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Review

Task-specific design of skeletal muscle: balancing muscle structural composition

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Abstract

Skeletal muscle fibers are composed of three structural elements, each contributing a unique aspect of muscle function, yet each 'competing' in a sense for space inside the cell. The volume occupied by myofibrils determines the force of contraction, the volume of sarcoplasmic reticulum sets the rate of onset and relaxation of a fiber's contraction and hence contraction frequency, and the volume of mitochondria sets the level of sustained performance. The entirety of functional outcomes in muscle, from sustained isometric to high frequency contractions, and from high power output to high endurance, are all primarily attributable to shifts in the proportions (and relationships) of those three structures. This paper examines and reviews these components of muscle first to identify and summarize structure–function 'rules', and second to examine the balance between sometimes competing demands. In particular, we focus on those muscles in which power, endurance and frequency are all simultaneously high (flight muscles), and examine how muscle has 'solved' problems of space and energy demand. From these results and observations it would appear that for flight to have evolved in small animals, the double packing of inner mitochondrial membranes may be expected in animals under 50–80 g in mass, and asynchronous muscle is structurally essential for flight in small insects with wing beat frequencies above about 100 Hz.

Keywords: Myofibrils; Sarcoplasmic reticulum; Mitochondria; Muscle structure; Asynchronous muscle; Symmorphosis; Rattlesnake; Hummingbird; Insects

1. Introduction

All skeletal muscles actively produce force during contraction, however the functional output or consequence of the force produced can vary widely among and within animals. One of those functions is high-power ballistic shortening, in which all the fibers of a muscle are recruited and shorten, maximizing both the active cross-sectional area of the muscle as well as the contraction velocity, maximizing power (force × velocity) output. Examples of this kind of muscle use includes a frog jumping, or the explosive burst speed necessary to avoid predation, e.g. in fishes [20], or capture of prey in cheetahs [14]. However, even during locomotion, muscles often produce force without actively shortening and thus without producing work. In these muscles, the force produced is essential to store elastic recoil potential energy that is subsequently recovered. This mechanism of muscle force production has been described in a number of animals and seems to be prevalent among the terrestrial vertebrates [4,8,10,19,24,26]. Thus, muscles may regularly act as one component of a biomechanical spring producing force either isometrically (maintaining a constant length), or even eccentrically (lengthening).

In addition to whether force production is accompanied by shortening, muscles also vary in their duration of force production and relaxation. While 'anti-gravity' muscles involved in maintaining posture sustain isomet-

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ric (tetanic) contractions, many skeletal muscles must function at high frequencies without undergoing tetany. For example, muscles involved in flight and sound production in vertebrates exceed 40 Hz in hummingbird flight muscle [27], 90 Hz in rattlesnake tailshaker [22], and 200 Hz in sonic muscle of the blowfish [21]. Among the invertebrates the highest frequency flight muscles approach 500 Hz in the smallest insects [3,6]. Thus postural muscles are characterized by a prolonged rate of onset of force production while this time course is minimized in flight and noise-making muscles.

Superimposed on this diversity of outcomes of work, power and force production among muscles is an equally broad distribution of durations over which various muscles are active. In those muscles active over prolonged periods, fuel and oxygen must be supplied beyond levels contained within the cell such that ATP can be re-synthesized aerobically.

This broad diversity of both contractile and metabolic properties characteristic of different skeletal muscles is not the consequence of any unique structural features of the muscles themselves. While a very small volume of a muscle fiber may be devoted to fuel storage, either in the form of lipids or glycogen (usually combined these are under 3% of the entire muscle cell volume), the remainder of the myocyte is composed of relatively few structural components. It is the distribution or balance of these structures that is the subject of this paper.

The amount of force produced by the fiber is a function of the relative area devoted to the contractile fibers, which are the most abundant element of most muscles, often comprising up to 90% or more of the fiber volume. Additionally, the volume of mitochondria within the cell determines the sustainable ATP production and hence sustainable intensity of muscle activity. Finally, the time required for both the excitation (i.e. time to peak tension) and relaxation of muscle are determined largely by the abundance of sarcoplasmic reticulum/t-tubule system (SR) within the fiber. As the volume density of SR is increased within the fiber, the average diffusion distance between the SR Ca^{2+} source/sink and the thin filaments is reduced. As this diffusion distance decreases so does the time course of Ca^{2+} concentration change required for excitation–contraction coupling and likewise for muscle relaxation. Muscles that contract at high frequencies must have an abundance of SR specifically to minimize the Ca^{2+} diffusion distance and hence diffusion times.

We see, therefore, that the diversity of functional outcomes common to skeletal muscle, from sustained isometric to high frequency contractions, and from maximized power output to maximized endurance, are all accomplished solely by shifting, quantitatively and/or qualitatively, the proportions and relationships of those three structures common to all muscle: myofibrils, mitochondria and SR. There may be very few machines of any kind, that accomplish such a broad range of tasks with what amount to primarily rearrangements of the same few structures. Are there apparent 'rules' of design that can be applied to investigate how these rearrangements result in the suite of functional outcomes we see in skeletal muscle?

The concept of symmorphosis, first defined by Taylor and Weibel 15 years ago, has been an intriguing hypothesis quantitatively linking structure and function. Generally, it has been applied on a systems level, examining the match for example in capacity in the cascade of structures making up the respiratory system, from lungs to mitochondria [25]. When viewed through this perspective, it appears that the respiratory system structures are 'designed' in such a way that at each level, the amount of structure present is well matched to the maximal oxygen flux through the system. However, we can also ask if the concept of symmorphosis is applicable to individual tissues or cellular ultrastructure. Muscle may be particularly well-suited to this approach because so few structures are responsible for such a large range of functional outcomes. In this paper we examine the matching of skeletal muscle structure and function and focus primarily on two questions: How are the functional properties of skeletal muscle linked to the underlying structural foundation and what structural compromises are found in those most 'extreme' muscles, e.g. those with sustained high power and high frequency?

2. Muscle fiber composition

Muscle fibers are comprised of myofibrils, SR and mitochondria in varying proportions. Because these three structures collectively make up essentially 100% of the fiber, every muscle fiber must be identifiable as a point on a single plane of a three dimensional graph depicting these muscle components (Fig. 1). Because of this spatial requirement, any increase in one structure (and its concomitant function), must be to some extent at the expense of another. Space alone would seem to dictate that those muscle fibers with the greatest force output cannot be the same as those with the greatest frequency of use or the highest aerobic capacity.

The basic arrangement of myofibrils, the thick and thin filaments of striated muscle, as well as the size of the sarcomere and the spacing of myosin heads are all essentially invariant across taxa, suggesting an early and conserved evolution. In fact, the structural composition and arrangement of myofibrils is so consistent that any (rare) deviation from this pattern (for example, increased sarcomere length) is argued to be evidence for specific muscle adaptation [9]. The consequence of this remarkable structural consistency is that muscles in general also share the functional attribute of maximum force per myosin head (about 5.3 pN), and conse-

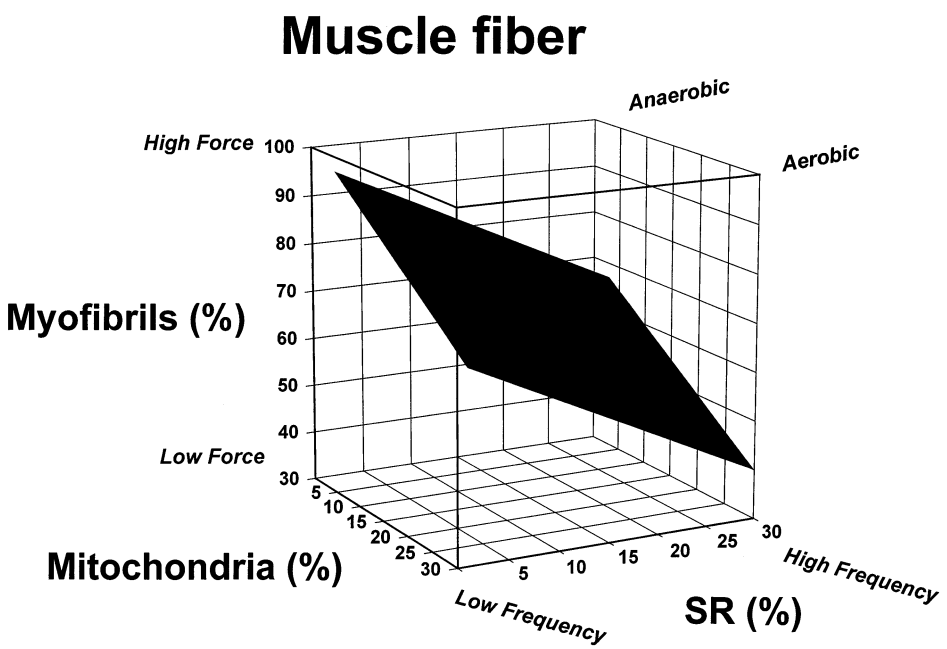

Fig. 1. Skeletal muscle is a composite of three different components, together these must comprise 100% (less a small volume devoted to lipid and glycogen fuel). Each of these structural elements within a muscle fiber is primarily responsible for one aspect of muscle function; myofibrils determine the maximum force (or power if the muscle shortens), SR sets the maximum frequency of contraction, and the mitochondria set the aerobic ATP synthetic rate. In this three-dimensional graph, all muscle cells must exist as a point somewhere on the depicted plane. In a sense, the only way one function can be increased is at the expense of another. In the smallest flying animals, where the demand is high for power, endurance and frequency, muscles have adaptations of stretch stimulation and densely-packed mitochondrial membranes, reducing the volume of the cell devoted to SR and mitochondria respectively. See text for details.

quently maximum (isometric) force per cross-sectional area of muscle (about 300 kN m^{-2}) [1]. Thus, the maximum force is relatively invariant among the vertebrates and is largely a function of the cross-sectional area of myofibrils within any given muscle fiber. In contrast, the kinetics of crossbridge formation do vary among different muscles; those muscles that shorten vary in power output as they vary in shortening velocity V_{max} [20]. Nonetheless, the cross-sectional area of the muscle fiber devoted to myofibrils determines the maximum force that the fiber is capable of producing (Fig. 1).

2.1. Sarcoplasmic reticulum and associated structures

Sarcoplasmic reticulum and associated structures (SR) determine the time required for calcium to diffuse into, as well as to be removed from, the cytoplasm. The time to peak tension as well as the time required for relaxation are both functions of the Ca^{2+} time transient within the fiber [21], which is itself determined primarily by diffusion distance from SR to muscle fiber. Fast contracting fibers, even if used ballistically, must have abundant SR to insure that the entire fiber is activated simultaneously, which maximizes power as force is applied nearly simultaneously. The volume of the cell devoted to SR is lowest in those that are slow at contracting and relaxing, such as postural muscles; and SR is highest, and thus Ca^{2+} diffusion distances lowest, in those fibers that are the fastest at contracting and relaxing. Nowhere is this more evident than in muscles that contract at high frequencies. Hummingbird flight muscle (40 Hz) contains just over 10% SR [27,28], while that of the rattlesnake tailshaker (90 Hz) is 26% SR [22]. The volume of the fiber devoted to SR determines the maximum contraction and relaxation frequency that the fiber is capable of attaining (Fig. 1).

2.2. Mitochondria

Mitochondria determine the magnitude of the sustainable performance of the muscle. There are sufficient high energy phosphate supplies within the muscle in the form of phosphocreatine (PCr) to fuel relatively few contractions; the concentration of PCr is several times that of ATP in muscle cells. Through the creatine kinase reaction, PCr within the muscle cell is an extremely effective buffer, maintaining ATP concentrations constant during muscle use [5]. However, because PCr is rapidly depleted, for longer durations ATP must be re-synthesized by oxidative phosphorylation in the mitochondria. Among the mammals, the volume of mitochondria is an accurate predictor of an animal's total skeletal muscle aerobic capacity, both peak and sustainable rates of oxidative phosphorylation (Fig. 2). On average, mitochondria in mammalian muscle are

capable of consuming a maximum (in vivo) of 5 mlO_2 cm^{-3} min^{-1} [11]. This figure is roughly the equivalent of the production of 1.3 mMol of ATP cm^{-3} min^{-1}, if we assume a P/O_2 of six, not unlikely at the highest rates of oxidative phosphorylation. This 'textbook' value corresponds to a net yield of 36 ATP from the complete catabolism of a glucose molecule, which requires six oxygen molecules. If we further assume that the hydrolysis of 1 mMol of ATP yields 60 J of energy (at physiological concentrations within the cell) [1,16], this rate of ATP synthesis works out to a maximum power yield (energy/time) of about 1.3 W per cubic centimeter of mitochondria. Muscles cannot utilize all of that energy, as there is a maximum efficiency of about 50% considering the conversion of energy liberated from ATP hydrolysis into work [20]. Consequently, from the measurements of mitochondrial volume density and maximum oxygen uptake we predict that the maximum oxidative power output of the muscle is roughly 0.67 W cm^{-3} of mitochondria. This number is similar to that reported by Pennycuick and Rezende [17] who used a method of estimating the 'power density of mitochondria' to calculate the mitochondrial power output of about 0.9 W ml^{-1} of mitochondria for pigeon flight muscle.

Thus, a measurement of mitochondrial volume alone is sufficient to make an estimate of a muscle's maximum aerobic ATP synthetic rate and hence maximum aerobic performance in mammals and likely most birds (but see below). In terms of oxygen uptake, the maximum is about 5 mlO_2 cm^{-3} (of mitochondrial volume) min^{-1} or about 1.3 mMol of ATP $cm^{-3} \cdot min^{-1}$. It is worth noting that the sustainable ATP synthetic rate is about 80% of this maximum [5,12], thus about 4 mlO_2 cm^{-3} min^{-1} or 1 mMol of ATP cm^{-3} min^{-1}.

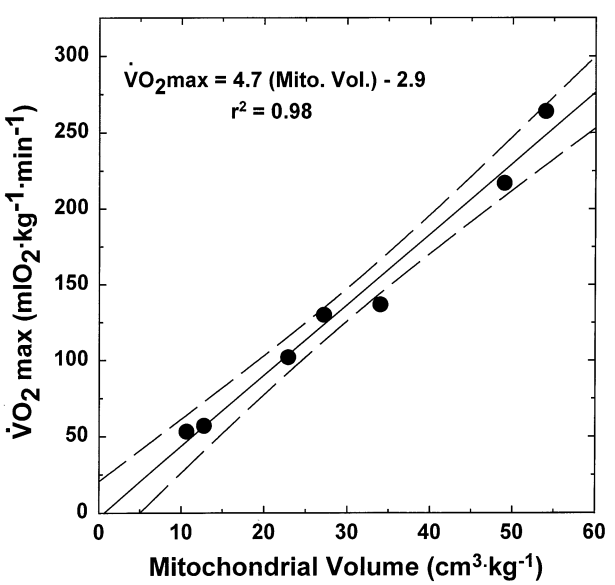

Fig. 2. When mean skeletal muscle mitochondrial volume is plotted as a function of maximum weight-specific oxygen uptake ($\dot{V}_{O_2}max$) among the mammals, the resultant regression has a slope of 4.7 mlO_2 cm^{-3} min^{-1} and an intercept near zero. Hence, at $\dot{V}_{O_2}max$ mammalian mitochondria consume a consistent maximum of 4.7 mlO_2 cm^{-3} min^{-1}. The 95% confidence intervals are shown as dashed lines [20].

3. Structural adaptations in muscles selected for high power, high frequency, high aerobic demand: saving space and energy.

In many flying animals, for example hummingbirds and insects, the sustained, weight-specific power requirements of flight muscles are extreme. These animals have the combined demand of high power outputs required to provide sufficient lift for flight. In fact, for flight to occur, it seems that the minimum volume of myofibrils is 50% of the cell volume [6,7]. However these small flyers operate at the highest wing beat frequencies and their high power requirements are sustained.

3.1. Mitochondria

The mass of the flight muscles in hummingbirds collectively account for roughly 25% of the total body mass. The volume of oxygen used per kilogram body mass per minute, $\dot{V}_{O_2}$ measured as whole animal oxygen uptake during hovering flight, is 850 mlO_2 kg^{-1} min^{-1}. When expressed per unit mass of flight muscles this works out to the flight muscle mass-specific oxygen uptake is 3400 mlO_2 kg^{-1} min^{-1}. As the flight muscles are composed of about 35% mitochondria, this value is the equivalent of a mitochondrial oxygen uptake of 7–10 mlO_2 cm^{-3} min^{-1} [23,27], or double the value consistently obtained for mammalian mitochondria. In other words, if hummingbird mitochondria were identical to those of mammals, the flight muscle would have to be 2/3 mitochondria, not leaving sufficient volume of myofibrils to generate the lift necessary for flight. How have hummingbirds managed such high aerobic power? The packing of mitochondrial inner membranes (cristae) in hummingbirds is double that of mammalian mitochondria [23,27,28], hence these mitochondria have the same oxygen uptake per unit of inner mitochondrial membrane as those of mammals and other vertebrates. When corrected for temperature, evidence suggests that all skeletal muscle mitochondria have the identical rate of O_2 uptake (or ATP synthetic rate) when calculated per unit area of inner mitochondrial membrane. At a muscle temperature of 30°C, this works out to about 30000 O_2 molecules μm^{-2} s^{-1} (Fig. 3) [22]. It remains a mystery how double packing of inner membranes (the site of electron transport) is accomplished without sacrificing the space in the matrix (the site of Krebs cycle enzymes), or for that matter, why this 'double packing' is not the norm for mitochondria in general [22].

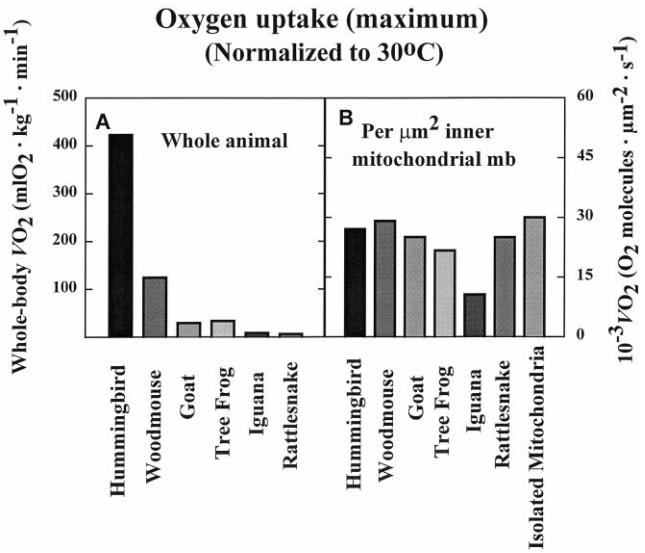

Fig. 3. (A) When maximum oxygen uptake ($\dot{V}_{O_2}$max) is compared across the vertebrates, the resultant values span nearly two orders of magnitude. (B) However, when $\dot{V}_{O_2}$max is plotted as a function of total inner mitochondrial membrane surface area, all these animals have roughly the same oxygen uptake, about 30000 O_2 molecules $\mu m^{-2}\ s^{-1}$ when normalized to 30°C body temperature [22].

Because of the high aerobic demands of flight, we can predict that flying animals may also require 'double-packed' mitochondria in order to provide sufficient power to fly. Among bats, birds and insects, the maximum, and hence non-sustainable, rate of oxygen consumption ($\dot{V}_{O_2}$max in $mlO_2\ kg^{-1}\ s^{-1}$), varies as 3.2 $M_b^{-0.35}$ [14]. In general, the flight muscles of bats, like those of hummingbirds, make up about 25% of the total body weight [26]. In bats, as in birds, the maximum density of mitochondria in the flight muscles is about 35% [15], presumably to allow for sufficient volume of myofibrils necessary to provide lift. Using these equations and what seem to be reasonable assumptions, we calculate that the maximum (whole body) weight-specific rate of oxygen uptake supported by 'standard' mammalian mitochondria at a 35% density is 7.5 $mlO_2\ kg^{-1}\ s^{-1}$, equivalent to 30 $mlO_2 \cdot kg^{-1}\ s^{-1}$ in the flight muscles, which would be reached in a 50 g bat. Thus, we can predict that any bats below this body size should have a space-conserving mechanism such as double packing of mitochondria. Likewise, all flying insects have densely-packed mitochondria as well, functionally allowing more space to be devoted to myofibrils [3].

Temperature also has a big impact on the rate of mitochondrial ATP synthesis. Because mitochondria have an apparent Q_{10} of about 2.2 [2], for every 10°C increase in body temperature there is a 2.2-fold increase in ATP synthetic rate; effectively equivalent to doubling the volume density of mitochondria within the cell. Hence, many insects are unable to fly because they are unable to produce adequate ATP to power flight, without warming up [6]. Thus, the synthetic rate of 30000 O_2 molecules $\mu m^{-2}\ s^{-1}$ shown in Fig. 3 corresponds to about 70000 O_2 molecules $\mu m^{-2}\ s^{-1}$ at a more typical avian or mammalian muscle temperature of 40°C.

3.2. Myofibrils

Because thick and thin filament structure (and hence, force production) is conserved across taxa, there seems to be a minimum myofibrillar density required for specific tasks. As mentioned above, flight muscles are composed of no less than 50% myofibrils, presumably because of the high power requirement of flight. In contrast, noise-making muscles may be composed of a much lower density of myofibrils, about 31% in rattlesnake tailshaker and only 22% in cicadas [13], as the amount of force required is apparently much less than that required for flight. As a consequence, noise-making muscles have less demand for ATP used in crossbridge formation, and more space for ATP producing mitochondria and much more space and energy devoted to the regulatory SR [7].

However, flight muscles in the smallest insects operate at just as high frequencies as noise-making muscles with the requirements of high power output. Perhaps the greatest single space- and energy-saving adaptation in skeletal muscle was the development of asynchronous or fibrillar muscle. It seems that this muscle found in the fastest-contracting insect flight muscles evolved subsequent to the evolution of synchronous striated muscle [18]. Asynchronous muscle does not require the release (and re-uptake) of Ca^{2+} for each contraction, rather it is stimulated to contract when stretched. By utilizing this stretch-stimulation, insects possessing this type of muscle save space and energy twice. The first is a direct savings of space that would otherwise be occupied by SR. For example, synchronous noise-making muscles in cicadas have about 35% SR in order to function at 220 Hz [13]. In contrast, asynchronous muscles can operate at higher frequencies with an order of magnitude reduction in SR. The second savings is a direct saving of energy and an indirect saving of space. The cycling of Ca^{2+} back into the SR is energetically expensive, perhaps requiring more energy than the contractile fibers in some of the fastest synchronous muscles [21]. Just as one ATP is required per crossbridge formed, one ATP is also required for each two Ca^{2+} ions pumped back into the SR [1]. Thus, in these muscles, nearly 100% of the mitochondrial ATP synthesis goes directly into contractile costs.

The final mechanism available to save space and energy, thus allowing the highest possible sustained power outputs, occurs in the fastest frequency muscles, i.e. flight and noise-making muscles. The number of

ATPs required per contraction is reduced to a minimum when the number of crossbridge cycles is likewise minimized. This may be an inevitable consequence of high frequencies as the time required for a single crossbridge to form is roughly equal to the time that the calcium is elevated in these high frequency muscles [1,21]. The result is first that the strain (shortening) is at a theoretical minimum of about 2% [1], and second that the cost per contraction is likewise minimized in these muscles [6].

In summary, the highest frequency flight muscle must be an asynchronous muscle operating at a high temperature, with minimum strain. By incorporating all of these energy- and space-saving features, if the muscle were composed of equal volumes of mitochondria and myofibrils, the predicted maximum frequency at 40°C muscle temperature (assuming dense-packed mitochondrial membranes) is about 600 Hz, similar to the highest frequencies found in the smallest midges [3].

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