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### **A9–MECHANICS AND ENERGETICS OF MUSCULAR FUNCTION: A TRIBUTE TO ROGER WOLEDGE**

Organised by A.M. Wilson and N.A. Curtin

#### **A9.1 Economy increases during isometric contraction**

N.A. Curtin, T. West, M. Ferenczi, M. Irving, H. He, Y-B. Sun & R.C. Woledge.

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Does the energetic cost (ATP use) change during an isometric contraction? To answer this question we measured ATP use by the actomyosin, which produces force, in permeabilised (membranes removed) and intact fibres from dogfish white muscle. Permeabilized fibres contain no functional ion pumps and thus all its ATP use is due to actomyosin. In permeabilized fibres ATP turnover was measured with MDCC-PBP, a fluorescent Pi binding protein which gave good time resolution at the start of contraction, and with a linked enzyme assay which measured ATP use in the steady state. In intact fibres, heat and work output was measured, giving continuous records of energy cost during the entire contraction. The results agreed in showing that the rate of ATP turnover by actomyosin (1) was greatest at the very start of contraction and (2) decreased substantially to about 20% of its initial value in a few seconds, even though isometric force was constant (West et al, in press). Thus the economy, force per ATP, increased during contraction. This effect is likely to be relevant to locomotion because an increase in the economy of force production by actomyosin probably also occurs continuously during intermittent stimulation with movement which is the usual pattern during walking, running, hopping and flying. Reference: West et al. (in press) J. Physiol.

#### **A9.2 Why are slow-twitch muscles more efficient than fast-twitch muscles?**

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Fast-twitch muscles are less efficient than slow-twitch muscles; that is, less of the chemical free energy pro-

duced by ATP hydrolysis is converted into mechanical work by the myosin cross-bridges in fast muscles. The purpose of the analysis to be presented is to determine the basis of this difference in efficiency.

The starting point of the analysis is the observation that, when a muscle shortens, the net amount of thin filament movement produced per ATP split along that filament is  $< 1$  nm. This movement is the ratio of the movement produced by each cross-bridge per ATP-splitting cycle (or cross-bridge step size) to the number of cross-bridges attached along the thin filament at any instant. If the numbers of cross-bridges attached during shortening in mouse fast- and slow-twitch muscles are predicted from the mechanical and energetic properties of the muscles and a 2-state cross-bridge model, it is found that when operating at maximal efficiency (shortening against a load of  $\sim 0.4P_0$ ), cross-bridge step size is  $\sim 10$  nm in both muscle types. The difference in peak muscle efficiency is due to there being more cross-bridges attached simultaneously in the fast-twitch muscle. The cross-bridge kinetics that allow more cross-bridges to be attached at a given load result in a higher shortening velocity at that load, thus greater power output. Therefore, this scheme can explain the inverse relationship between power output and efficiency.

#### **A9.3 Energetics, mechanics and isometric economy of the anterior compartment muscle in human legs**

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Dynamic measurements of isometric force, fiber pennation, muscle volume and chemical changes during and after contraction were made to quantify economy of contractions in intact human anterior compartment leg muscles. Twitch mechanical responses by peroneal nerve stimulation at a rate of 3 per sec and concentrations of PCr, ATP, Pi and ADP and pH were measured by  $^{31}\text{P}$  NMR spectroscopy before, during and after the 90 sec

interval of stimulation. The decrease in PCr during stimulation was quantitatively explained by three processes, each separately measured: a PCr breakdown rate proportional to the developed integral of fiber force times time, ATP resynthesis during the stimulation by glycolysis, and in the case of aerobic experiments, by ATP resynthesis by oxidative phosphorylation during the stimulation. There was a larger range (up to three fold) in the economy among the subjects. These results show that differences in isometric economy can be detected in the anterior compartment muscles among a small number of normal human subjects who were recreationally active but without specific training. The range of economy measured (ATPase in units of mM ATP per sec divided by stress in units of kN/m<sup>2</sup> of muscle fiber cross section area) ranged from 0.012 to 0.026. These values agree with measurements in single skinned human fibers maximally activated by Ca<sup>2+</sup> when corrected for differences in temperature. These results provide evidence that standard biochemical and physiological tools can provide quantitative information on muscle energetics at a cellular level from macroscopic measurements in intact human muscle. (Acknowledge the many contributions from current and former members of my laboratory, and support from NIH Grant AR41928 and NASA NSBRI IHF00202)

#### **A9.4 New energetics approaches to elucidating mechanisms of contraction and relaxation**

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Vertebrate muscle performs mechanical work over a wide range of frequencies (<1 Hz to >200 Hz). We have demonstrated that specific molecular modifications must be made to permit operation at different frequencies. The kinetics of the cross-bridges and the kinetics of Ca<sup>2+</sup> pumping are major determinants of twitch speed. Because cross-bridge force generation and Ca<sup>2+</sup> cycling involve rapid utilization of ATP, measurement of ATP utilization during contraction can serve as an important window on these mechanisms. Using potent SR-Ca<sup>2+</sup> pump blockers (TBQ, CPA), has permitted direct measurement of cross-bridge ATPase which, in turn, has permitted the determination of cross-bridge attachment and detachment rate constants (e.g., Rome *et al.* 1999 PNAS).

Recently, we have shown that BTS serves as an analogous cross-bridge blocker (Young *et al.* 2003 AJP). Hence the ATP utilization of SR-Ca<sup>2+</sup> pumps can be directly measured in skinned fibers from which one can

determine the total Ca<sup>2+</sup> pumping rate as well as the pump turnover rate.

Utilizing BTS in intact fibers has opened up additional opportunities to study Ca<sup>2+</sup> release and reuptake. Although Ca<sup>2+</sup> transients are very useful measurements of free [Ca<sup>2+</sup>], they do not measure the amount of Ca<sup>2+</sup> released. Using a combination of high energy phosphate and recovery metabolism measurements, we are able to determine how much Ca<sup>2+</sup> is released per stimulus as well as the fate of the released Ca<sup>2+</sup>. This information, coupled with the muscle stimulation pattern in calling toadfish, for instance, permits unprecedented insights into muscle Ca<sup>2+</sup> usage and budgeting during normal motor behavior.

#### **A9.5 Considerations on mechanical work production in skeletal muscle**

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Tendons, aponeuroses, and fibres are often considered mechanically in series, therefore it is assumed that forces in these structures are the same, or at least, that they are in constant proportion. These arguments have led to force calculations (e.g., 1, 2), and work determination (3) that are mechanically incorrect, and have led to positive work creation in passive structures, thereby violating basic laws of thermodynamics. Here, we demonstrate that tendons, aponeuroses and fibres are not arranged in series. In fact, the forces in tendons and aponeuroses depend critically on the elastic and shear modulus of the incompressible muscle, and they cannot be related to each other in an intuitive way. Furthermore, we present experimental results emphasizing the viscoelastic behaviour of tendon and aponeurosis and demonstrate, in the cat medial gastrocnemius, that the proximal aponeurosis shortens upon force production and elongates during force relaxation. Therefore, when related to the tendon force, it appears as if the aponeurosis produces mechanical work. However, this is a misinterpretation of the mechanics of contraction which has led, in some instances, to overestimations of the work produced by passive elements.

References:

1. Ettema JC *et al.* 1990, Multiple Muscle Systems, Springer Verlag, p. 57–68
2. Gottlieb GL, 1996, Exercise and Sport Sciences Reviews, Williams and Wilkins 24:1–34
3. Roberts TJ *et al.* 1997, Science 275: 1113–1115

#### **A9.6 Scaling of muscle mechanical power output**

G.N. Askew, University of Leeds, UK

The traditional view of muscle performance is that maximum muscle mass specific power decreases with

increasing body mass. This is based on the assumption that stress, strain and therefore work are constant across species. Limb cycle frequency ( $n$ ) decreases with increasing body mass ( $M_b$ ), typically  $\propto M_b^{-1/3}$ , and power should scale as  $\propto M_b^{-1/3}$  or as  $n^1$ .

The mechanical power output of the flight muscles of several species in the pheasant family (Phasianidae) was recently determined in birds performing escape take-off flights. Grey partridges (*Perdix perdix*), red legged partridges (*Alectoris rufa*), ring necked pheasants (*Phasianus colchicus*) and peafowl (*Pavo cristatus*) were filmed and the power output of their flight muscles estimated from the power required to move the centre of mass and the additional aerodynamic components. These data were combined with data for blue-breasted quail (*Coturnix chinensis*; Askew *et al.*, 2001). The power output was constant across a range of body masses that covers two orders of magnitude (0.04–4.5 kg). A decrease in the wingbeat frequency with increasing body mass, resulted in an increase in the mass specific work generated per wingstroke, scaling as  $M_b^{-1/3}$ . This is not consistent with the earlier predictions. The validity of the assumptions upon which these hypotheses were based will be discussed, with evidence drawn from other muscle driven systems.

Askew, G.N., Marsh, R.L. and Ellington, C.P. (2001). *J. Exp. Biol.* 204, 3601–3619.

### **A9.7 Passively augmented performance in terrestrial, aquatic and aerial locomotion in humans**

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Musculo-skeletal systems and body design in general evolved to effectively move and travel in specific environments. Humans have always aspired to reach higher movement power and to locomote safely and fast even through unfamiliar media (air, water, snow, ice). Through ingenuity starting a couple of millennia ago, they invented a variety of passive tools that helped to compensate for the limitations in body design. This contribution is about many of those tools (e.g. halteres used by athletes in ancient Greece, bows, fins, skates, bicycles, human powered submarines and airplanes), whose common characteristic is to rely on muscular force only. The energy cascade from metabolic fuel to final movement is described, with particular emphasis to the steps where some energy saving and/or power enhancement is viable. Swimming will be used to illustrate the efficiency breakdown in complex locomotion, and the advantage of using fins. A novel graphical representation of world records in many different terrestrial and aquatic locomotion, with a suggested method to esti-

mate their metabolic cost (energy per unit distance), helps to appreciate the success of the involved tools.

References: Minetti A.E. Passive tools for enhancing muscle-driven motion and locomotion. *J. Exp. Biol.* (in press 2004).

### **A9.8 Comparative energetics and mechanics of bird flight**

Matthew W. Bundle

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Empirical investigations of the energetic cost of flight have revealed two qualitative types of power curves. For some species of birds the energy required to fly across a range of speeds is strongly speed dependant, and is typically referred to as a U-shaped power curve. Although in the minority, these results agree well with classic aerodynamic theory. However, the majority of species flown during metabolic studies have generated power curves that are largely independent of speed, commonly referred to as flat power curves. These latter power curves are generally attributed to measurement artifact, due to the inescapable reality of overcoming the higher drags that must be present at very low and very fast flight speeds.

We asked whether birds have evolved two strategies to fly across a range of speeds, and measured the metabolic cost of flight across the respective speed ranges of four avian species (Magpies, Budgerigars, Cockatiels, and American Kestrels). Under identical experimental methodology and at the speeds for which these measurements were possible, we observed both flat and U-shaped power curves. To test the hypothesis that in-flight wing and tail surface area changes are responsible for the observed power curve differences, we also measured 3D flight kinematics, and *in-vivo* muscle function and force generation during flight. These new data will provide meaningful insight to the link between the energetics and biomechanics of bird flight.

### **A9.9 Measuring the dynamics of muscle function in vivo: comparisons of design and function across locomotor modes**

A. Biewener (Harvard University)

The physical requirements for movement when running compared with flying or swimming are quite distinct. Whereas limb muscles may generally be designed to minimize work output when they contract during steady level locomotion on land, they must perform useful mechanical work to move animals up and down hills and to propel them through the air or water. Although

the mass-specific work of muscles should be independent of architecture, muscle-tendon designs that favor more economical force generation and facilitate elastic energy recovery might constrain the ability to modulate changes in work and control limb position. By obtaining *in vivo* recordings of muscle length change, force and activation, we can begin to address how the architecture of muscles and their tendons influences their roles under the dynamic conditions of varying kinds of locomotor movement, and whether tradeoffs in design exist. Studies of muscles that power flight in birds compared with limb muscles that support the running gaits of terrestrial locomotion suggest that these muscles all likely benefit from being activated while being stretched or held nearly isometric. This allows the muscles to develop force rapidly and to high levels, whether or not they subsequently shorten to perform work. Although reducing energy costs during steady locomotion is important, the capacity for modulating work output to change speed, to maneuver, and to accommodate changes in substrate conditions is likely equally important to the roles and designs of muscles and their tendons.

#### **A9.10 Energy saving mechanisms in the locomotion of large animals**

A.M. Wilson (The Royal Veterinary College and University College London)

The mass specific cost of transport (amount of energy used to transport 1kg of body mass one metre) declines with increasing animal size. This talk will address the mechanisms used to minimise the cost of transport in horses.

The energetic work of locomotion is often conceptually subdivided into the work of moving the centre of mass and the work of moving the limbs relative to the centre of mass. Large animals typically have muscle tendon units with short muscle fibres and long tendons. Much of the length change therefore occurs through the energetically efficient elastic deformation of the series elastic component. This helps to minimise the energetic cost of moving the centre of mass. How the animal uses a predominantly passive limb to achieve locomotion under varying conditions is of interest.

It is also advantageous for an animal to maximise duty factor. This minimises limb force and hence the volume

of tissue required to resist the ground reaction force. Rapid limb protraction is also aided by elastic mechanisms. The motion of the trunk is used to load an elastic biceps muscle which is then used to initiate limb protraction.

The sequence of foot falls used may also help to minimise the work of locomotion either by enabling more effective limb placements and stance phases or by reducing the energetic cost of external work. We are investigating this by comparing the mechanics of locomotion in horses using different gaits at the same speed.

#### **A9.11 Muscle energetics: what have we learned since A.V.Hill?**

Roger C Woledge

By 1939 Hill had established the amount and time course of energy production by muscle both during contractions of various durations in the much longer recovery period that follows. He had also identified the important influence of the speed of shortening on the rate of energy output. He was able to show that the likely origin of the heat in the recovery period, which is at least half of the total energy output, was oxidative. However he could not identify the processes producing the energy output during contraction itself, since the chemical nature of the processes driving contraction, that is splitting of ATP and its resynthesis by the creatine kinase reaction were only discovered in the 1940s and 50s. The challenge was then to show whether these reactions could account for all the energy produced in muscle contraction. Energy balance studies showed that they did not; therefore other exothermic processes must also be occurring. These might be: (1) the reactions associated with the activation of the contractile machinery by calcium, in particular the binding of calcium to parvalbumin (2) partial reaction cycles in the actomyosin system. Recent work has compared the production of Pi by skinned muscle fibres with the time course of energy release by actomyosin in intact fibres. With reasonable assumptions about the heats of reaction of the steps in the actomyosin cycle a quantitative kinetic model can now be suggested for the origin of the energy liberated by the actomyosin system during contractions of live muscle fibres.