Chapter 10
Regulation and Integration of Metabolism During Physical Activity

The survival of our ancestors was highly dependent on hunting, gathering, and fighting, behaviors that demanded intense physical activity. A sedentary lifestyle in that environment would certainly result in the elimination of the individuals. This situation imposed a selective pressure directed to adaptations of human physiology to a high capacity of physical activity, resulting in the development of a very efficient locomotor system, in which the skeletal muscles correspond to about 40 % of the body mass and account for a great proportion of the average energy consumption of the organism. The present-day sedentarism is dissonant with the human genetic background selected to favor a physically active lifestyle and probably consists in one of the main causes of the increasing incidence of modern chronic diseases, such as hypertension, obesity, and insulin resistance.

Muscles are tissues specialized in producing force and movement due to an amazing ability to convert the chemical energy of ATP phosphate bonds to mechanical work. This energy interconversion is performed by an array of proteins that forms a very organized structure inside the cells. The muscle mechanical activity may change very fast so that the energy sources and the metabolic pathways used to maintain cellular functions need to be finely regulated.

In this chapter, we will discuss the metabolic adaptations to physical exercise, with special attention to the metabolism of the skeletal muscle cells. We will start with a brief review of the structure of the contractile apparatus and the mechanism of muscle contraction, followed by the description of the energy sources and the metabolic pathways involved in muscle activity during physical exercise. We finish the chapter with the mechanism of action and the main metabolic effects of adrenaline, the major hormone secreted during exercise.

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10.1 Muscle Contraction

Muscles are used either for locomotion or for the movements associated to the functions of the internal organs and are classified in three groups according to the type of movement they generate (Fig. 10.1). The skeletal muscles can be contracted voluntarily allowing the body to move and to maintain the posture, while rhythmic involuntary muscle contractions, such as heart contraction or peristalsis and other autonomous motilities, are performed by cardiac and smooth muscles, respectively.

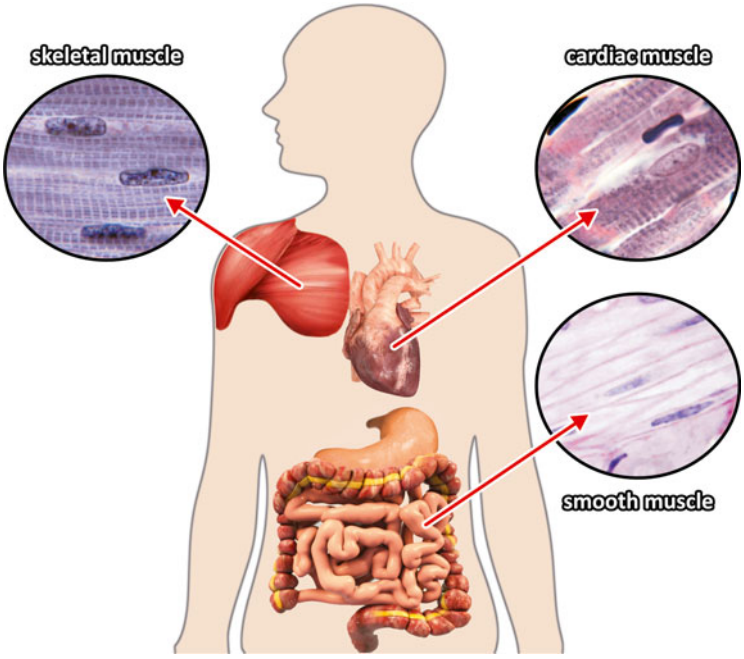


Fig. 10.1 The three types of muscle tissues. The details show histological images of skeletal, cardiac, and smooth muscles (Reprinted with the permission of Instituto de Histologia e Biologia do Desenvolvimento, Faculdade de Medicina, Universidade de Lisboa, FMUL)

10.1.1 Structural Organization of the Contractile Apparatus

In this chapter, we will focus our attention in skeletal muscles. This tissue is composed of parallel bundles of large multinucleated cells called muscle fibers. The fibers have 10–100 μm diameter and sometimes can extend over the full length of the muscle, reaching several centimeters. Most of the intracellular volume of the muscle fiber is occupied by 2 μm-thick myofibrils formed by the contractile array of proteins (Fig. 10.2a). Observed at the light microscope, the fiber presents a typical pattern that alternates light and dark bands caused by a regular arrangement of molecules of different densities (Fig. 10.2b).

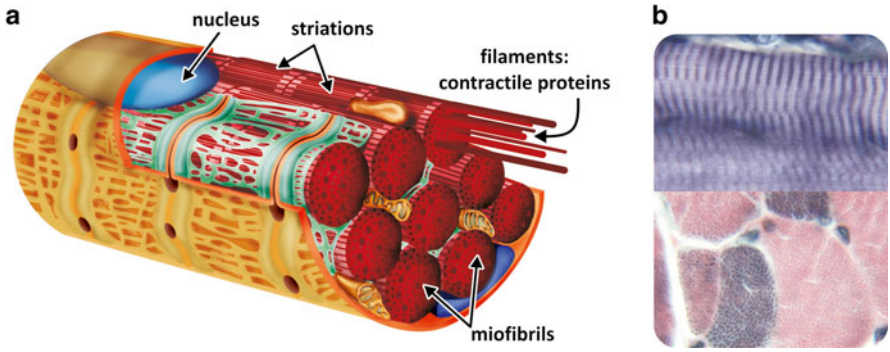


Fig. 10.2 (**a**) Schematic representation of a muscle fiber with the nucleus (*blue*) in the periphery of the cell and myofibrils formed by the contractile proteins. (**b**) Histological image of longitudinal (*top*) and transversal (*bottom*) sections of skeletal muscle tissue showing the striated pattern (Reprinted with the permission of Instituto de Histologia e Biologia do Desenvolvimento, Faculdade de Medicina, Universidade de Lisboa, FMUL)

The organization of the contractile proteins in the myofibril explains the striated pattern observed at the light microscope: they are arranged in an ordered structure forming thin and thick filaments, clearly seen by electron microscopy, as firstly observed by Hugh Huxley in the 1950s (Fig. 10.3). The different regions were named according to their characteristics. The dense regions are called A (from anisotropic) bands, while the less dense regions are named I (from isotropic) bands. A dark line in the medium point of each I band is also observed and is designated the as Z line (from the German word *zwischenscheibe*, which means "the disk in between"). A denser line is observed in the middle of the A band, called M line (from the German word *mittelscheibe*, which means "the disk in the middle"). In the resting muscle, the central region of the A band shows a lighter area, which is called H zone (from the German word *heller,* which means "brighter"). The I band is a region containing only thin filaments, while the A band contains both type of filaments. In the resting muscle, the thin filaments do not reach the center of the A band, explaining the H zone, which contains only thick filaments (Fig. 10.3).

The striated pattern occurs in skeletal and cardiac muscles, which are called striated muscles, while the smooth muscles do not present striations.

The thin filaments are inserted in the Z line and are composed of three proteins: actin, tropomyosin, and troponin. The thick filaments are formed by a protein named myosin (see Box 10.1). The region comprised between the Z lines is called sarcomere and corresponds to the contractile unit of the myofibril. It is important to mention that the components of muscle cells are usually designated by a specific nomenclature having the prefix *sarco* (from a Greek root meaning "flesh"): the plasma membrane is known as sarcolemma, the cytoplasm is known as sarcoplasm, and the endoplasmic reticulum is called sarcoplasmic reticulum.

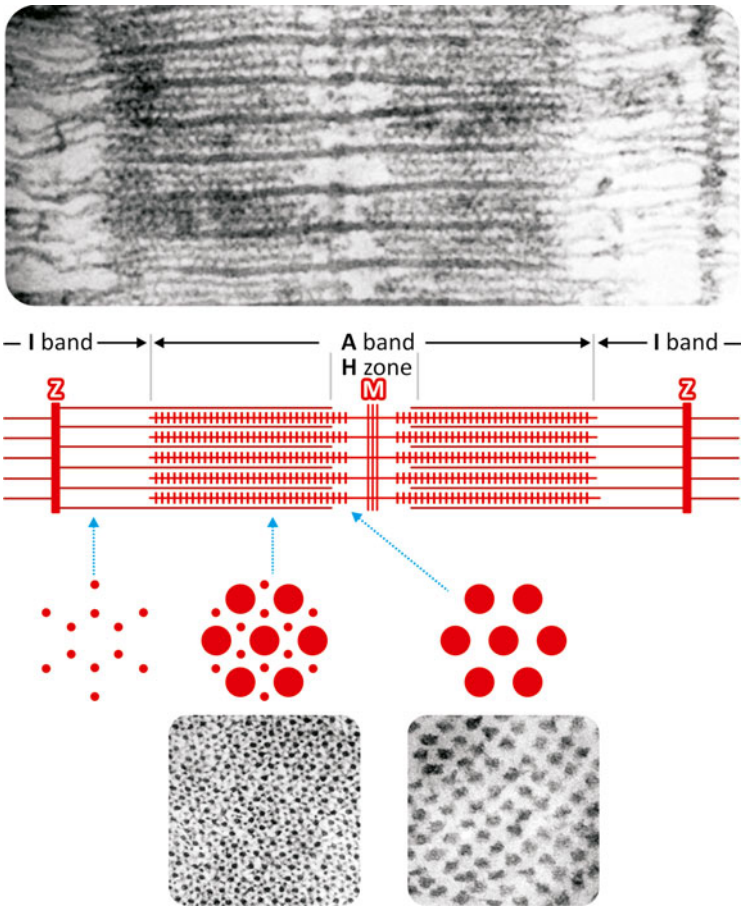


Fig. 10.3 Electron micrographs of a skeletal muscle (*top*) longitudinal section showing the thin and the thick filaments and (*bottom*) transverse sections of A band and H zone (Reproduced with permission from Huxley, J. Biophys. Biochem. Cytol. 3:631–648, 1957). The schematic representation shows filaments' organization in the region in between two Z lines and transverse sections of the I band (*left*), the A band (*medium*), and the H zone (*right*). The region of 2–3 μm in between two Z lines is called sarcomere, which is the contractile unit of the myofibril

Box 10.1: Isolation of Myosin

Since the middle of the nineteenth century, it was known that the disruption of muscle cells resulted in the precipitation of an insoluble material in a much higher amount than that observed for homogenates of other tissues. Still in the nineteenth century, this insoluble material was called "myosin" by Wilhelm Kuehne, but only in the 1940s, with the studies performed mainly by Albert Szent-Gyorgyi, this term was specifically used to name the proteic

(continued)

Box 10.1 (continued)
component that could be solubilized from the insoluble material by treatment with a high ionic strength solution (0.6 M KCl, an ionic strength much higher than the physiological 0.15 M). Another proteic component could be solubilized from the remaining insoluble material of muscle homogenate by lowering the ionic strength below the physiological range, and this protein was named "actin" (due to its ability to activate the ATPase activity of myosin, as it will be explained in the next section). It is important to note that both myosin and actin are insoluble at the physiological ionic strength, forming the organized filaments seen by electron microscopy.

10.1.1.1 The Main Proteic Components of the Contractile Apparatus

Myosin represents up to 65 % of the total protein that constitute the myofibrils. A landmark finding regarding the role of myosin in contraction came from the work of W. A. Engelhardt and M. N. Lyubimova, who demonstrated that it displays enzymatic activity hydrolyzing ATP. It was also shown that the volume of myosin in its insoluble state (at ionic strength lower than 0.6 M; see Box 10.1) contracted after addition of ATP, leading to the supposition that the hydrolysis of ATP by myosin would be the driving force for muscle contraction. This is indeed the basis for the chemical–mechanical energy conversion during muscle contraction, as we will discuss in the next section.

The first electron microscopy images of isolated myosin filaments showed a very peculiar morphology. They present typical projections regularly spaced from each other along the extension of the filament, except for the central part, which is known as the "bare zone" (Fig. 10.4a). The projections are the contact points with the thin filaments.

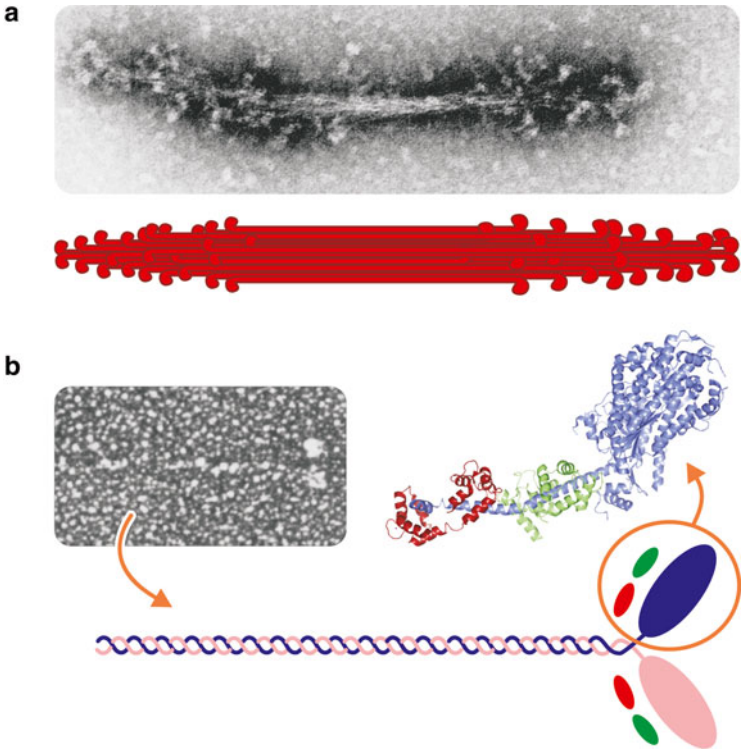


Fig. 10.4 Structure of the myosin filament. (**a**) Electron micrograph of a reconstituted myosin filament (Reproduced with permission from Huxley, Science 164:1356–1366, 1969) and its schematic representation. (**b**) Electron micrograph of an isolated myosin molecule (Reproduced from Slayter & Lowey, Proc. Natl. Acad. Sci. USA 58:1611–1618, 1967) and a schematic representation of its structure, which is composed of two heavy chains (*purple* and *pink*) associated to four light chains (*green* and *red*). The three-dimensional structure of the head, or S1 subfragment, is also shown, with the light chains highlighted in *green* and *red* (PDB 1DFL)

The morphology of the thick filament can be explained by the structure of myosin units. Each myosin unit in the thick filament shows a golf club shape, with two well-defined regions: a globular "head" from which a "tail" of about 150 nm long extends (Fig. 10.4b). It is a hexameric protein containing two identical heavy chains (with ~220 kDa each) and four light chains (each with ~20 kDa). The head can be separated from the whole myosin by brief digestion with proteases. The generated fragment, containing the N-terminal end of the heavy chains associated with the light chains, is called subfragment 1 (S1) and comprises the ATPase activity and the actin-binding site. The tail is composed of the C-terminal region of the two heavy chains intertwined to form a coiled coil.

Actin is the major component of the thin filament and corresponds to about 20–25 % of the muscle proteins. Actin is a protein of 42 kDa that is called G-actin

(in a reference of its globular structure) in its monomeric form. To form the thin filaments, actin monomers polymerize in a helical structure, in which it is named F-actin (Fig. 10.5).

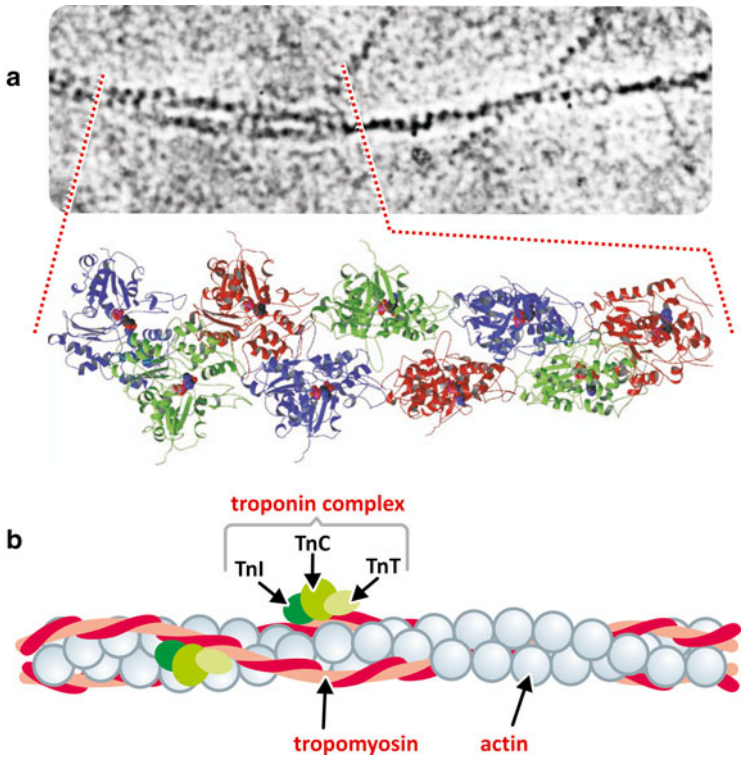


Fig. 10.5 Structure of the thin filament and its components. (**a**) Section of an electron micrograph from the article that firstly described the organization of an actin filament (Reproduced from Hanson & Lowy, J. Mol. Biol. 6:46–60, 1963, with permission from Elsevier), with the correspondent molecular structure determined by X-ray crystallography showing 13 actin monomers arranged on six left-handed turns repeated every 36 nm (Reproduced with permission from Geeves & Holmes, Ann. Rev. Biochem. 68:687–728, 1999). (**b**) Schematic representation of an actin filament with tropomyosin and troponin bound

Other proteins are associated to the thin filament. Among them, the most important are the tropomyosin and troponin, both involved in the regulation of contraction. Tropomyosin is a dimeric protein of 65 kDa that associates to actin as twisted α-helices that interacts in a tail-to-head manner forming long rods along the thin filament (Fig. 10.5b). Troponin is a globular protein of 78 kDa composed of three subunits, Tn-I, Tn-C, and Tn-T. Tn-I (I from inhibiting) binds actin fixing the tropomyosin-troponin complex on the actin surface and blocking myosin binding to thin filament. Tn-C (C from calcium binding) binds calcium ions (Ca^{2+}), the main

regulator of contraction (see Sect. 10.1.3). Tn-T (T from tropomyosin binding) promotes the association of the two other subunits of troponin and the binding of them to tropomyosin (Fig. 10.5b).

10.1.2 Mechanism of Muscle Contraction

After the discovery that the contractile apparatus was constituted mainly by two proteins, actin and myosin, many hypotheses have emerged to explain the contraction, most of them suggesting that muscle shortening during contraction was due to changes in protein structure leading to a more packed folding or coiling of the filament. However, the visualization of two separate sets of longitudinal filaments that overlap in certain regions in a series of studies performed during the 1950s led to a completely new model to explain the process.

10.1.2.1 The Sliding Filaments Model

The theory accepted today to explain myofibril shortening during muscle contraction was proposed in 1954, independently by H. E. Huxley and J. Hanson, and A. F. Huxley and R. M. Niedergierke, both studies published simultaneously. These scientists observed that either in contracted muscles or after their complete stretching, the length of the A band remained constant, while the length of the I band or the H zone varied according to the extent of contraction (Fig. 10.6). Based on these observations, they proposed that contraction occurred through the sliding of the filaments along each other.

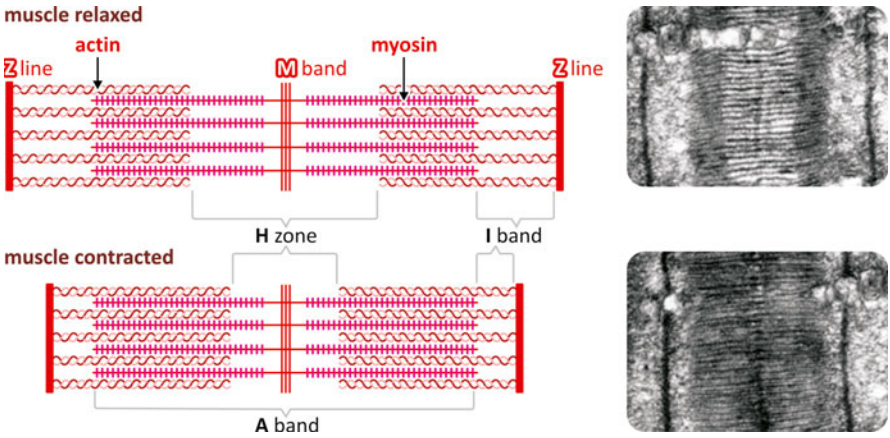


Fig. 10.6 Schematic representation of the slide of the filaments showing the relaxed and the contracted pattern with the corresponding electron micrographs (Reproduced with permission from Huxley. J. Biophys. Biochem. Cytol. 3:631–648, 1957)

One important finding that contributed to the elucidation of the mechanism that allows the filaments to slide was the observation that when the thin filaments were incubated with myosin S1, these subfragments form crossbridges with the thin filament in two orientations: perpendicular to the filament or with an inclination of approximately 45°. When inclined, all myosin heads show the same orientation on one side of the Z line but the opposite orientation on the other side of the Z line, which means that on each side of the Z line, the myosin heads point to opposite directions (Fig. 10.7a). This information could be correlated to the fact that during contraction the thin filaments on each side of the Z line are pulled to the center of the sarcomere.

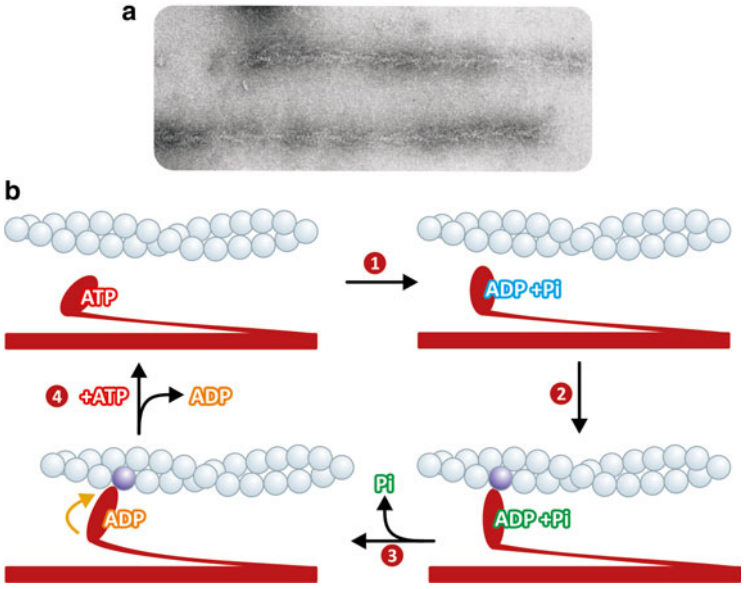


Fig. 10.7 (**a**) Actin filaments "decorated" with myosin heads, which show an arrowhead appearance (Reproduced with permission from Huxley, Science 164:1356–1366, 1969). (**b**) Schematic representation of actomyosin cycle: (1) myosin head hydrolyzes ATP to ADP and Pi; (2) myosin heads bind to the actin molecule in a perpendicular orientation, in a pre-force-generating state; (3) Pi is released, leading to a conformational change that makes myosin head to be at a 45° orientation in relation to the filaments, allowing myosin to perform work; (4) ADP is replaced by ATP leading to myosin dissociation from actin

The current knowledge on the molecular mechanism that leads to the sliding of the filaments is based on the model proposed by R. W. Lymn and E. Taylor, known as the Lymn–Taylor actomyosin ATPase cycle, according to which contraction occurs as myosin heads bind and detach repeatedly to the thin filaments with concomitant ATP hydrolysis, each cycle with the binding occurring in a position closer to the Z line. The sequence of events that are repeated in each cycle can be summarized as follows, starting with ATP binding to the myosin head for convenience (Fig. 10.7b):

1. Myosin hydrolyzes ATP to ADP and Pi, which remain tightly bound to the protein. The hydrolysis induces a structural change in the myosin head, which points to the thin filament in a perpendicular orientation.

2. Myosin heads bind to the actin molecule in a pre-force-generating state.
3. Binding of the myosin head to actin induces Pi to be released, leading to a conformational change that makes the myosin head to be at a 45° orientation in relation to the filaments. This conformational change allows myosin to perform work.
4. ADP dissociates from myosin being replaced by ATP, whose binding makes myosin heads detach from the actin.

Thus, as the cycle is repeated, the thick filaments move along the thin filaments in the direction of the Z line, so that the H zone becomes shorter and the Z lines get nearer to each other.

It is important to note that if ATP is not available, myosin keeps strongly attached to the thin filaments, in a condition known as *rigor*, as occurs after death, when all the body muscles become rigid, which is called *rigor mortis*. In living muscle cells, on the other hand, there is always an excess of ATP due to a constant cycle of hydrolysis and resynthesis and thus myosin heads remain bound to actin only for short periods of time.

10.1.3 Regulation of Muscle Contraction

Knowing that ATP is constantly hydrolyzed and resynthesized within the cells, so that its levels remain almost unchanged, one would ask how muscle contraction can be triggered exactly when it is required and how it is stopped when a specific task has already been performed.

The answer resides in the fact that the process is controlled by the central nervous system. This means that although the contraction is sustained by ATP hydrolysis, the ATP levels inside the muscle cells do not regulate the process extensively. Conversely, ATP concentrations are maintained high by different metabolic pathways that will be discussed in Sect. 10.2. Thus, it becomes clear that an additional player has to be called in to translate the brain signal to a biochemical response. This role is played by Ca^{2+}, whose concentration transiently increases in the sarcoplasm inducing the start of the contraction.

To understand how Ca^{2+} concentration is modulated in the sarcoplasm to control contraction, we need firstly to look at the morphological organization of the muscle fibers. One particularity of the skeletal muscle cell is that its sarcolemma invaginates perpendicularly to the length of the cell forming what is called the transverse (T) tubules (Fig. 10.8). Inside the cell, the myofibrils are surrounded by a system of membranous vesicles called sarcoplasmic reticulum (SR), which contains enlarged areas, known as the SR terminal cisternae, where Ca^{2+} is stored in a high concentration (~10^{-3} M). The SR terminal cisternae are connected to the T-tubules by a complex of two proteins, the dihydropyridine receptor (DHPR, inserted in the T-tubule membrane) and the ryanodine receptor (RyR, inserted in the terminal cisternae membrane) (Fig. 10.8).

A nerve impulse induces the opening of ion channels in the sarcolemma leading to an inflow of Na^+ into the cell. This causes membrane depolarization due to the dissipation of the membrane potential maintained by the Na^+/K^+-ATPase (see Box 10.2).

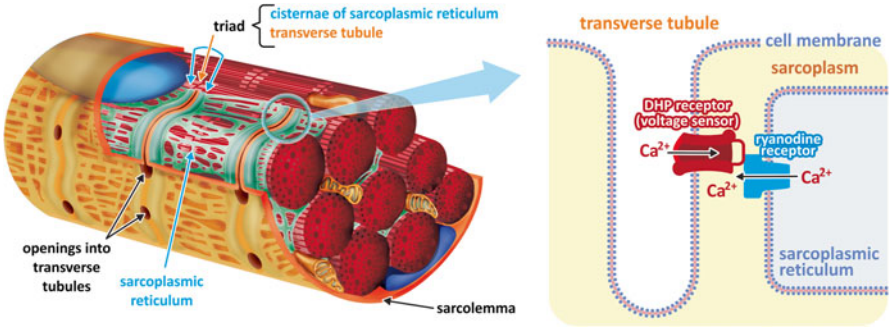
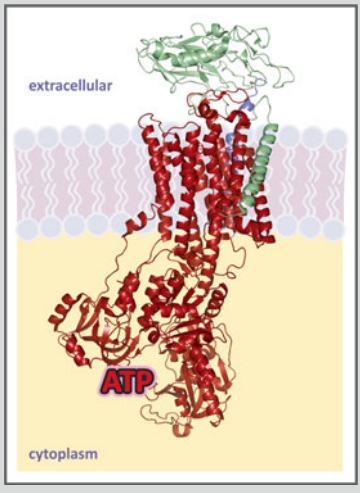


Fig. 10.8 Schematic diagram of a skeletal muscle fiber section showing the sarcolemma invaginations into the T-tubules and the sarcoplasmic reticulum with its terminal cisternae. Dihydropyridine receptor (DHPR), a voltage-dependent Ca^{2+} channel inserted in the T-tubule membrane, interacts with ryanodine receptor (RyR), also a Ca^{2+} channel, inserted in SR terminal cisternae membrane. Propagation of the action potential over the T-tubules activates DHPR, which in turn induces RyR opening, leading to Ca^{2+} release from SR lumen to the cytosol

Box 10.2: Na^+/K^+-ATPase and the Maintenance of Cellular Membrane Potential

In living cells, the distribution of ions inside and outside the plasma membrane is asymmetric, resulting in an electrical voltage between the two sides of the membrane, which is called membrane potential. Membrane potential is regulated by the combined action of ion channels, which depolarize the membrane, and ion pumps, which actively exchange ions across the membrane, restoring polarization. Membrane potential mainly arises from the exchange of Na^+ and K^+ through the activity of the Na^+/K^+-ATPase. This enzyme is an integral protein in the plasma membrane (see figure) that pumps 3 Na^+ out in exchange of 2 K^+ in, at the expense of ATP hydrolysis. Its activity accounts for a great part of cellular energy expenditure, being estimated that it is responsible for from 1/3 to 2/3 of ATP hydrolysis in the cells.



Crystal structure of Na^+/K^+-ATPase (PDB 3A3Y), with its three subunits, the α (catalytic, in *red*), β (in *green*), and regulatory (in *blue*). ATP is drawn in its binding site

Membrane depolarization propagates along the sarcolemma from the fiber surface to the T-tubules. DHPR is a voltage-dependent Ca^{2+} channel that is activated by the action potential propagation over the T-tubules. DHPR interaction with RyR in the closely apposed SR membrane causes RyR opening, leading to Ca^{2+} release from SR lumen to the cytosol. The increase of cytosolic Ca^{2+} concentration itself also activates RyR, causing further Ca^{2+} release, in a process known as Ca^{2+}-induced Ca^{2+} release. At higher concentrations in the sarcoplasm, Ca^{2+} binds to troponin, resulting in a conformational change that induces the displacement of tropomyosin, allowing myosin heads to bind actin and starting the contraction cycle (Fig. 10.9).

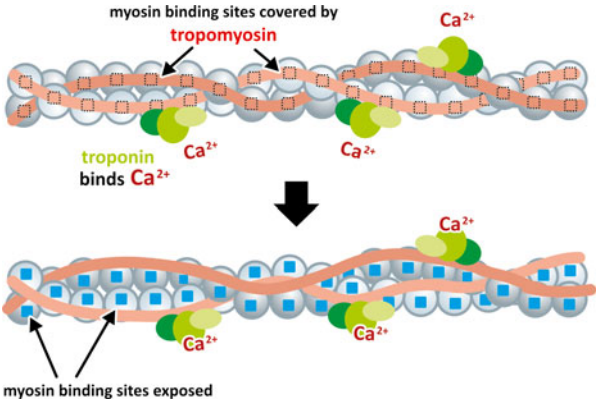


Fig. 10.9 Model for the Ca^{2+} regulation of contraction. At low concentration of Ca^{2+} in the sarcoplasm, the complex troponin–tropomyosin blocks the myosin-binding sites in actin. When Ca^{2+} is released from the SR and its concentration increases in the sarcoplasm, it binds troponin inducing a conformational change that ultimately leads tropomyosin to move away from the myosin-binding sites

When the nervous stimulus ceases, Ca^{2+} concentration in the sarcoplasm decreases due to the activity of Ca^{2+}-ATPase, an SR membrane enzyme that pumps Ca^{2+} from the cytosol to the SR lumen. Thus, in resting muscle, the concentration of Ca^{2+} in the sarcoplasm is maintained very low, in the range of 10^{-7} to 10^{-8} M, since almost all the intracellular Ca^{2+} is stored inside the SR. In this situation, the complex troponin–tropomyosin is bound to actin in a way that prevents myosin binding (Fig. 10.9). Thus, even in high ATP concentrations, its hydrolysis by myosin occurs in a very slow rate and the muscle remains relaxed.

10.2 Different Metabolic Profiles of the Skeletal Muscle Fibers

Skeletal muscles are used to perform very different kinds of activities. Some of these activities require that muscle cells work in their maximal capacity, such as when an elite athlete runs a 100 m sprint, but also in more usual situations, as when you have

to quickly run to take a bus that just started to leave the bus stop. Other activities demand muscle work for a long time, such as in running a marathon but also in prolonged walks, riding a bicycle, or cleaning the house. The different metabolic demands required in these diverse activities can be achieved due to the existence of distinct types of muscle fibers, characterized by specific metabolic adaptations that include the type of nutrient metabolized and the metabolic pathways used to synthesize ATP (Fig. 10.10).

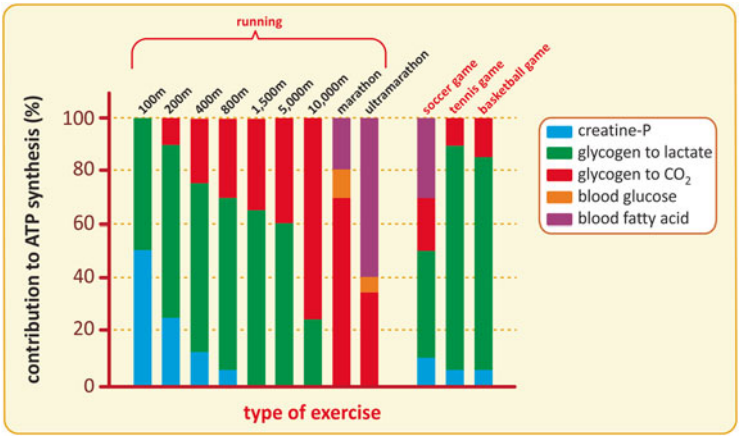


Fig. 10.10 Contribution of distinct metabolites to ATP synthesis during different types of exercise. The transfer of the phosphate group of phosphocreatine to ADP is the fastest way to regenerate ATP in muscle cells (see next section for details), but the content of phosphocreatine is limited and sustains only short duration exercises. Muscle glycogen is the main energy source used for ATP synthesis during short- or medium-duration exercises. Depending on the type of exercise, muscle glycogen may be used anaerobically (for instance, in short-distance runs), generating lactate as the end product, or aerobically (as in long-distance runs), being oxidized to CO_2. Aerobic metabolism becomes gradually more important in long-duration exercises, with also an increasing requirement of fatty acid oxidation as glycogen is depleted. The use of blood glucose by muscle during exercise is almost irrelevant, especially because its transport into the muscle cells is dependent on insulin (see Sect. 8.4). This guarantees glucose availability for the cells that use this nutrient preferentially or exclusively, such as brain cells or erythrocytes, respectively. Ball games, such as soccer or tennis, may be long in duration but they consist in short and intense runs alternating with resting periods, which makes them having metabolite use profiles closer to short-distance running (Based on data from Newsholme & Leech, Functional biochemistry in health and disease, chap. 13. P. 291, 2010)

From the physiological point of view, there are two major types of muscle fibers: the red or slow-twitch fibers and the white or fast-twitch fibers. They are classified according to the expression profile of myosin heavy chain isoforms, which correlate with their physiological role and their biochemical adaptations. Slow-twitch fibers are also known as type I fibers due to the predominant expression of type I myosin heavy chain, while fast-twitch fibers are called type II fibers due to the predominance of myosin heavy chains type IIa and IIb. There is an association between fiber

type and mitochondrial content, with type IIb fibers tending to have the lowest and type I fibers the highest abundance of mitochondria.

Slow-twitch fibers are adapted to prolonged work and are very resistant to fatigue, although they provide relatively low force upon contraction. Their metabolism is mainly dependent on oxidative phosphorylation and thus requires an adequate supply of O_2. These cells are very rich in mitochondria and myoglobin and are irrigated by a large amount of blood vessels. The cytochromes in the mitochondria and the high content of myoglobin inside the cells, as well as the hemoglobin in the surrounding blood, give to red muscle its characteristic red color. Myoglobin has a much higher affinity for O_2 than hemoglobin (see Sect. 3.3.3) and thus receives O_2 from blood to supply the aerobic activity of these muscle fibers. The fatty acids are the main nutrient used by red fibers, but they can also use ketone bodies and degrade glycogen and glucose aerobically.

Fast-twitch fibers are adapted to work at low levels of O_2. These cells contain a low number of mitochondria and are less supplied by blood vessels than type I fibers. Their metabolism is mainly anaerobic, using muscle stocks of glycogen as the major metabolic substrate. Although this type of fiber contracts quickly and powerfully, it undergoes fatigue very rapidly as the low ATP yield provided by anaerobic metabolism cannot sustain the ATP demand for long periods of contraction.

There is a genetic component that determines the amount of red and white muscles in the body, but exercise training influences the expression profile of contractile proteins resulting in changes in the proportion of fiber types in the muscles.

10.3 Overview of ATP Synthesis in the Muscle Cells

ATP consumption in muscle cells may increase 100-fold from resting to vigorous activity. During intense contractile activity, ATP is hydrolyzed to ADP and Pi mainly as a result of three ATPase activities: (a) the ATPase activity of myosin head, which is directly involved in the sliding of the filaments and thus in the contraction; (b) the Na^+/K^+-ATPase activity that maintains the Na^+/K^+ gradient across the sarcolemma and T-tubules, allowing the membrane potential to be restored; and (c) the SR Ca^{2+}-ATPase activity, responsible for pumping Ca^{2+} against the concentration gradient from the sarcoplasm into the lumen of SR. The high rate of ATP hydrolysis during contraction demands that ATP levels are continuously restored within the muscle cells.

ATP is supplied to muscle cells through different pathways depending on the type of the fiber (Fig. 10.11). In type I fibers, oxidative phosphorylation is the major mechanism of ATP synthesis, with the fatty acids being the main metabolic substrate used. In contrast, in type II fibers most of the ATP synthesized comes from the substrate-level phosphorylation in glycolysis, being the muscle glycogen the main source of glucose-6-phosphate for glycolysis, and lactates the major end product of this pathway.

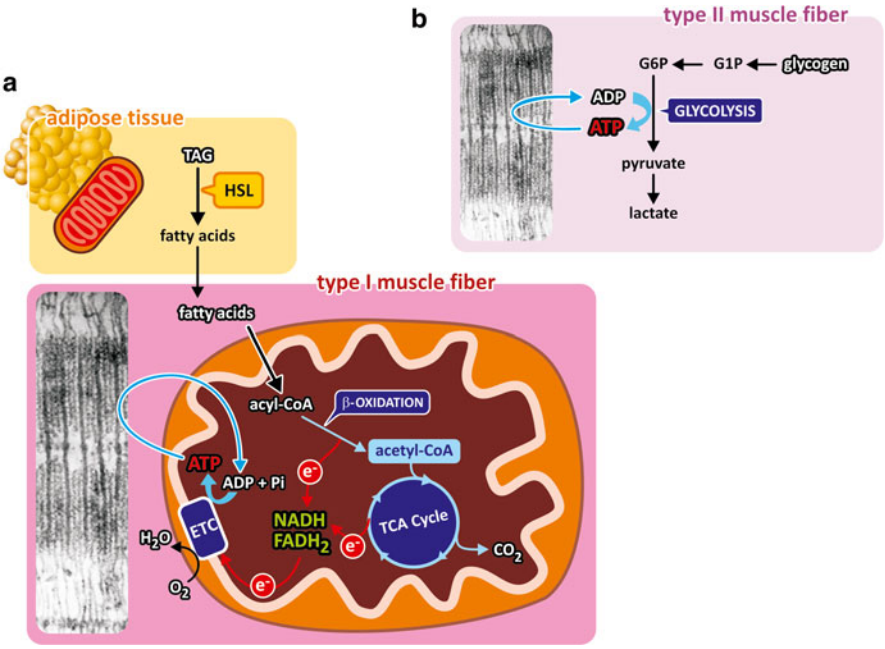


Fig. 10.11 Main metabolic pathways for ATP synthesis that sustain contraction in muscle fibers. (**a**) Type I fibers use fatty acids as the main metabolic substrate. The fatty acids are mobilized by the hydrolysis triacylglycerols (TAGs) coming mainly from the adipose tissue adjacent to muscles, in a reaction catalyzed by the enzyme hormone-sensitive lipase (HSL). Fatty acids are oxidized through the β-oxidation pathway followed by complete oxidation of the resultant acetyl-CoA in TCA cycle. The electrons transferred to the coenzymes NADH and $FADH_2$ are then transported in the electron transport chain, ultimately reducing O_2 to H_2O. Electron transport promotes the formation of an electrochemical gradient that is the driving force for ATP synthesis. (**b**) Type II fibers use muscle glycogen as the main metabolic substrate. Glycogen degradation forms glucose-1-phosphate (G1P), which is converted in glucose-6-phosphate (G6P), which in turn enters glycolysis. ATP is formed by substrate-level phosphorylation in glycolysis. In low availability of O_2, the product of glycolysis, pyruvate, is reduced to lactate

Additionally, skeletal muscle contains another mechanism to transiently and rapidly generate ATP. This is possible due to a large amount of phosphocreatine (10–30 mM) in muscle cells (Fig. 10.12). The enzyme creatine kinase catalyzes the transfer of phosphate group of phosphocreatine to ADP, regenerating the ATP hydrolyzed in contraction. The resulted creatine can be phosphorylated again during recovery, when the ATP levels increase in the cells. The same enzyme, creatine kinase, catalyzes the reverse reaction using ATP to phosphorylate creatine, restoring the levels of phosphocreatine in muscle cells.

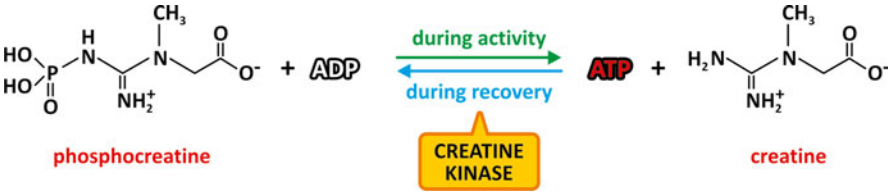


Fig. 10.12 Creatine kinase catalyzes the reversible reaction of phosphate transfer from phosphocreatine to ADP, generating ATP and creatine during muscle contraction, or from ATP to creatine, forming phosphocreatine and ADP, during recovery

10.4 Muscle Cell Metabolism During Physical Activity

Due to the extremely high demand for ATP imposed by the contractile activity during intense exercise, a rapid metabolic adaptation to regenerate ATP is required in muscle cells, in which the available substrates are driven to the catabolic pathways. In this section we will discuss the main metabolic steps that are regulated in muscle cell to maintain ATP concentration within the adequate ranges required for cellular functions.

10.4.1 Role of the Cellular Energy Charge in the Muscle Cell Metabolism

Before focusing specifically on muscle cells, we will start by discussing a basic level of metabolic regulation that occurs in all the cells of the body. The idea of such metabolic control emerged in the decade of the 1960s, when Daniel E. Atkinson proposed that the regeneration and the expenditure of ATP would be regulated by the cellular energy balance itself. This concept has proven to be true for all the cells, at least as a first level of metabolic control. To formalize this idea, Atkinson developed a parameter to describe the cellular energy status based on the relative concentrations of the adenine nucleotides within the total cellular pool in a given moment or situation. He termed this parameter as the "energy charge of the adenylate system," whose value represents half of the average number of anhydride-bound phosphates per adenosine moiety (Box 10.3). A value of energy charge of 0 would mean that only AMP is present in the cell, whereas if all the adenine nucleotide were in the form of ATP the cellular energy charge would be 1. In most cells, the energy charge value ranges from 0.8 to 0.95.

Box 10.3: Atkinson's Concept of Cellular Energy Charge
Daniel E. Atkinson proposed that the energy stored into the cell in the form of adenine nucleotides, referred by him as the adenylate system (AMP+ADP+ATP), resembles an electrochemical storage cell in its ability to accept, store, and supply energy. Based on this view, the adenylate system is fully discharged when all adenylate is in the form of AMP and fully charged when only ATP is present, with the number of anhydride-bound phosphates per adenosine moiety varying from 0 to 2. To have a parameter varying from 0 to 1, he divided the number of anhydride bonds per adenosine nucleotide by 2. Since ATP contains 2 of anhydride-bound phosphate groups and ADP contains 1, Atkinson defined the cellular "energy charge" as the actual concentrations of ATP+½ADP in the total adenylate system:

$$\frac{[\mathrm{ATP}]+½[\mathrm{ADP}]}{[\mathrm{ATP}]+[\mathrm{ADP}]+[\mathrm{AMP}]}$$

It is important to note that during intense contraction, AMP accumulates as a result of the combination of ATP hydrolysis (which generates ADP and Pi) and the reaction catalyzed by the enzyme adenylate kinase (which converts two molecules of ADP in one ATP and one AMP). Therefore, each ATP is ultimately converted in one AMP and two Pi molecules (Fig. 10.13a). Considering the reaction catalyzed by adenylate kinase at equilibrium, Atkinson represented the variation of the concentrations of AMP, ADP, and ATP as a function of the energy charge (Fig. 10.13b). Observing this graph, it becomes clear that AMP is a very sensitive indicator of metabolic status, since its concentration varies in a much greater amplitude when compared to ADP concentration variation (during exercise, AMP concentration may rise more than 100-fold, while no more than a 10-fold increase is observed for ADP concentration).

AMP is indeed an important activator of the pathways for ATP synthesis in muscle cells. It regulates muscle metabolism acting as an allosteric modulator of many enzymes, such as activating the muscle isoform of the glycogen phosphorylase (GP) and the glycolytic enzyme phosphofructokinase (PFK) and inhibiting fructose-1,6-bisphosphatase (see Sect. 10.4.2 for details).

Besides acting through its direct binding to several enzymes in different metabolic pathways, another regulatory role has been attributed to AMP: it is the activator of an important regulatory enzyme, the AMP-activated protein kinase (AMPK; note that this is not the *cyclic AMP-dependent protein kinase*, PKA, but an enzyme activated by the 5′-AMP).

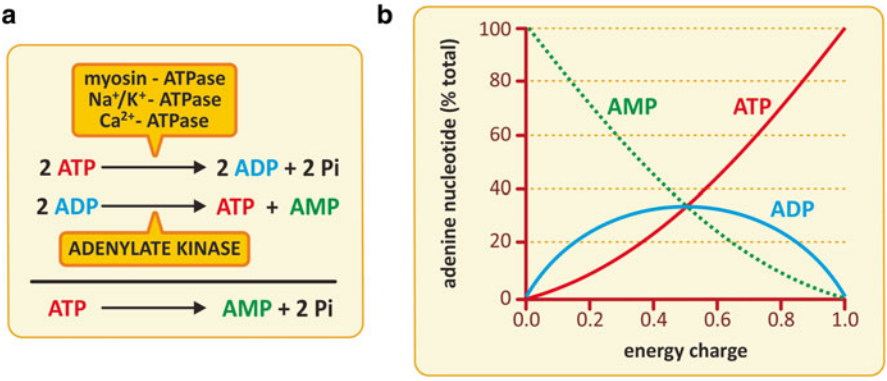


Fig. 10.13 (**a**) AMP production in muscle cells. ATP is hydrolyzed to ADP and Pi by the ATPase activities of myosin, Na^+/K^+-ATPase and Ca^{2+}-ATPase. The resulting ADP may be converted to ATP and AMP by the action of the enzyme adenylate kinase. (**b**) Considering the reaction catalyzed by adenylate kinase at equilibrium (using the calculated equilibrium constant of 0.8), the concentrations of the adenine nucleotides in the cell can be represented as a function of the cellular energy charge (Reproduced from Oakhill et al. Trends Endocrinol. Metab. 23:125–132, 2012, with permission from Elsevier)

10.4.1.1 The AMP-Activated Protein Kinase: A Cellular Energy Sensor

AMPK is a heterotrimeric protein composed of one catalytic subunit (α) and two regulatory subunits (β and γ) (Fig. 10.14a). In the γ-subunit, there are four adenine nucleotide-binding sites (sites 1–4). Site 2 seems to be unoccupied in mammalian enzymes and site 4 has a non-exchangeable AMP molecule bound. This indicates that only sites 1 and 3 are involved in AMPK regulation, exchanging ATP for ADP or AMP as the cellular energy charge varies. The N-terminal end of the β-subunit is myristoylated, being this modification important for nucleotide binding to γ-subunit.

AMPK activity is regulated mainly through phosphorylation/dephosphorylation of the Thr172 in the catalytic subunit (Fig. 10.14b). The main protein kinases involved in AMPK phosphorylation are the LKB1 complex and the Ca^{2+}/calmodulin-dependent kinase kinase-β (CaMKKβ). Phosphorylation of Thr172 results in an increase of more than 100-fold in AMPK activity. On the other hand, its dephosphorylation catalyzed by phosphatases, such as the protein phosphatase 2C (PP2C), leads to AMPK inactivation.

Binding of ADP or AMP to site 3 of the γ-subunit facilitates AMPK phosphorylation and inhibits its dephosphorylation, maintaining the enzyme in its active state, while binding of ATP to this site causes the opposite effect. This is an interesting example of a posttranslational modification that is modulated allosterically, since

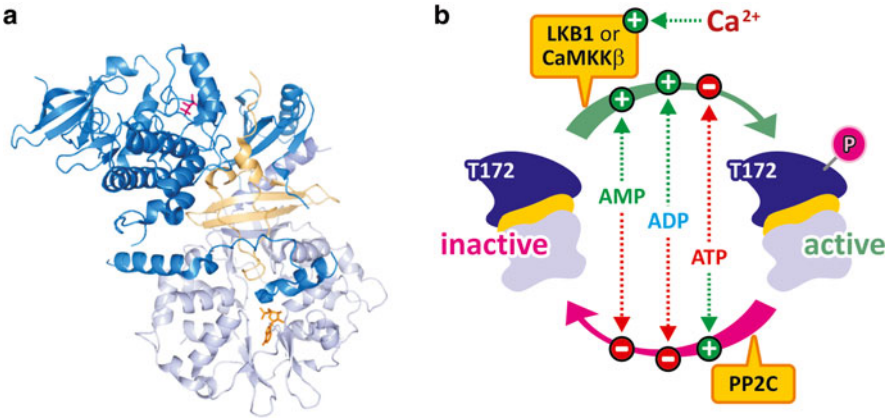


Fig. 10.14 (**a**) Structure of mammalian AMPK. The structure represents a composition of the available structures of the rat α-subunit (*blue*), with the phosphorylated Thr shown in *red*; the human β-subunit (*light yellow*); and the rat γ-subunit (*lilac*), with an AMP molecule bound shown in *orange* (PDB 4CFH). (**b**) Schematic representation of AMPK regulation. The increase in ADP/AMP concentration triggers Thr172 phosphorylation by the upstream kinases LKB1 and CaMKKβ and simultaneously inhibits Thr172 dephosphorylation by phosphoprotein phosphatase 2C (PP2C). ATP antagonizes this ADP/AMP effect. AMP acts directly as an allosteric activator of AMPK

binding of the adenine nucleotide to γ-subunit makes the enzyme a better or a worse substrate to the kinases or phosphatases that will introduce or remove the phosphate group in Thr172.

Additionally, when AMPK is phosphorylated, binding of AMP to the site 1 of the γ-subunit further activates the enzyme, although this direct allosteric activation results in only two- to fivefold stimulation of AMPK activity.

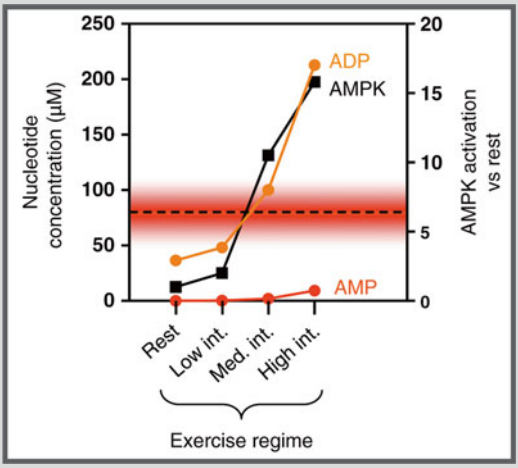
It is also interesting to note that AMPK activation/inhibition is not controlled by extracellular signals (e.g., hormones), as occurs with most of the regulatory kinases, but by the intracellular status. Therefore, AMPK can be seen as an adenylate charge-regulated protein kinase, since it detects and reacts to changes in the adenine nucleotide ratio.

It is interesting to mention that although it is unquestionable that protein phosphorylation by AMPK is an important mechanism of control of the energy metabolism in response to changes in cellular energy charge, restoring cellular ATP levels by switching off the anabolic pathways and switching on the catabolic pathways, recent evidence suggest that the actual physiological activator of this enzyme seems to be ADP rather than AMP (see Box 10.4).

In muscle cells, the main metabolic pathways regulated by AMPK are the β-oxidation of fatty acids and the glucose uptake via its transport by GLUT4 (see next section for details).

Box 10.4: ADP and the AMPK Activation

Recent studies that measured the nucleotide-binding affinity to AMPK revealed that the binding constants (K_d) of AMP and ADP to the site 3 of AMPK γ-subunit are 80 and 50 μM, respectively. Although during exercise AMP concentration rises dramatically (up to more than 100-fold over the resting levels), the highest AMP concentration reached (about 10 μM) is much lower than the K_d value. On the other hand, ADP concentration, although showing a much more modest increase (from 36 to 200 μM), overcomes the K_d value by up to fivefold. These observations led Bruce E. Kemp and colleagues to propose that ADP, rather than AMP, plays a dominant role in activating AMPK. These authors also showed that upon exercise AMPK activation clearly correlates with the increase in ADP concentration (see figure), further supporting this new view. This makes the historical name of this kinase as well as the extensive discussion in the literature regarding the role of AMP in this regulation somewhat confusing.



AMPK activation and the concentrations AMP and ADP in human muscle cells after exercises of different intensities. The *black dashed line* indicates the measured K_d for AMP binding at site 3 with the standard deviation indicated by a *red zone*. (Reproduced from Oakhill et al. Trends Endocrinol. Metab. 23:125–132, 2012, with permission from Elsevier)

10.4.2 Metabolic Pathways for ATP Synthesis in the Skeletal Muscle

10.4.2.1 Fatty Acid Oxidation in Skeletal Muscle

Fatty acids are the preferential nutrient used by muscle cells. These molecules are taken up by muscle fibers from blood or mobilized from TAG accumulated either inside the myocytes themselves or in the adipocytes dispersed between or along the fibers (Fig. 10.15).

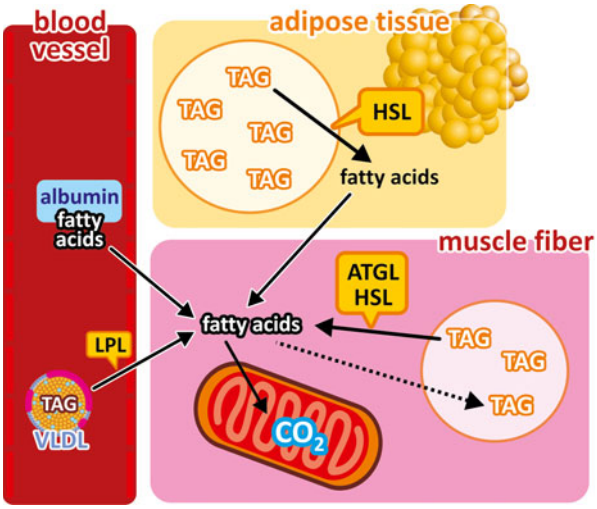


Fig. 10.15 Sources of fatty acids for muscle metabolism: Fatty acids mobilized from the adipose tissue circulate in association with albumin and are delivered to the muscle cells. VLDL transports the de novo synthesized TAG from the liver to the peripheral tissues, including muscles. VLDL-associated TAGs are hydrolyzed by the enzyme lipoprotein lipase (LPL) at the surface of the endothelium cells of the vessels that irrigate the muscles and the resulting fatty acids are transported into the fibers. The enzyme adipose triglyceride lipase (ATGL) hydrolyzes the TAG molecules stored inside the myocytes, generating DAG, which in turn is further hydrolyzed by the muscle isoform of the hormone-sensitive lipase (HSL). Finally, the TAGs stored in the adipocytes associated to the fibers are also mobilized yielding fatty acids to the muscle cell metabolism

Albumin-bound fatty acids circulate in the blood after mobilization from the adipose tissue, being the main source of fatty acids for muscle metabolism. Fatty acid can also be obtained from blood through the circulating VLDL. This lipoprotein is the main carrier of TAG in the blood in the postabsorptive state (see Sect. 8.3.2). VLDL-associated TAG is hydrolyzed by lipoprotein lipase (LPL), an enzyme present at the luminal site of endothelium cells of the capillary bed of muscles, making the resulting fatty acids available to skeletal muscle during exercise (Fig. 10.15).

Muscle cells contain a certain amount of intracellular stores of TAG, especially in type I fibers, representing a potential energy source for muscle metabolism during aerobic exercise. Intramyocellular TAGs are probably formed through the reesterification of the excess of fatty acids that are taken up from blood. It is important to note that although an increasing number of evidence support the importance of intramyocellular TAG mobilization during muscle activity, it is still difficult to unequivocally distinguish between intracellular and intercellular TAGs (those located in the adipocytes associated with the muscle cells), which should also have a role in supplying muscle cells of fatty acids during exercise. The hydrolysis of intramyocellular TAGs is attributed to the activity of two lipases, the adipose triglyceride lipase (ATGL), which catalyzes the hydrolysis of TAG to diacylglycerol (DAG), and the muscle isoform of the hormone-sensitive lipase (HSL), which exhibits a higher specificity for DAG than TAG (Fig. 10.15).

The reactions of fatty acid oxidation are described in detail in Sect. 7.4. Briefly, fatty acids are firstly activated in the cytosol by esterification with coenzyme A (CoA) in an ATP-dependent reaction catalyzed by the acyl-CoA synthetase (ACS). The resulting acyl-CoA molecules are transported into mitochondria where they undergo β-oxidation, generating acetyl-CoA, $FADH_2$, and NADH (Fig. 10.16). To be transported into mitochondria, acyl-CoA molecules are firstly converted to their acyl-carnitine derivatives that are then translocated across the mitochondrial membrane and reconverted to acyl-CoA molecules in the matrix.

The main regulation site of fatty acid oxidation is the transfer of the acyl group from CoA to carnitine, a reaction catalyzed by the carnitine/palmitoyl transferase I (CPT-I). This enzyme is located at the outer mitochondrial membrane and is strongly inhibited by malonyl-CoA. Therefore, a decrease in malonyl-CoA concentration in sarcoplasm increases the transport of the fatty acids into mitochondria, favoring their oxidation.

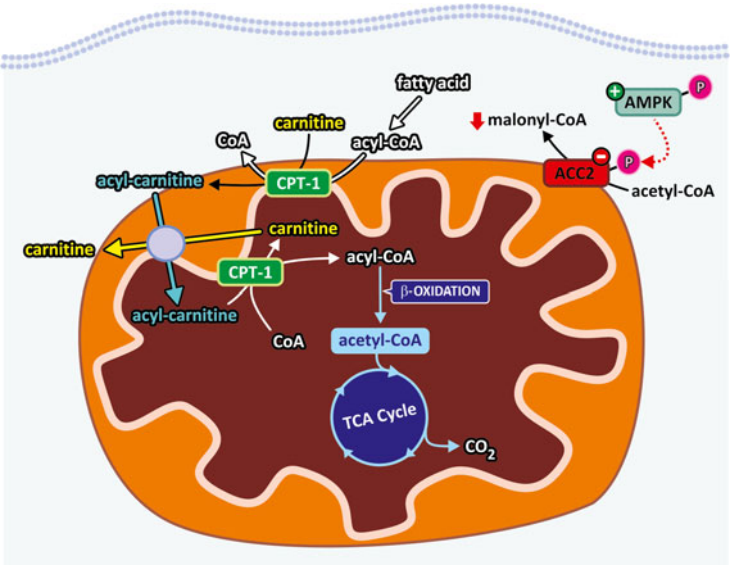


Fig. 10.16 β-oxidation of fatty acids is activated during contraction due to the decrease in the levels of malonyl-CoA, a potent inhibitor of the transport of the acyl-CoA into the mitochondrial matrix. Activated AMPK phosphorylates the isoform 2 of the acetyl-CoA carboxylase (ACC2), inhibiting the conversion of acetyl-CoA in malonyl-CoA. To be transported into the mitochondrial matrix, the acyl group of acyl-CoA is firstly transferred to carnitine, in a reaction catalyzed by the carnitine/palmitoyl transferase I (CPT-I). The acyl-carnitine is then transported across the inner mitochondrial membrane through the carnitine-acyl-carnitine transporter. In the matrix, the acyl group is transferred to coenzyme A by carnitine/palmitoyl transferase II (CPT-II), regenerating the acyl-CoA, which undergoes β-oxidation. Since malonyl-CoA is a potent inhibitor of the CPT-I, the decrease in its concentration allows the transport and the subsequent oxidation of the acyl-CoA

Malonyl-CoA is produced by the carboxylation of acetyl-CoA in the cytosol (see also Sect. 8.3.1). In muscle cells, this reaction is catalyzed by the isoform 2 of the enzyme acetyl-CoA carboxylase (ACC2), which is associated to the outer mitochondrial membrane (Fig. 10.16). ACC2 is inhibited by the phosphorylation of its Ser219 and Ser220, catalyzed by AMPK. Therefore, the activation of AMPK during contraction (see previous section) causes the decrease in the concentration of malonyl-CoA in sarcoplasm due to ACC2 inhibition, resulting in the activation of fatty acid β-oxidation (Fig. 10.16).

10.4.2.2 Insulin-Independent Glucose Uptake in the Skeletal Muscle

Glucose uptake in muscle cells occurs mainly through the isoform 4 of the glucose transporters (GLUT4). In the absence of specific stimuli, GLUT4 is sequestered in intracellular vesicles, restricting the use of blood glucose by the muscle cells. Insulin, the hormone secreted when the concentration of glucose in the blood increases, is the major signal that induces the exposure of GLUT4 on the cell surface, leading to a robust increase in glucose uptake by GLUT4-containing cells (see Sect. 8.4). In the muscle cells, contraction also regulates the migration of GLUT4-containing vesicles to the plasma membrane, allowing an increase in glucose uptake from blood during intense exercise even in the absence of insulin signaling.

The mechanisms by which contraction stimulates the glucose uptake in muscles are not completely understood, but several evidence support that AMPK is involved. The decrease in the cellular energy charge due to the intense ATP hydrolysis during contraction activates AMPK, and this can be correlated to GLUT4 exposure on the cell surface. The signaling pathway that links AMPK activity to GLUT4 translocation has not been elucidated yet, but it seems to involve the phosphorylation of AS160 (AKT substrate 160; see Sect. 8.4) (Fig. 10.17).

As mentioned in Sect. 10.1.3, upon stimulation by a nerve impulse, the membranes of the transverse tubules depolarize causing an increase in cytoplasmic Ca^{2+} concentration due to the release of this ion from the SR stores. Ca^{2+} activates the Ca^{2+}/calmodulin-dependent protein kinase kinase (CaMKK), one of the upstream kinases that phosphorylates and activates AMPK (see Sect. 10.4.1.1). Therefore, during exercise, the increase in the glucose uptake by the muscle cells occurs through a coordinated action of ADP/AMP and Ca^{2+}, which work simultaneously on the activation of AMPK: while the increase in ADP/AMP concentration makes AMPK susceptible to the action of the CaMKK, the elevation of Ca^{2+} concentration in the sarcoplasm makes this kinase active to phosphorylate and activate AMPK (Fig. 10.17).

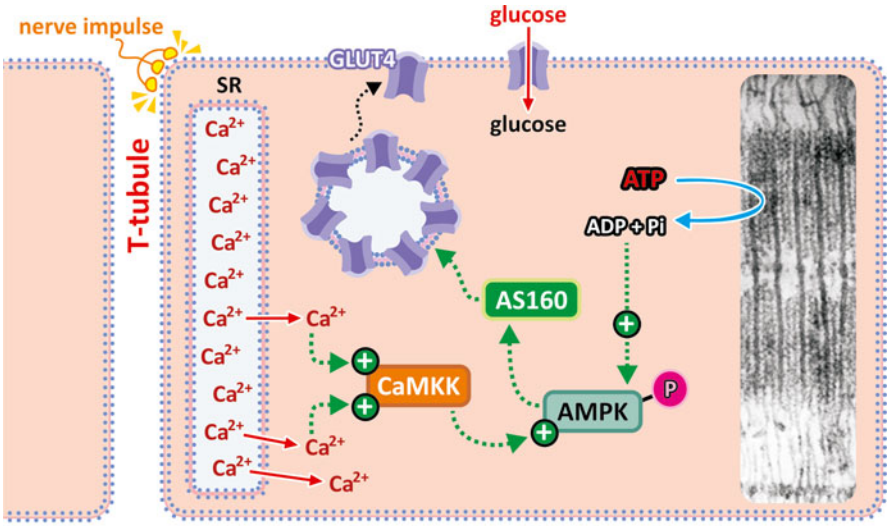


Fig. 10.17 Increase in glucose transport into the muscle cells during exercise. Intense contraction leads to a high rate of ATP hydrolysis, increasing the concentration of ADP (and AMP due to adenylate kinase activity). ADP/AMP binding to AMPK facilitates its phosphorylation and activation. Simultaneously, T-tubule membrane depolarization caused by nerve impulses induces the release of Ca^{2+} from SR, increasing its concentration in the sarcoplasm and resulting in the activation of the CaMKK, which phosphorylates AMPK. Probably through phosphorylation of AS160 by AMPK, the GLUT4 vesicles migrate to the cell surface, leading to an increase in glucose uptake

10.4.2.3 Glycogen Degradation in the Skeletal Muscle

Glycogen content in muscle cells corresponds to 1–2 % of the net weight of muscles. It consists of an important energy store especially because it can be used either aerobically or anaerobically, although in this latter case it can sustain vigorous activity only for a short period of time.

The existence of an energy source that can be use independently of the amount of O_2 available is especially important for the type II muscle fibers as they are less irrigated by blood vessels and contain a low number of mitochondria, depending largely on the anaerobic metabolism as the mechanism of ATP synthesis (see Chap. 6 for review of the mechanisms of ATP synthesis).

The detailed structure of glycogen granules and the reactions for glycogen degradation have already been presented in Sect. 9.2.1. Although in that chapter special attention was given to the liver cells, the reactions per se are the same as those that occur in the muscle cells. The differences between glycogen metabolism in the liver and muscle cells consist basically of the mechanisms of regulation of the synthesis and degradation pathways. Thus, in this section we will give only a brief description of the glycogen degradation pathway, focusing our discussion on the aspects of the regulation of the glycogen metabolism that are characteristic of the muscle cells.

Glycogen degradation depends on the activity of two enzymes, the glycogen phosphorylase (GP) and the debranching enzyme. GP sequentially removes the terminal glucose unit from the nonreducing ends of the glycogen molecule by a phosphorolysis reaction, yielding glucose-1-phosphate. When a branched point is reached, further degradation depends on the activity of the debranching enzyme.

The major site of control of glycogen degradation is the regulation of GP activity. This enzyme exists in two interconvertible conformational states, called GP*a*, the catalytically active form, and GP*b*, the less active form (see Sect. 9.2.2). In resting muscle, the predominant form is the GP*b*, which is converted to the active form by adrenaline-mediated phosphorylation, the main hormonal control that acts on muscle cells. The action of adrenaline on muscle cells (detailed in Sect. 10.4) results in the activation of the enzyme phosphorylase kinase, which catalyzes the phosphorylation of GP. Phosphorylation maintains GP in the active form, favoring glycogen degradation (Fig. 10.18).

In addition to the regulation by phosphorylation, two allosteric modulators activate GP in muscle cells: Ca^{2+} and AMP.

As described in Sect. 10.1, the nervous stimulus to contraction induces Ca^{2+} release from SR to the sarcoplasm, where the concentration of this ion increases greatly. Ca^{2+} binds to the phosphorylase kinase, activating this enzyme and leading to the phosphorylation of GP to its active form (Fig. 10.18). This coordinates the

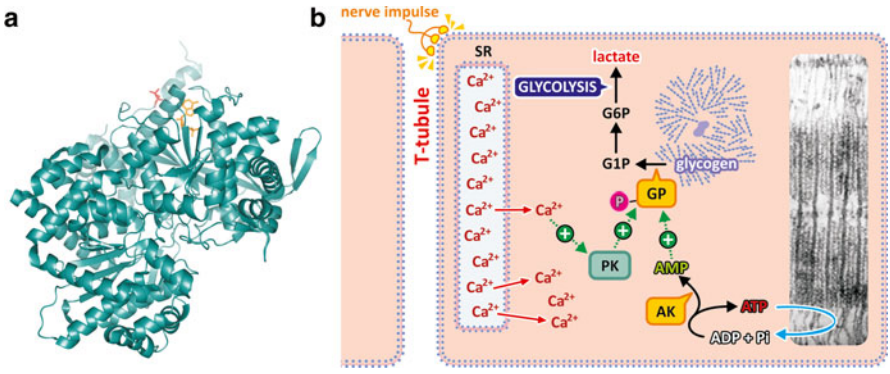


Fig. 10.18 (**a**) Structure of the human muscle GP (PDB 1Z8D) showing the phosphorylated in Ser14 (*pink*) and the AMP (*yellow*) in its binding site. (**b**) Activation of the glycogen degradation in contracting muscle. Intense contraction leads to a high rate of ATP hydrolysis, increasing the concentration of ADP, which may be converted to AMP and ATP by the adenylate kinase (AK). AMP binds to GP and facilitates glucose-1-phosphate (G1P) release from the active site, speeding the reaction of glycogen phosphorolysis. Simultaneously, T-tubule membrane depolarization caused by nerve impulses induces the release of Ca^{2+} from SR, increasing its concentration in the sarcoplasm and resulting in the activation of the phosphorylase kinase (PK), which phosphorylates and activates GP. G1P resulted from the glycogen degradation is converted to glucose-6-phosphate (G6P) by the phosphoglucomutase (PGM). G6P enters glycolysis, which in anaerobiosis generates lactate as the end product

first intracellular signal that induces contraction (Ca^{2+} release) to the mobilization of glycogen as an energy source for the process.

The other allosteric activator of glycogen degradation is AMP, which acts directly on GP, favoring glucose-1-phosphate release from the active site of the enzyme and speeding the GP reaction (Fig. 10.18). In resting muscle, ATP, which is in higher concentrations, replaces AMP in the allosteric site, inactivating the enzyme and inhibiting glycogen degradation.

The direct end product of glycogen degradation is glucose-1-phosphate (together with a small amount of glucose that is the product of the glucosidase activity of the debranching enzyme; see Sect. 9.2.1). Glucose-1-phosphate is then converted to glucose-6-phosphate by the action of the enzyme phosphoglucomutase (Fig. 10.18). It is important to remind that muscle cells lack the enzyme glucose-6-phophatase, an enzyme whose expression is restricted to the liver and kidneys. Thus, glucose-6-phosphate in muscles enters glycolysis, generating ATP to support muscle contraction.

Glycogen stores mobilized during muscle activity are replenished in muscle cells after carbohydrate ingestion. The increase in blood glucose after a carbohydrate-rich meal induces insulin secretion, whose action on muscle cells promotes glucose uptake through GLUT4 and its conversion to glycogen through the activation of the enzyme glycogen synthase (GS). The detailed reactions and regulatory aspects that take place in this situation are described in Sects. 8.2 and 8.4.

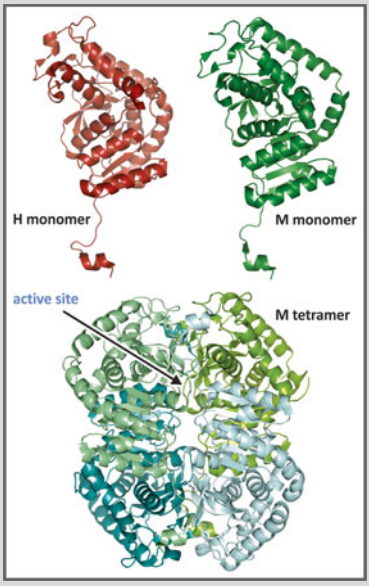
10.4.2.4 Glycolysis in the Skeletal Muscle

The glycolytic pathway in the muscle cells is also activated during physical activity. This occurs mainly by hormonal regulation, through adrenaline-induced phosphorylation of the muscle isoform of the bifunctional enzyme, which results in the activation of its phosphofructokinase-2 (PFK-2) activity, leading to an increase in the concentration of fructose-2,6-bisphosphate (see next section). Fructose-2,6-bisphosphate strongly activates the glycolytic enzyme phosphofructokinase-1 (PFK-1), increasing the metabolic flux through glycolysis.

Once glycolysis proceeds rapidly and if there is not enough O_2 available, pyruvate must be converted to lactate to allow NADH produced in glycolysis to be reoxidized (see Sect. 6.1.2 for details). The conversion of pyruvate to lactate is catalyzed by lactate dehydrogenase (LDH), an enzyme that is expressed as different isoforms depending on the tissue. The LDH isoform expressed in the skeletal muscles has a high affinity for pyruvate, making it possible a high glycolytic flow during contraction, especially in anaerobiosis (see Box 10.5).

Box 10.5: LDH Isoforms

LDH is a tetrameric enzyme that can be formed by a combination of two types of polypeptide chains: the M (from muscle) and the H (from heart) chains (see figures, PDB 1I10 and 1I0Z). Thus, there are five possible different isoforms of LDH (MMMM—see figure at the bottom—MMMH, MMHH, MHHH, HHHH). The detection of H isoform in the plasma can be used for diagnosis of heart infarction (see Sect. 3.3.4.1). The M subunits confer to the enzyme a lower K_M to pyruvate, favoring the reduction of pyruvate to lactate even when the concentration of pyruvate is low. This gives to skeletal muscle cells a high capacity of performing lactic fermentation with the pyruvate produced in glycolysis, generating a high glycolytic flow during contraction. The presence of H subunits favors the oxidation of lactate to pyruvate. Therefore, cells expressing the H chain-containing isoforms can use the lactate as a metabolic substrate, converting it to pyruvate, which in turn can be oxidized, as occurs in the heart tissue, for example. This is also important in liver cells, where lactate is converted to pyruvate to enter gluconeogenesis (see Sect. 9.3.2). The close similarity of the structures of the M and H subunits suggests that the different K_M observed for each isoforms results from variations in charge surface distribution on the active site.



10.5 Hormonal Regulation During Physical Activity: Role of Adrenaline

Adrenaline (also known as epinephrine) is the major hormone secreted when the organism is confronted with different stimuli processed in the central nervous system as indicative of an acute stressful situation, for which the capacity to perform an intense physical activity to deal with dangerous situations—the "fight-or-flight" response—was favored during evolution. Additionally, the physical activity itself promotes adrenaline secretion in a way dependent on the duration or the intensity of the exercise.

Adrenaline acts on almost all the tissues in the body, triggering many physiological and metabolic responses that prepare the organism for action. It promotes the dilatation of bronchioles, which increases the O_2 uptake, and a wide-range effect on the circulatory system, with the increase in the heart rate and blood pressure, and changes in blood flow patterns, leading to a decrease in the peripheral circulation and a reduction in the digestive system activity. These effects guarantee O_2 delivery to different organs, especially to the brain, allowing an increase in alertness. Nutrient availability is also tightly controlled by adrenaline, with the activation of glucose production by the liver and the mobilization of TAG in the adipose tissue. Finally, adrenaline prepares the skeletal muscle for contraction, with the activation of ATP-generating pathways, either through the anaerobic use of muscle glycogen or through the aerobic use of fatty acids. In this section, we will focus on the metabolic effects of adrenaline on the muscle, liver, and adipose tissues.

10.5.1 Molecular Mechanisms of Adrenaline Action

Adrenaline belongs to a group of substances known as catecholamines. It is synthesized by the adrenal gland, from which its name is derived. The adrenal glands are localized at the top of the kidneys and can be divided in two regions, the cortex, which secretes steroid hormones such as the glucocorticoids (see Sect. 9.4.2), and the medulla, where adrenaline is produced, more specifically in the chromaffin cells (Fig. 10.19A). The pathway for the adrenaline synthesis starts with the amino acid tyrosine and consists in four enzymatic steps, detailed in Fig. 10.19B.

Adrenaline secretion is triggered by a direct stimulus from the sympathetic nervous system that propagates through preganglionic nerve fibers reaching the adrenal gland (Fig. 10.19A). The concentration of adrenaline in the blood may increase more than 50-fold upon stimulation of the adrenal gland, but since the half-life of this hormone is too short, about 2 min, the effects of adrenaline on the body may be seen as acute and short-term responses to stress, which is compatible with an evolutionary adaptation to deal with "fight-or-flight" situations.

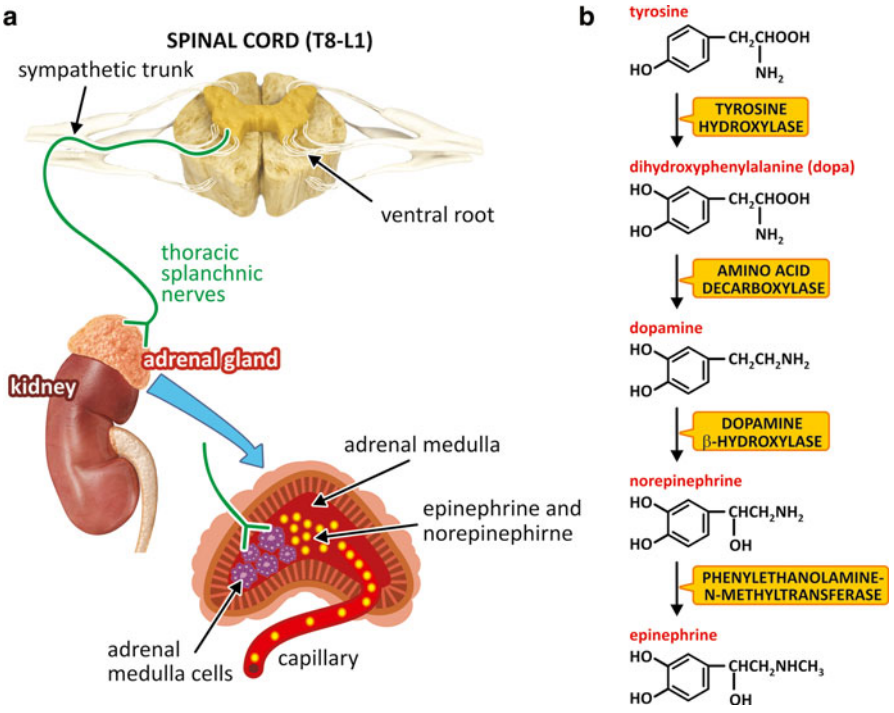


Fig. 10.19 (**a**) Representation of the adrenal gland, showing the two distinct parts: the cortex and the medulla. The medulla chromaffin cells produce and secrete adrenaline and noradrenaline. The stimulus for hormone secretion comes from the sympathetic nervous system and is transmitted to the adrenal gland through thoracic nerve fibers. (**b**) Metabolic pathway for adrenaline synthesis: the enzyme tyrosine hydroxylase converts the amino acid Tyr to L-dopa, which is decarboxylated by the enzyme aromatic amino acid decarboxylase, generating dopamine. The enzyme dopamine β-hydroxylase transforms dopamine in noradrenaline, which is converted to adrenaline by the action of the phenylethanolamine-*N*-methyl transferase (PNMT)

10.5.1.1 Cellular Receptors for Adrenaline

The action of adrenaline on its target tissues depends on the binding of the hormone to receptors present on the cell surface. There are different types of receptors for adrenaline divided in two main classes, the α- and the β-adrenergic receptors, which are in turn subdivided in α1 and α2, and β1, β2, and β3 subtypes, respectively.

The different, and sometime antagonic, effects of adrenaline on each tissue, such as the relaxation of the smooth muscles in the airways that increases the respiratory rate and the contraction of the muscles of the arterioles that causes vasoconstriction, can be explained by a tissue-specific expression of the different types of receptors. The different responses occur because each receptor subtype is coupled to distinct signaling systems, whose activation upon hormone binding triggers distinct intracellular responses (Fig. 10.20).

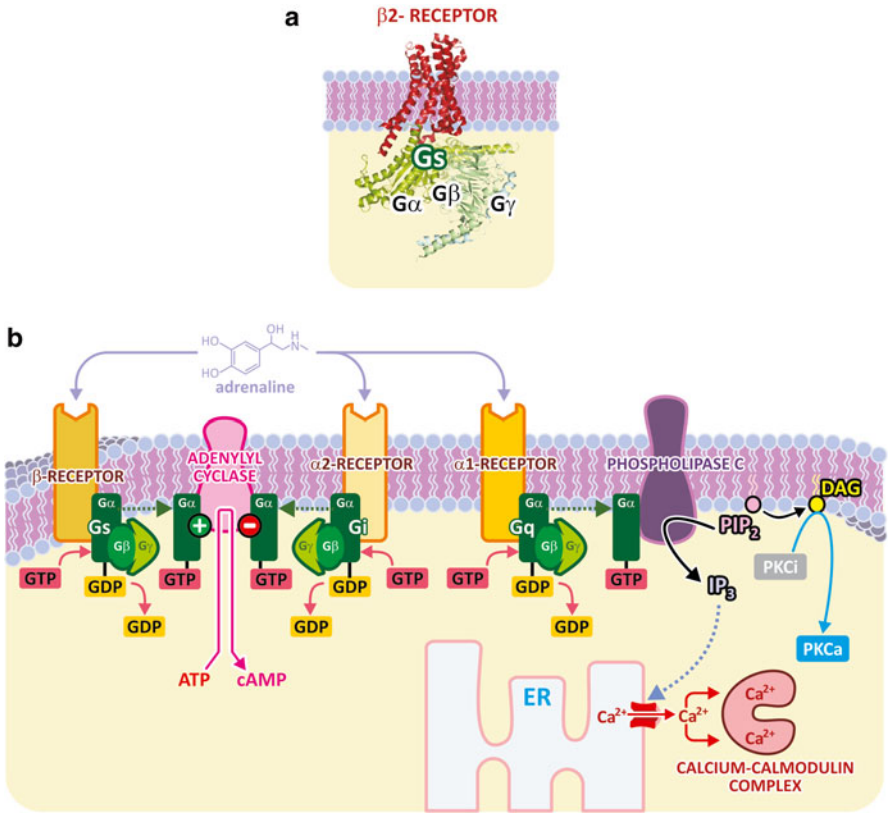


Fig. 10.20 (**a**) Crystallographic structure of the β2-receptor (*red*) bound to the trimeric G_s protein, with α (*green*)-, β (*light green*)-, and γ (*blue*)-subunits (PDB 3SN6). (**b**) Schematic representation of adrenaline signaling pathway through the different types of adrenergic receptors showing the different G protein α-subunits: G_s activates adenylate cyclase, while G_i inhibits this enzyme; G_q activates phospholipase C. β-adrenergic receptors are bound to G_s protein. When adrenaline binds to the receptor, the GDP bound to G protein is replaced by GTP and the G protein α-subunit moves on the membrane surface until it reaches adenylate cyclase, activating this enzyme, which converts ATP into cAMP. cAMP activates PKA leading to the phosphorylation of different targets in the cell. α2 receptor is bound to G_i, which inhibits the adenylate cyclase, leading to opposite effects of those mediated by G_s-coupled receptors. α1-adrenergic receptor is bound to G_q protein. When adrenaline binds to the receptor, the GDP bound to G protein is replaced by GTP and the G protein α-subunit moves on the membrane surface until it reaches the phospholipase C, which hydrolyzes the phosphatidylinositol (PIP_2) in DAG and IP_3. DAG activates PKC and IP_3 induces the release of Ca^{2+} from the SR stores to the cytoplasm

All the adrenergic receptors belong to the superfamily of G protein-coupled receptors (see also Sect. 9.4.1). They contain seven transmembrane helices and are bound to the G protein, a trimeric protein composed of α-, β-, and γ-subunits that is associated to the internal face of the plasma membrane (Fig. 10.20). The G protein α-subunit binds GDP, which maintains the protein in its inactive form, in

which it is associated to β- and γ-subunits. Upon hormone binding to the receptor, GDP is replaced by GTP, causing the α-subunit to dissociate from βγ-subunits and to move on plasma membrane until reaching a target enzyme, which may be the adenylate cyclase or the phospholipase C, depending on the type of G protein. The α-subunit has an intrinsic GTPase activity that terminates the signaling pathway through the conversion of the bound GTP in GDP, leading its reassociation to βγ-subunits.

The three subtypes of β-adrenergic receptors are linked to G_s ("s" from stimulatory G protein), which is a G protein type that activates the adenylate cyclase. The signaling pathway mediated by G_s-coupled receptors is the same as that involved in the glucagon mechanism of action, detailed in Sect. 9.4.1. Briefly, upon activation by the G_s subunit, adenylate cyclase catalyzes the conversion of ATP in cyclic AMP (cAMP), leading to an increase in the intracellular concentration of this molecule (Fig. 10.20). cAMP promotes the activation of the cAMP-dependent protein kinase (PKA), which phosphorylates several enzymes, modulating their activities. The β2-adrenergic receptors mediate the main effects of adrenaline on energy metabolism, which will be the focus of the next sections.

The α1-adrenergic receptors are mainly expressed in the smooth muscles, causing vasoconstriction and the decrease of the gastrointestinal tract motility. They are coupled to the type G_q of the G proteins, which activates the phospholipase C, an enzyme that hydrolyzes the phosphatidylinositol in the plasma membrane generating diacylglycerol (DAG) and inositol triphosphate (IP_3). DAG activates the protein kinase C (PKC), which mediates the smooth muscle contraction through the phosphorylation of proteins and ion channels. IP_3 binds to SR inducing Ca^{+2} release from the SR stores to the cytosol, stimulating muscle contraction (Fig. 10.20).

The α2 receptors are coupled to the type G_i, a G protein that inhibits the adenylate cyclase, leading to opposite effects of those mediated by G_s-coupled receptors.

10.5.2 Effects of Adrenaline on Energy Metabolism

10.5.2.1 Effects of Adrenaline on the Adipose Tissue Metabolism

The main effect of adrenaline on the adipose tissue is the activation of lipolysis, with the release of fatty acid in the bloodstream, leading to an increase of their availability to be used as an energy source. Lipolysis is activated through the PKA-mediated phosphorylation of two adipocyte proteins, the perilipin present on the surface of the lipid droplets and the hormone-sensitive lipase (HSL), which catalyzes the hydrolysis of the ester linkages of the TAG molecules. Phosphorylation activates the HSL and promotes a conformational change in perilipin that allows HSL recruitment to the surface of the lipid droplet, where it gains access to the TAG molecules (Fig. 10.21; see also Sect. 7.4.1 for details).

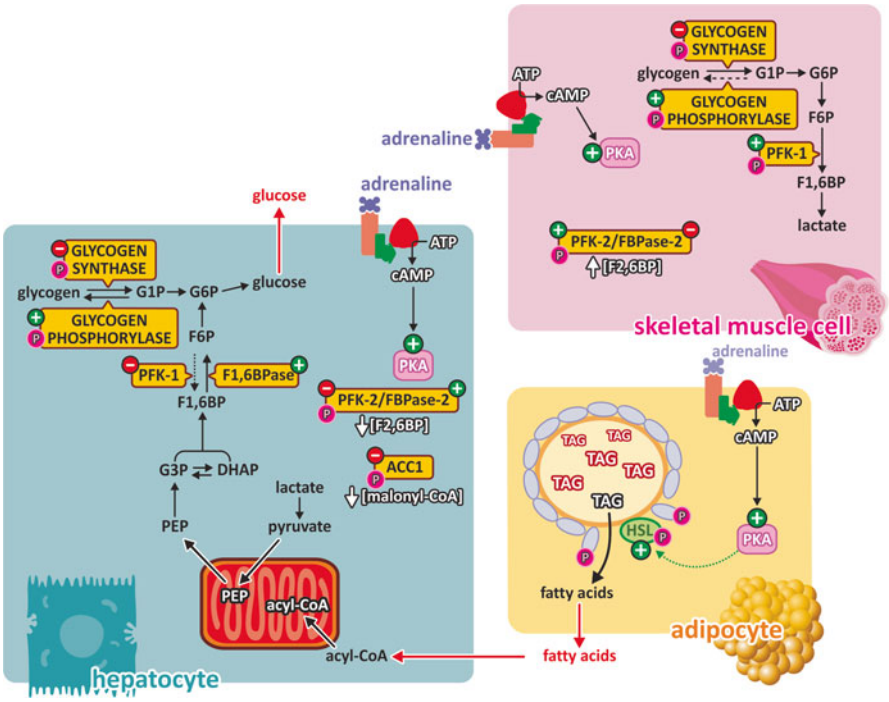


Fig. 10.21 Effects of adrenaline on energy metabolism. In liver, muscle, and adipose tissue cells, adrenalin binds to β-adrenergic receptors (*orange*) leading to G protein (*green*)-mediated adenylate cyclase (*red*) activation. The intracellular concentration of cAMP increases leading to the activation of PKA (*pink*), which phosphorylates different enzymes. In the adipocytes, PKA targets are perilipin (*light blue*) and HSL, resulting in the mobilization of TAGs. In the hepatocytes, the targets are (a) GP and GS, leading to glycogenolysis; (b) the bifunctional enzyme, leading to a decrease in the concentration of fructose-2,6-bisphosphate and activation of gluconeogenesis; and (c) ACC1, leading to the decrease in malonyl-CoA concentration and thus to the inhibition of fatty acid synthesis and the activation of the acyl-CoA transport into the mitochondrial matrix where they undergo β-oxidation. In the muscle cells, PKA targets are GP and GS, leading to glycogenolysis, and the muscle isoform of the bifunctional enzyme, in this case leading to an increase in fructose-2,6-bisphosphate concentration and activation of glycolysis

Adrenaline also interferes with the activity of the lipoprotein lipase (LPL), an enzyme involved in the transfer of lipids from the lipoproteins, especially from chylomicrons and VLDL, to the adipocyte, where they are stored after ingestion or de novo synthesis. The effects of adrenaline on LPL involve the inhibition of the translation of its mRNA, ultimately leading to a decrease in the lipid uptake by the adipose tissue. This makes the lipids available for use by the muscle cells.

10.5.2.2 Effects of Adrenaline on the Liver Metabolism

The main hepatic metabolic response upon adrenaline binding is the increase in glucose release in the bloodstream. This occurs both through the degradation of the liver glycogen and through the gluconeogenic pathway. Additionally, fatty acid synthesis is inhibited, allowing the incoming fatty acids to undergo β-oxidation. The effects of adrenaline on the liver metabolism are the result of a coordinated regulation of different metabolic pathways through the modulation of the activity of their key enzymes by phosphorylation promoted directly or indirectly by PKA.

Figure 10.21 provides a schematic overview of the adrenaline effects on liver metabolism, the results of PKA-induced phosphorylation being (a) the activation of GP and the inhibition of GS, leading to glycogenolysis; (b) the activation of the F2,6BPase activity of the bifunctional enzyme, leading to a decrease in the concentration of fructose-2,6-bisphosphate and the consequent activation of F1,6BPase and inhibition of PFK-1 with the activation of gluconeogenesis and the inhibition of glycolysis; and (c) the inhibition of ACC1, leading to the decrease in malonyl-CoA concentration and the consequent inhibition of fatty acid synthesis and the activation of the acyl-CoA transport into the mitochondrial matrix where they undergo β-oxidation. A more detailed description of the regulation of each of these key enzymes can be found in Sect. 9.4.1, since the effects of adrenaline on the liver metabolism are the same as those induced by glucagon (both β-adrenergic and glucagon receptors are G_s-coupled receptors).

10.5.2.3 Effects of Adrenaline on Muscle Metabolism

The effects of adrenaline in the muscle cells result in glycogen degradation and in a strong activation of glycolysis, adaptations that are especially important for the anaerobic metabolism of the type II fibers.

The adrenaline-mediated regulation of glycogen metabolism is similar to that occurring in the liver cells. PKA phosphorylates the phosphorylase kinase, which in turn phosphorylates and activates GP. Simultaneously, PKA-mediated phosphorylation inactivates GS. This results in intense glycogen degradation, yielding glucose-6-phosphate to the glycolytic pathway (Fig. 10.21).

Glycolysis is activated by the increase in the concentration fructose-2,6-bisphosphate, a potent activator of the glycolytic key enzyme PFK-1. Fructose-2,6-bisphosphate is synthesized through the phosphorylation of fructose-6-phosphate by the phosphofructokinase-2 (PFK-2) activity of bifunctional enzyme (see Sect. 9.3.3 for more details). The isoform of the bifunctional enzyme expressed in muscle cells differs from the liver isoform in its regulatory phosphorylation site, resulting in an opposite effect of phosphorylation on the enzyme activity in each tissue. The effect of PKA-induced phosphorylation on muscle isoform is the activation of the PFK-2 activity and in the inhibition of the fructose-2,6-bisphosphatase (F2,6BPase) activity,

leading to the synthesis of fructose-2,6-bisphosphate and the consequent PFK-1 activation, increasing the metabolic flux through glycolysis (Fig. 10.21). Furthermore, the muscle isoform of PFK-1 is itself a substrate for PKA. Phosphorylated muscle PFK-1 binds to actin filaments, resulting in further activation of the enzyme, which also becomes insensitive to the inhibitory effects of ATP, citrate or lactate. Thus, as a consequence of adrenaline action, glycolysis is strongly stimulated in muscle cells, while in the liver this hormone activates of gluconeogenesis.

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