Skeletal muscle physiology

Philip M Hopkins

The skeletal muscles are the effector organs of the locomotor system. They are under voluntary control, although much of their activity is subconsciously regulated. Skeletal muscle and cardiac muscle are both described as striated muscle because of their striped microscopic appearance. This appearance results from the ordered and regular arrangement of the subcellular contractile elements. Unlike cardiac muscle, skeletal muscle has no intrinsic spontaneous activity because it lacks the ion channels responsible for spontaneous membrane depolarization. Therefore, the stimulus for physiological skeletal muscle activity is always derived from a nerve impulse. The great majority of skeletal muscle fibres receive their nerve inputs at single central swellings of the fibres known as motor endplates. A few muscles, notably some of the facial muscles, are more diffusely innervated along the length of their fibres; such multifocal innervation may explain why these muscles respond with a more pronounced initial increase in tension after administration of succinylcholine.

However, irrespective of the type of innervation, the charge density arriving at the motor nerve terminal is insufficient to directly activate the much larger muscle fibres. The electrical neuronal impulse is amplified at the neuromuscular junction, the mechanism of which is beyond the remit of this review. The resulting generation of the endplate potential is the first step in muscle contraction.

Electrical events in muscle contraction

Muscle fibres are excitable cells. The cell membrane (sarcolemma) contains the ion channels and pumps necessary to maintain a very negative resting membrane potential and the voltage gated ion channels necessary for generation of an action potential. As with all excitable cells, the membrane potential of muscle cells at any time is a function of the net electrochemical gradients of ions that the membrane is permeable to at that time. This is largely determined by the instantaneous permeability of the sarcolemma to ions that are unevenly distributed across it, which in turn is dependent on which ion-selective membrane channels are open. The potential difference between intracellular and extra-cellular compartments when concentration and electrochemical gradients of a permeable ion are balanced is known as the equilibrium potential, which can be calculated for an ion, X, using the Nernst equation:

$$E_X = \frac{RT}{zF} \ln \frac{[X]_{out}}{[X]_{in}}, \qquad \text{Eq. 1}$$

where, E_X = equilibrium or Nernst potential for ion X; $[X]_{out}$ = extracellular concentration of X; $[X]_{in}$ = intracellular concentration of X; R = universal gas constant; T = absolute temperature; z = valency of ion; and F = Faraday constant.

At 37°C and converting from natural to base 10 logs, $RT/F = 61.5$. The Nernst potential can, therefore, be calculated for the major ions (Table 1).

The resting membrane potential in skeletal muscle cells is similar to that in neurons, i.e. −70 to −90 mV. Unlike nerve cells, where the resting membrane potential is predominantly a result of K^+ permeability, skeletal muscle cell resting membrane potential receives a significant contribution from Cl^- conductance. The importance of this Cl^- current became apparent when the excitability associated with myotonia congenita was found to be a result of chloride channel mutations. The physiological relevance of the Cl^- current stems from a need to maintain muscle activity during repeated stimulation. When muscle contracts, there is leakage of K^+ from the cell. With repeated activity there is run-down of the K^+ concentration gradient across the

Table 1 Plasma and cytoplasmic concentrations of various ions and the resulting Nernst potential (E_X)

Ion	Plasma (mM)	Cytoplasm (mM)	E_X (mV)
Na^+	145	12	+67
K^+	4	140	−95
Ca^{2+}	1.2	10^{-4}	+125
Mg^{2+}	1.5	0.8	+8
Cl^-	115	4	−90

Key points

Skeletal muscle constitutes 40% of muscle mass. Derangement of muscle function can have profound systemic effects.

Physiological skeletal muscle contraction requires generation and spread of a membrane action potential, transduction of the electrical energy into an intracellular chemical signal that, in turn, triggers myofilament interaction.

Intracellular cytoskeletal proteins, cell membrane structures and the associated glycoprotein extracellular matrix are important for maintenance of cell architecture and force transmission.

Smooth and graded changes in force of contraction are achieved through summation of responses to successive stimuli and recruitment of motor units.

Sustained muscle contraction requires de novo synthesis of ATP, which is principally aerobic or anaerobic depending on muscle fibre type.

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sarcolemma. Without the Cl^- current to maintain resting membrane potential, the muscle would not repolarize sufficiently to regenerate the active state of the channels responsible for generation of succeeding action potentials.

A skeletal muscle action potential is generated when the motor endplate potential is sufficient to raise the surrounding sarcolemmal potential above the threshold for activation of the voltage gated Na^+ channels that are abundant throughout the sarcolemma. When these channels are activated, the membrane is rapidly depolarized towards the Nernst potential for Na^+ (Table 1). However, the peak potential achieved is approximately +30 mV. The Nernst potential is not achieved for two main reasons. First, just as the Na^+ channels are activated by membrane voltage changes, a process of inactivation is also initiated as the membrane potential becomes less negative. Inactivation is a slower mechanism than activation, so the Na^+ current continues to flow for a short period after the onset of inactivation, but not sufficiently to reach the Nernst potential. The second factor limiting the upstroke of the action potential is the voltage activation of rectifying potassium channels. Their activation is initiated also during the upstroke of the action potential but (in a similar way to Na^+ channel inactivation) there is a slight delay in channel opening. The resulting K^+ current, in addition to limiting the peak of the action potential, is also principally responsible for repolarization.

Once an action potential has been generated, it spreads as a wave over the sarcolemma. Skeletal muscle sarcolemma is characterized by invaginations called transverse- or t-tubules that run perpendicular to the surface of the cell deep into its body. By passing down the t-tubular membrane, the action potential is carried to the structures responsible for transducing an electrical into a chemical signal that will trigger activation of the contractile elements.

Excitation-contraction coupling

This term encompasses the processes by which the surface membrane action potential leads to interaction of the intracellular contractile elements. The t-tubular membrane contains the highest density in the body of binding sites for dihydropyridine compounds, such as nifedipine. This finding was initially assumed to suggest that arrival of the action potential in the t-tubules opened voltage-gated Ca^{2+} channels. We now know that, although the t-tubular dihydropyridine receptors show marked amino acid homology with L-type voltage gated Ca^{2+} channels of other tissues, they do not function as Ca^{2+} channels. Their role in skeletal muscle is that of voltage sensors. When the action potential arrives in the t-tubule, the change in membrane potential leads to a conformational change in the α-subunit of the dihydropyridine receptor. This subunit consists of four transmembrane domains each of six segments (Fig. 1). The first and sixth segments of adjacent domains are linked by alternating extracellular and cytoplasmic loops of amino acids. The voltage-induced conformational change results in the projection of the cytoplasmic loop between the second and third transmembrane domains deeper into the cytoplasm. Here, charged amino acid residues of the cytoplasmic loop come into proximity with those of a protein projecting towards the cytoplasmic surface of the t-tubule from the adjacent terminal swelling of the sarcoplasmic reticulum.

The sarcoplasmic reticulum is the intracellular store of Ca^{2+} and the protein that projects from the surface of its terminal cistern towards the t-tubule is its Ca^{2+} release channel (Fig. 2). This Ca^{2+} channel is frequently referred to as the ryanodine receptor, after the plant alkaloid that was used to isolate and characterize it. The ryanodine receptor gene is the principal gene implicated in malignant hyperthermia.

The result of the interaction between the dihydropyridine and ryanodine receptors is the opening of the channel pore of the latter. Ca^{2+} floods out of the sarcoplasmic reticulum down its concentration gradient into the cytoplasm where it acts as the trigger for contraction.

Contractile structures

Muscle contraction results from the interaction of two filamentous proteins, actin and myosin. When actin and myosin are mixed

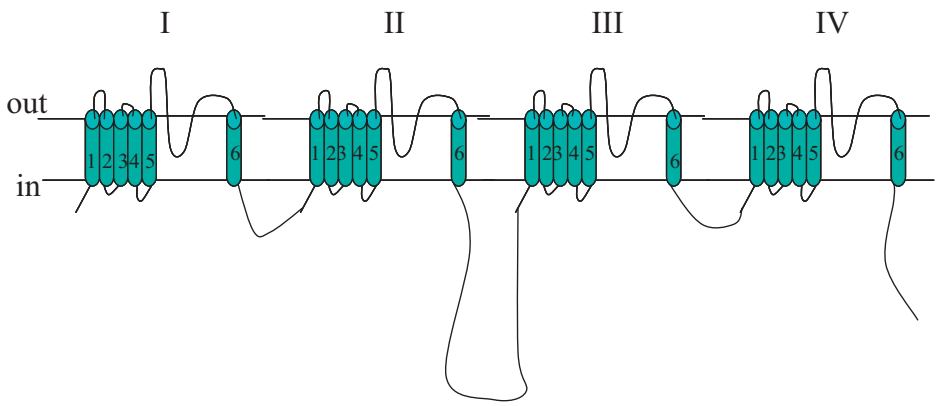

Fig. 1 The α1 subunit of the dihydropyridine receptor. Four transmembrane domains (I–IV) are each made up of six segments (1–6). The cytoplasmic loop between the sixth segment of domain II and the first segment of domain III interacts with the ryanodine receptor protein to cause Ca^{2+} release from the sarcoplasmic reticulum.

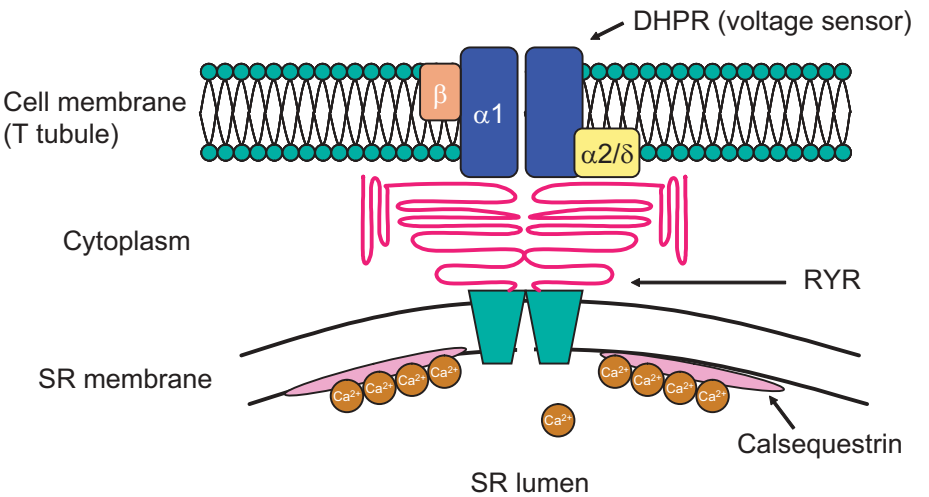

Fig. 2 The key structures involved in excitation–contraction coupling. The change in t-tubular membrane potential causes a change in conformation of the α1 subunit of the dihydropyridine receptor (DHPR). This in turn causes a change in conformation of the ryanodine receptor (RYR), which is the Ca^{2+} release channel of the sarcoplasmic reticulum (SR), resulting in opening of the channel and release of Ca^{2+} from the SR where it is stored bound to calsequestrin.

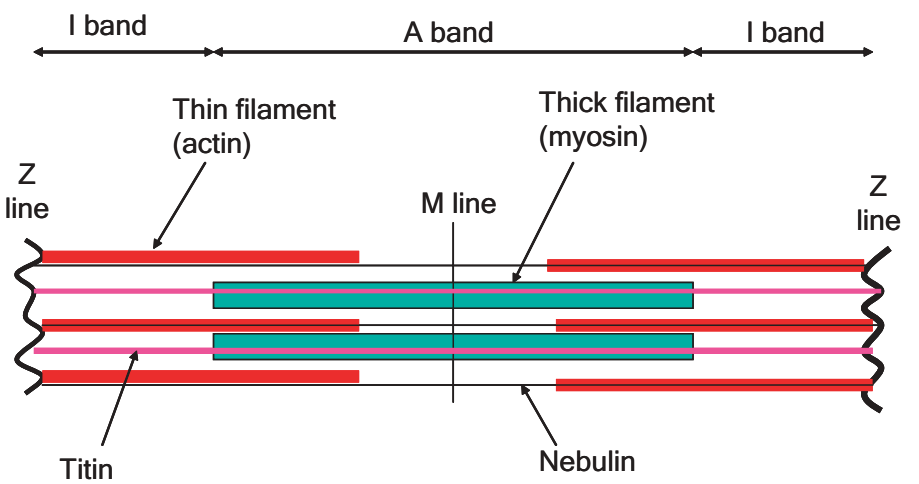

Fig. 3 A schematic diagram of the sarcomere showing the arrangement of thick and thin filaments and their major supporting proteins. The A and I bands were named from their appearances on microscopy where the A band appears dark (anisotropic) relative the lighter I band (isotropic).

as the only two components in an *in vitro* system strong bonding occurs between them. In skeletal muscle, myosin and actin are organized into a regular pattern within sarcomeres, which are the contractile units. Each sarcomere is formed between adjacent sheets of Z proteins (seen as Z lines on microscopy) that are structural proteins running transversely across the fibre. Projecting perpendicularly from each of the two facing sheets of Z proteins towards the centre of the sarcomere are thin myofilaments that are mainly composed of two helically coiled actin filaments. The thin filaments interdigitate with thick filaments that also run parallel to the long axis of the fibre but lie in the central part of the sarcomere. The thick filaments are formed by bundles of myosin filaments. The architecture of the sarcomere is maintained by the Z proteins and other important structural proteins (Fig. 3). M line proteins form a structural mesh at the midline of the sarcomere where they anchor directly the tails of myosin filaments and indirectly, through connecting proteins (principally nebulin), the central end of the thin filaments. The thick filaments are further supported by titin, the largest polypeptide described in vertebrates. Each titin molecule spans half the sarcomere between the Z and M proteins. The sarcomeric architecture is anchored within the cytoskeleton of the cell, which is formed principally by a mesh of actin chains: actin is, therefore, the most abundant protein in skeletal muscle.

In the ultrastructural cross-section of the parts of the sarcomere where thin and thick filaments overlap, it can be seen that each thick filament is surrounded by six thin filaments. Myosin molecules consist of a long tail with a globular head. Within the

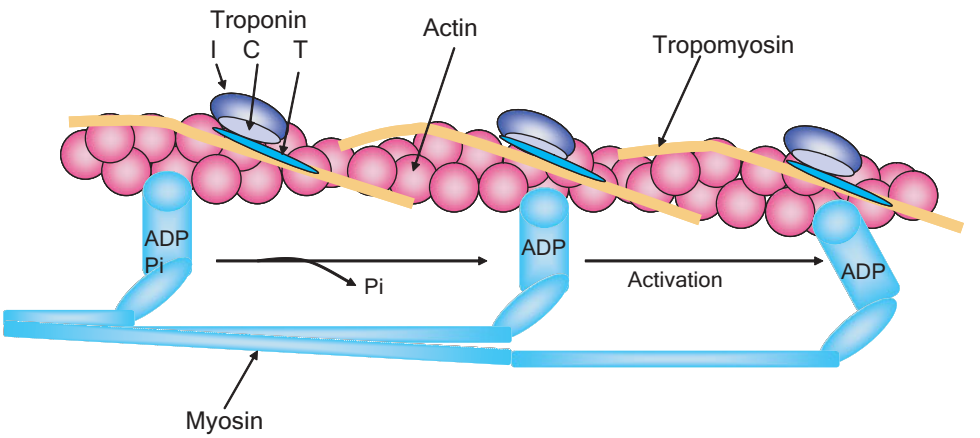

Fig. 4 Detail of the interaction between thin and thick filaments. Three myosin heads are shown depicting progressively three stages, moving from left to right, of the power stroke (note that this is not a realistic depiction as, in life, the stages would be coordinated, occurring simultaneously in all the myosin heads). All the stages shown occur after binding of Ca^{2+} to troponin C and subsequent exposure of the myosin binding sites on actin. This permits binding of myosin (left-hand myosin head), followed by release of inorganic phosphate (middle myosin head) and subsequent activation of hinging of the myosin head (right-hand myosin head).

thick filament, the myosin heads are arranged so that they project radially outwards towards the thin filaments. The myosin heads contain the binding sites for actin, but at rest actin–myosin binding is prevented by regulatory proteins on the thin filaments. Tropomyosin is a filamentous protein than runs in the groove formed by the two actin strands. Furthermore, adjacent to each myosin-binding site on the actin chains there is a troponin complex, composed of T (tropomyosin-binding), I (inhibitory), and C (Ca^{2+}-binding) subunits. Actin–myosin binding is prevented by the combination of tropomyosin and troponin-I.

When Ca^{2+} is released from the sarcoplasmic reticulum into the cytoplasm, Ca^{2+} allosterically binds to troponin-C resulting in conformational changes of the other troponin subunits that expose the myosin-binding sites on the actin chain.

At rest, the myosin head is complexed with ADP and inorganic phosphate. Binding of myosin to actin displaces the inorganic phosphate, which in turn leads to pivoting of the myosin head at its junction with the tail of the molecule, thereby moving the thin filament towards the centre of the sarcomere. This ratchet-like movement of the thin filaments towards the M proteins pulls together adjacent Z sheets and shortens the sarcomere (Fig. 4). But the shortening of the sarcomere produced by one of these power strokes would be minute and so multiple cycles of actin–myosin interaction need to occur. To achieve this ATP is required. The conformational change inherent in pivoting of the myosin head favours substitution of ATP for ADP on the myosin head. The myosin head has ATPase activity and the energy released by this enzymic activity is sufficient to overcome the actin–myosin bonds, returning the myosin to its resting shape bound to ADP and inorganic phosphate, with potential to bind to a further actin site in the continued presence of high cytoplasmic concentrations of Ca^{2+}.

Therefore, actin–myosin interaction continues as long as cytoplasmic Ca^{2+} concentration remains sufficiently high. Muscle relaxation will occur when Ca^{2+} removal from the cytoplasm exceeds Ca^{2+} release into it. Under physiological conditions the majority of Ca^{2+} is taken back up into the sarcoplasmic reticulum through the action of the SERCA (sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase) pump. As cytoplasmic Ca^{2+} concentration falls, Ca^{2+} dissociates from troponin C, the myosin binding sites on the actin chains become unavailable allowing the sarcomeres to return to their resting length. Crucial to relaxation is titin, which behaves like a spring, becoming tightly coiled during sarcomere shortening and subsequently recoiling when the actin–myosin bonds are released.

Force transmission

Until 20 yr ago, it was thought that the force of contraction generated within the sarcomeres was transmitted directly in series through to the musculotendinous insertions of the fibre. It is now apparent that a significant proportion of the force generated by a fibre (>70% in some experiments) depends on transmission of the force successively through structural intracellular proteins to the sarcolemma and extracellular matrix. The extracellular matrix around each fibre forms the endomysium, the connective tissue that binds adjacent fibres and supports the capillary network of the muscle. The endomysium is in continuity with thicker connective tissue, the perimysium that holds groups of fibres within fascicles. All the fascicles of a muscle are further contained within even thicker connective tissue, the epimysium, which is continuous with the outer layer of the tendon.

Crucial to the efficient transmission of forces from the sarcomere to the extracellular matrix are the integrin and dystroglycan complexes that link the latter to the cytoskeletal elements of the sarcomere. The importance of the dystroglycan complex (Fig. 5) became apparent with the discovery that several of the hereditary dystrophies result from abnormal components of this complex.

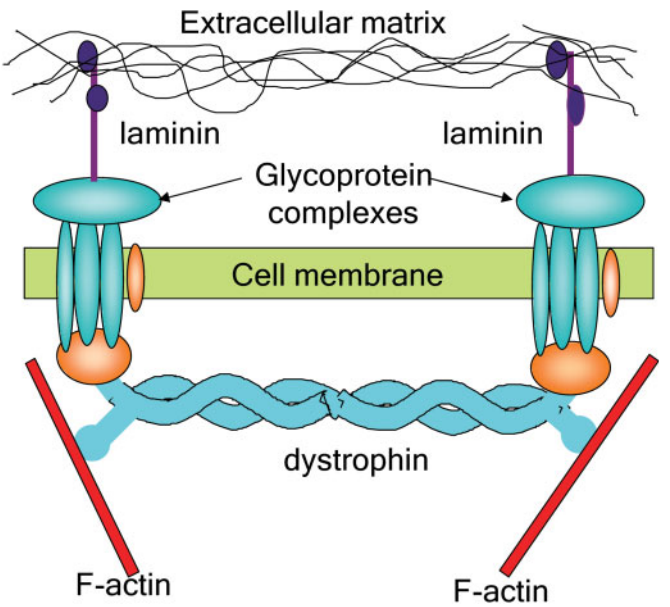

Fig. 5 The dystroglycan complex, which forms a crucial structural and functional link between the intracellular cytoskeleton (actin) and the extracellular matrix.

There are as yet no disease associations with abnormalities of the integrin complex but attempts at introducing mutations of integrin complex genes in transgenic mice have all proved incompatible with life.

Determinants of force of contraction

For a single muscle fibre the force of contraction is proportional to the number of actin–myosin bonds formed. This will be optimal when the initial sarcomere length is such that all myosin heads are overlapped by thin filaments. If the sarcomere is stretched too far, the central myosin heads will be redundant. If the sarcomere is too short, the distance between the actin and myosin binding sites increases and their alignment may also be distorted, both of which will reduce the efficiency of contraction.

However, unlike in cardiac muscle, skeletal muscle fibres are maintained near their optimal length in their working range; therefore, the Frank-Starling length–tension relationship is not a major factor in skeletal muscle physiology. Consequently, the force generated by a single skeletal muscle fibre will be a function of both the cross-sectional area and the length of the fibre. The same relationship applies to the muscle as a whole. Of course, it would not be very useful if each muscle could contract only at its maximum force. Graded muscular contraction is achieved through two main mechanisms: summation and recruitment.

Summation

Summation of skeletal muscle fibre contractions is possible because the absolute refractory period of the sarcolemma is considerably less than the duration of raised cytoplasmic Ca^{2+} concentration and subsequent tension generation. If a second stimulus is applied to the muscle before it has fully relaxed from the first, the response to the second stimulus will add to the residual response of the first stimulus. This summation reaches a peak when the second stimulus occurs ~20 ms after the first, corresponding to a stimulus frequency of 50 Hz. Between stimulus frequencies of 20–50 Hz the summed responses form a smooth ramped increase in tension, or tetanic response. The usual firing frequency of vertebrate motor neurons is within the tetanic range.

Recruitment

Single motor neurons innervate multiple muscle fibres. A motor neuron and the muscle fibres it innervates are collectively called a motor unit. The number of muscle fibres within a motor unit varies within and between muscles. The smallest motor units, containing as few as 3–10 muscle fibres, are found in muscles used for fine intricate movements. Much larger motor units, containing up to several hundred muscle fibres, are predominant in muscles used for gross vigorous movements.

When a muscle is required to produce a progressive increase in tension, initially, when the load applied to the muscle is small, the smallest motor units within the muscle are used. As the load increases larger and larger motor units are recruited, so that when the load is the maximum attainable by that muscle, all its motor units will be operating.

Energy for contraction

We have seen that ATP is required for significant tension to develop and it is also crucial for muscle relaxation. However, ATP is a relatively unstable compound and the instantaneously available ATP is able to maintain contraction for <1 s. Muscle has a specialized means of storing high-energy phosphate in the form of creatine phosphate. ATP is derived from creatine phosphate by the action of creatine kinase but the so-derived ATP is sufficient to maintain contraction for only a further 5–8 s. More prolonged contractile activity requires synthesis of ATP by intermediary metabolism. Aerobic metabolism of 1 mole of glucose produces 38 moles of ATP but even maximal oxygen delivery is insufficient to meet the demands of vigorous muscle activity. Anaerobic metabolism is less efficient in that 1 mole of glucose produces only 2 moles of ATP, but the ATP produced is more readily available. However, this is at the expense of a build-up of lactate, which is an important factor in the development of muscle fatigue.

Muscle fibre-types

The diameter of muscle fibres varies from 10 to 100 μm. Most human muscles contain a mixture of fibres within this range. The thinner fibres are type I fibres and they are adapted for sustained activity requiring submaximal tension generation. The thickest fibres (type IIb) are adapted for short bursts of near-maximal activity. Muscles containing a predominance of type I fibres

Table 2 Physical, contractile, and metabolic characteristics of different types of muscle fibre

	Type I	Type IIa	Type IIb
Diameter	Small	Intermediate	Large
Motor unit size	Small	Intermediate	Large
Recruitment	Early	Intermediate	Late
Contraction	Slow	Fast	Fast
Twitch	Long	Short	Short
Ca^{2+} sequestration	Slow	Rapid	Rapid
Capillaries	Abundant	Intermediate	Sparse
Glycolytic capacity	Low	Intermediate	High
Oxidative capacity	High	High	Low
Myoglobin	High	Intermediate	Low
Glycogen	Low	Intermediate	High
Myosin ATPase	Slow	Fast	Fast

appear a deeper red colour than those with few type I fibres because type I fibres have a high myoglobin content. Myoglobin is pigmented because of a haem moiety that is responsible for its oxygen-binding capability. Myoglobin provides a storage capacity for oxygen within muscle cells; its affinity for oxygen is greater than that of haemoglobin, which aids oxygen delivery to muscle, but is such that oxygen is released for aerobic metabolism when demand is increased. Type IIa fibres are intermediate in size and myoglobin content. Other physical and metabolic characteristics of the different fibre types are described in Table 2.

Further reading

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Please see multiple choice questions 1–5.