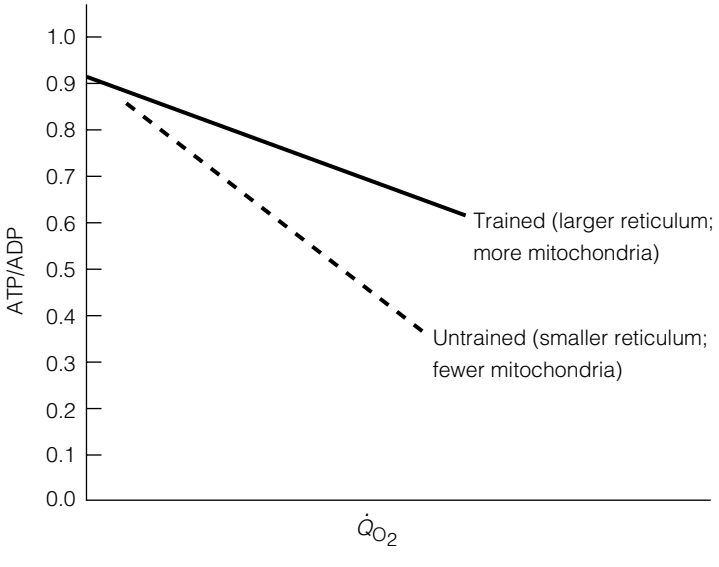


Figure 6-14 Increasing the mitochondrial mass in a muscle, or any cell, allows a given rate of mitochondrial oxygen consumption $\dot{Q}_{O_2}$ to be accomplished at a higher ATP/ADP ratio. This increased sensitivity of respiratory control is thought to down regulate glycolysis, thus allowing for greater lipid oxidation at a given $\dot{Q}_{O_2}$ and $\dot{V}_{O_2}$.

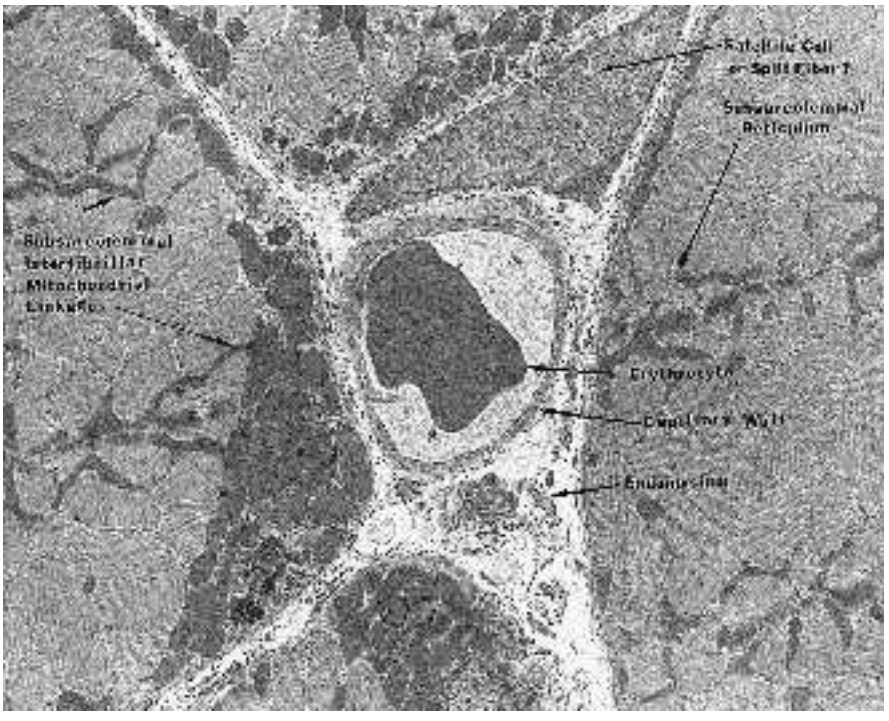


Figure 6-15 Electron micrograph of rat deep (red) vastus thigh muscle showing linkages between subsarcolemmal and interfibrillar mitochondria. Micrograph courtesy of S. P. Kirkwood, E. A. Munn, L. Packer, and G. A. Brooks.

(Figures 6-3, 6-4, 6-5, 6-13, and 6-15), it appears that the mitochondrial reticulum is continuous from the subsarcolemmal area to deep regions within a muscle cell.

■ The Training Adaptation and Coordination of Mitochondrial and Nuclear Genes

Despite remarkable discoveries in the areas of mitochondrial energetics and biogenesis, we do not yet know the signal for the proliferation of the mitochondrial reticulum in response to endurance training. As well, through advances of "modern biology" we are coming to know why and how muscle mitochondrial mass increases in response to endurance exercise training, chronic electrical stimulation, and intermittent interruption of blood flow. It has been suspected that things such as Ca^{++} oxygen free radicals, hypoxia, ADP, and inorganic phosphate affect the balance of synthesis and degradation of mitochondrial constituents, including proteins and phospholipids. We hope that in the not-too-distant future, the signals and mechanisms of action of mitochondrial genesis will be understood, as such knowledge will have wide clinical application.

Initial progress in the field of mitochondrial biogenesis is attributable to investigators such as Booth, Williams and Essig, and their associates (Booth and Thompson, 1991; Williams, 1986; Williams et al., 1986; Essig and McNabney, 1991). More recently, David Hood of York University has moved the field ahead. Progress in the field has been complicated by the fact that while the mitochondrial reticulum contains multiple copies of the

small (16,659 nucleotide) circular mitochondrial DNA (mtDNA) molecules; mtDNA codes for only 13 mRNA, 22 tRNA, and 2 rRNA molecules. Mitochondrial DNA is remarkably well-conserved across species and in gametes is carried on the X chromosome. Hence, mitochondrial characteristics are inherited from the mother. Because mtDNA is so small, only 13 mitochondrial proteins are coded for in the mitochondria and most are encoded in the nucleus. From long experience we know that while endurance training increases muscle mitochondrial mass and enzymatic activity (Table 6-1), mitochondrial composition and specific activity are preserved (Table 6-2). As well, we know that the mtDNA copy number/mitochondrial mass ratio is constant whether there are increases due to training or decreases due to casting, denervation, or other forms of inactivity (Williams, 1986). As a result, we can deduce that there is exquisite coordination of mitochondrial and nuclear genes in assembling the larger mitochondrial reticulum that comes from endurance training.

Because mitochondrial adaptations occur only in muscles made to contract, it is believed that local signals in the tissue, and not circulating factors, are responsible for coordinating mitochondrial biogenesis in working muscle. At present, two factors, calcium ion (Ca^{++}) and ATP turnover, appear to be most responsible for the mitochondrial biogenesis following exercise. However, many metabolites and processes change in muscles when they contract, and at present no one candidate stands out to explain all that is known to happen in response to exercise. For a more detailed review of this and related issues, readers are referred to the review of David Hood (2001). As shown in Figure 6-16, Ca^{++} released from the sarcoplasmic reticulum (SR) activates kinases (e.g., protein kinase C, PKC) and phosphatases (e.g., calcinurin) that are translocated into the nucleus to affect activation of transcription factors which influence the expression of nuclear genes encoding for mitochondrial proteins. As well, Ca^{++} enters mitochondria, binds to and activates the TCA cycle dehydrogenases, and affects energy status of the organelle by affecting the mitochondrial chemiosmotic gradient ($\Delta\psi$).

Activation of mitochondrial biogenesis by increased energy demand and ATP turnover probably does not result from a change [ATP], but [AMP] does rise during physical activity (see Figure 3-7). Activation of AMP-active protein kinase (AMPK, discussed more in Chapter 7) increases expression of mitochondrial proteins (Winder et al., 2000). As well, activation of other protein kinases such as PKC and mitogen-activated protein (MAP) kinase may be involved in phosphorylation and activation of transcription factors such as nuclear respiratory factor-1 (NRF-1). Other transcription factors thought to be important are peroxisome proliferator-activated receptors-α and -γ (i.e., PPAR-α and PPAR-γ). Transcription factors such as NRF-1 and PPAR-α bind to upstream regulatory regions of genes, such as those coding for subunits of cytochrome oxidase (COX) and enzymes of mitochondrial free fatty acid uptake (e.g., CPT-1, Chapter 7), respectively. However, at this point our understanding of the complex regulation of mitochondrial biogenesis is incomplete; for instance, not all 10 genes coding for subunits of COX have NRF-1 binding sites.

In addition to a growing, but still incomplete, understanding of the transcription of nuclear genes coding for mitochondrial proteins, there are even wider gaps in other aspects of our knowledge of the regulation of mitochondrial biogenesis. For instance, much of the reticulum consists of phospholipids, but their synthesis and organization into the lipid bilayer sheets forming the reticular network is little studied. Similarly, little is known about stability of mRNA stability, even whether or not physical activity affects stability of messages for translation of mitochondrial proteins. Fortunately, from studies on yeast more is known about how proteins synthesized by ribosomes in the cytosol are imported into mitochondria.

Frequently, mitochondrial precursor proteins are synthesized with charged signal sequences that in the cytosol interact with accompanying proteins (chaperones) such as the 70-kDa heat shock protein (HSP70). Together with its chaperone, the mitochondrial precursor protein complex is drawn to an outer membrane import receptor complex, termed

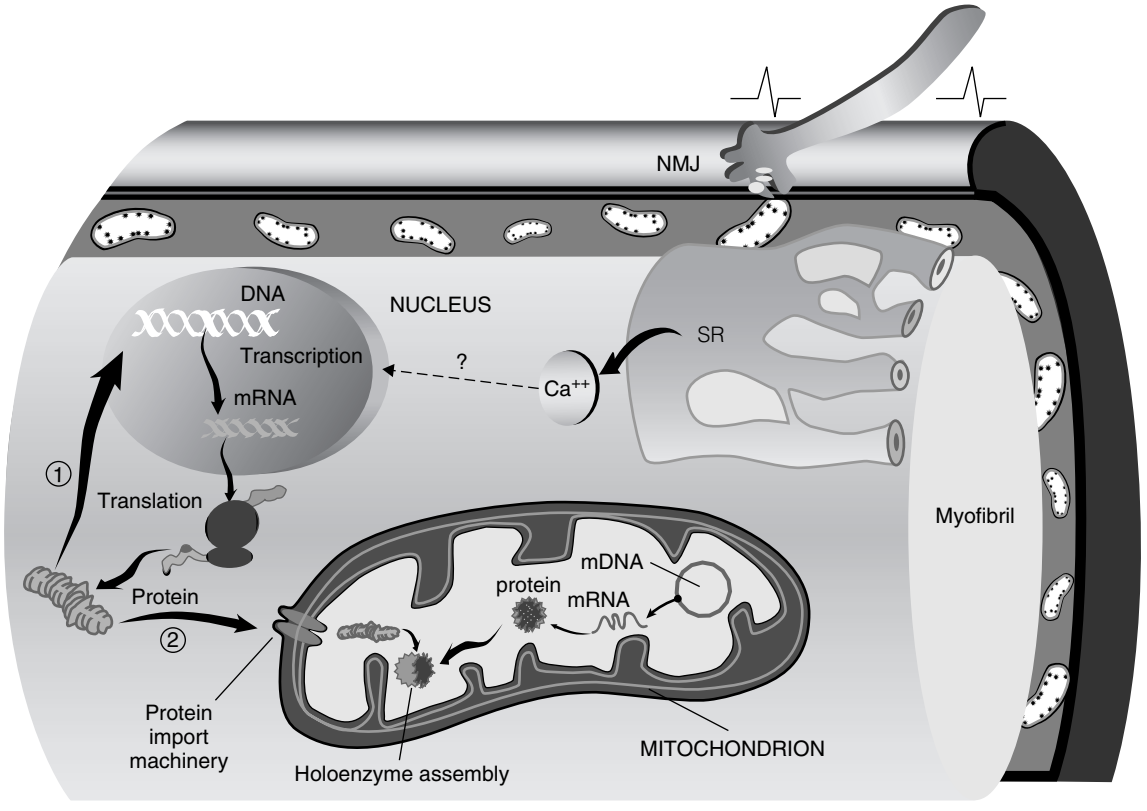


Figure 6-16 Overall synopsis of mitochondrial biogenesis in a muscle cell as described by David Hood, York University, Toronto. Muscle fiber (cell) stimulation by action potentials from the neuromuscular junction (NMJ) result in electrical activity in the sarcolemma that is coupled to the release of calcium from the sarcoplasmic reticulum (SR). Calcium ion (Ca^{++}) acts in several ways. First, Ca^{++} is a second messenger to activate phosphatases and/or kinases, which are ultimately translocated to the nucleus to affect the activation of transcription factors and which influence the expression of nuclear genes encoding mitochondrial proteins. Messenger RNA (mRNA) produced by transcription is translated into protein in the cytosol, which can be either translocated back to the nucleus (transcription factor) or chaperoned to the protein import machinery and taken up by the organelle. Within mitochondria it may act as a single protein subunit or be combined with other subunits to form a multi-subunit enzyme (holoenzyme) (e.g., cytochrome *c* oxidase that has 10 subunits). Some subunits of the holoenzyme may be derived from the mitochondrial genome (mtDNA), which also undergoes transcription and translation to synthesize 13 proteins that are essential components of the electron transport chain. As well, in working muscle Ca^{++} released as part of the excitation-contraction process (Chapter 18) activates TCA cycle dehydrogenases, and the mitochondrial chemiosmotic gradient ($\Delta\psi$), events that affect the mitochondrial energy status and respiratory rate. Courtesy of D. Hood and the American Physiological Society.

the *translocase of the outer membrane ("Tom") complex.* If the protein is to enter the mitochondrial matrix, the chaperone complex next traverses the *translocase of the inner membrane ("Tim") complex.* Function of the Tim complex is facilitated by phospholipids unique to the inner mitochondrial membrane such as cardiolipin. Several factors including charge determine whether the protein is eventually oriented in the outer or inner mitochondrial membrane or drawn to the matrix by the mitochondrial $\Delta\psi$. In general, the more positive the charge on the chaperone-protein complex the more strongly it is drawn to the matrix where the mitochondrial $\Delta\psi$ exerts a negative charge. Once at the appropriate site, the prosthetic-targeting group and chaperone are cleaved by mitochondrial-processing peptidase (MPP), leaving the free mitochondrial protein.

So far as the replication of mtDNA is concerned, again it is known that the mtDNA copy number/mitochondrial mass ratio is constant. The mitochondrial transcription factor (Tfam) is a nuclear-encoded transcription factor synthesized outside the reticulum that interacts with HSP70 for translocation into the matrix. In the matrix, Tfam interacts with heavy-strand promoters (HSP) and light-strand promoters (LSP) within the D-loop region of mtDNA and stimulates replication of mtDNA. Replication of mtDNA requires presence of DNA polymerase-γ (poly-γ), a single-stranded binding protein (SSB), Tfam, and a mitochondrial RNA-processing endoribonuclease (RNase MRP), and the latter may hold a key to understanding how the nuclear and mitochondrial genomes are coordinated. Ordway, Williams, and their associates (1993) studied the expression of a small RNA encoded within the nucleus that is an essential subunit of RNase MRP. The nuclear mitochondrial RNA-processing RNA (MRP-RNA) generates primers for mtDNA replication. Thus, mtDNA copy number is highly correlated expression of MRP-RNA, SSB (Schultz et al., 1998) and Tfam (Gordon and Hood, et al., 2001), but not poly-γ, which is present in abundance and not rate limiting.

In closing this section, it is necessary to emphasize that this is a rapidly developing area in which new discoveries are continually emerging. Students interested in the topic are urged to consult reviews by Hood (2001), Hoppeler and Flück (2003), and others as they emerge. Because this is a such a dynamic area due to the development of new technologies as well as opportunities for advancing basic and applied sciences, it is also a fecund area of investigation for young scientists.

■ What Is the Mitochondrial Oxygen Partial Pressure During Exercise?

For years, it has been known that the critical mitochondrial oxygen tension is very low, on the order of 1 mm Hg (Figure 6-17). The critical oxygen tension is the partial pressure below which there is insufficient oxygen for mitochondria to achieve maximal rates of respiration. A major technical limitation in measuring critical oxygen tension has been the difficulty of measuring oxygen tension in working skeletal muscle as well as the diurn right tfficulty of understanding the relationship between working muscle P_{O_2} and lactate formation. With dog muscle

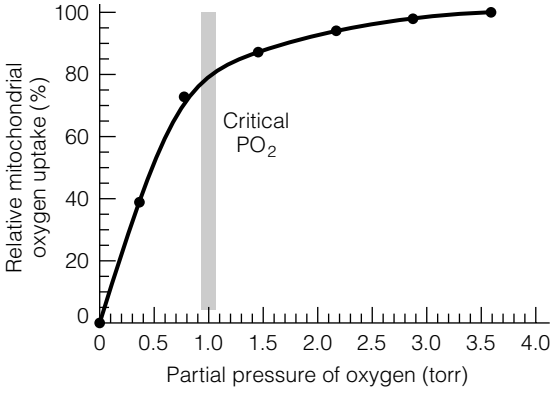


Figure 6-17 Relationship between oxygen consumption and partial pressure of oxygen in mitochondria isolated from heart. The critical mitochondrial P_{O_2} is the pressure below which maximal respiratory rate (V_{max}) cannot be maintained. Modified from Rumsey and Wilson et al. (1990). As discussed by Gladden (1996), the critical mitochondrial P_{O_2} may even be lower ($\cong$ 0.5 torr). Used with permission of the American Physiological Society.

preparations, first Jöbsis and Stainsby (1968) and then Connett and associates (1984) provided data that working muscles maintain a P_{O_2} above the critical oxygen tension while lactate is released. In other words, lactate was formed under fully aerobic conditions (see Figure 5-15). Unfortunately, the techniques used on isolated muscles in anesthetized dogs were not readily transferable to human exercise studies.

More recently, Richardson and associates (1998) utilized classical (a-v) lactate balance measurements for measuring lactate release along with NMR spectroscopy to measure myoglobin saturation in the working human quadriceps muscle. Using the myoglobin spectra from muscle, and knowing the shape of the myoglobin dissociation curve, Richardson and colleagues were able to calculate the intramuscular P_{O_2}. Their results for intracellular P_{O_2} and net lactate release during progressive exercise to maximum are portrayed in Figure 6-18. At rest, for healthy subjects breathing normal air muscle P_{O_2} is quite high, very close to that in the venous effluent from the limb, approximately 40 torr. However, when exercise starts, muscle P_{O_2} falls dramatically to approximately 4 torr, a value well above the critical mitochondrial P_{O_2}. Moreover, the intracellular P_{O_2} (triangles in Figure 6-18) was well maintained above the critical mitochondrial P_{O_2} as power output increased.

In contrast to intramuscular P_{O_2}, which fell rapidly at exercise onset, as has been seen many times before, muscle lactate release (circles in Figure 6-18) changed little at exercise onset. However, at approximately a power output corresponding to 65% of $\dot{V}_{O_2max}$, coinciding with a rise in arterial epinephrine, muscle lactate release began a steep rise, again an observation made previously. Thus, as predicted from the model of an intramuscular lactate shuttle, during both rest and exercise lactate is formed and released under fully aerobic conditions. During easy to moderate intensity exercise (i.e., between 50% and 65% $\dot{V}_{O_2max}$), muscles do not release lactate, probably because clearance by oxidation in the muscle balances production (see Figure 5-28). However, when the glycolytic flux and lactate production exceeds that which can be cleared by mitochondria, then the excess glycolytic flux is released as lactate.

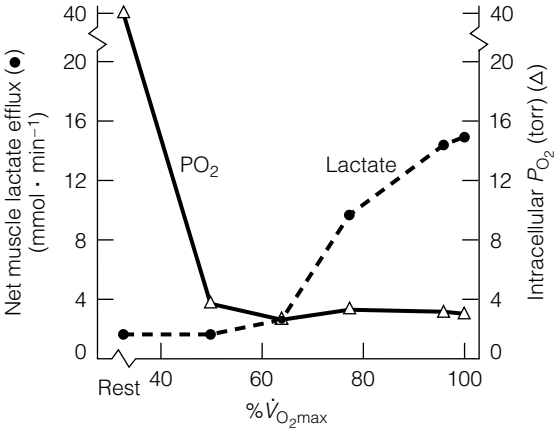


Figure 6-18 Muscle intracellular P_{O_2} (determined from NMR myoglobin spectroscopy) and net lactate release (determined from arterial-venous difference measurements) in resting and exercising men. Note that when exercise starts, muscle P_{O_2} falls but remains well above the critical mitochondrial O_2 tension of 1 torr. In contrast, resting muscle releases a small amount of lactate, and muscle net lactate release does not change until a power output eliciting 65% of $\dot{V}_{O_2max}$ is achieved. Lactate is formed under fully aerobic conditions, during both rest and exercise. Modified from Richardson et al. (1998) with inclusion of resting values from parallel experiments. Used with permission.

Before closing this section, it needs to be acknowledged that at the present time investigators are pushing the limits of technology in applying NMR spectroscopy to contracting human skeletal muscle. In their efforts at the University of California at Davis, Thomas Jue and the late Paul Molé also used NMR and myoglobin saturation measurements to measure P_{O_2} in working human muscle. Their data show a *less* pronounced decline in muscle P_{O_2} at exercise onset than evident in Figure 6-18. However, the values during hard and maximal exercise (≅4 torr) are similar. With continued efforts, investigators will soon agree on the shape of the curve relating muscle P_{O_2} and power output. For the present, however, it is probable that the results showing lactate production in muscle during sub-

maximal exercise when P_{O_2} is well above the critical mitochondrial P_{O_2} will be confirmed.

Students interested in reading more about this exciting contemporary research are encouraged to read Bruce Gladden's review on the subject that appeared in the *Handbook of Physiology*, Section 12, 1996.

■ Free Radicals, Reactive Oxygen Species (ROS), Oxidative Damage, Cell Signaling and Protection

Although one of the coauthors (GAB) was a coinvestigator of the first paper on free radical formation during exercise (Davies et al., 1982), this fourth edition of *Exercise Physiology* is the first to include a presentation of the topic. Thanks to the contributions of many investigators, a reasonable presentation is now possible.

A free radical is an atom or molecule with an unpaired electron in the outer valence, and oxygen is a molecule susceptible to becoming a superoxide ($O_2^{\bullet -}$). Based in initial work by Boveris et al. (1976), there emerged concern that electron leak during physical exercise when the rate of oxygen consumption is raised could produce significant amounts of superoxide during exercise. Moreover, it soon became recognized that working muscle had the potential to form other reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), nitric oxide (NO^-), and hydroxyl radicals (HO^-). Because of the unpaired electron in the outer valence shell, free radicals are potentially dangerous as they are likely to react indiscriminately with any molecule nearby including DNA, RNA, proteins, and lipids. Indeed, it has been found that free radicals are involved in the death of pancreatic β-cells in Type 1 Diabetes, tissue reperfusion injury after surgery, and some forms of cancer. Further, free radicals are damaging because the electron can move from one molecule to the next, thus propagating a cascade of damage. A prevalent, but unsubstantiated, theory of aging holds that free radicals are the cause. This theory led some biochemists to become concerned that physical exercise was damaging and caused premature aging while some commercial entities became very interested in selling vitamins and other forms of "antioxidants" to millions of potential athletic and aging customers.

Fortunately, as recognized immediately by exercise physiologists, exercise is healthful, increases vigor, and prolongs life. Insistence by physiologists that the effects of exercise are contrary to those attributed to it by the *chicken littles* of chemistry and pill merchants has led to really important discoveries of the roles of radicals in cellular functioning.

Because of their ephemeral nature and characteristics of high reactivity and short half-life, it is very hard to know how many radicals are formed during exercise. Certainly, ROS are formed but whether they are damaging or provide signals that induce protective adaptations to stress is another matter. Likely, free radicals are important as they promote adaptive processes. Cells have several lines of defenses to quench and protect against radicals; these are enzymes, water and lipid-soluble antioxidants (glutathione and vitamins).

Enzymes

Cells contain three main antioxidant enzymes, one of which occupies two cell domains. These are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX).

SOD. As implied by its name, superoxide dismutase dismutates (breaks up) superoxide radicals:

$$2\ O_2^{\bullet -} + 2\ H^+ \rightarrow H_2O_2 + O_2$$

Mammals possess two isoforms of SOD, the manganese catalyzed SOD (Mn-SOD) in mitochondria, and the copper-zinc catalyzed SOD (Cu-Zn-SOD) that exists in the cytosol. Hence, Mn-SOD provides the first line of defense against oxygen radicals produced in exercise, with the Cu-Zn-SOD and other mechanisms in backup position.

Muscles of untrained individuals possess sufficient SOD that superoxide-induced damage is below the level of detection even in weekend warriors.

However, since endurance training can double the mitochondrial mass, training also doubles the level of SOD. Hence, regular exercise provides protection against ROS, not just during the activity itself, but throughout the day.

CAT. Catalase functions to degrade hydrogen peroxide to water and oxygen:

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

Catalase is widely distributed in mammalian cells, with highest concentrations in peroxisomes and mitochondria. Endurance training is not known to have a physiologically significant effect on CAT activity.

GPX. The enzyme glutathione peroxidase catalyzes the reduction of hydrogen peroxide (H_2O_2) and requires reduced glutathione (GSH, the SH referring to the sulfhydryl group that quenches radicals). Glutathione disulfide (two glutathione molecules) and water are the products:

$$2\ GSH + H_2O_2 \rightarrow GSSG \text{ and } 2\ H_2O$$

Like SOD, GPX exists in cytosol and mitochondria where GSH exists in significant quantities. *In vivo,* function of GPX requires function of a counterenzyme to restore the level of GSH after conversion to GSSG, and this enzyme is glutathione reductase (GR). GPX is catalyzed by selenium ion (Se^{2-}), but in excess Se^{2-} can by itself act as a pro-oxidant and is of concern as an environmental toxin and pollutant. Also like the case with SOD, endurance exercise training increases muscle GPX activity and GSH content, rendering the tissue highly resistant to oxidant damage.

In addition to enzymatically-mediated antioxidant defenses, cells have antioxidant defenses which include vitamins and vitamin-like substances that quench free radicals. Of particular interest are vitamin E (α-tocopherol and other tocopherols), vitamin A, and vitamin C. Vitamin E is lipid soluble, so it exists in mitochondrial and other cellular membranes, and the others are water soluble and exist throughout cells and tissues. Vitamins manage (quench) free radicals by accepting the unpaired electron from the valence shell, thus making the vitamin-electron products radicals themselves. However, such complexes are weak radicals, accommodate and quench the electron in their molecular structure, and hence are unlikely to propagate damage to an adjacent molecule.

In summary on this section, as described by Powers and colleagues (1999) it is fair to conclude that we are evolved to be well protected against free radical damage during exercise and, further, that regular physical exercise affords additional protection. As well, radicals give rise to protective mechanisms, such as increasing expression of HSP70, an event that is involved in mitochondrial biogenesis and the protection of cell proteins against ROS. True, ROS and other free radicals can be damaging in excess, but regular exercise and good dietary practices leave our skeletal muscles (Powers et al., 1999) and hearts well protected against radical damage (Powers et al., 2001).

SUMMARY

Oxygen supplied to active muscle by the lungs, heart, and blood supports cellular production of ATP. Through the process of cell respiration in mitochondria, derivatives of carbohydrates as well as fats and proteins can yield substantial sources of potential energy for phosphorylating ADP to ATP. At present, we know that red skeletal muscle fibers are more abundant in mitochondria than are pale muscle fibers. Further, we know that the oxidative capacity of muscle improves greatly in response to endurance training because of an elaboration of the mitochondrial reticulum. Thus, *endurance training makes skeletal muscle more like the heart* in terms of its oxidative capacity. The training-induced increase in

muscle mitochondrial mass and other related training adaptations, such as increased capillarity, significantly increase the ability of working muscle to extract oxygen from the blood coursing through it. Thus, improving the muscle mitochondrial mass through training serves to increase $\dot{V}_{O_2max}$. However, the major result of increasing the muscle mitochondrial content through training is that respiratory control and lactate clearance capacity in skeletal muscle are improved. Improved respiratory control allows for muscle glycogen sparing, increased intramuscular lactate clearance, and increased lipid metabolism to occur during submaximal exercise. Mitochondrial electron transport chain activity and oxygen consumption can lead to formation of superoxide free radicals and other reactive oxygen species (ROS). However, muscles and muscle mitochondria are well protected against oxidative damage. At present, our knowledge of why and how muscle cells increase their mitochondrial content in response to endurance exercise is incomplete, but important progress in understanding mitochondrial biogenesis is being made.

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