Review

Energy-saving mechanisms in muscle: the minimization strategy

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Summary

Many mechanisms reduce the cost of muscle activity. Here, we describe a set of specializations that reduce the cost of contraction in the high-frequency twitches that are used by a wide variety of animals for either sound production or flight. Minimizing the cost of these contractions means that cellular ATP production can meet ATP demand and sustain the high contractile rate. Two classes of specialization are found that minimize the contractile cost. The first class reduces the muscle work required per contraction. Light appendages such as rattles, insect limbs and membranous wings that require little work for movement are used in high-frequency contractions. The second set of specializations involves processes that minimize energy use. High-frequency muscles tend to have a lower cross-bridge content, fewer attached cross-bridges and shorter length changes per contraction. The result is low muscle-specific forces (stress), small length changes (strain) and rapid contraction times that suggest that these muscles push the lower limit of contractile function. The consequence of function at this lower extreme of contraction is to minimize the contractile cost of high-frequency muscles. Thus, specializations that permit rapid contractions at a low rate of ATP use per twitch are the basis of a minimization strategy for energy saving in muscles contracting at high frequency.

Key words: sound production, flight, muscle, energetics.

Introduction

Muscle exercise is the most costly activity for animals. A single twitch can raise ATP use more than 100-fold compared with the flux in resting muscle (Kushmerick, 1983). Many species of sound-producers and insect fliers use very high frequency contractions (50–200 Hz) that dramatically elevate ATP demand (contraction and twitch are used interchangeably to represent a single limb or wing cycle). Meeting the high demand of sustained contractions involves a number of well-known specializations that maximize ATP supply: high mitochondrial volume and density of inner membranes (i.e. cristae) and even an elevated operating temperature of the muscle (Conley and Lindstedt, 1998; Lindstedt et al., 1998). However, just as important to ATP balance are the strategies for reducing contractile demand. Here, we argue for a unique class of specialization that permits high-frequency contractions but has the consequence of reducing ATP demand and minimizing the cost per contraction.

Energy-saving mechanisms

High-frequency contractions are used by a wide variety of animals for sound production or flight. Minimizing the cost of these contractions is necessary for cellular energy production to meet energetic demand and sustain the high-frequency contractions. For example, rattlesnake tailshaker muscle contains 30 % mitochondria by volume that can maximally generate $4 \mu\text{mol ATP g}^{-1}\text{s}^{-1}$ (i.e. $13 \mu\text{mol ATP ml}^{-1}$ mitochondria $\text{s}^{-1} \times 0.3 \text{ ml mitochondria ml}^{-1}$ muscle). This capacity for aerobic ATP flux is far less than the $14 \mu\text{mol ATP g}^{-1}\text{s}^{-1}$ needed for the 70 Hz contraction rate at the $0.2 \mu\text{mol ATP g}^{-1}\text{twitch}^{-1}$ contractile cost typical of mammalian muscle (Blei et al., 1993; Schaeffer et al., 1996). Thus, minimizing muscle ATP use per twitch is necessary if ATP supply is to meet ATP demand and permit the high-frequency contractions typical of sound-producers (Conley and Lindstedt, 1996). Two sets of characteristics are found that minimize the contractile cost. The first set involves minimizing muscle work per contraction. Light appendages such as rattles, insect limbs and membranous wings permit high-frequency contractions because they require little mechanical work for movement. A second set minimizes muscle ATP use (Conley and Lindstedt, 1996). We show here that muscles specializing

in high-frequency contractions have a lower cross-bridge content and appear to cycle fewer cross-bridges per twitch. The result of these characteristics is low muscle-specific forces and rapid contraction times that keep ATP use to a minimum.

Light appendages/low work

Sound-producers and fliers use very light limbs that require little mechanical work and correspondingly low muscle energy expenditure. The rattlesnake tailshaker/rattle system offers a good example of how energy minimization is achieved by reducing mechanical and muscle work. The hollow and thin-walled structure of the rattle of rattlesnakes appears to be ideally designed to minimize rattling work. In addition, several specializations of muscle function ensure that contractile costs are very low for rattling; as a result, the cost of tailshaking is among the lowest per contraction of any vertebrate muscle (Conley and Lindstedt, 1996). These specializations can best be illustrated by analyzing why the cost of rattling does not change with temperature and contraction frequency.

Minimizing work

Tailshaking by the rattlesnake has a constant contractile cost of rattling per twitch despite the fact that the mechanical work of rattling is expected to increase at higher rattling frequencies. Brad Moon and Jo Hopp in my laboratory resolved this paradox by considering the rattle movement during tailshaking as an inverted pendulum (Moon et al., 2002). The work (W) required per swing of the pendulum increases as the frequency (f) of the swings (or rattles) increases at a constant swing arc (ϑ):

$$W \propto f^2\vartheta^2 . \qquad (1)$$

The increased work results because the pendulum must move faster per swing at a high frequency. Thus, an increase in rattling frequency from 23 to 70 Hz would be expected to increase mechanical work by ninefold. Two factors contribute to reducing the increase in work. The first is that the rattle does not move through the same arc at all frequencies. High-speed video recordings of rattling revealed that the displacement of the rattle during each swing (ϑ) declines as frequency increases. A kinematic analysis of the rattle revealed the second factor reducing the increase in work: the rattle is not a stiff pendulum but rather an articulated appendage. Thus, the rattle acts like a beaded string rather than a stiff rod. The smaller rattle displacement at higher frequencies and the loose linking between the rattle segments means that the work required to move the rattle increased approximately fourfold compared with approximately ninefold expected for a stiff rod moving through a fixed arc. Consequently, the increase in mechanical work with frequency is greatly reduced. However, this still leaves the question of how the contractile cost of tailshaking per twitch could remain constant, despite increased frequency (and temperature), when the mechanical cost is expected to increase.

To answer the question of how cost can remain constant despite a rise in mechanical work requires an analysis of how the tailshaker muscle uses energy in the generation of force. Rearrangement of the relationship between work and force over distance in equation 2 shows that, to achieve a greater rattle mechanical work, the muscle must increase peak force production (F):

$$F \propto W/\vartheta . \qquad (2)$$

Moon et al. (2002) found that a peak force increase is achieved without increasing cost by a trade-off of physiological properties. Specifically, they found that peak tailshaker force production increases as the duration of the twitch decreases at higher frequency and temperature. This trade-off was discovered using direct measurement of tailshaker force production *in vivo*, which showed that twitch duration at 30°C (approximately 70 Hz) was one-tenth of that at 10°C (approximately 23 Hz). This same measurement showed an increase in peak force production with no net change in the integral of force over the twitch period. At 10°C, force production was spread over a long twitch time and resulted in a lower peak force. At 30°C, twitch duration dropped, restricting force production to a shorter time and resulting in a higher peak force. Simply stated: the higher peak force production resulted from the same total force generation squeezed into a shorter period. This finding resolves the paradox of constant contractile cost but greater peak force at higher temperature and frequency. Thus, rattling achieves the higher force production needed to accelerate the rattle and meet the work demands at higher temperatures and frequencies by the trade-off of twitch time for peak force. The end result is greater peak force and more rattle work at higher frequency (and higher temperature) without a change in the force/time integral and contractile cost.

Minimizing contractile costs

High-frequency contractions necessitate reduced cost per contraction for two reasons. The first is that a reduced contractile cost accompanies the muscle changes needed to achieve fast contractions. For example, rattlesnake tailshaker muscle can operate at nearly 100 Hz, yet its maximum contraction velocity is not very different from that of frog muscle (Rome et al., 1996). Instead of a faster cross-bridge cycling rate to achieve high contraction rates, the tailshaker muscle has a number of specializations characteristic of super-fast muscle (Rome et al., 1996, 1999), including a large sarcoplasmic reticulum content (Schaeffer et al., 1996) for Ca^{2+} recycling that results in rapid Ca^{2+} removal and fast muscle relaxation. One consequence of these rapid contractile kinetics is fewer cross-bridge cycles and, thereby, lower ATP use per twitch, as shown below.

The second reason that high-frequency contractions necessitate reduced contractile cost is the need to balance ATP supply to demand. Since the cell has a finite volume for structures involved in the ATP balance, any increase in the volume of the structures supplying ATP comes at the expense of the structures using ATP. Three cellular structures are
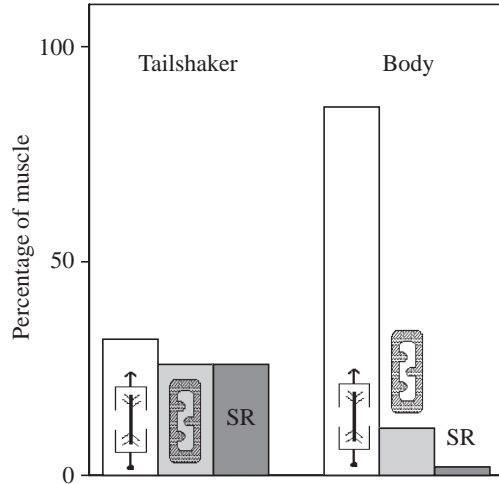
Fig. 1. The percentage of contractile elements (myosin ATPase; open columns), mitochondria (pale shaded columns) and sarcoplasmic reticulum (SR; dark shaded columns) in rattlesnake tailshaker muscle and body muscle. Data from Schaeffer et al. (1996).

involved in the ATP balance and vie for space in the cell: mitochondria (ATP supply), sarcoplasmic reticulum (Ca^{2+}-ATPase) and contractile elements (myosin ATPase). The consequence of maximizing ATP supply to sustain high-frequency contractions is increased mitochondrial volume density and a decreased fraction of the muscle available for ATP use (Conley and Lindstedt, 1998; Lindstedt et al., 1998). Thus, the simple fact of increasing the volume of the cell devoted to ATP supply means less volume available for the structures using ATP.

This trade-off is illustrated for the rattlesnake body and tailshaker muscles in Fig. 1 (Schaeffer et al., 1996). Nearly 85 % of the body muscle is made up of contractile elements (i.e. actin and myosin), with a small volume fraction dedicated to mitochondria and sarcoplasmic reticulum. In contrast, the tailshaker muscle has a large increase in the volume of mitochondria and a greater sarcoplasmic reticulum content for

rapid Ca^{2+} cycling. Since the cell volume is a zero-sum game, the higher proportion of mitochondria and sarcoplasmic reticulum comes at the expense of actin and myosin content, which drops from 85 to 30 % of cell volume. This shift means that only one-third of the muscle cell is actually made up of contractile elements in the tailshaker muscle! This lack of contractile area is directly reflected in a reduction in the ability to generate force by the muscle. For example, frog muscle with 83 % of cell volume as actin and myosin (Mobley and Eisenberg, 1975) generates maximal muscle stress at an isometric tension of $200\,kN\,m^{-2}$ (Bagshaw, 1993), but this falls in tailshaker muscle with 30 % of cell volume as actin and myosin to approximately $63\,kN\,m^{-2}$ (Martin and Bagby, 1973). A similar difference in maximal twitch tension between the body musculature and the sound-producing muscles has been reported in two species of gray tree frog (Marsh, 1999).

The reduction in actin and myosin content in sound-producing muscle is not as great in insect flight muscle, which must generate sufficient lift for flight. Fig. 2 compares the cellular composition of insect muscle from two types of flight muscle and a muscle involved in sound production. One characteristic that distinguishes flight from sound-producing muscle is the higher proportion of actin and myosin (>40 %), presumably to meet the force production needs of flying. Another characteristic that distinguishes the two types of flight muscle is the proportion of sarcoplasmic reticulum. Asynchronous muscle (e.g. bee flight muscle at 220 Hz) relies on a stretch activation mechanism to trigger contraction rather than a direct link between a nerve impulse and a twitch. As a result, the proportion of the cell volume devoted to Ca^{2+} pumping for muscle relaxation drops from more than 30 % in synchronous muscle (Fig. 2A) to a few per cent (Fig. 2B). This fall in sarcoplasmic reticulum content affects ATP balance in two ways: by nearly eliminating Ca^{2+} cycling costs but also by providing space for additional mitochondrial volume, which permits ATP supply to increase. For lower-frequency flight muscle and muscle involved in sound production, an ATP balance can be achieved with a high proportion of sarcoplasmic
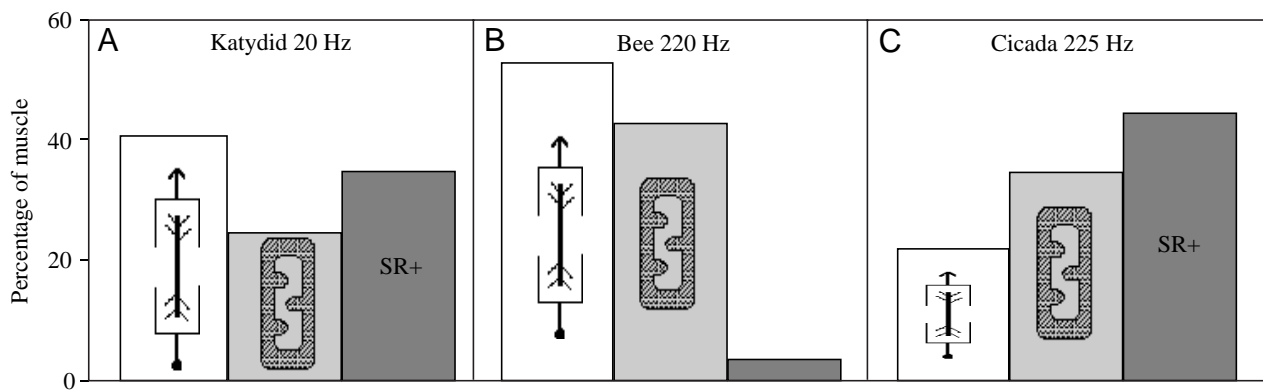

Fig. 2. Proportions of contractile elements (myosin ATPase; open columns), mitochondria (pale shaded columns) and sarcoplasmic reticulum (SR; dark shaded columns) in synchronous muscle (A), asynchronous muscle (B) and singing muscle (C) from insects. The frequencies noted reflect the typical contraction rates for flying or singing for the muscle illustrated. Data from Casey and Ellington (1989), Josephson and Young (1985) and Ready (1986).

reticulum, permitting direct coupling between nerve activation and contraction.

Reduced cross-bridge cycling

The two major changes with higher-frequency contractions – a shorter contraction time and a reduced cross-bridge content – will directly affect contractile ATP use. The major cost of contraction is the cycling of cross-bridges, 60–70 % of the total costs in synchronous muscle (see Rall, 1985) and nearly all the costs in asynchronous muscle. A reduced content of contractile elements will mean fewer cross-bridges to cycle and less use of ATP with each twitch. There is evidence for two additional factors that minimize cross-bridge cycling and the associated cost in high-frequency muscle: fewer attached cross-bridges per twitch and small length changes resulting from the shorter contraction time.

Fewer attached cross-bridges

A small proportion of attached cross-bridges with each twitch has been reported recently for toadfish muscle generating its characteristic 'whistle' (at 200 Hz). Only 10 % of the available cross-bridges attach with each twitch compared with nearly 70 % in the trunk muscles of this fish and typical of locomotory muscles in general (Rome et al., 1999). The impact of the low proportion of attached cross-bridges and low contractile content is a very low maximal force per cross-sectional area (see below).

Low strain

A final factor related to cross-bridge cycling is the muscle length change. The total length change of the muscle with each twitch is termed the strain. Larger strains are accomplished by more cross-bridge cycles and, presumably, higher costs with each twitch. The few muscles studied from flying species show a clear relationship between strain and frequency. Birds flying at a flapping frequency of few hertz have strains as high as 32 % of muscle length (Biewener et al., 1998), while strain drops to a few per cent (as low as 2 %) in insect flight muscle (Gilmour and Ellington, 1993). Fig. 3 shows that, as frequency increases and the period of each contraction decreases, strain falls and appears to reach a minimal value (i.e. 2 %) above a sustained contraction frequency of 100 Hz. This figure clearly demonstrates that muscle strain is not constant, as has been assumed in simple models of muscle function (see Pennycuick and Rezende, 1984).

Does the 2 % strain represent a true minimal value? Such a minimum would be defined by the cycle of a single cross-bridge. Since a cross-bridge cycle covers approximately 20 nm in intact frog muscle (Bagshaw, 1993) and sarcomere length is 2.3 µm in tailshaker muscle (K.E.C. and S.L.L., unpublished observation), then a 1 % length change per half-sarcomere and 2 % overall per twitch defines a single cross-bridge cycle in intact muscle (shorter cross-bridge length changes are reported for isolated myosin in *in vitro* motility assays unconstrained by the three-dimensional structure of the intact muscle; Molloy et al., 1995). The similarity between the minimal measured strain and the distance for a cross-bridge cycle suggests that high-frequency contractions may be at the limit of contractile function, with each twitch representing a single cycle of the cross-bridges (see below).
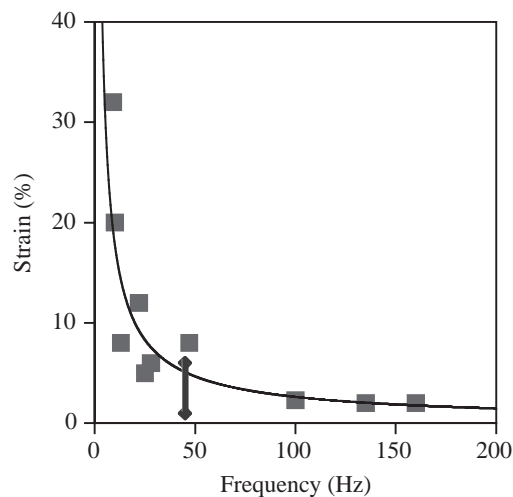

Fig. 3. Percentage of the muscle's length change in each twitch (i.e. strain) in flying and singing muscle as a function of contraction frequency. The vertical line represents the range of strain values found for tailshaker muscle. Data are from Biewener et al. (1998), Ellington (1991), Gilmour and Ellington (1993), Girgenrath and Marsh (1999), Josephson and Stevenson (1991) and Moon et al. (2002).
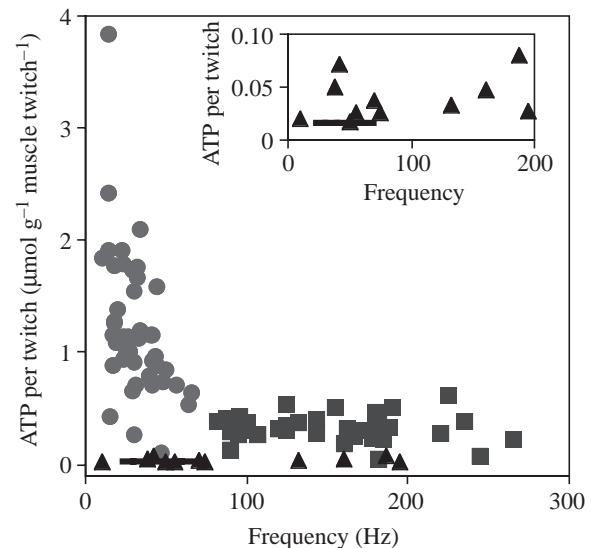

Fig. 4. Cost per contraction in fliers and sound-producers as a function of contraction frequency. The circles are for fliers with synchronous muscle, the squares are for fliers with asynchronous muscle and the triangles are for singing species. All data are for insects, and the line is the range of frequency used by the tailshaker muscle of the rattlesnake (reprinted with permission from *Nature* **383**, 71. © 1996 Macmillan Magazines Ltd). The inset shows the data for singing species and the rattlesnake (horizontal bar).

Minimizing contractile costs

Fig. 4 shows that the consequence of the minimization of contractile function is a decrease in ATP cost per twitch as frequency increases in insect flight and sound-producing muscles. The highest cost per twitch occurs in synchronous fliers that contract at low frequency, but cost rapidly drops off with frequency to reach a plateau above 80 Hz in the asynchronous fliers. Above 100 Hz, there is no change in cost with frequency, but there is a striking difference in the cost per contraction for asynchronous fliers (approximately $0.3\,\mu\mathrm{mol\,g^{-1}\,twitch^{-1}}$) compared with sound producers ($<0.05\,\mu\mathrm{mol\,g^{-1}\,twitch^{-1}}$). This minimal cost per contraction suggests that the sound-producing muscles may represent muscles that have adapted to function at the contractile limit. Let us use the anatomical and functional properties of these muscles to evaluate the basis of these costs and to test whether this minimal cost represents the functional limit of contraction in muscle.

Muscle at the contractile limit

The properties of sound-producing muscles are indicative of greatly reduced contractile function: minimal cross-bridge content, a low fraction of attached cross-bridge and small length changes during a twitch. Our hypothesis is that these contractions represent the contractile limit of muscle function; specifically, a single cross-bridge cycle per twitch. The low strains of 2 % in high-frequency muscle are consistent with the length change of a single cross-bridge cycle per twitch. A simple calculation allows us a test of this hypothesis.

Model of muscle contractile energetics

Power output per unit volume of muscle (P^*) can be predicted from a simple model based on muscle stress (σ), strain (ε) and twitch frequency (f) modified from Ellington (1991) and Pennycuick and Rezende (1984):

$$P^* = \sigma \varepsilon f. \qquad (3)$$

This model can be expressed in terms of energy input per twitch (ATP/twitch) based on the factors responsible for muscle stress and strain. Two factors responsible for muscle stress are the cross-bridge content ([CB]) and the fraction of attached cross-bridges (f_{CB}). For muscle strain, the underlying factor is the number of cross-bridge cycles (N_{CB}) in each twitch. Finally, the contractile cost relates to the number of cross-bridges cycling in each twitch assuming 1 ATP per cross-bridge cycle, (ATP/CB)=1:

$$\mathrm{ATP/twitch} = [\mathrm{CB}] f_{CB}\, N_{CB}(\mathrm{ATP/CB}). \qquad (4)$$

This equation allows us to evaluate contractile cost on the basis of the number and function of the cross-bridges in a muscle. The asynchronous muscle of flying bees is a good place to start since we can ignore Ca^{2+} cycling costs because the stretch activation mechanism requires little Ca^{2+} cycling. In addition, information on the content and cycling of cross-bridges is available for flying bees (Casey and Ellington, 1989) using frog muscle as a standard (Bagshaw, 1993).

Calculating contractile cost from muscle properties

The first property needed for this calculation is [CB] in bee muscle, which can be calculated by adjusting the [CB] of frog muscle ($240\,\mu\mathrm{mol\,l^{-1}}$ myosin heads) for the difference in actin and myosin content of these muscles. Correcting for the lower actin and myosin content in bee (57 %) compared with frog (83 %) muscle reduces [CB] to $160\,\mu\mathrm{mol\,l^{-1}}$ for the bee muscle. The second property is the fraction of cross-bridges that attach in a twitch (f_{CB}). The conventional estimate is that 70 % of the cross-bridges are attached in an isometric contraction (f_{CB}=0.7) (Bagshaw, 1993; Rome et al., 1999). This assumption of an isometric contraction may be correct given the low strain (2 %) reported for the flight muscle of these bees (Gilmour and Ellington, 1993) and suggests that the cross-bridges undergo a single cycle per twitch (N_{CB}=1). Using these values in equation 4 yields a contractile cost of $116\,\mu\mathrm{mol\,l^{-1}\,ATP\,twitch^{-1}}$ (chemical concentration is expressed per total muscle water with the assumption that $1\,\mathrm{ml}{=}1\,\mathrm{g}$) or $0.116\,\mu\mathrm{mol\,ATP\,g^{-1}\,twitch^{-1}}$. This value is remarkably close to the mean measured value of $0.12\,\mu\mathrm{mol\,g^{-1}\,twitch^{-1}}$ reported for these bees (assuming ATP:O_2=6). This agreement between a simple model of ATP use by the flight muscle and measured energy use supports our assumptions of cross-bridge attachment (70 % of cross-bridges attach per twitch) and cross-bridge cycling (one cross-bridge cycle per twitch). The implication of one cross-bridge cycle per twitch is that fliers have pushed cross-bridge cycling to one limit of contractile function. Have sound-producers approached another limit by reducing the proportion of attached cross-bridges in each twitch from 70 % to only 10 % (Rome et al., 1999)?

Sound production and the limits of contractile function

The lowest reported contractile cost is for tailshaking by the rattlesnake (Conley and Lindstedt, 1996). We can evaluate the basis of this minimal contractile cost using the anatomical and energetic information available for the tailshaker muscle (Fig. 1) (Schaeffer et al., 1996). The 30 % actin and myosin content in tailshaker muscle translates to $85\,\mu\mathrm{mol\,l^{-1}}$ cross-bridges. If only 10 % of the cross-bridges attach (f_{CB}=0.1) and go through one cycle (N_{CB}=1) with each twitch, then $8.5\,\mu\mathrm{mol}$ cross-bridge $\mathrm{l^{-1}}$ cycle with each twitch for a cost of $0.0085\,\mu\mathrm{mol\,ATP\,g^{-1}\,twitch^{-1}}$. For synchronous muscle, we also need to take into account that $2\,Ca^{2+}$ are required to activate each cross-bridge. The minimum cost of recycling Ca^{2+} is given by the stoichiometry of the sarcoplasmic reticulum Ca^{2+} ATPase: 1 ATP per $2\,Ca^{2+}$. Thus, each cross-bridge cycle requires 1 ATP for the myosin head and 1 ATP to recycle Ca^{2+}. This equal partitioning of costs between Ca^{2+} and cross-bridge cycling (50 %/50 %) is close to the 35–45 % for 'activation costs' (and 55–65 % for cross-bridge cycling) recently reported for toadfish muscle (Rome and Klimov, 2000). Thus, Ca^{2+} cycling requires an additional $0.0085\,\mu\mathrm{mol\,ATP\,g^{-1}\,twitch^{-1}}$ for a total cost of $0.017\,\mu\mathrm{mol\,ATP\,g^{-1}\,twitch^{-1}}$, which is, again, remarkably close to the measured contractile cost per twitch of $15\,\mu\mathrm{mol\,ATP\,twitch^{-1}}$ or $0.015\,\mu\mathrm{mol\,ATP\,g^{-1}\,twitch^{-1}}$ (Conley

and Lindstedt, 1996). These calculations indicate that the minimal contractile cost during rattling reflects the tailshaker muscle operating at the limits of contractile function: one cross-bridge cycle per twitch and attachment of only 10 % of available cross-bridges. Thus, the tailshaker muscle and sound-producers in general have minimized contractile cost by pushing muscle to the lower limits of contractile function.

Contractile cost versus *muscle properties*

This simple calculation approach allows us to evaluate the relative importance of the factors underlying muscle stress and strain in determining contractile cost. We can use equation 4 to estimate the maximum contractile cost for comparison with the minimal cost in the sound-producers. The upper end of [CB] ($240\,\mu\mathrm{mol\,l^{-1}}$) and cross-bridge attachment ($f_{CB}=0.7$) are represented by frog muscle. The highest strain shown in Fig. 3 is 32 %, which results in $N_{CB}=16$ ($N_{CB}=1$ for 2 % strain). The resulting cross-bridge cost of $2688\,\mu\mathrm{mol\,l^{-1}}$ or $2.6\,\mu\mathrm{mol\,ATP\,g^{-1}\,twitch^{-1}}$ plus an additional 30 % for Ca^{2+} cycling (typical 'activation cost' in vertebrate muscle; Rall, 1985) yields $3.5\,\mu\mathrm{mol\,ATP\,g^{-1}\,twitch^{-1}}$. This upper limit of costs is very close to the highest value shown in Fig. 4 (i.e. $3.8\,\mu\mathrm{mol\,ATP\,g^{-1}\,twitch^{-1}}$). Thus, the range of anatomical and functional properties found among muscles results in the full range of measured contractile costs.

We can now use equation 4 to estimate the relative importance of each muscle property in determining cost. Surprisingly, the smallest relative change in cost comes from the range in [CB], which varies threefold between frog ($240\,\mu\mathrm{mol\,l^{-1}}$) and sound-producers ($85\,\mu\mathrm{mol\,l^{-1}}$). A larger contribution comes from cross-bridge attachment, which changes sevenfold from the minimal 10 % cross-bridge attachment in sound-producing muscle ($f_{CB}=0.1$) to the value of 70 % thought to be typical of most vertebrate and insect flight muscle ($f_{CB}=0.7$). Finally, the largest contribution comes from muscle strain (or the number of cross-bridge cycles), which varies 16-fold from the minimal value in sound-producers and fliers (2 %) to the highest value in a flying bird (32 %; Biewener et al., 1998). The contribution of muscle strain to cost is apparent in the similarity in the shape of the data plots in Figs 3 and 4, in which the large decline in strain with frequency is directly reflected in a sharp drop in contractile cost. Similarly, the role of cross-bridge attachment is reflected in the difference in cost above a frequency of 100 Hz in Fig. 4: the nearly 10-fold difference in cost between asynchronous fliers ($f_{CB}=0.7$) and sound-producers ($f_{CB}=0.1$) is close to the sevenfold difference in cross-bridge attachment.

These results demonstrate a remarkable correspondence between the measured contractile costs of muscle and those predicted on the basis of a simple model of cross-bridge content and function. Of course, each assumption of this simple model can be challenged, such as the proportion of cross-bridges attached, a single cross-bridge cycle per twitch, etc. However, what is remarkable is how well this simple model predicts the range of contractile costs, even with these assumptions. This predictive power illustrates two points. First,

it appears that the model quantitatively accounts for the major ATP-using processes in active muscle. Thus, the contribution of energy-recovery mechanisms, such as elastic storage, is probably close to the estimate (approximately 10 %; Dickinson and Lighton, 1995) for insect flight muscle contracting at high frequency (>100 Hz). Second, the minimal contractile cost measured for sound-producing muscle reflects muscle working close to the functional limit of the cross-bridges. Thus, the constraints imposed on muscle to achieve high frequency – rapid contractile kinetics and high sustained ATP demand – result in a reduction in cross-bridge content and function that minimizes ATP use per contraction. The result is a reduction in muscle function to the lower limits of contraction, with the consequence of achieving a minimal cost per contraction in sound-producers such as the rattlesnake.

Concluding remarks

Here, we show that high-frequency sound-producers and fliers have a unique strategy for saving energy: minimizing contractile cost. We argue that the very nature of high-frequency contractions constrains the use of energy. The large cell volume needed for ATP supply reduces the volume fraction available for ATP demand. Further, the nature of high-frequency contractions requires short, rapid twitches that reduce cross-bridge cycling. The result is low muscle-specific forces (stress), small length changes (strain) and rapid contraction times, which suggest that these muscles are at the lower limit of contractile function. The end result is a minimal demand for ATP per twitch that keeps total contractile costs down and permits the high sustained contractile frequency required for flight and sound production by small animals. Thus, it does not appear that high-frequency muscles have evolved to reduce costs *per se*. Instead, it appears that the muscle specializations needed to achieve high-frequency contractions in sound-producers and insect fliers approach the limits of contractile function, with the consequence of minimizing the cost of contraction.

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