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Biomechanics

Passive skeletal muscle can function as an osmotic engine

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Muscles are composite structures. The protein filaments responsible for force production are bundled within fluid-filled cells, and these cells are wrapped in ordered sleeves of fibrous collagen. Recent models suggest that the mechanical interaction between the intracellular fluid and extracellular collagen is essential to force production in passive skeletal muscle, allowing the material stiffness of extracellular collagen to contribute to passive muscle force at physiologically relevant muscle lengths. Such models lead to the prediction, tested here, that expansion of the fluid compartment within muscles should drive forceful muscle shortening, resulting in the production of mechanical work unassociated with contractile activity. We tested this prediction by experimentally increasing the fluid volumes of isolated bullfrog semimembranosus muscles via osmotically hypotonic bathing solutions. Over time, passive muscles bathed in hypotonic solution widened by 16.44 ± 3.66% (mean ± s.d.) as they took on fluid. Concurrently, muscles shortened by 2.13 ± 0.75% along their line of action, displacing a force-regulated servomotor and doing measurable mechanical work. This behaviour contradicts the expectation for an isotropic biological tissue that would lengthen when internally pressurized, suggesting a functional mechanism analogous to that of engineered pneumatic actuators and highlighting the significance of three-dimensional force transmission in skeletal muscle.

1. Introduction

Muscle contraction is a three-dimensional process, involving energy flow in directions other than the muscle's line of action. Individual muscle fibres, for example, necessarily undergo changes in radius as they stretch or shorten because they are filled with a nearly incompressible fluid. For some engineered pneumatic actuators, such three-dimensional deformations are at the core of the forceful shortening process. McKibben pneumatic actuators, for example, convert radial bulging and volumetric expansion into longitudinal shortening [1,2]. McKibben actuators comprise an elastic cylindrical bladder reinforced by a braided sleeve of helically oriented fibres. When pressurized internally, the expansion of the elastic bladder widens the sleeve radially, causing the sleeve, and the actuator as a whole, to forcefully shorten.

Biological muscles are not McKibben actuators. Force production in skeletal muscle results from the interaction of actin and myosin proteins operating within muscle sarcomeres and is not the product of volumetric expansion. However, muscles share some important features with McKibben actuators. Both experience radial expansion accompanying their shortening, and both are wrapped by a 'sleeve' of fibrous material. In skeletal muscle, a robust network of collagen fibres in the extracellular matrix (ECM) surrounds fibres and fascicles, in an arrangement analogous to the fibre sheath and bladder arrangement of a McKibben actuator [3]. Some ancient theories of muscle

contraction, such as the one proposed by Erasistratus circa 250 B.C., postulated a McKibben-like action for muscle in which volumetric expansion of cells causes forceful shortening of the tissue [4]. These theories were dismissed early and succeeded by the modern sliding filament theory of muscle contraction [5–7]. Recent observations, however, indicate that interactions between the ECM and fluid can influence muscle force output under both active and passive [8–10] conditions. These studies suggest that fluid pressures within a muscle, through interactions with the collagenous ECM, can be converted into longitudinal forces oriented along the line of action of the muscle, a process reminiscent of the mechanism underlying pneumatic McKibben actuators.

Increasing the volume of an isotropic biological structure, such as a single isolated cell, would be expected to result in forceful expansion in all directions. Here, however, we test the prediction that volumetric expansion can drive forceful shortening of skeletal muscle tissue as a whole. Using hypotonic bathing solutions, we drove water into isolated skeletal muscles held at constant tension via a servomotor. We calculated mechanical work done by muscles as their fluid volumes were increased and report the effects of volume increase on muscle length and width. The behaviour of muscle is compared to that of a mathematical model of a simplified pneumatic McKibben actuator.

2. Methods

Osmotic perturbations were performed on isolated left semimembranosus muscles of the American bullfrog [*Rana catesbeiana* (Shaw, 1802)] ($n = 8$). The semimembranosus was chosen for its relatively parallel-fibred architecture, large size and superficial location [11,12]. All use was approved by the Brown University Institutional Animal Care and Use Committee. Animals were euthanized using an isoflurane overdose followed by double-pithing. Muscles were carefully removed by dissection and attached to a servomotor (305B, Aurora Scientific Inc., Ontario, Canada) via a Kevlar thread and chain connected to the distal semimembranosus tendon. The proximal end of each muscle was left attached to a sliver of hip bone held stationary in a custom clamp. Isolated muscles were bathed in a chamber of physiological Ringer's solution which could be removed and replaced without touching or displacing the muscle. A video camera (Flare 12MCX, IO Industries) oriented perpendicular to the superficial aspect of the muscle belly recorded frontal views of muscles during all experiments.

Hypotonic Ringer's solutions have been used previously to increase muscle fluid volume, as they create an osmotic gradient that causes water to enter muscle tissue over time [8,13–15]. Two different Ringer's solutions were used in the present study: a standard Ringer's solution isotonic to amphibian muscle tissue (115 mmol l^{-1} NaCl, 2.5 mmol l^{-1} KCl, 1.0 mmol l^{-1} $MgSO_4$, 20 mmol l^{-1} imidazole, 1.8 mmol l^{-1} $CaCl_2$, 11 mmol l^{-1} glucose, pH 7.9) and a diluted Ringer's solution hypotonic to amphibian muscle tissue (23 mmol l^{-1} NaCl, 0.5 mmol l^{-1} KCl, 0.2 mmol l^{-1} $MgSO_4$, 4.0 mmol l^{-1} imidazole, 1.8 mmol l^{-1} $CaCl_2$, 2.2 mmol l^{-1} glucose, pH 7.9). Ca^{2+} was left undiluted in the hypotonic solution, as changing Ca^{2+} concentration resulted in sporadic twitching of isolated muscles. All measurements were conducted at room temperature of 21.4–21.6°C and a thermocouple was used to monitor the temperature in the Ringer's solution.

Muscles were first placed in isotonic Ringer's solution and regulated at a constant stress of 0.14 N cm^{-2} via subtle changes in length prescribed by the servomotor (in force control mode). This stress could be reliably maintained by the servomotor without the risk of passively overstretching the muscle. Muscle cross-sectional areas were approximated from measurements of the muscle width midway along the length of the muscle, assuming an ellipsoidal cross-sectional shape where a and b are the ellipse semi-major and semi-minor axes, respectively.

$$A = \pi ab \tag{2.1}$$

Muscles remained in isotonic solution for a period of 30 min, over which they settled into a steady, unchanging length. The isotonic solution was then replaced with hypotonic solution, and motor position and muscle width were measured for 1 h. Muscles were returned to isotonic Ringer's solution for an additional hour following the hypotonic perturbation.

Length and force data were sampled at a frequency of 1000 Hz with a PowerLab DAQ and LabChart software (PowerLab 16/35, ADInstruments, Colorado Springs, CO, USA). Muscle width was measured post-experiment from the video recordings using ImageJ, at a location approximately one-third of the muscle length away from the attachment of the hip. Muscle width was used as an indicator of changes in muscle volume.

All length and width data were normalized to the initial length (L_0) and width (W_0) at the regulated force in isotonic Ringer's solution. Length and width strain were calculated using the following equations, where L and W are the length and width of the muscle at each data collection point:

$$\varepsilon_L = \frac{L - L_0}{L_0}, \tag{2.2}$$

$$\varepsilon_W = \frac{W - W_0}{W_0}. \tag{2.3}$$

Work was calculated as the product of the prescribed force and measured length change in the longitudinal direction.

3. Results

We found that muscle shortened forcefully over time when placed in a hypotonic solution and re-lengthened to a value close to resting length upon return to an isotonic Ringer's solution (figure 1*a*). Increases in muscle width coincided with decreases in muscle length, while force was maintained constant throughout the trial (figure 1*b*,*c*). The shapes of both the length and width over time curves showed a plateau towards the end of the 1 h hypotonic treatment. This was expected, as the concentration difference between the hypotonic Ringer's solution and the muscle's internal ionic composition should decay exponentially with time.

Skeletal muscle and engineered McKibben muscles show similarities in both structural organization and strain patterns (figure 2). Both muscles and McKibben actuators involve a fibrous sleeve surrounding a fluid-filled compartment or compartments (figure 2*a*,*c*). As muscle volume was perturbed osmotically, there was an approximately linear relationship between width strain and longitudinal strain (figure 2*b*). Average maximum longitudinal strain was $-2.13 \pm 0.75\%$ (mean ± s.d.). Average maximum width strain was $16.44 \pm 3.66\%$. Average maximum work done in the shortening direction was 0.155 ± 0.056 mJ. A simple model of McKibben muscles also shows a linear relationship between width strain and longitudinal strain (figure 2*d*), which varies depending on the initial angle of fibres in the sleeve. Raw data are available and a detailed description of the model is included as electronic supplementary material [16].

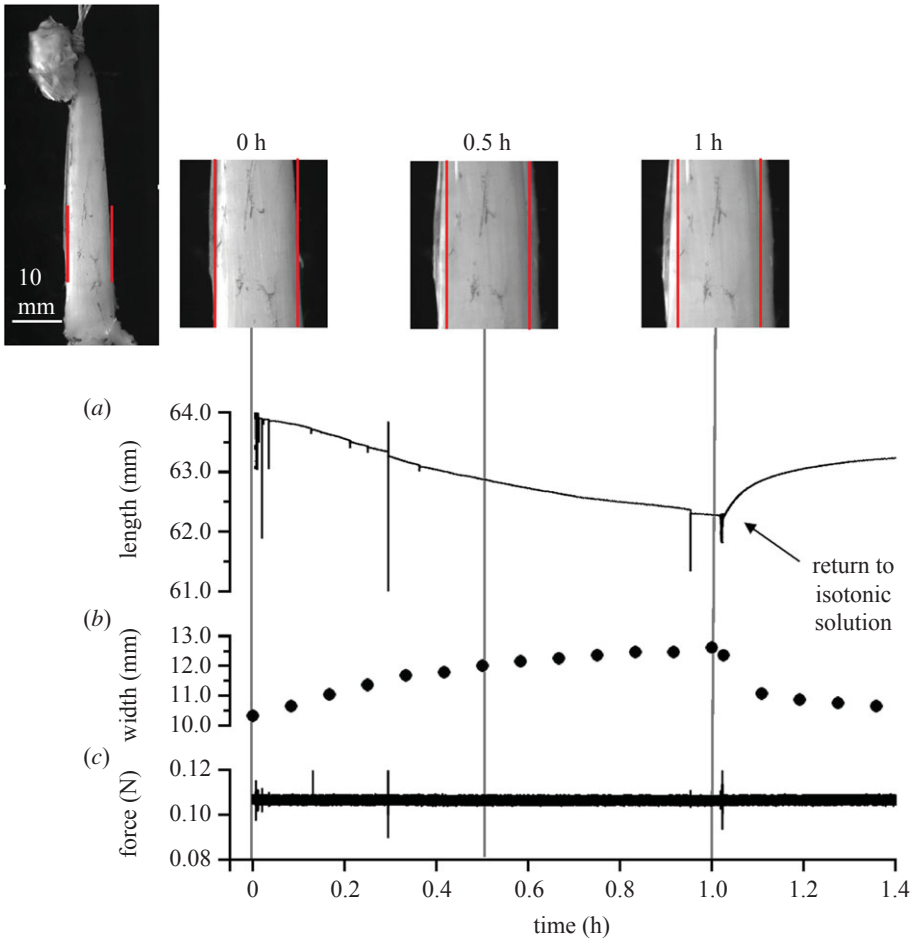

Figure 1. Representative data from an isolated semimembranosus muscle bathed in hypotonic solution (20% normal concentration) for 1 h. Inset images taken at 0, 0.5 and 1 h in hypotonic solution demonstrate muscle width changes. Red vertical lines denote baseline muscle width (excluding extraneous connective tissue) at the introduction of hypotonic solution. Muscle length decreases (*a*) and muscle width increases (*b*) with time. Muscle reverts to its original length and width upon return to isotonic solution. Passive muscle force is held constant throughout the experiment (*c*). The spikes in length at 0.25 and 0.95 h were due to spontaneous twitches in the muscle.

4. Discussion

We found that as a muscle takes in water from a surrounding hypotonic bath, it swells radially and shortens longitudinally. These results demonstrate that muscle tissue can shorten passively under constant force, with all input energy being derived from the movement of water into the muscle, hence the term 'osmotic engine'. This result is surprising, as volume increases are typically associated with increases in both the width *and* length of flexible biological tissues. Leeches, for example, elongate and widen simultaneously as they swell during feeding [17,18]. Our results suggest that muscle can transmit strains in the radial direction to strains in the longitudinal direction. We propose that interactions between intramuscular fluid and extracellular collagen fibres mediate this process, via a functional mechanism analogous to shortening in a McKibben actuator. As a McKibben actuator is pressurized and swells radially, its reinforcing fibres reorient to become more perpendicular to the long axis of the actuator, ultimately decreasing its length (figure 2*c*). A simple mathematical model of a McKibben actuator with the dimensions of an isolated semimembranosus muscle shows a similar qualitative relationship between length and width with volume increase to that observed in biological muscle (figure 2*d* and electronic supplementary material, figure S1).

A growing body of evidence suggests that muscles and McKibben actuators share functional properties under specific sets of conditions. Mathematical models of muscle ECM morphology suggest that fluid–ECM interactions serve to load collagen fibres in tension during longitudinal stretching of passive skeletal muscle [3,19]. In accordance with these models, passive muscle tension has been shown empirically to vary with intramuscular fluid volume [8,9], and active contractile force has been shown to be influenced by variations in intramuscular fluid pressure [10]. These behaviours are replicated by simple physical models of fibre-wound cylinders mechanically similar to McKibben actuators [8–10]. The current work lends novel support to the idea that fluid–ECM interactions can influence both muscle force and shape.

Biological muscle is far more complex than the idealized McKibben-like treatment described here. Collagen in biological muscle, for example, is wavy and extensible while the fibres of our simple McKibben mathematical model do not change in length. Uncrimping of collagen fibres may provide an alternative mode of volume accommodation that is not accounted for by our mathematical model. Additionally, ECM morphology varies greatly both between and within organisms, and across spatial scales within individual muscles [20]. The behaviour of our simple mathematical

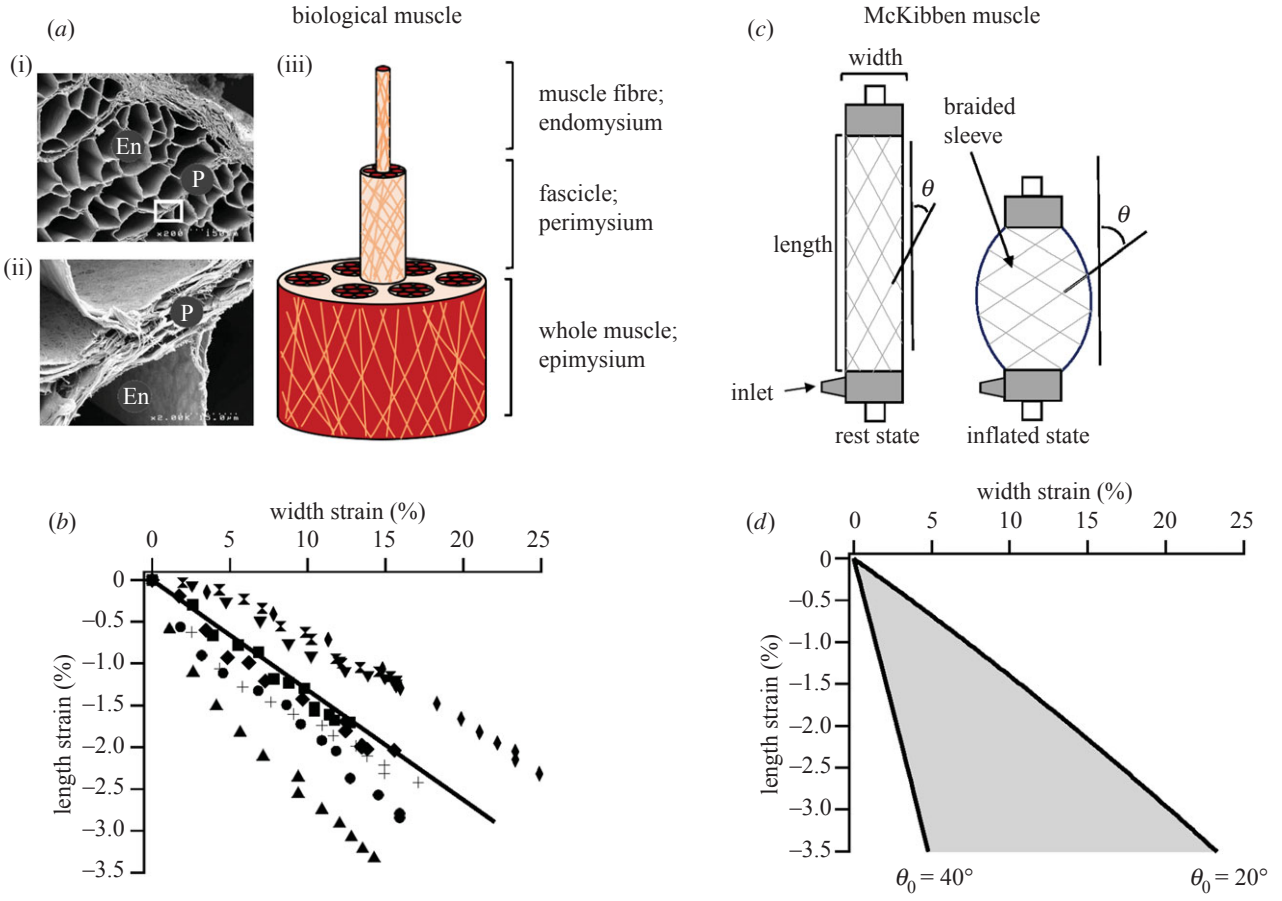

Figure 2. Skeletal muscles and McKibben actuators share similarities in structure as well as in their strain response to a change in volume. (*a*) Scanning electron micrograph showing cross-sectional views of decellularized frog muscle endomysium (En) and perimysium (P) adapted from Sleboda *et al.*, 2020 with permission from John Wiley & Sons [20]. Diagrammatic representation of skeletal muscle, showing the tissue's hierarchical structure, and the extracellular matrix elements that wrap it at each level of organization. (*b*) Longitudinal and width strain measured in all semimembranosus muscles ($n = 8$). Marker shapes correspond to different individuals and the solid line shows a mean. (*c*) Schematic of a McKibben actuator, showing an idealized braided sleeve which is analogous to the ECM of biological muscle. (*d*) Idealized McKibben muscle model prediction of shape change for an actuator with the same dimensions as the frog semimembranosus, for a range of initial collagen fibre angles.

model is sensitive to certain assumptions such as the initial wrapping angle of collagen within the muscle (figure 2*d*), which in the current study are only approximations. Furthermore, unlike the idealized McKibben actuator, biological muscle is viscoelastic, anisotropic, and almost always irregularly shaped. Future experiments that measure volume-dependent passive muscle properties while disrupting the mechanical properties of extracellular collagen may confirm its role in mediating this energy transmission.

The work done by our 'osmotic engine', 0.051 J kg^{-1} muscle mass, is exceedingly small compared to the mass-specific work done by an active frog hindlimb muscle which can be as much as 49.8 J kg^{-1} *in vivo* [21]. We do not mean to suggest that muscles behave exactly like McKibben actuators *in vivo*. However, the principle of translating radial to longitudinal strains may play a role in a variety of *in vivo* activities. While muscle is thought to be isovolumetric on the timescale of an individual contraction, muscles do undergo volume changes of up to 17% during states such as prolonged exercise [14,22]. During more typical muscle function, spatially inhomogeneous intramuscular pressures develop causing fluid movement and significant local radial bulging [23–26]. Our results suggest that this local radial bulging may contribute to overall muscle shortening, depending on collagen fibre geometry. More broadly, the current experiments suggest that off-axis forces acting on muscle from external surrounding tissues in the muscle compartment or internal pressure may appreciably affect work produced in the shortening direction, not just force. This observation is consistent with results from experiments that show both off-axis work and a reduction in muscle line-of-action work when transverse loads are applied [27–29].

Fibre-wound cylinder models of skeletal muscle have expanded our biomechanical view of muscle as more than just a uniaxial motor. The transmission of force and strain within muscle tissue between contractile elements and internal elastic structures is complex and three-dimensional. Fluid–ECM interactions may provide pathways for intramuscular energy flow not accounted for by most conceptual models of muscle mechanics and are a promising area for further research.

Ethics. All procedures were approved by the Brown University IACUC no. 18-12-0002.

Data accessibility. Data are available on Dryad Digital Repository: doi:10.5061/dryad.280gb5mp5 [16].

Authors' contribution. E.S.W. drafted the manuscript and collected and analysed data. E.S.W., D.A.S. and T.J.R. conceived of the study

and edited the manuscript. All authors gave final approval for publication and agree to be held accountable for the content of this article.

Competing interests. We declare we have no competing interests.

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