

RESEARCH ARTICLE

The energetic benefits of tendon springs in running: is the reduction of muscle work important?

 Natalie C. Holt^{1,*}, Thomas J. Roberts² and Graham N. Askew^{1,‡}
ABSTRACT

The distal muscle-tendon units of cursorial species are commonly composed of short muscle fibres and long, compliant tendons. It is assumed that the ability of these tendons to store and return mechanical energy over the course of a stride, thus avoiding the cyclic absorption and regeneration of mechanical energy by active muscle, offers some metabolic energy savings during running. However, this assumption has not been tested directly. We used muscle ergometry and myothermic measurements to determine the cost of force production in muscles acting isometrically, as they could if mechanical energy was stored and returned by tendon, and undergoing active stretch–shorten cycles, as they would if mechanical energy was absorbed and regenerated by muscle. We found no detectable difference in the cost of force production in isometric cycles compared with stretch–shorten cycles. This result suggests that replacing muscle stretch–shorten work with tendon elastic energy storage and recovery does not reduce the cost of force production. This calls into question the assumption that reduction of muscle work drove the evolution of long distal tendons. We propose that the energetic benefits of tendons are derived primarily from their effect on muscle and limb architecture rather than their ability to reduce the cyclic work of muscle.

KEY WORDS: Economy, Elastic, Force, Metabolic, Myothermic

INTRODUCTION

In animal locomotion, natural selection is expected to favour musculoskeletal adaptations that minimize metabolic energy expenditure, whilst meeting the mechanical demands of movement. Muscle-tendon units composed of short muscle fibres and long compliant tendons, found in the distal limbs of many cursorial species, are commonly thought to provide such an adaptation for running (Alexander and Bennet-Clark, 1977; Alexander et al., 1982; Dimery et al., 1986; Taylor and Heglund, 1982; Roberts et al., 1997). Tendons act as springs during running (Alexander et al., 1982; Dimery et al., 1986), storing and recovering mechanical energy with each step to accommodate the cyclical fluctuations in the mechanical energy of the centre of mass in this ‘bouncing’ gait (Cavagna et al., 1964). As passive elastic structures, tendons do not consume metabolic energy in their role as running springs. By contrast, muscles consume significant metabolic energy to generate mechanical energy. Thus it is intuitive, and widely accepted, that runners save metabolic energy by bouncing along on springy

tendons (Alexander et al., 1982; Taylor and Heglund, 1982; Roberts et al., 1997; Alexander, 2002).

The idea that tendons save energy by reducing cyclic muscle work during locomotion is supported by empirical studies showing that muscle work is metabolically expensive (Fenn, 1923; Hill, 1938; Beltman et al., 2004). A muscle that shortens to perform work whilst producing force consumes energy at two to three times the rate of a muscle producing force isometrically (Smith et al., 2005). Because of this high cost of muscle work, most efforts to relate the metabolic cost of running to the cost of muscle function have focused exclusively on the work done to raise and reaccelerate the centre of mass raise in each step (Cavagna et al., 1964; Heglund et al., 1982; Taylor and Heglund, 1982; Minetti and Alexander, 1997; Minetti et al., 1999). The efficiency of running calculated using this approach has been shown to be as high as 73% in running humans (Heglund et al., 1982), and even to exceed 100% in galloping horses (Minetti et al., 1999). The fact that this efficiency is so much higher than the 20–30% efficiency reported in isolated muscles doing work (Hill, 1950; Smith et al., 2005) has been taken as strong evidence that some of the work of running is done by tendons, and that this provides metabolic energy savings.

One limitation of the work-based approach to analysing locomotor energetics is that it does not account for the energy consumed by muscles that produce force but not work, such as occurs when a muscle operates isometrically or is lengthened while active. An alternative is an approach that focuses on the force that must be produced to support body weight. For a runner moving on level ground at constant speed, each step involves cyclic work but there is no net change in the mechanical energy of the body. Thus, only negligible net work must be done to overcome minimal wind resistance or losses to friction. Regardless of any cyclic work, all runners must exert a force on the ground, equal to one body weight when averaged over a complete stride, to support the body. While muscle or tendons can do cyclic work, only muscle activity can produce the force required to support bodyweight over the course of a stride. Because the force requirement of body support is fixed at one body weight regardless of the runner or the speed, the metabolic energy cost of the stance phase of running should be directly proportional to the cost of force production, that is, the metabolic energy used per unit of force developed.

How do tendons influence the cost of force production during running? Fig. 1 depicts a thought experiment designed to address this question. In the first scenario, the presence of a compliant tendon and ‘perfect’ elastic energy storage and recovery allows the muscle to operate isometrically, as tendon stretches to store mechanical energy and then recoils to return it (Fig. 1A). In the second scenario, the tendon is rigid and all of the cyclic work must be performed by the muscle; it first actively lengthens to absorb mechanical energy and then shortens to produce it (Fig. 1B). In both scenarios, the muscle must generate the same average force, but its mechanical function is fundamentally different. If tendons save

¹School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, Leeds LS2 9JT, UK. ²Department of Ecology and Evolutionary Biology, Brown University, Providence, RI 02912, USA.

*Present address: Department of Ecology and Evolutionary Biology, University of California Irvine, Irvine, CA 92612, USA.

‡Author for correspondence (g.n.askew@leeds.ac.uk)

Received 18 August 2014; Accepted 22 October 2014

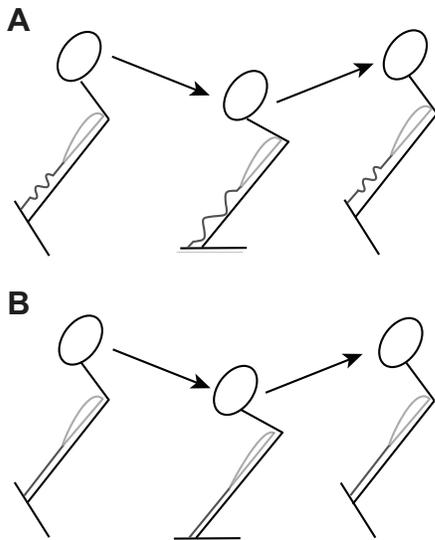


Fig. 1. Schematic representation of the trajectory of the centre of mass and the cycling of mechanical energy over the course of a running step. (A) Muscle (light grey) is operating in series with a long, compliant tendon (dark grey). Tendon stretches and recoils to absorb and return mechanical energy. (B) Muscle is operating in series with a rigid tendon. Muscle is actively stretched and then actively shortens to absorb and generate mechanical energy.

metabolic energy by reducing muscle work, we would expect the metabolic cost to be higher in scenario B than in scenario A. It is clear from our existing understanding of the energetics of muscle function (Fenn, 1923; Hill, 1938; Beltman et al., 2004) that the cost of force production during shortening (in the second half of Fig. 1B) should be higher than that of isometric force production (Fig. 1A). However, when muscle is lengthened while active, it will produce high forces (Katz, 1939), and require little metabolic energy (Curtin and Davies, 1973; Beltman et al., 2004), thus the cost of force production during the lengthening portion of the cycle in Fig. 1B will be low. The total energy consumed to produce one body weight of force in these two scenarios will depend on the relative cost of producing force isometrically, whilst shortening and whilst being stretched in dynamic cycles where the muscle undergoes rapid length changes during periods of activation and deactivation. It is difficult to predict these costs from current models of muscle energetics. But if the idea that tendons reduce metabolic energy cost by reducing muscle work is to be supported, then the total cost of the cycle depicted in scenario B should be less than that depicted in scenario A.

In this study we aimed to reproduce, *in vitro*, the muscle behaviour depicted in Fig. 1 to address the question of whether force production is energetically cheaper when cyclic work is performed by the muscle or by an in-series tendon. We used muscle ergometry and myothermic measurements to determine the metabolic cost of force production in isolated frog iliofibularis muscles, with negligible tendon, operating either isometrically or whilst being actively stretched and then shortening. To mimic the scenario where muscle ‘replaces’ the action of tendon, we designed stretch–shorten cycles that absorbed and produced equal amounts of mechanical energy. For comparison, muscles also underwent cycles where they either actively shortened and produced mechanical energy or were stretched while active and absorbed mechanical energy (Fig. 2). These four conditions are henceforth referred to as isometric, stretch–shorten, shorten and stretch cycles, respectively. If isometric

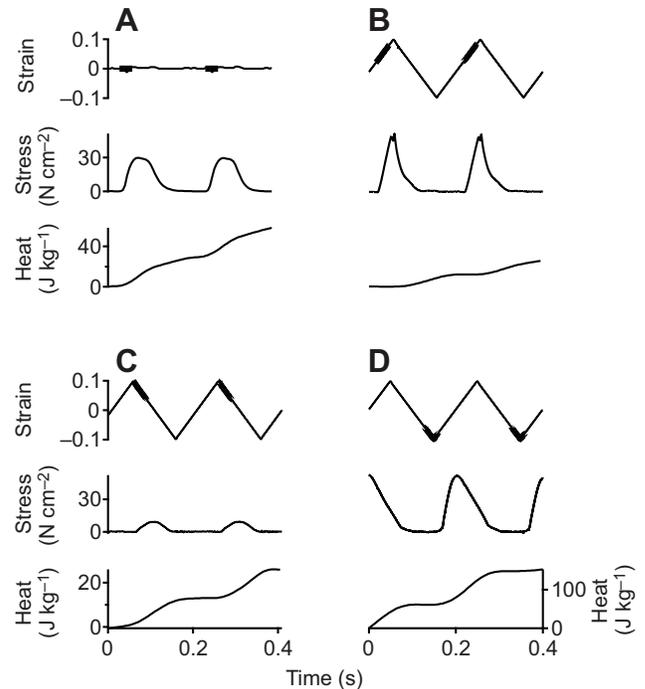


Fig. 2. Imposed strain and stimulation pattern, stress generated and heat produced. Isometric (A), stretch–shorten (B), shorten (C) and stretch (D) cycles. The stimulation period is shown by the black bars superimposed on the strain trace. Stimulus duration was the same in all conditions. Any delays between the rise of force and heat production in cycles with active stretch were probably a result of the transient storage of mechanical energy in the preparation (Linari et al., 2003). Note that because of large differences in the heat produced in different cycles, the heat axes have different scales.

muscle action with the absorption and return of mechanical energy in compliant tendons is the most economical way to meet the mechanical demands of running, the cost of force production should be lower in isometric cycles than in stretch–shorten cycles.

RESULTS

All results are presented as mean \pm s.e. The mass of the iliofibularis muscle was 15.2 ± 0.6 mg ($N=24$). Optimum muscle length (L_0) was 13.5 ± 0.2 mm ($N=24$) and fibre length, determined from digestion of a muscle fixed at L_0 , was $0.93 L_0$ ($N=1$). The basic contractile properties of the iliofibularis muscle were determined in a subset of five muscles; maximum isometric stress (P_0) was 42.6 ± 3.6 N cm $^{-2}$ ($N=5$) and maximum shortening velocity (V_{max}) was $5.8 \pm 0.2 L_0 s^{-1}$ ($N=5$).

Net work, heat and force

Net work varied with cycle type as intended (Figs 3 and 4). No work was done in the isometric cycles because of the absence of any length change (Fig. 3A). In stretch–shorten cycles, the lengthening period of the cycle involved 15.9 ± 1.8 J kg $^{-1}$ of work done on the muscle by the ergometer, followed by 16.2 ± 2.0 J kg $^{-1}$ of work done by the muscle during shortening. This resulted in a net work output over the course of a whole cycle that was not significantly different from zero (0.27 ± 0.29 J kg $^{-1}$) ($P=0.39$) (Fig. 3B). In shorten cycles, work was predominately done by the muscle (Fig. 3C) whereas in stretch cycles, work was predominately done on the muscle (Fig. 3D). Heat produced by the muscle ranged from 15.1 ± 2.7 J kg $^{-1}$ in shorten cycles, to 52.2 ± 8.8 J kg $^{-1}$ in stretch cycles (Fig. 4). It should be noted that the large amount of heat produced in stretch

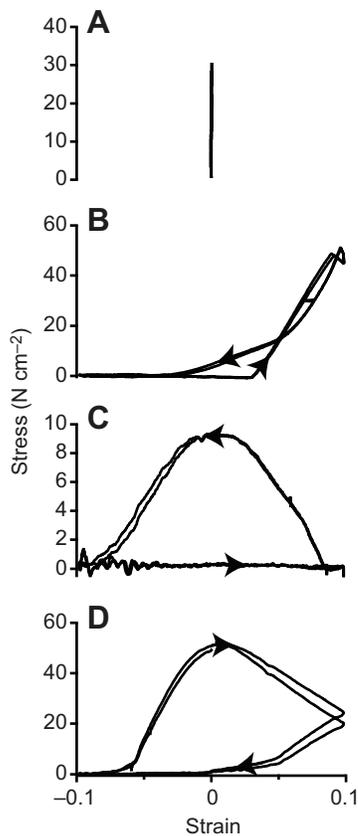


Fig. 3. Example work loops for all four conditions. Isometric (A), stretch–shorten (B), shorten (C) and stretch (D) cycles. Net work is zero in both isometric and stretch–shorten conditions, but is achieved in different ways. Note that in order to visualize the work loops, the stress axes have different scales. Arrows indicate the direction of muscle length change.

resulted primarily from the degradation of work done on the muscle by the ergometer, to heat (Fig. 4).

The cost of a cycle, calculated as the sum of net work done by the muscle and heat produced during the cycle, varied from $30.4 \pm 5.0 \text{ J kg}^{-1}$ in isometric cycles to $14.3 \pm 6.3 \text{ J kg}^{-1}$ in stretch cycles (Fig. 4). However, given the variation in force production the values of cost alone are difficult to interpret. Muscle stress profiles (Fig. 2), peak stress and the force–time integral varied considerably with cycle type as we would expect from the force–velocity relationship. Peak stress was highest in cycles where the muscle was actively stretched, $43.5 \pm 4.1 \text{ N cm}^{-2}$ and $45.6 \pm 6.3 \text{ N cm}^{-2}$ in stretch and stretch–shorten cycles respectively, and lowest in shorten cycles, $12.8 \pm 1.4 \text{ N cm}^{-2}$. Whereas peak stress and the force–time integral would ideally be the same across all conditions, the variation in the inherent force-producing capability of the muscle under different mechanical conditions means that this cannot be achieved without altering other parameters. This would make the results impossible to interpret. Hence, we believe that using different levels of force and normalising for this by using the metric of the metabolic cost per unit of force produced provides the most informative measure.

The cost of force production and efficiency

The cost of force production, defined as the ratio between the cost of a cycle and the force–time integral, was significantly different between conditions ($P < 0.001$; F -statistic, 21.9; d.f., 3) (Fligner–Killeen test for homogeneity of variances, $P = 0.9$). *Post hoc* tests showed significant differences between isometric and shorten

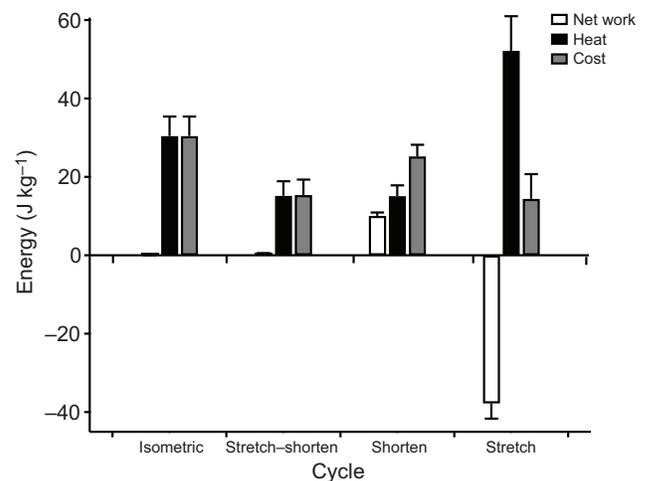


Fig. 4. Net work done (white bars), heat produced (black bars) and the cost of a cycle (grey bars) in all four conditions. The cost of a cycle is given by the sum of the net work and heat; hence, when net work is zero (isometric and stretch–shorten cycles), heat is equal to cost. $N = 11, 6, 7$ and 6 for the four conditions, respectively. Results are means \pm s.e.

and shorten and stretch cycles ($P > 0.05$). As expected, shorten cycles had the highest cost of force production ($58.3 \pm 6.3 \text{ mJ N}^{-1} \text{ s}^{-1}$) and stretch cycles the lowest ($8.3 \pm 3.5 \text{ mJ N}^{-1} \text{ s}^{-1}$). The cost of isometric cycles ($20.1 \pm 3.6 \text{ mJ N}^{-1} \text{ s}^{-1}$) was intermediate between shorten and stretch cycles, as has been observed previously (Beltman et al., 2004). However, most importantly, there was no detectable difference between the cost of force production in isometric and stretch–shorten cycles ($P = 0.86$) (Fig. 5).

In cycles where muscle work was done (shortening and stretch–shorten) we can calculate an efficiency for work production as the ratio between the work done during shortening (16.2 J kg^{-1} in stretch–shorten cycles and 10.5 J kg^{-1} in shorten cycles) and the cost of the cycle (note that for the stretch–shorten cycle this calculation ignores the work done on the muscle during lengthening, an approach that is common among studies of *in vivo* energetics; Cavagna et al., 1964; Heglund et al., 1982; Taylor and Heglund, 1982; Minetti and Alexander, 1997; Minetti et al., 1999). The efficiency of doing work was significantly higher ($P < 0.05$; t -

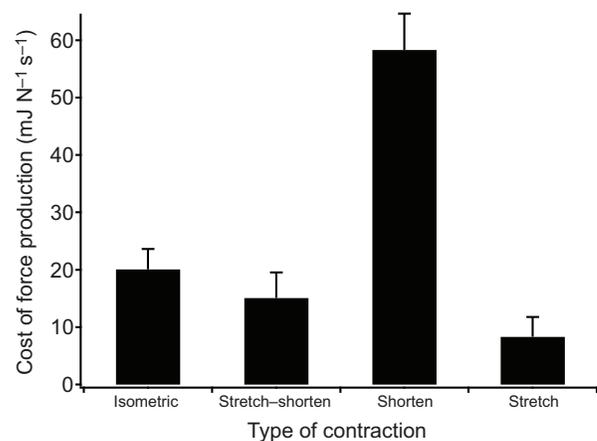


Fig. 5. The metabolic cost of force production in all four conditions. $N = 11, 6, 7$ and 6 for the four conditions, respectively. There are significant differences between shorten and isometric and shorten and stretch conditions ($P < 0.05$), but not between isometric and stretch–shorten conditions ($P = 0.86$). Results are means \pm s.e.

statistic, -3.2 ; d.f., 5.5) in stretch–shorten ($135.8 \pm 28\%$) than in shorten ($45.1 \pm 6.3\%$) cycles. Although these values of efficiency appear high, it should be noted that they are measures of initial mechanical efficiency (Barclay and Weber, 2004); that is the ratio between the work done and the sum of the net work done and the heat produced during the cycle. As such, they do not take any account of heat produced, and therefore the inefficiencies, in the recovery processes. Values of initial mechanical efficiency have been shown to be double those of net mechanical efficiency, the ratio between work done and the sum of all heat produced during and after a contraction (Barclay and Weber, 2004; Smith et al., 2005). However, although these values of efficiency are high, there is no reason to assume that the efficiency of the recovery processes would vary between conditions and therefore reasonable to conclude that net efficiency is higher in stretch–shorten cycles than it is during shortening cycles.

DISCUSSION

The primary aim of this study was to determine the relative costs of force production in isometric and stretch–shorten cycles that replicated muscle function with and without mechanical energy absorption and return in a compliant tendon. These results show that there was no detectable difference in the cost of force production when muscle performed isometric or stretch–shorten cycles ($P=0.86$) (Fig. 5). This suggests that force can be produced as economically when the muscle undergoes an active length change and does work, as when it operates isometrically. The lack of a detectable difference between the cost of force production in isometric and stretch–shorten cycles is probably a combination of the well-established low cost of force production during stretch (Curtin and Davies, 1973; Beltman et al., 2004) and the relatively low cost of doing work during shortening in stretch–shorten cycles (Lou et al., 1998; Lou et al., 1999). There may be multiple factors contributing to the low cost of work (and high efficiency) seen in stretch–shorten cycles. Force is high and stimulation has ended when the muscle begins to shorten and do work (Fig. 2). Hence, work is done by the muscle during relaxation from a point of high force generation. The energetics of this kind of dynamic muscle action, when the muscle is in the process of activating during energy absorption and deactivating during work production, are not well understood. However, it is conceivable that the cost of force production could be much lower, and the efficiency higher, for the shortening portion of a stretch–shorten cycle compared with a cycle in which the muscle starts from a point of zero force production and shortens whilst being stimulated. Data from dogfish muscles support this idea; the cost of a cycle involving shortening and work production approached that of an isometric cycle when the shortening occurred during muscle relaxation (Lou et al., 1998; Lou et al., 1999). There is also potentially an effect of an active stretch on efficiency during shortening. Active stretch has been shown to slightly increase the efficiency of subsequent active shortening (Trinh and Syme, 2007) and whilst there is negligible tendon in our preparation, there is still potential for storage and return of elastic energy in the muscle tissue itself during stretch–shorten cycles which may contribute to the low cost of force production.

The methods used here to determine that force can be produced as economically when a muscle is actively stretched and shortens as when it operates isometrically necessarily differ from the *in vivo* action of muscles during running in several important ways. We deliberately used a muscle preparation with minimal compliance and we excluded any external tendon from the preparation. *In vivo*

muscles that operate isometrically with mechanical energy being absorbed and returned by tendon will have a lot of series compliance. We varied muscle net work by systematically varying the phase of stimulation. This resulted in varying levels of force production in our conditions. The demand for force production to support bodyweight during running would be constant regardless of muscle work. We imposed a defined strain cycle on the muscle; this constrained muscle shortening and lengthening velocity, making our measurements of energy consumption specific to these velocities. We assumed that mechanical energy is cycled either entirely within the tendon (isometric cycles) or entirely within the muscle (stretch–shorten cycles). In reality, the absorption and production of mechanical energy is likely to be distributed between these structures with long compliant tendons reducing rather than eliminating muscle work.

However, the muscle preparation and experimental approach do allow us to address an assumption that is implicit in many models of the energetic benefits of tendons: that replacing muscle work with work done by tendon recoil will reduce the cost of muscular action, and therefore improve locomotor economy. Using a muscle with minimal compliance and negligible external tendon allowed us to reliably replicate muscle function with (isometric) and without (stretch–shorten) the presence of a compliant tendon. Had we used a compliant muscle tendon unit, it would have been impossible to control and determine the length change undergone by the muscle fibres themselves, and therefore impossible to draw conclusions about the energetic benefits of the reduction of muscle work by tendons. Although all efforts were made to ensure we used the most relevant metric of energy consumption (cost of force production) and to select physiologically realistic strain parameters, these factors are somewhat irrelevant to our ability to test the assumption that replacing muscle work with mechanical energy cycling in long, compliant tendons reduces metabolic energy consumption. Under our previous assumptions, we would not have predicted that force could be produced for the same metabolic cost in a stretch–shorten cycle as in an isometric cycle at any level of force, under any length change condition. Our observation that a muscle can produce force for the same metabolic cost regardless of whether cyclic work is done by muscle or by tendon calls into question the widely held belief that tendons reduce locomotor costs by reducing muscle work.

Our conclusions regarding the relative cost of force production rely on muscle heat measurements to determine the metabolic energy consumed during muscle activity. The use of regional heat measurements to estimate whole muscle energy consumption (e.g. Hill, 1949; Woledge et al., 1985; Barclay et al., 1993; Barclay and Weber, 2004; Holt and Askew, 2012) is dependent on the assumption of uniform heat production throughout the muscle. It has been hypothesized that non-uniformity in sarcomere strain arising during active stretch and/or relaxation (Hill, 1949; Linari et al., 2003; Woledge et al., 1985) and uneven distribution of strain across entire muscles (Hill, 1949; Ahn et al., 2003; Carr et al., 2011). This could be of concern here because we measured heat production in approximately 30% of the muscle. Heterogeneity in heat production may have contributed to the variability in our measurements (Fig. 5) and it might be beneficial to repeat these experiments using a variety of techniques. However, the observation that our methods produce results for the cost of force production in shorten, stretch and isometric cycles that are similar to those using other muscle preparations and a chemical rather than myothermic method (Beltman et al., 2004) support the validity of our conclusions.

Implications for our understanding of the energetic benefits of long distal tendons

The finding that force can be produced as economically when the muscle undergoes an active length change as when it operates isometrically somewhat contradicts our current understanding of the energetic benefits of long, compliant tendons. It has been argued that muscle-tendon units with short fibres and long, compliant tendons reduce the metabolic cost of legged, terrestrial locomotion through both a reduction in muscle fibre length and the work muscle is required to do (Alexander and Bennet-Clark, 1977; Alexander et al., 1982; Dimery et al., 1986; Taylor and Heglund, 1982; Roberts et al., 1997; Biewener et al., 1998; Roberts et al., 1998). However, our findings imply that the reduction of muscle work may not necessarily result in metabolic energy savings, and suggest that the energetic benefits of long, compliant tendons may largely be due to the reduction in muscle fibre length they allow over evolutionary time. Shorter muscle fibres not only reduce the cost of force generation due to a reduction in muscle volume to cross-sectional area ratio (Roberts et al., 1998), but also concentrate muscle mass proximally within the limb, thereby reducing inertia and therefore swing costs (Steudel, 1990; Marsh et al., 2006; Browning et al., 2007; Tickle et al., 2010). Hence, we propose that the energetic benefits of short muscle fibres, rather than reduced muscle work, drove the evolution of long, compliant tendons in the distal limbs of cursorial species.

Although we may conclude from these findings that effects on muscle fibre length, rather than work, underpin the energetic benefits of long, compliant tendons, muscle fibre length and muscle work are somewhat interdependent. A reduction in muscle fibre length will limit the ability of a muscle to absorb and regenerate mechanical energy. Hence, over the course of evolution, a decrease in muscle fibre length may mean that muscle cannot accommodate the fluctuations of mechanical energy associated with the movements of the centre of mass. Mechanical energy then has to be stored and returned by compliant tendons rather than absorbed and regenerated by muscle. The evolution of long, compliant tendons may therefore have resulted in a reduction in work done by muscle. However, we would now argue that this reduction in muscle work is secondary to a reduction in muscle fibre length, rather than providing an energetic benefit in its own right. This may explain why isometric muscle function is not ubiquitous; some muscle fibres undergo active length changes despite the presence of long, compliant tendons (Daley and Biewener, 2003; McGuigan et al., 2009).

Linking the mechanics and energetics of locomotion

The findings presented here may help us to resolve some of the issues in our understanding of the link between the mechanics and energetics of locomotion. It has been demonstrated that the metabolic cost of terrestrial locomotion can be explained largely by the cost of producing force, rather than by the cost of doing work (Kram and Taylor, 1990). Empirical studies show a good correlation between the cost of force production and metabolic cost (Kram and Taylor, 1990; Griffin et al., 2003; Gabaldón et al., 2008), but this model is challenged by the fact that it ignores the metabolically expensive muscle work, which has been observed in muscles active during running (Carrier et al., 1998; Gillis and Biewener, 2002; Daley and Biewener, 2003; Gillis et al., 2005; McGuigan et al., 2009; Roberts et al., 2007). The finding that the cost of force production is not significantly different in isometric and stretch-shorten cycles suggests that force can be produced for a similar cost under a wider range of conditions than previously

assumed, and so may explain how the cost of force production can explain the cost of locomotion, even though some muscles perform work.

Early studies exploring the relationship between the mechanics and energetics of running showed that the efficiency of the mechanical work done during running vastly exceeded the efficiency of work production in isolated muscles. In humans, the efficiency of work production during steady, level running has been measured as 40–50% (Cavagna et al., 1964) and 73% (Heglund et al., 1982); significantly greater than the 20–30% efficiency of isolated skeletal muscle (Hill, 1950; Smith et al., 2005). This discrepancy between whole body and expected muscle efficiency led to the conclusion that some of the mechanical work of the body must be supplied by the return of elastic energy stored in compliant elements (Cavagna et al., 1964; Heglund et al., 1982). However, the finding that muscle efficiency is much higher in stretch-shorten than in shorten cycles calls into question the assumption of a constant muscle efficiency of 25% and suggests that although the storage and recovery of elastic energy in tendons may be significant, is not necessarily required to explain high locomotor efficiencies reported previously (Cavagna et al., 1964; Heglund et al., 1982).

MATERIALS AND METHODS

Animals and tissue preparation

All experiments were performed on intact iliofibularis muscles from African clawed frogs (*Xenopus laevis* Daudin 1802). Animals were kept according to local and Home Office guidelines and maintained at 20°C. They were killed using a Schedule 1 approved method and iliofibularis muscles were dissected out of the hind limb in oxygenated (95% O₂, 5% CO₂) Ringer's solution (88.4 mM NaCl, 2.5 mM KCl, 23.8 mM NaHCO₃, 0.6 mM NaH₂PO₄, 1.8 mM MgCl₂, 1.8 mM CaCl₂, 5 mM D-glucose, 5 mM sodium pyruvate; pH 7.4) (Fischmeister and Hartzell, 1987) at 3°C. The iliofibularis muscle was chosen for this study because examination showed it to be parallel fibred, and therefore contain minimal series elasticity. Thus, we could be confident that the length change of the ergometer provided a relatively accurate indication of the length change of the muscle fibres.

Muscle Physiology

We used muscle ergometry and myothermic measurements to determine the cost of force production during cyclical activation of muscle where force was generated isometrically (isometric) (Fig. 2A and Fig. 3A), or whilst the muscle was stretched while active and then shortened, resulting in no net work being done (stretch-shorten) (Fig. 2B and Fig. 3B). For comparison, muscles also underwent cyclical activations where they either actively shortened and predominately did work (shorten) (Fig. 2C and Fig. 3C) or were stretched while active and predominately had work done on them (stretch) (Fig. 2D and Fig. 3D).

Isometric and force-velocity properties

Muscles from a subset of five frogs were used to determine the isometric and force-velocity properties of the muscles. These procedures have been reported in greater detail previously (Holt and Askew, 2012). Briefly, muscles were suspended in a Perspex flow-through muscle chamber circulated with oxygenated Ringer's solution at 20°C. The muscle was anchored to the base of the chamber using a stainless steel clip at one end and connected to an ergometer using 5.0 silk suture and a length of lightweight silver chain at the other. Supra-maximal stimulus pulses (0.25 ms square-wave pulses at 120 Hz) were generated (S48, Grass, W. Warwick, USA; UIISO model 236; Hugo Sachs Elektronik, March-Hugstetten, Germany) and delivered via platinum plate electrodes. Isometric twitches were performed at a range of lengths and optimal length for maximum twitch force generation (L_0) determined. All subsequent muscle actions were then performed at L_0 or within a range of strains that would allow for force production on the plateau of the length-tension curve. To construct a force-velocity curve, after-loaded isotonic contractions were performed at a range of loads and shortening velocity was determined. The

Table 1. Strain and stimulation parameters for the four conditions

	Isometric	Stretch–shorten	Shorten	Stretch
Cycle frequency (Hz)	5	5	5	5
Strain amplitude (\pm)	0	0.1	0.1	0.1
Stimulus duration (ms)	30	30	30	30
Stimulus phase ^a (ms)	n.a.	–45 to –47	0	80

^aPeak length is defined as time 0 and stimulus phase is defined as the timing of the onset of stimulation relative to peak length. n.a., not applicable.

theoretical unloaded maximal shortening velocity (V_{\max}) was calculated by extrapolating to zero force (Marsh and Bennett, 1986). Following length–tension and force–velocity characterization, the same muscles were used to determine the approximate parameters required to elicit the desired isometric, stretch–shorten, shorten and stretch cycles.

Strain and stimulation pattern

Muscles from the same five frogs were subjected to varying strain patterns and stimulation phases to achieve the desired mechanical outputs (Table 1). For the isometric condition, the muscle was held at a constant length (L_0) and repeatedly stimulated (Fig. 2A and Fig. 3A). For the other conditions, the work loop technique (Josephson, 1985) was used to impose a cyclical strain and stimulation pattern on the muscle. A strain amplitude of ± 0.1 was chosen because it would allow for force production over a range close to the plateau of the length–tension curve. Such strains are also within the range of muscle strains recorded in terrestrial walkers and runners *in vivo* (Carrier et al., 1998; Gillis and Biewener, 2002; Daley and Biewener, 2003; Gillis et al., 2005; McGuigan et al., 2009). Cycles were performed at 5 Hz because this would result in a shortening velocity of $0.34 V_{\max}$; this will have provided relatively high mechanical power output and efficiency (Rome et al., 1988; Barclay et al., 1993) and is representative of shortening velocities used *in vivo* (Rome et al., 1988). To produce the three conditions involving muscle length change, the phase of stimulation relative to the strain pattern was adjusted to produce the desired mechanical output. For stretch–shorten cycles, phase had to be precisely adjusted for each muscle in order to achieve negligible net work (Table 1).

Myothermic measurements

Muscles from a further 19 frogs were used to determine the metabolic cost of force production for each of the four cycles described above. Procedures for myothermic measurements have been reported previously in greater detail (Holt and Askew, 2012). Briefly, muscles were placed in a thermopile chamber (20°C) and positioned so they were sitting over the active region of an antimony–bismuth thermopile (4 mm long, containing 16 thermocouples with a Seebeck coefficient of $56.8 \mu\text{V K}^{-1}$ couple⁻¹). The muscle was held, using aluminium foil clips and stainless steel hooks, between a fixed point and an ergometer (series 300B-LR; Aurora Scientific Inc., ON, Canada) and the cycles described above performed in a randomized order. This arrangement allowed force, length and temperature to be measured in isometric, stretch–shorten, shorten and stretch cycles. Muscle performance under isometric and work loop conditions in both the flow-through and thermopile chambers were similar, indicating that the myothermic setup did not adversely affect the initial condition of the muscle. Muscle performance was monitored during myothermic experiments and the experiment terminated once net power had fallen below 80% of its initial value. The nature of the thermopile preparation meant that it was not always possible to achieve all cycles in each muscle before muscle performance dropped below this level, however, as many as possible were performed in each individual.

The thermopile output during cyclical muscle activations was converted to a temperature change using the Seebeck coefficient and the number of active thermocouples. This temperature change was corrected for heat loss and converted to a measure of heat using the heat capacity of the preparation (Wolledge et al., 1985). The Seebeck coefficient, rate of heat loss and heat capacity of the preparation were determined using the Peltier heating method (Kretzschmar and Wilkie, 1972; Kretzschmar and Wilkie, 1975; Wolledge et

al., 1985). Stimulus heat was determined by measuring the heat produced during the stimulation of a muscle that had been rendered inexcitable by leaving it in water overnight. Stimulus heat constituted less than 3% of the heat produced; no correction was therefore required. The average heat produced per cycles was calculated for the second and third cycles.

Analysis

The average peak stress, force–time integral and net work done by the muscle per cycle were calculated from the force and length recordings and muscle cross-sectional area in the second and third cycles of each series. The cost of a cycle was calculated as the sum of the heat produced and the net work done during a cycle with work done on the muscle denoted as negative net work. The cost of force production was calculated as the ratio between the cost of a cycle and the force–time integral. For stretch–shorten and shorten cycles, efficiency was calculated as the ratio between work done and the sum of the net work done and heat produced. A Fligner–Killeen test was performed to assess homogeneity of variances in the different groups. A one-way ANOVA was used to compare the cost of force production in isometric, stretch–shorten, shorten and stretch cycles. A *post hoc* Tukey's honest significant differences test was used to examine where any differences arose. A *t*-test was used to compare efficiencies in stretch–shorten and shorten cycles.

Acknowledgements

We would like to thank Chris Barclay for supplying thermopiles, Nancy Curtin for her assistance with thermopile setup, Roger Wolledge, Andy Biewener and Ivo Ros for their comments.

Competing interests

The authors declare no competing financial interests.

Author contributions

T.J.R. and G.N.A. conceived the study. All authors designed the experiments. N.C.H. and G.N.A. performed research. N.C.H. analysed data. All authors wrote the paper.

Funding

This work was funded by a Biotechnology and Biological Sciences Research Council Doctoral Training Grant (BB/D526488/1) awarded to the University of Leeds; the research was supported by Biotechnology and Biological Sciences Research Council (BBSRC) research grant BB/C503703/1 to G.N.A. and by a National Institutes of Health grant AR055295 to T.J.R. Deposited in PMC for release after 12 months.

References

- Ahn, A. N., Monti, R. J. and Biewener, A. A. (2003). In vivo and in vitro heterogeneity of segment length changes in the semimembranosus muscle of the toad. *J. Physiol.* **549**, 877–888.
- Alexander, R. M. (2002). Tendon elasticity and muscle function. *Comp. Biochem. Physiol.* **133A**, 1001–1011.
- Alexander, R. M. and Bennet-Clark, H. C. (1977). Storage of elastic strain energy in muscles and other tissues. *Nature, Lond.* **265**, 114–117.
- Alexander, R. M., Maloiy, G. M. O., Ker, R. F., Jayes, A. S. and Warui, C. N. (1982). The role of tendon elasticity in the locomotion of the camel (*Camelus dromedarius*). *J. Zool.* **198**, 293–313.
- Barclay, C. J. and Weber, C. L. (2004). Slow skeletal muscles of the mouse have greater initial efficiency than fast muscles but the same net efficiency. *J. Physiol.* **559**, 519–533.
- Barclay, C. J., Constable, J. K. and Gibbs, C. L. (1993). Energetics of fast- and slow-twitch muscles of the mouse. *J. Physiol.* **472**, 61–80.
- Beltman, J. G. M., van der Vliet, M. R., Sargeant, A. J. and de Haan, A. (2004). Metabolic cost of lengthening, isometric and shortening contractions in maximally stimulated rat skeletal muscle. *Acta Physiol. Scand.* **182**, 179–187.
- Biewener, A. A., Konieczynski, D. D. and Baudinette, R. V. (1998). In vivo muscle force-length behavior during steady-speed hopping in tammar wallabies. *J. Exp. Biol.* **201**, 1681–1694.
- Browning, R. C., Modica, J. R., Kram, R. and Goswami, A. (2007). The effects of adding mass to the legs on the energetics and biomechanics of walking. *Med. Sci. Sports Exerc.* **39**, 515–525.
- Carr, J. A., Ellerby, D. J. and Marsh, R. L. (2011). Differential segmental strain during active lengthening in a large biarticular thigh muscle during running. *J. Exp. Biol.* **214**, 3386–3395.
- Carrier, D. R., Gregersen, C. S. and Silverton, N. A. (1998). Dynamic gearing in running dogs. *J. Exp. Biol.* **201**, 3185–3195.
- Cavagna, G. A., Saibene, F. P. and Margaria, R. (1964). Mechanical work in running. *J. Appl. Physiol.* **19**, 249–256.

- Curtin, N. A. and Davies, R. E. (1973). Chemical and mechanical changes during stretching of activated frog skeletal muscle. *Cold Spring Harb. Symp. Quant. Biol.* **37**, 619-626.
- Daley, M. A. and Biewener, A. A. (2003). Muscle force-length dynamics during level versus incline locomotion: a comparison of in vivo performance of two guinea fowl ankle extensors. *J. Exp. Biol.* **206**, 2941-2958.
- Dimery, N. J., Alexander, R. M. and Ker, R. F. (1986). Elastic extension of leg tendons in the locomotion of horses (equus-caballus). *J. Zool.* **210**, 415-425.
- Fenn, W. O. (1923). A quantitative comparison between the energy liberated and the work performed by the isolated sartorius muscle of the frog. *J. Physiol.* **58**, 175-203.
- Fischmeister, R. and Hartzell, H. C. (1987). Cyclic guanosine 3',5'-monophosphate regulates the calcium current in single cells from frog ventricle. *J. Physiol.* **387**, 453-472.
- Gabaldón, A. M., Nelson, F. E. and Roberts, T. J. (2008). Relative shortening velocity in locomotor muscles: turkey ankle extensors operate at low $V/V_{(max)}$. *Am. J. Physiol.* **294**, R200-R210.
- Gillis, G. B. and Biewener, A. A. (2002). Effects of surface grade on proximal hindlimb muscle strain and activation during rat locomotion. *J. Appl. Physiol.* **93**, 1731-1743.
- Gillis, G. B., Flynn, J. P., McGuigan, P. and Biewener, A. A. (2005). Patterns of strain and activation in the thigh muscles of goats across gaits during level locomotion. *J. Exp. Biol.* **208**, 4599-4611.
- Griffin, T. M., Roberts, T. J. and Kram, R. (2003). Metabolic cost of generating muscular force in human walking: insights from load-carrying and speed experiments. *J. Appl. Physiol.* **95**, 172-183.
- Heglund, N. C., Fedak, M. A., Taylor, C. R. and Cavagna, G. A. (1982). Energetics and mechanics of terrestrial locomotion. IV. Total mechanical energy changes as a function of speed and body size in birds and mammals. *J. Exp. Biol.* **97**, 57-66.
- Hill, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. B* **126**, 136-195.
- Hill, A. V. (1949). The energetics of relaxation in a muscle twitch. *Proc. R. Soc. B* **136**, 211-219.
- Hill, A. V. (1950). The dimensions of animals and their muscular dynamics. *Sci. Prog. Lond.* **38**, 209-230.
- Holt, N. C. and Askew, G. N. (2012). The effects of asymmetric length trajectories on the initial mechanical efficiency of mouse soleus muscles. *J. Exp. Biol.* **215**, 324-330.
- Josephson, R. K. (1985). Mechanical power output from striated-muscle during cyclic contraction. *J. Exp. Biol.* **114**, 493-512.
- Katz, B. (1939). The relation between force and speed in muscular contraction. *J. Physiol.* **96**, 45-64.
- Kram, R. and Taylor, C. R. (1990). Energetics of running: a new perspective. *Nature* **346**, 265-267.
- Kretzschmar, K. M. and Wilkie, D. R. (1972). A new method for absolute heat measurement, utilizing the Peltier effect. *J. Physiol.* **224**, 18P-21P.
- Kretzschmar, K. M. and Wilkie, D. R. (1975). The use of the Peltier effect for simple and accurate calibration of thermoelectric devices. *Proc. R. Soc. B* **190**, 315-321.
- Linari, M., Woledge, R. C. and Curtin, N. A. (2003). Energy storage during stretch of active single fibres from frog skeletal muscle. *J. Physiol.* **548**, 461-474.
- Lou, F., Curtin, N. A. and Woledge, R. C. (1998). Contraction with shortening during stimulation or during relaxation: how do the energetic costs compare? *J. Muscle Res. Cell Motil.* **19**, 797-802.
- Lou, F., Curtin, N. A. and Woledge, R. C. (1999). Elastic energy storage and release in white muscle from dogfish scyliorhinus canicula. *J. Exp. Biol.* **202**, 135-142.
- Marsh, R. L. and Bennett, A. F. (1986). Thermal dependence of contractile properties of skeletal muscle from the lizard *Sceloporus occidentalis* with comments on methods for fitting and comparing force-velocity curves. *J. Exp. Biol.* **126**, 63-77.
- Marsh, R. L., Ellerby, D. J., Henry, H. T. and Rubenson, J. (2006). The energetic costs of trunk and distal-limb loading during walking and running in guinea fowl *Numida meleagris*: I. Organismal metabolism and biomechanics. *J. Exp. Biol.* **209**, 2050-2063.
- McGuigan, M. P., Yoo, E., Lee, D. V. and Biewener, A. A. (2009). Dynamics of goat distal hind limb muscle-tendon function in response to locomotor grade. *J. Exp. Biol.* **212**, 2092-2104.
- Minetti, A. E. and Alexander, R. M. (1997). A theory of metabolic costs for bipedal gaits. *J. Theor. Biol.* **186**, 467-476.
- Minetti, A. E., Ardigó, L. P., Reinach, E. and Saibene, F. (1999). The relationship between mechanical work and energy expenditure of locomotion in horses. *J. Exp. Biol.* **202**, 2329-2338.
- Roberts, T. J., Marsh, R. L., Weyand, P. G. and Taylor, C. R. (1997). Muscular force in running turkeys: the economy of minimizing work. *Science* **275**, 1113-1115.
- Roberts, T. J., Chen, M. S. and Taylor, C. R. (1998). Energetics of bipedal running. II. Limb design and running mechanics. *J. Exp. Biol.* **201**, 2753-2762.
- Roberts, T. J., Higginson, B. K., Nelson, F. E. and Gabaldón, A. M. (2007). Muscle strain is modulated more with running slope than speed in wild turkey knee and hip extensors. *J. Exp. Biol.* **210**, 2510-2517.
- Rome, L. C., Funke, R. P., Alexander, R. M., Lutz, G., Aldridge, H., Scott, F. and Freadman, M. (1988). Why animals have different muscle fibre types. *Nature* **335**, 824-827.
- Smith, N. P., Barclay, C. J. and Loiselle, D. S. (2005). The efficiency of muscle contraction. *Prog. Biophys. Mol. Biol.* **88**, 1-58.
- Studel, K. (1990). The work and energetic cost of locomotion. I. The effects of limb mass distribution in quadrupeds. *J. Exp. Biol.* **154**, 273-285.
- Taylor, C. R. and Heglund, N. C. (1982). Energetics and mechanics of terrestrial locomotion. *Annu. Rev. Physiol.* **44**, 97-107.
- Tickle, P. G., Richardson, M. F. and Codd, J. R. (2010). Load carrying during locomotion in the barnacle goose (*Branta leucopsis*): the effect of load placement and size. *Comp. Biochem. Physiol.* **156A**, 309-317.
- Trinh, M. and Syme, D. A. (2007). Effects of stretch on work and efficiency of frog (*Rana pipiens*) muscle. *J. Exp. Biol.* **210**, 2843-2850.
- Woledge, R. C., Curtin, N. A. and Homsher, E. (1985). *Energetic Aspects of Muscle Contraction*. London: Academic Press.