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**SYMPOSIUM ON  
'EXERCISE, A STIMULUS FOR METABOLISM AND A  
CHALLENGE TO NUTRITION'**

**The biochemistry and physiology of the muscle cell**

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Muscle tissue is unique in that it has the capacity to increase its metabolic activity by a very large factor when it changes from the resting to the active state. For example, Hill (1964) showed that the heat generated by the frog *rectus abdominis* muscle in the tetanized state is 2500 times greater than when the muscle is at rest. The maximum energy output of muscle during activity varies with the type of muscle and the species. When compared with the flight muscles of certain birds and insects the cardiac and skeletal muscles of man have a relatively low rate of energy transduction (Table 1). Later speakers in the symposium will discuss in more detail specialized aspects of nutrition and metabolism as they relate to muscle performance. I wish in this introduction to outline some aspects of the biochemistry and physiology of skeletal muscle to provide a background to their discussions.

The large increase in catabolic metabolism that occurs on stimulation requires a highly-specialized control system to bring about the several-hundredfold changes

Table 1. *Metabolic rates of various muscles (from Weiss-Fogh, 1952)*

Species	Muscle	Metabolic rate	
		kJ/kg per h	kcal/kg per h
Man	Leg	250-2500	60-600 (Max)
	Heart	250-420	60-100 (Max)
Humming bird	Wing	3000-4200	700-1000 (Short flight)
Locust	Wing	1670-3350	400-800 (Av)
Fruit fly	Wing	2700	650 (Av)
Blow fly	Wing	7100	1700 (Av)
Bee	Wing	10 000	2400 (Av)

Max, maximum; Av, average.

in activity that occur with key enzymes such as the actomyosin ATPase. Despite its size the increase is extremely rapid, taking milliseconds in the fast muscles and involving large changes in the distribution of calcium ions within the cell.

In addition to its ability to vary the rate of the transduction of energy over a wide range, skeletal muscle is an extremely adaptable tissue in that it responds by changes in mass to the activity pattern and state of nutrition. Indeed, it acts as a major protein store during starvation and malnutrition. Factors which affect muscle mass do not usually involve a change in muscle cell number or of phenotype but rather of size. Other factors such as type of innervation, hormones and disease, however, can produce modifications in gene expression resulting in changes of cell type.

### *Muscle fibre types*

Although the major portion of the metabolism associated with exercise occurs in skeletal muscle, cardiac muscle and smooth muscle, particularly that of the vascular system, also contribute. The nutrition of all these types each with their particular requirement must be considered during exercise. In all muscles the mechanochemical process is very similar, but differences exist between the three types in their dependence on the various metabolic pathways for ATP production. Features characteristic of the individual muscle types are also apparent in the mechanism of regulation, particularly in the finer tuning required for the specialized contractile activity of each muscle type.

Skeletal muscle exists in two main forms, slow twitch, type I, and fast twitch, type II fibres. These types are distinguished by their speed of contraction and dependence on anaerobic and aerobic metabolism.

Further subclassifications such as types IIA and IIB (Table 2) have been proposed on the basis of differences in the fast twitch muscles in histochemical staining for glycolytic and oxidative enzymes. Both of the subclasses of the type II fibres contain the myosin and troponin isoforms corresponding to fast twitch muscle, but the metabolism of the IIA subclass is more like that of type I fibre in that it has greater capacity to carry out aerobic metabolism than the type IIB fibre.

Table 2. *Types of human skeletal muscle fibres (from Saltin et al. 1977)*

	Type I (slow twitch)	Type IIA (fast-twitch oxidative)	Type IIB (slow-twitch glycolytic)
ATPase activity after pre-incubation at pH 10.3	-	+++	+++
ATPase activity after pre-incubation at pH 10.3 and pH 4.6 - 4.8	-	-	+++
Speed of contraction	Slow	Fast	Fast
Glycolytic capacity	Low	Moderate	High
Oxidative capacity	High	Moderate	Low
Glycogen store	Moderate-high	Moderate-high	Moderate-high
Triacylglycerol store	High	Moderate	Low
Capillary supply	Good	Moderate	Poor

The two main fibre types are under different neuronal control and in the muscles of some species, e.g. rat, guinea-pig and rabbit, the different fibre types are concentrated in bundles which make up parts of, or, in some cases, the whole muscle. In humans, the fibre types are usually mixed throughout the muscle. The relative proportions of the two types do vary, however, in different muscles and in different regions of the same muscle.

Although the major difference between these two muscle cell types lies in the relative dependence on anaerobic (type IIB) and aerobic (type I, type IIA) metabolism to produce ATP, all the evidence suggests that the mechanism of ATP utilization is identical in both types of fibre. The energy transduction system, however, will operate faster in type II fibres to accommodate the higher velocity of shortening of this fibre type.

### Energy transduction

The characteristic feature of the contractile system is that it is composed of two filaments, that containing myosin, the A filament, and that composed principally of actin, the I filament, in striated muscle (Fig. 1). The basic design of the system is similar in striated and smooth muscle. In the former tissue, the filament system is organized into myofibrils and the amount of myosin is about 2.5 times greater than that of actin, whereas in smooth muscle, the two-filament systems are not organized in such a regular manner and there is about three times as much actin as myosin.

*Actin filament.* The filament is composed of actin monomer subunits of molecular weight 42 000 that are oblate ellipsoid in shape (Engleman & Padron, 1984). The subunits are arranged in a double helical arrangement with a repeat of 37 nm along the axis and approximately fourteen subunits per turn so that each myosin head binds with the same orientation to the axis of the filament. In striated muscle the proteins tropomyosin and the troponin complex are also associated with the I filament. They are not essential for the contractile process *per se*, but are

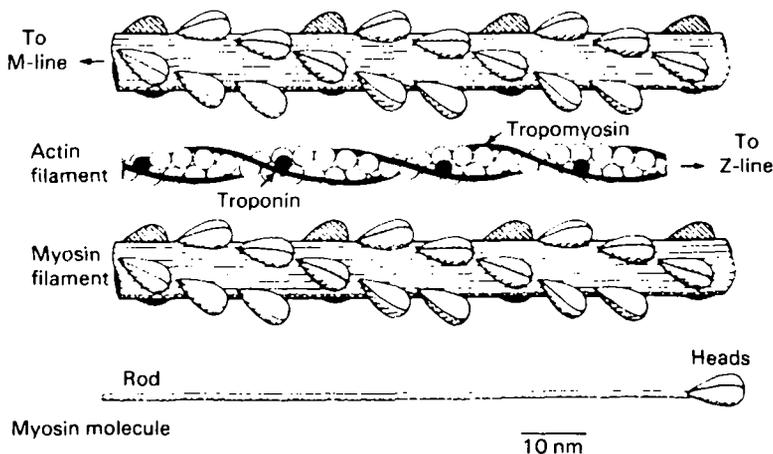


Fig. 1. Scheme of the I and A filament system in striated muscle. Myosin molecule illustrated at same scale for comparison (from Squire, 1983).

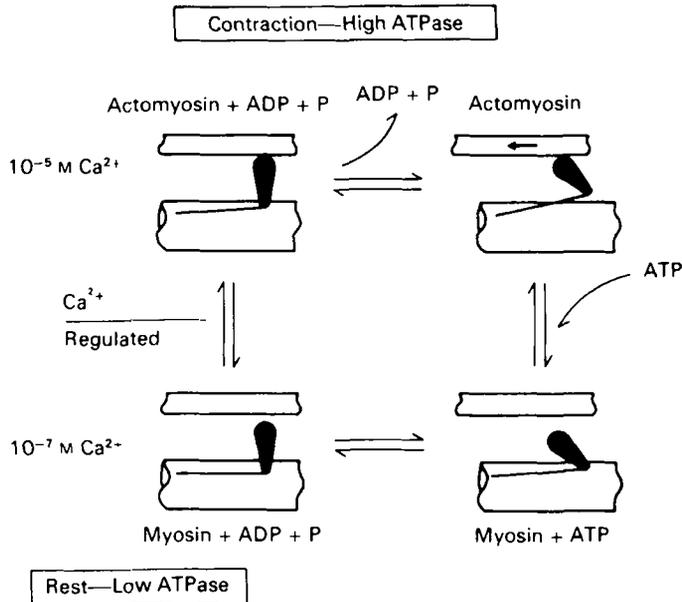


Fig. 2. Schematic representation of the cross-bridge cycle (from Lymn & Taylor, 1971).

essential for the regulation of the MgATPase of the actomyosin complex by Ca. As is the case with most of the myofibrillar proteins, actin, although a strongly-conserved protein, occurs in a number of isoforms that are characteristic for the muscle cell type. Nevertheless, unlike the other myofibrillar proteins, the isoforms of actin present in fast and slow skeletal muscles are identical (Vanderkerckhove & Weber, 1979).

**Myosin filament.** The myosin molecules are arranged in regular bipolar fashion in the A filament so that on each half of the A filament there are three myosin heads per 14.3 nm along the axis of the filament. The A filament has a bare region of 200–300 nm in the centre that is free of myosin heads. It is the interaction of the myosin heads with the actin monomers in the I filament leading to the hydrolysis of ATP that produces movement between the filaments and thus results in the generation of force (sliding filament hypothesis).

Expressed in its simplest form, the events presumed to occur during contraction are illustrated in Fig. 2. On the basis of kinetic studies it was suggested (Lymn & Taylor, 1971) that in resting muscle the enzyme site on the myosin head is saturated with ADP and inorganic phosphate, the products of the rapid hydrolysis of MgATP<sup>2-</sup>. The products of hydrolysis dissociate very slowly from the myosin when it does not interact with actin. On stimulation interaction with actin occurs, the products are lost and the energy of hydrolysis up to now stored in the myosin molecule, causes conformational changes to take place that involve re-adjustment of the interaction between the two molecules. This results in a lateral movement of the actin with respect to the myosin of 5–10 nm. After the loss of products, ATP binds to the myosin causing dissociation of the complex. ATP is rapidly

hydrolysed at the enzymic site and the cycle is completed with the myosin head returning to its original conformation with associated ADP and inorganic phosphate. The nature of the conformational changes that lead to relative movement of the two filaments is far from clear even today. Hinge-like movements at the region where the head of the myosin molecule joins the tail or even within the tail itself have been invoked. A more likely explanation is that the conformational change involves relative movement between the domains of the myosin head. As outlined above it is assumed that the cross-bridge cycle involves an attachment–detachment state of the myosin head. It is by no means clear that this is the case. Recently considerable evidence has accumulated suggesting that the myosin may not actually detach from the actin in the relaxed state but that it exists in two states both in close contact with the latter protein. Contraction would then occur when the myosin changes from one state to the other either as a consequence of, or associated with, the dissociation of the products of enzymic hydrolysis from the active site. Most of the schemes proposed for the cross-bridge cycle involve only one of the two heads of the myosin molecule. Presumably two heads are essential for the contractile function of myosin but as yet there is no clear indication of their role.

The tension developed on contraction will depend on the number of cross-bridges actively engaged in cycling in unit time, but all the evidence suggests that the cross-bridge movement is asynchronous. The number involved is very large for it can be calculated that in 10 mm<sup>3</sup> striated rabbit muscle there is about 300 km of A filament.

Unlike actin, myosin in skeletal muscle exists in a number of isoforms that are characteristic of the type of fibre in which they occur. The myosin molecule consists of two heavy chains of relative molecular weight ( $M_r$ ) 200 000 and four light chains of  $M_r$  15 000–25 000 made up of two each of the so-called alkali and P light chains (Fig. 3).

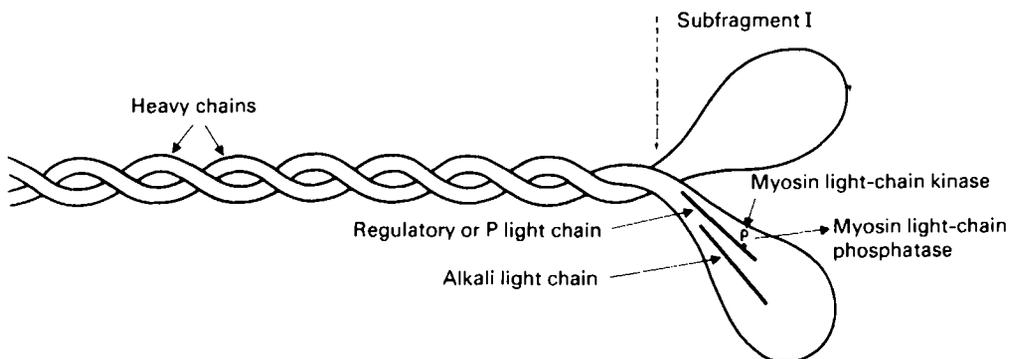


Fig. 3. Schematic representation of the myosin molecule. The polypeptide chains in the tail region are largely  $\alpha$ -helical in conformation. Both heads, which are probably identical, possess light chains although they are shown to be present in one only in the diagram. A number of studies have indicated that both light chains may be located in the neck region of the molecule. P, the approximate position of the serine residue on the P light chain that is phosphorylated by myosin light-chain kinase.

Both heavy and light chains exist in a number of isoforms produced by different genes or in some cases by different routes of RNA processing. In general the forms of myosin present in type I fibres have lower ATPase activity than those present in type II fibres. Thus in skeletal muscle the enzymic activity of the myosin correlates with the maximum speed of shortening (Barany, 1967). For reasons which are not clear, each type I and type II fibre contains at least two isoforms of myosin as judged by their light chain composition.

*Regulation of contraction.* The ability of muscle to control its metabolic activity over a very wide range and within a time-scale of milliseconds requires that it possesses a highly-effective regulatory system. For this purpose, it has adapted the system involving  $\text{Ca}^{2+}$  and specific target proteins that bind  $\text{Ca}^{2+}$  with high affinity that is widely distributed in all types of cell. When  $\text{Ca}^{2+}$  are bound to the target protein conformational changes occur that can be transmitted to other components within the system. The contractile system makes up about 60% of the total cell mass in skeletal muscle and its regulation involves large fluxes of  $\text{Ca}^{2+}$  within the cytoplasm. This requires an expenditure of energy and the regulation of contraction makes a significant call on the ATP produced by the muscle cell which cannot be used for mechanical work. It is estimated that in fast skeletal muscle, depending on the work rate, as much as 20–30% of the ATP produced may be required to maintain the necessary  $\text{Ca}^{2+}$  gradients involved in the contracted and stimulated states (Kushmerick *et al.* 1969). The importance of the effective functioning of the regulatory system in muscle cannot be overestimated.

Indeed, fatigue may be a consequence of the reduced efficiency of the  $\text{Ca}^{2+}$  pump in the sarcoplasmic reticulum rather than the unavailability of ATP for the contractile system. This would be the case if the fall in pH associated with increased lactic acid concentration had, as some experimental evidence suggests, a greater effect on the  $\text{Ca}^{2+}$  pump than on the cross-bridge cycle.

Regulation in striated muscle depends on one important effect of actin on the myosin ATPase. The substrate for the myofibrillar ATPase is  $\text{MgATP}^{2-}$  for in muscle the free  $\text{Mg}^{2+}$  concentration is in the mM range and probably does not change markedly during contraction.  $\text{MgATP}^{2-}$  is a very poor substrate for myosin, but in the presence of actin as actomyosin the rate of hydrolysis is speeded up several hundred times, i.e. to the rate observed in intact stimulated muscle. With actomyosin alone in the absence of the other components of the I filament, tropomyosin and the troponin complex, the rate of MgATPase is high and it is unaffected by changes in the  $\text{Ca}^{2+}$  concentration of the order observed in changing from the resting to the contracted state. If, however, the actomyosin system also contains tropomyosin and troponin the MgATPase becomes sensitive to low levels of  $\text{Ca}^{2+}$ . In the absence of  $\text{Ca}^{2+}$  ( $\ll 10^{-7}$  M), the MgATPase is low and similar to that of myosin alone. If the  $\text{Ca}^{2+}$  concentration is increased to  $10^{-5}$  M the MgATPase rises to the high value associated with contraction. Thus, in resting muscle ( $\ll 10^{-7}$  M  $\text{Ca}^{2+}$ ), the tropomyosin and the troponin complex in some way prevent actin from activating the MgATPase of the myofibril.

The troponin complex contains three well-defined components: troponin T which interacts with tropomyosin, troponin I which has inhibitory activity on the actomyosin MgATPase in the presence of tropomyosin, and troponin C. Troponin C is a specific  $\text{Ca}^{2+}$ -binding protein unique to striated muscle although it bears a marked structural resemblance to the widespread  $\text{Ca}^{2+}$ -binding protein found in all cells, calmodulin. Sequence evidence suggests that both proteins have evolved from the same primitive gene and troponin C has been selected out for specialized function in striated muscle. In resting muscle, troponin C does not bind  $\text{Ca}^{2+}$  but when the sarcoplasmic  $\text{Ca}^{2+}$  concentration rises to  $10^{-5}$  M the cation-binding sites of troponin C are occupied by  $\text{Ca}^{2+}$ . In this condition, the tropomyosin–troponin complex system no longer inhibits the MgATPase of actomyosin and the cross-bridges cycle rapidly.

The precise manner in which the binding of  $\text{Ca}^{2+}$  to troponin C changes the tropomyosin–troponin complex system from inhibiting the actomyosin interaction is not known. A popular theory is the so-called steric hypothesis which proposes that at low  $\text{Ca}^{2+}$  concentrations the tropomyosin filaments physically block the interaction of actin with myosin by covering the appropriate binding site on each actin monomer. According to this hypothesis, on contraction the tropomyosin is considered to move further into the groove in which it lies on the actin filament and thus expose the site on actin with which myosin can interact and rapidly hydrolyse  $\text{MgATP}^{2-}$ . Such a mechanism would require that the binding of  $\text{Ca}^{2+}$  to troponin C initiates a series of conformational changes in the  $\text{Ca}^{2+}$  binding-protein that are transmitted through the troponin complex and lead to movement of the tropomyosin filament.

As with the theory of cross-bridge mechanism involving a cycle of attachment followed by detachment of the myosin head, the experimental evidence does not completely support the steric hypothesis as originally proposed. For example, such a mechanism would require a big difference in the binding constant of myosin to the I filament in the absence and presence of Ca. In those *in vitro* experiments which attempt to simulate the situation *in vivo* this does not appear to be the case. Clearly when the precise mechanism of the interaction of actin and myosin during the cross-bridge cycle is understood, the role of tropomyosin and the troponin complex in its regulation will be apparent.

Like myosin, tropomyosin and the troponin complex also exhibit differences in isoform composition in each of the two main fibre types. For example, the troponin C found in slow type I fibres differs from that in fast type II fibres and appears in some species at least to be identical with troponin C from cardiac muscle. It may be of functional significance that slow skeletal muscle troponin C contains only three effective  $\text{Ca}^{2+}$  binding sites compared with four in the fast skeletal muscle isoform. The  $\text{Ca}^{2+}$  binding sites fall into two classes, sites I and II,  $\text{Ca}^{2+}$  specific, and sites III and IV  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  specific. Sites I and II are considered to be the sites that have to be filled with  $\text{Ca}^{2+}$  to trigger contraction. In the slow skeletal muscle isoform, site I is imperfectly formed and as it is not able to bind  $\text{Ca}^{2+}$  with high affinity, contraction will occur where site II only is filled. This implies that

Table 3. *Isoforms of I-filament proteins in skeletal and cardiac muscle cells (from Perry et al. 1984).*

Muscle ...	Skeletal		Cardiac
	Type I fibres (slow)	Type II fibres (fast)	
Protein			
Actin	Skeletal	Skeletal	Cardiac
Troponin C	Slow	Fast	Slow
Troponin I	Slow	Fast	Cardiac
Troponin T	Slow	Fast*	Cardiac
Tropomyosin	$\alpha, \beta, \gamma, \delta$	$\alpha, \beta$	$\alpha (\beta)$
No. of genes	7-8	6	5-6

\*There is evidence of several forms of troponin T in chicken fast skeletal muscle (T. Hirabayashi, personal communication; Wilkinson *et al.* 1984).

contraction is triggered in type I fibres when one  $\text{Ca}^{2+}$  is bound per molecule troponin C compared with two in type II fibres.

#### *Genetic control of muscle phenotypes*

The fact that all the myofibrillar proteins exist as a number of isoforms gives flexibility to the contractile and regulatory function of the myofibril and thus accommodates the physiological characteristics of different muscle types. With the exception of actin the type I and type II fibres contain unique sets of myofibrillar proteins that are the products of different genes (Table 3). All skeletal muscle cells have the capacity to synthesize isoforms present in type I and type II fibres, but in normal adult muscle only the set of genes expressing the protein isoforms characteristic of type I or type II fibres are active in a cell. This implies coordinated gene expression of a kind that is little understood. The type of innervation is clearly important for by cross innervation type I fibres can be converted to type II fibres and vice versa (for review, see Perry *et al.* 1984). It is not the innervation *per se* that controls gene expression in adult muscle at least, but the frequency of the impulses that reach the muscle, for interconversion of muscle fibre types can be obtained by subjecting muscles to direct electrical stimulation at the frequencies characteristic of slow and fast nerves. Other factors, such as denervation, disease, hormones and development can bring about changes in the isoform pattern of muscle and hence the phenotype. Whether activity *per se* can do this is somewhat more controversial. It is now well established that the fibre type composition of elite athletes is correlated with the type of physical activity in which they excel, i.e. sprinters have a high proportion of type II fibres, whereas marathon runners have a predominance of type I fibres. The high proportion of a particular fibre type in an individual muscle has been largely ascribed to genetic factors. The majority of studies provide little evidence of interconversion between slow and fast twitch muscle fibres (for review see Schantz *et al.* 1982). Nevertheless, recent evidence shows that a period of protracted training of the

endurance type on muscles that are untrained can produce a decrease of 13% in type II and a corresponding increase in intermediate type I fibres (Schantz & Henriksson, 1983). Thus under certain circumstances, extensive training can produce some changes in gene expression, but the mechanism of this is uncertain. It is possible that training can bring about changes in the firing pattern of the nerves involved, but hormonal influences generated by the training schedule may also play a part.

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