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REVIEW

Perturbing the muscle work loop paradigm to unravel the neuromechanics of unsteady locomotion

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ABSTRACT

Muscle function during movement is more than a simple, linear transformation of neural activity into force. The classic work loop technique has pioneered our understanding of muscle, but typically only characterizes function during unperturbed movement cycles, such as those experienced during steady walking, running, swimming and flying. Yet perturbations away from steady movement often place greater demands on muscle structure and function and offer a unique window into muscle's broader capacity. Recently, studies in diverse organisms from cockroaches to humans have started to grapple with muscle function in unsteady (perturbed, transient and fluctuating) conditions, but the vast range of possible parameters and the challenge of connecting *in vitro* to *in vivo* experiments are daunting. Here, we review and organize these studies into two broad approaches that extend the classic work loop paradigm. First, in the top-down approach, researchers record length and activation patterns of natural locomotion under perturbed conditions, replay these conditions in isolated muscle work loop experiments to reveal the mechanism by which muscle mediates a change in body dynamics and, finally, generalize across conditions and scale. Second, in the bottom-up approach, researchers start with an isolated muscle work loop and then add structural complexity, simulated loads and neural feedback to ultimately emulate the muscle's neuromechanical context during perturbed movement. In isolation, each of these approaches has several limitations, but new models and experimental methods coupled with the formal language of control theory give several avenues for synthesizing an understanding of muscle function under unsteady conditions.

KEY WORDS: Unsteady locomotion, Neuromechanics, Muscle force production, Tendon elasticity, Closed loop feedback control, Perturbation

Introduction

Muscles enable locomotion, a complex and highly dynamic behavior that is critically important to survival. Indeed, to move around in the natural world, animals must continuously alter speed, maneuver and negotiate changing environmental features (e.g. currents, rough terrain, wind). As such, under realistic conditions, muscles operate at steady-state (see Glossary) only briefly, if at all. Instead, muscles must constantly change length, contraction velocity and activation (see Glossary) to meet the demands of dynamic external loads. This insight has sparked a shift in locomotion neuromechanics research over the last decade decidedly toward unsteady (see Glossary) conditions (Biewener and Daley, 2007). Indeed, in addition to being relevant to natural locomotion behavior, perturbations (see Glossary), transients, maneuvers and other unsteady conditions are likely to place the greatest demands on locomotor systems and could help reveal the factors that enable or limit extreme performance (Dickinson et al., 2000; Rome, 2006; Syme and Josephson, 2002; Wilson et al., 2018). Ultimately, elucidating mechanisms that facilitate locomotion stability and maneuverability demands investigation into how muscles contribute when an organism's movement must change with the environment and sensory input (Cowan et al., 2014).

Despite its diverse roles in movement, muscle is composed of elements that are common and often well characterized across species. Skeletal muscle is composed of hierarchically structured and well-ordered active matter (in the physics sense), producing internal stress within a regular lattice of proteins (Millman, 1998; Sponberg, 2017). Within this lattice are regularly spaced thick and thin filaments, which contain myosin and actin, respectively. When activated, the motile heads of myosin bind to actin, creating 'crossbridges' that span the filaments and ratchet along to produce force and contraction. However, there is much more to muscle's myofilament lattice than just actin and myosin, and there is a growing appreciation of the contributions of other proteins, most notably titin and similar molecules (kettin, projectin, sallimus; Glasheen et al., 2017; Herzog et al., 2015; Hessel et al., 2021; Hooper and Thuma, 2005; Lindstedt and Nishikawa, 2017; Powers et al., 2021; Yuan et al., 2015). The proteins are organized into 1–10 µm contractile units, called sarcomeres, which themselves are organized in series and parallel within a large muscle cell, or fiber. Despite a large diversity of molecular variation in both structure and kinetics (there are more than 30 classes and 2000 variants of myosin alone), the sarcomere is highly conserved throughout vertebrates and invertebrates (Hooper and Thuma, 2005; Huxley and Simmons, 1971; Kawai and Brandt, 1980; Odronitz and Kollmar, 2007; Powers et al., 2021). For an excellent modern review of the molecular structure and cellular basis for contraction in muscle, see Powers et al. (2021).

Approaches to measuring muscle performance have changed as the questions explored have moved from testing the biophysical basis of contraction to understanding the function of muscle during locomotion (see Box 1). Physiological function is often still defined in the context of a muscle that is static in length (isometric) or load (isotonic). However, the work loop paradigm (Josephson, 1985a; Ahn, 2012) is a now classic approach to characterizing the function of muscle when it undergoes periodic changes in length and force, as during most types of steady locomotion involving the cyclical movement of limbs. During periodic movement, muscles serve versatile functions from brakes and motors to struts and springs (Dickinson et al., 2000).

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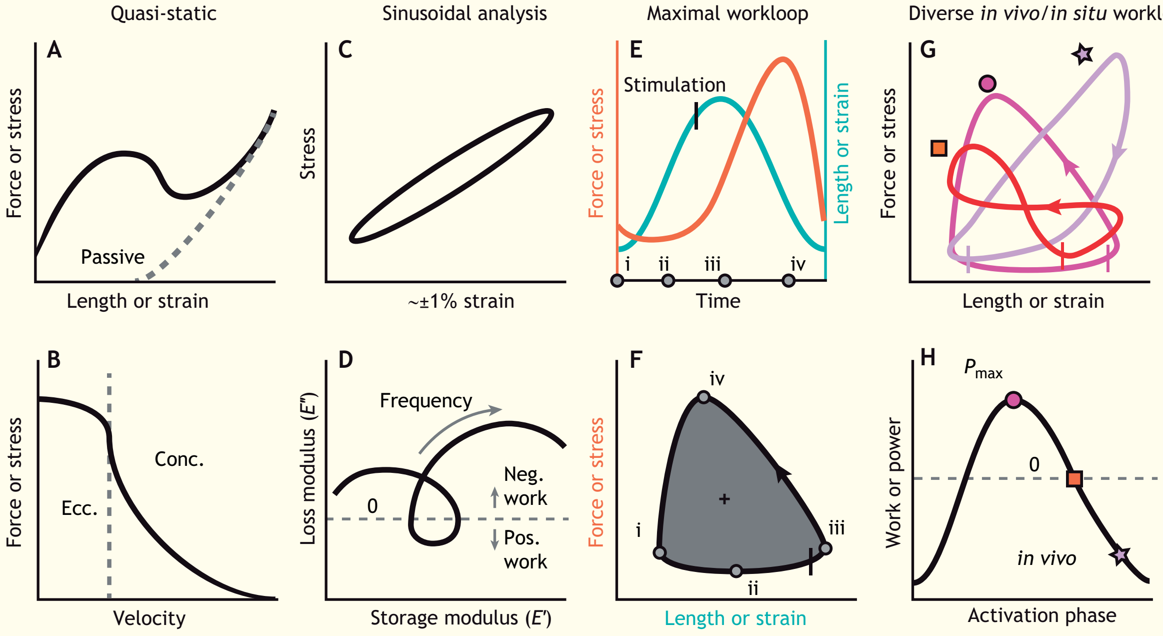
Box 1. Approaches to assessing muscle function

Even today, physiological characterizations of muscle performance typically begin with measurements that hold most properties constant (A,B) – the isometric, twitch and tetanus and length–tension curves (i.e. force–length curve), and isotonic, force–velocity curves (see Glossary for all terms). These cover both the active lengthening (ecc., eccentric) and shortening (conc., concentric) regimes. Such measures typically do not reflect physiologically realized conditions, especially during locomotion, but are useful for parameterizing models, especially the nearly ubiquitous Hill-type model (see Glossary; Hill, 1938; Zajac, 1989). They also were part of original experiments establishing the sliding filament model, which shows that thick and thin filaments slide axially, changing the overlap of crossbridge forming regions (Gordon et al., 1966).

Even before this, Machin and Pringle (1959, 1960) appreciated the dynamic nature of muscle, and characterized very fast insect flight muscle under oscillatory conditions. Low amplitude sinusoidal analysis under constant activation fits an energy storage modulus (slope, E′) and loss modulus (area within the curve, E′) to elliptical stress–strain curves (C, a Lissajous figure). Plotting these moduli over a range of frequencies (D, a Nyquist plot) shows how the linear material behavior of muscle changes but does not necessarily scale to large strains (but see Nguyen and Venkadesan, 2021 preprint for a modern extension). Such approaches helped to test biophysical models of molecular function, explicitly fitting the frequency dependence of the small sinusoidal oscillation to the underlying rate equations of myosin binding (Iorga et al., 2012; Kawai and Brandt, 1980; Kawai and Schachat, 1984).

In his landmark paper, Josephson (1985a) connected sinusoidal inputs to whole-muscle function and introduced the classical work loop method (Box 2). Originally, work loops aimed to find the strain and activation conditions (E) that generated maximum work or power (F; i–iv show corresponding points in time). More recently, work loops have explored a large range of strain and stimulation conditions (G) (Dickinson et al., 2000; Nishikawa et al., 2018). These *in vivo* and isolated muscle studies revealed that muscles can act to increase mechanical energy (a motor, circle), dissipate energy (a brake, star), store and return energy (a spring, square) or transmit energy to other structures like tendons (a strut) (H; P_{max} indicates maximum mechanical power).



This versatility underlies the role of muscle in steady movement of diverse species including guinea fowl (Daley and Biewener, 2003; Higham and Biewener, 2008), fish (Rome et al., 1993; Shadwick et al., 1999; Young and Rome, 2001), flies (Tu and Dickinson, 1994), frogs (Richards and Biewener, 2007), turkeys (Gabaldón et al., 2004; Roberts et al., 1997), cockroaches (Ahn and Full, 2002; Ahn et al., 2006; Full et al., 1998), mice (James et al., 1995), rats (Ettema, 1996), katydids (Josephson, 1985a), scallops (Cheng and DeMont, 1997; Marsh and Olson, 1994), songbirds (Elemans et al., 2008), moths (George and Daniel, 2011; Sponberg and Daniel, 2012; Tu and Daniel, 2004), humans (Ishikawa and Komi, 2008) and many others. The same muscle can even adopt different functions (Box 1) depending on the strain cycle (see Glossary), phase of activation and other parameters (Ahn et al., 2003; Hedrick et al., 2003; Higham and Biewener, 2008; Josephson, 1985b; Roberts et al., 1997). Work loops capture the diverse functions of muscle, and this energetic versatility far exceeds the flexibility of our best-engineered actuators even if muscle is not singularly the best at efficiency, energy density, power or speed (Carpi et al., 2011; Kornbluh et al., 2004; Madden et al., 2004; Mirvakili and Hunter, 2018).

Despite remarkable progress in understanding the versatility of muscle function under steady and periodic conditions (reviewed in Biewener and Daley, 2007; Dickinson et al., 2000; Nishikawa et al., 2018; Ting and Chiel, 2017), the muscle-level mechanisms that determine unsteady locomotion performance remain unclear. In freely moving animals, perturbations are the norm, not the exception, and muscle force production is strongly shaped by neural and mechanical feedback (see Glossary) (Dickinson et al., 2000; Holmes et al., 2006; Koditschek et al., 2004; Revzen et al., 2009). Neuromuscular activation can change during a perturbation, but the force a muscle produces on the body also depends on its mechanical state – its strain and strain rate – and also its strain history and loading (Josephson, 1999). The already large parameter space of muscle strain and activation explodes when we consider the range of possible perturbation conditions. These issues make it very

Glossary

Activation

Intensity of activity of the actin–myosin contractile machinery, i.e. crossbridge cycling. Activation occurs owing to release of calcium from the sarcoplasmic reticulum after stimulation of neuromuscular synapses or, in some experiments, direct electrical stimulation of the muscle. Activation may depend on more than just stimulation owing to history effects within the sarcomere (e.g. delayed-stretch activation, shortening deactivation). Contrast with stimulation.

Closed loop

The presence of feedback. A dynamic system has feedback if the output affects the input. This can be natural (neural reflexes, force affecting strain) or can be artificially produced in an experiment, e.g. an interactive virtual-reality game. In the context of muscle experiments, ‘closed loop’ usually refers to the fact that the force (output) of the muscle affects the strain (input), such as when the muscle is behaving against a real or virtual load.

Force–length (or length–tension) curve

A fundamental property of skeletal muscle characterized by several isometric contractions where steady force is measured at constant lengths. At intermediate lengths, active force development is highest and corresponds to the maximal overlap between myosin and actin. At long lengths, active force declines owing to reduced overlap. At short lengths, active force declines owing to disruption of myosin–actin spacing.

Force–velocity curve

A fundamental property of skeletal muscle characterized by several isotonic contractions. At light loads, muscle shortening velocity is fast, and at heavy loads muscle shortening velocity is slow. If a load is applied that is greater than the peak force a muscle can produce, then the muscle actively lengthens, i.e. there is negative shortening velocity.

Hill-type muscle model

Based on the early work by Hill (1938) and later by others (Wilkie, 1956; Zajac, 1989), a Hill-type model of muscle includes a contractile element that captures the active force production of muscle. It also includes a passive parallel spring and a series spring element. In terms of a mathematical function, the force from a Hill-type muscle typically depends only on the activation, length and velocity of the muscle at that particular point in time (no history dependence).

Linear material

A material (e.g. linear elastic) with outputs (e.g. stress) that can be defined as a linear function of its inputs (e.g. strain).

Linear system

A system whose outputs scale and superimpose with the inputs. If input A alone produces output Y and input B produces output Z, then in a linear system an input of 2A will produce 2Y and an input of A+B will produce an output of Y+Z.

Mechanical feedback

Changes in muscle force not mediated by changes in neural activation. Changes in muscle force output that come from the force directly alter the strain that the muscle uses. Can also include effects from a changing environment on the mechanical states of a muscle (e.g. slipping, deformation).

Muscle–tendon unit (MTU)

A tissue complex that encompasses the contractile cells (i.e. muscle fibers) that convert chemical energy into force and mechanical energy, and the elastic connective tissue, i.e. tendon and aponeuroses, that transmit mechanical energy to an animal’s body.

Neural feedback

Changes in muscle force owing to altered neural activation mediated through reflex or central nervous system pathways (e.g. proprioception, vision, vestibular).

Open-loop

A dynamic system that does not use feedback to determine the output, e.g. watching a pre-recorded movie. In the context of muscle experiments, open-loop usually refers to cases where the strain is fully prescribed and the muscle produces a force depending on its instantaneous length, velocity, activation and history.

Perturbation

Any disturbance to the state that moves the system away from its current position. In biomechanics, this is typically a mechanical force or displacement that moves the animal, limb, joint or muscle away from its steady-state operating condition.

Steady-state

In biomechanics, conditions in which states of the system are held constant (static) or allowed to vary in a regular periodic pattern (steady-periodic). These include isometric, isotonic and steady work loop conditions.

Stimulation

Electrical impulses that are applied to elicit muscle contraction; may be delivered via peripheral nerves or directly to the muscle. Electrical stimulation typically results in calcium activation of the contractile proteins with muscle. Contrast with activation.

Strain

The relative change in length normalized to the length of the muscle ($\Delta L/L_0$). Strain rate or strain velocity refers to the rate at which the muscle’s length is changing.

Unsteady

Conditions under which the state of the system moves away from constant or steady-periodic conditions. These include perturbations, transients and fluctuations. Unsteady conditions usually arise from influences external to the muscle and frequently produce more extreme physiological demands (e.g. rates of strain, energy requirements, forces) than steady conditions.

difficult to link an individual muscle’s performance during unsteady conditions to the kinematics and kinetics of the animal’s whole-body movement. Assessing the function of muscle under unsteady conditions and attributing changes in function to specific neural, mechanical or molecular determinants of muscle force production requires consideration of the interplay of these factors across timescales and through feedback loops. But this complexity is not intractable.

Perturbations such as rapid stretches and quick releases have been used to probe muscle’s molecular and biophysical mechanisms, including in the classic experiments that established the basic molecular mechanism of force generation in muscle (Huxley and Simmons, 1971). In some static or slowly varying conditions, knowledge of a muscle’s instantaneous length, velocity and activation (i.e. Hill-type model – see Glossary) can yield reasonable predictions of its force and work output (Biewener et al., 2014; Dick et al., 2017; Lichtwark and Wilson, 2005; Sandercock and Heckman, 1997), but this predictability does not extend to many dynamic conditions (e.g. Ahn et al., 2006; Perreault et al., 2003; Ross et al., 2018) because of the complex interaction of strain and activation (Hessel et al., 2021; Lindstedt and Nishikawa, 2017). In one of the most careful and systematic analyses to date, Lee et al. (2013) examined the efficacy of a Hill-type model in predicting limb muscle force profiles during walking, trotting and running in pygmy goats, *Capra hircus*. A simple Hill-type model was unable to predict even steady, periodic force. Although better fits were achieved with an innovative two-element elaborated Hill model designed to capture the role of fast and slow motor units, even this model struggled to predict maximum force (up to 32% errors) or the proper phasing of force during the locomotor cycle; it could still only explain 51% of variation in *in vivo* conditions. Given the need to characterize muscle function under unsteady conditions, many investigations have now started to perturb the classic work loop paradigm to measure muscle function directly (Box 2).

Broadly, we see two paths along which investigations into unsteady muscle function have made significant progress (Fig. 1). The top-down approach (Fig. 1A) starts with the whole animal freely moving under conditions that produce unsteady variation, and then explores how this variation changes muscle performance by perturbing isolated muscle work loop conditions or performing *in vivo* measurements during perturbations. The bottom-up approach (Fig. 1B) starts with classic steady-state work loops and builds up unsteady function by allowing the output force and work to affect the input strain and activation parameters, by systematically adding structural and environmental feedback pathways. Here, we explore the strengths and limitations of each approach and how they are likely to integrate with complementary tools in the future. We end with a more synthetic view of how the two approaches could converge onto a more systematic framework.

A top-down approach: explore, reveal, generalize

The top-down research approach starts by understanding what conditions the muscle experiences during locomotion in an unsteady environment and how it influences the body. It then zooms in to understand how muscle achieves these changes. Broadly, the three stages of the top-down approach are to: (1) explore function of a muscle in terms of how it affects body dynamics during perturbations, (2) reveal mechanisms for this function shift by examining how the mechanical work output of muscle changes and (3) generalize conditions by systematically perturbing isolated or partially intact muscle preparations beyond the specific conditions realized during movement.

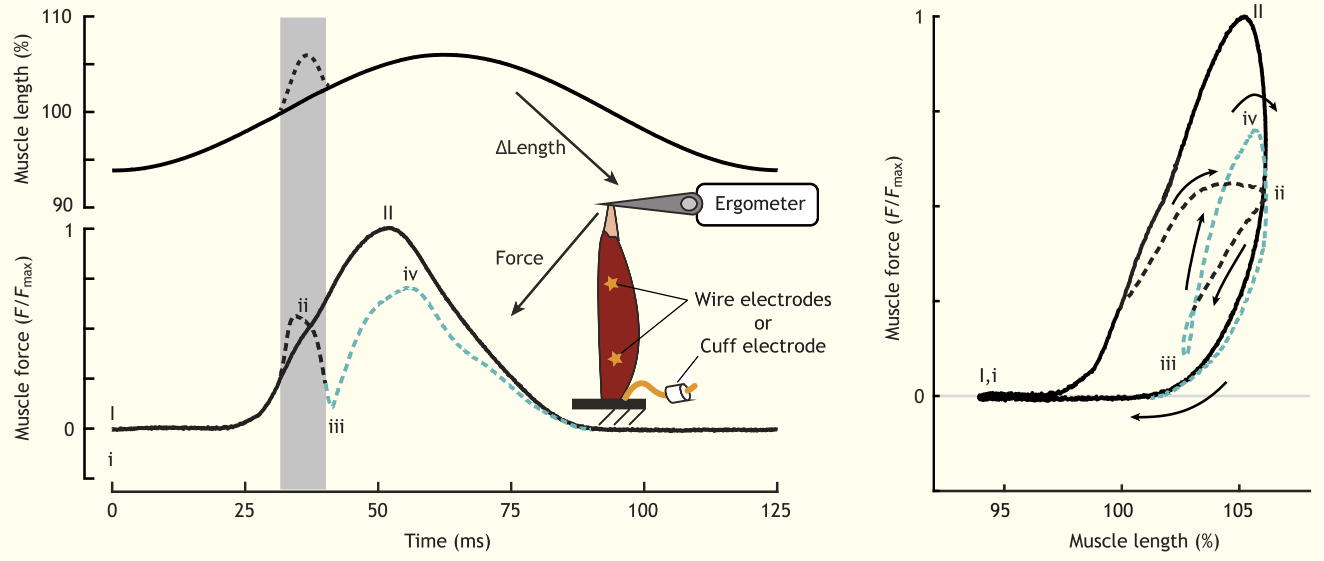
Step 1: Exploring muscle function under unsteady conditions with perturbations

Many studies have examined how muscle activation and body mechanics change from one steady behavior to another. For example,

Box 2. Steady versus unsteady work loops

A steady work loop measures the work done by the muscle or muscle–tendon unit (MTU, see Glossary) during periodically stimulated loops of length change (Box 1E,F; Josephson, 1985a, overviewed by Ahn, 2012). Work is the dot product of force and length change, so the area enclosed (integral) by a ‘loop’ trajectory in a force versus length plot is the net mechanical work. I and II indicate comparable points on the steady time series and work loop. Instantaneous work is $W=F\cdot\delta L$, where the instantaneous length change, δL, is often approximated by a small discrete difference in length (ΔL). Force is conventionally positive in the shortening direction, so length change should be as well. If a muscle is actively lengthened by an external load while producing force in the shortening direction, F is positive, ΔL is negative (lengthening) and work is negative. This makes sense, because negative work will resist the motion and dissipate energy. Mass-specific work (J kg^{-1}) is calculated by replacing force and length by stress (force per cross-sectional area, N m^{-2}) and strain (change in length normalized to rest length, unitless) and assuming a density (Box 1E,F). Dividing by the cycle period (or multiplying by frequency) gives mass-specific power (W kg^{-1}).

Unsteady conditions are deviations from steady-state (static or periodic, see Glossary) conditions and occur during perturbations. ii, iii and iv show equivalent points on the unsteady plots. The resulting work loop is no longer a simple loop in the force–length plot. The example here is a perturbation to a cockroach limb muscle where the muscle is suddenly pulled eccentrically (actively lengthened) and returned in the middle of the lengthening (grey region on left; data from Libby et al., 2020). Under steady conditions, this muscle has a net negative work loop (brake, solid line). Under the perturbed conditions, the muscle has multiple regimes (dashed lines). Nonetheless, net work is still the integral under the force–length curve. The difference in net work done during the perturbed and unperturbed work loop gives one measure of the change in function, but the within-cycle dynamics can be analyzed for more detailed interpretations. If the perturbation does not resolve within a single gait cycle, then the work ‘loop’ may not form a closed loop at all (e.g. Fig. 3D).



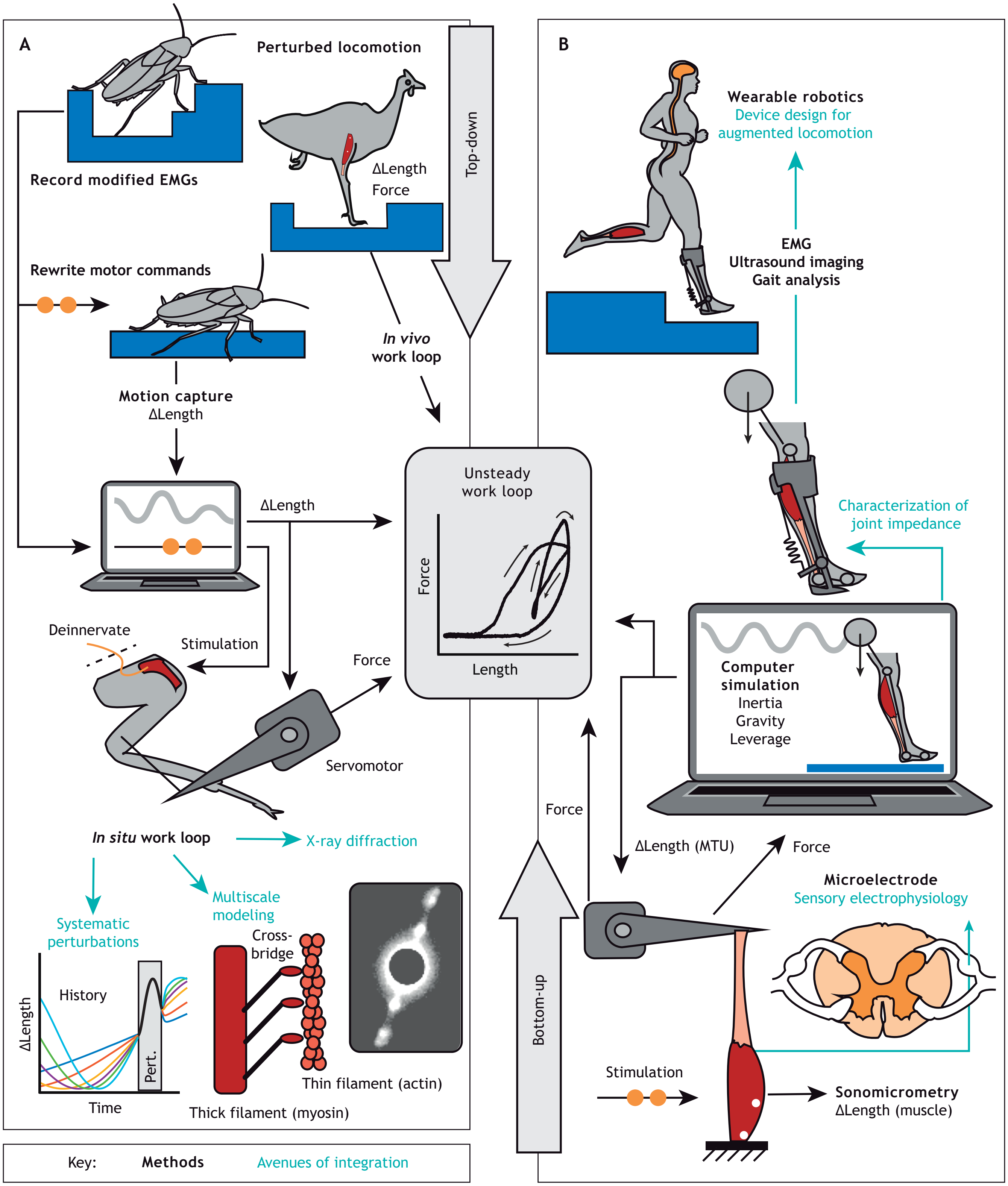


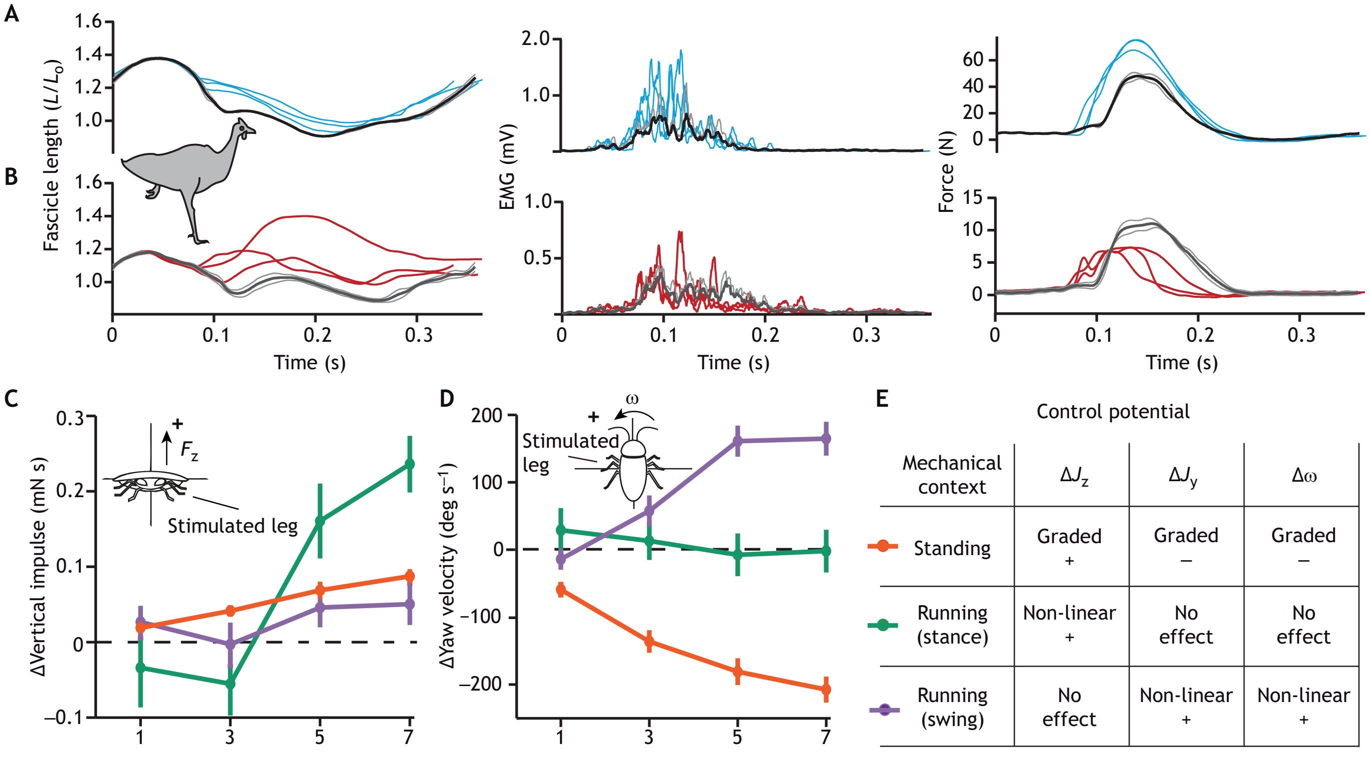
Fig. 1. Top-down and bottom-up approaches to perturbed work loops. (A) In the top-down approach, natural perturbations inform stimulation parameters for isolating the control potential of an individual muscle through recording and rewriting muscle activity via electromyography (EMG). Corresponding unsteady, *in situ* work loops reveal muscle function. Alternatively, recordings of muscle length and force can produce unsteady, *in vivo* work loops. Recent studies are extending the work loop paradigm by connecting it to systematic exploration of parameter space (e.g. testing history dependence from Libby et al., 2020), spatially explicit biophysical models (e.g. of actin and myosin containing filaments) and time-resolved X-ray diffraction (e.g. of a cockroach leg muscle during a work loop). (B) The bottom-up approach allows muscle to undergo perturbations in the context of simulated loads. Mechanical and neural feedback loops are iteratively incorporated into experiments to connect muscle function back to free locomotion. Eventually, this approach informs the design and integration of wearable robotics that augment or restore function. The key for producing an unsteady work loop (center) is to obtain the changes in length (Δlength) and the resulting force under appropriate neural stimulation (orange dots).

in turkeys (*Meleagris gallopavo*), the lateral gastrocnemius usually operates as an active strut, meaning it produces little net work and mostly transmits force to support body weight without changing length (Roberts et al., 1997). However, during inclined running, it can switch to dissipating energy on a decline or acting as a motor on an incline (Gabaldón et al., 2004). Cockatiels change the neural activity of their pectoralis muscles to modulate power across flight speeds without large changes to muscle strain patterns (Hedrick et al., 2003).

However, fewer studies have examined the behavior of muscle under transient perturbed conditions. One of the pioneering studies in this area showed how guinea fowl negotiate an unexpected drop when they step into a hole covered by tissue paper (Daley and Biewener, 2006; Daley et al., 2006). Muscle activation *in vivo* is frequently measured using electromyography (EMG) – recordings of the electrical potentials in a muscle – although calcium imaging is an alternative in small animals, especially those for which precise genetic techniques exist, such as *Drosophila melanogaster* (Lindsay et al., 2017), *Caenorhabditis elegans* (Butler et al., 2015) and *Mus musculus* (Rogers et al., 2007). In the guinea fowl studies, recordings from the gastrocnemius muscle showed that EMG patterns barely change when the bird hits the hole, but the muscle experiences a large change in length corresponding to changes in the kinetic energy of the body (Fig. 2A). This contrasts with the digital flexor (a more distal muscle), which does show significant changes in EMG activity and less change in strain (Fig. 2B). These differences in responses of specific muscles are likely to be due to different tendon architecture (Daley and Biewener, 2011).

Recording from many muscles simultaneously can show the coordinated patterns of correlated change that occur throughout the neuromechanical system. Gordon et al. (2015) extended the above studies by recording eight limb muscles spanning all leg joints in guinea fowl running along an obstacle treadmill. They found that when the birds visually anticipate obstacles, they use more anticipatory neural adjustments. In contrast, the birds' responses to very sudden obstacles are mediated by changes in muscle function that are more intrinsically mechanical, even if some neural modulation remains. In frogs, cats, moths and humans, the same groupings of muscle ('muscle synergies'; Bizzi et al., 2008; Ting, 2007), even as animals shift from swimming to hopping to walking (d'Avella and Bizzi, 2005; d'Avella et al., 2003), change stance (Torres-Oviedo et al., 2006; Torres-Oviedo and Ting, 2010), coordinate different flight turns (Putney et al., 2021 preprint) or experience disease (Cheung et al., 2009; Rodriguez et al., 2013; Ting et al., 2015). However, in most of these studies that consider many muscles, strain information is absent, making it hard to later interpret the mechanical work and power done by the muscle.

Another challenge with the approach of just recording muscle activation and strain is that these measures are only correlated with how the animal responds to the perturbation. Many different muscles could be involved in mediating the animal's response, so it



Mechanical context	Control potential ΔJ_z	Control potential ΔJ_y	Control potential $\Delta\omega$
Standing	Graded +	Graded –	Graded –
Running (stance)	Non-linear +	No effect	No effect
Running (swing)	No effect	Non-linear +	Non-linear +

Fig. 2. A muscle's control potential is neuromechanical and context dependent. (A,B) Recordings of muscle fascicle length, EMG and resulting force in two digital extensors in the leg of a guinea fowl during steady (black) and perturbed (colored) strides. One muscle (lateral gastrocnemius, A) primarily changes neural activation (EMG), whereas the other (digital flexor, B) primarily changes force due to mechanics (length change). Figures adapted from Daley and Biewener (2011), with permission. (C–E) The middle leg ventral femoral extensor (muscle 137) of the cockroach *Blaberus discoidalis* has the potential to control vertical impulse (F_z, vertical force; C) and yawing angular velocity (ω; D) around the center of mass (COM). Dashed lines denote no change from typical strides. Graded control potentials during standing (orange lines) give way to non-linear control of vertical acceleration during running (green lines). However, changing the timing (phase) of the added spikes (purple lines) eliminates the vertical control and introduces non-linear lateral plane control, but in the opposite direction of standing. (E) Summary of the control potential in each context. ΔJ_z, change in vertical impulse; ΔJ_y, change in lateral impulse; $\Delta\omega$, change in yaw angular velocity, all relative to unperturbed stride. Data from Sponberg et al. (2011a).

is tricky to infer how each muscle is causally involved. This limitation can be overcome by identifying a muscle's specific control potential through a set of stimulation (see Glossary) experiments (Sponberg and Daniel, 2012; Sponberg et al., 2011a; Srivastava et al., 2017). Control potential is the specific mapping of a change in neuromuscular activation (e.g. number of action potentials or firing rate) to the animal's response. A muscle's control potential frequently depends on biomechanical or behavioral context, just as neural encoding in sensory systems and the brain does. As an example, consider the ventral femoral extensor muscle of a cockroach, *Blaberus discoidalis*, which normally dissipates energy (Ahn and Full, 2002; Full et al., 1998). This muscle is a single motor unit (only one innervating motor neuron) and the number, but not the timing, of muscle action potentials to this muscle increases when the animal traverses very large obstacles (Sponberg and Full, 2008). But how does this muscle contribute to how the cockroach accomplishes this traversal?

To determine the muscle's control potential (Fig. 2C–E), Sponberg et al. (2011a) took an unperturbed cockroach and precisely altered the patterns of neuromuscular stimulation to this limb muscle by adding individual muscle potentials (‘spikes’) to mimic the pattern during a perturbation (Fig. 1A). When adding spikes while the animal is stationary, the muscle acts like a linear motor. It gradually accelerates the animal vertically (the vertical impulse, Fig. 2C) and produces a rotation (Fig. 2D) around the cockroach's center of mass. However, adding one to three spikes to the muscle in a running cockroach does not produce any significant response in body dynamics. But, as soon as one adds a fifth spike, there is a large burst of acceleration greater than any that occurs in the stationary animal (Fig. 2C), but the animal still does not turn (Fig. 2D). When the authors changed the timing of the spikes, but kept the pattern the same, they could switch the effect, causing the animal to turn, but not accelerate vertically. This turn was in the opposite direction to the animal's rotation when stationary. This means that the muscle response is highly non-linear during perturbed running, and the muscle's function – even with the same pattern of motor commands – depends on the behavior and biomechanical context (Fig. 2E).

Step 2: Perturbed work loops reveal mechanisms for a single muscle's diverse function

The first stage of the top-down approach establishes the effect of the muscle on body dynamics either correlationally or causally, and records the length change and activation a muscle experiences during a perturbation. The next stage examines the mechanical work output of the muscle itself by replaying the strain and stimulation conditions recorded from the perturbed locomotor conditions into a work loop. Muscle (or muscle tendon unit – MTU, see Glossary) forces and length change can be measured *in vivo* with surgically implanted tendon buckles and sonomicrometry crystals, respectively (Biewener and Gillis, 1999; Biewener et al., 1988, 1998a,b; Roberts et al., 1997). One of the first applications of this combination of recordings was performed in some of the guinea fowl experiments over unexpected drops. Large variations in the work output of the gastrocnemius during step-downs and step-ups were largely accounted for by changes in muscle strain owing to different leg lengths (measured as hip height, Fig. 3A) (Daley et al., 2009).

Alternatively, mechanical work is also sometimes measured at the scale of the whole joint. Even though joint work arises from multiple muscles and may obscure effects such as antagonistic co-activation of two muscles, it can show important patterns of function (Fig. 3B). For example, in the perturbed running guinea fowl, there is a proximal-to-distal gradient of control. The proximal muscles around the hip joint are largely invariant to perturbations, but the more distal joints, such as the tarsometatarso-phalangeal (TMP), shift from positive to negative net work (dissipation) depending on biomechanical context (Daley et al., 2007).

Finally, muscle function can also be examined in a reduced or partially intact experimental preparation, such as unsteady work loops (Box 2) on isolated muscle. In a study complementary to Sponberg et al. (2011a), the experimenters extracted the limb kinematics, inferred muscle strain patterns under the perturbed conditions and replayed the feedback into unsteady work loop experiments (Sponberg et al., 2011b) (Fig. 3C). Muscle function under these perturbed conditions was highly dependent on the locomotor behavior. When the cockroach considered above is stationary, the muscle studied acts like a simple linear motor, consistent with its graded acceleration of the body (Fig. 2C,E). In running, adding one or three spikes does not change the muscle strain pattern significantly, but at five spikes and above, the duty factor (the portion of the stride that the muscle shortens) increases non-linearly (Fig. 3C). This causes a positive mechanical feedback loop (see Glossary) that amplifies positive work in the muscle. The increased force further increases duty factor, allowing more time for even more force to develop. It is not until the antagonistic muscles (flexors) produce sufficient force that the swing period commences. Even then, the extensor muscle still has high stress when the leg begins to swing back, and this causes it to dissipate energy (negative work), pushing the leg back into stance and letting it do more positive work at the beginning of the next stride (Fig. 3D). Unsteady work loops could also explain the turning that was produced when the muscle was stimulated at different times (Fig. 2D,E), because the muscle produces positive work at a different phase in the stride cycle when the limb is positioned to turn the body (Sponberg et al., 2011b).

Step 3: Systematically generalizing the conditions of perturbed work loops

In the third stage of the top-down approach, the perspective is broadened to understand a wider range of muscle function than what is realized in the specific experiments of earlier stages. Doing so, we can systematically explore how muscle responds to carefully designed perturbations. Even if Hill-type muscle models cannot predict force over full gait cycles, they might be able to predict short-term perturbation response properties. Libby and colleagues used the perturbed work loops based on the earlier cockroach studies cited above to test whether Hill-type muscle parameters were sufficient to predict the immediate response of muscle to rapid changes in length during periodic contractions (Libby et al., 2020). They designed perturbations that were exactly the same under all Hill-muscle contractile conditions (length, velocity and activation), but differed only in the history (the strain trajectory) leading up to the perturbation. Despite identical perturbations, the different histories produce up to four-fold differences in the amount of energy that the muscle dissipates during the perturbation. The enhancement of dissipation is potent; a single muscle in the leg of a cockroach can dissipate energy equivalent to the entire kinetic energy of the running animal during a 5 ms perturbation response.

Whereas the work loops in the previous section explicitly tested how muscle performed mechanical work under conditions that mimic those *in vivo*, these perturbed work loops were designed to test a particular hypothesis of muscle biophysical function. Perturbations can also include changes in muscle function owing to injury, aging or disease. In a clever translational study, Bukovec et al. (2020) examined the potential effects of Duchenne muscular

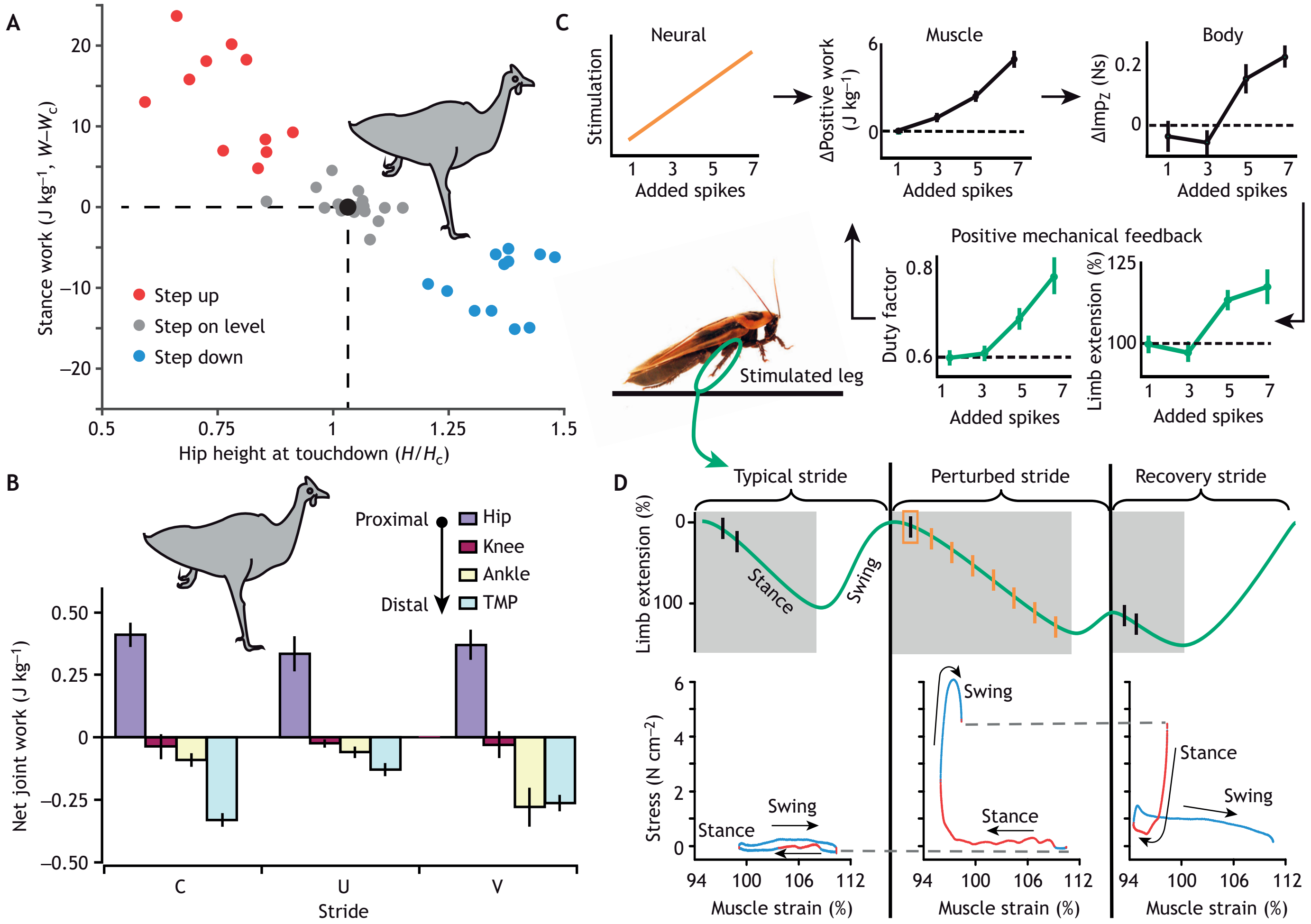


Fig. 3. Perturbed work loops reveal mechanisms of context dependence. (A) Work output from the gastrocnemius of guinea fowl undergoing unexpected perturbations is largely determined by the muscle strain. The longer the limb extension (measured as hip height at touchdown normalized to control), the more negative the net mechanical work (*W*) produced by the muscle compared with the control stride (W_c). Figure redigitized with webplotdigitizer and reproduced from Daley et al. (2009), with permission. (B) Net mechanical work analyzed at the joint level rather than muscle level shows that there is a proximal–distal gradient in control, because the work output of the more distal joints (ankle and TMP) vary more during unexpected perturbations. C, control stride; U, invisible drop perturbed stride; V, visible perturbation stride. Figure reproduced from Daley et al. (2007) with permission; only one strategy shown. (C) During running, a cockroach leg muscle that is normally a brake (Full et al., 1998) transitions to being a non-linear motor. A steady, linear increase in neural stimulation (orange) causes a non-linear increase in muscle work and, ultimately, vertical acceleration (positive *z* impulse, Imp_z). This transformation arises because of a positive feedback loop between muscle force and limb kinematics (duty factor and limb extension, green) that amplifies work when activation exceeds three added spikes. (D) This feedback can be dissected using corresponding unsteady work loops. Black ticks denote timing of typical spikes during a steady stride; orange ticks denote added spikes during the perturbed stride, triggered on the first spike. During a typical running stride, the muscle normally dissipates a small amount of energy (first work loop; negative instantaneous work, blue; positive, red). When perturbed, the extended stance phase enables some positive work. However, the high negative force during the second half of the perturbed strides also reduces the duration of the swing phase. This causes a second region of positive work at the onset of stance in the recovery stride. Dashed grey lines connect one stride to the next (perturbed work loops are not necessarily closed trajectories). Data from Sponberg et al. (2011b).

dystrophy on human soleus muscle function, by mimicking the human gait in neuromechanical simulation, scaling the results to a mouse model, and performing work loops with these parameters in an *ex vivo* mouse muscle prep containing a genetic knockout of dystrophin.

Others have suggested using systematic perturbations of muscle as a system identification tool (Kiemel et al., 2016 preprint; Roth et al., 2014). A system identification approach attempts to describe the input (stimulation and strain) to output (force) relationship with a systematic set of perturbations, usually such that the relationship can be generalized to other conditions. This is challenging in periodic systems because the perturbation response can depend on the phase or time in the cycle. The first case study of this used harmonic transfer functions (HTFs – a linear, but time-periodic system identification method) to show that body muscles in a lamprey, *Ichthyomyzon unicuspis*, are highly non-linear with activation-dependent stiffness and damping consistent with the importance of history effects (Tytell et al., 2018). As a result, no one HTF can describe the response of the muscles, at least in this system. This might be frustrating from the standpoint of having one simple predictive relationship, but it illustrates the inescapable interdependence of length, activation and force even for small deviations from steady conditions. Interestingly, when multiple antagonistic muscles act together, the identification of a linear system (see Glossary) improves, and the response of the whole lamprey body is better captured by the HTFs.

A bottom-up approach: incorporate, close the loop, contextualize

The bottom-up research approach starts with understanding how an isolated muscle's activation and strain pattern influences its force and work output (e.g. through the classical work loop approach). Then, in three stages, the bottom-up approach systematically layers on additional elements to (re)create the conditions that a muscle would experience *in vivo* during real-world movement. These are to: (1) incorporate structure that realizes the physical connections through which muscle forces are applied *in vivo* to create or modify motion of the limb or body, (2) 'close the loop' around the biological actuator/transmission system by incorporating the mechanical and neural feedback loops that alter muscle's force dynamically as its strain changes with limb or body configuration and (3) emulate context by (re)creating the physical conditions consistent with ongoing perturbations characteristic of a real-world, unsteady environment (Fig. 1B). Regarding point 2, in the term 'work loop,' 'loop' refers to the closed trajectory of force and length whose area indicates net mechanical work. 'Loop' is also used in the context of feedback loops where the output of a system affects its input. When we discuss 'closing' or 'opening' loops we mean it in this latter context.

Incorporating structures around muscle into the work loop paradigm

Most muscles attach to the skeleton through elastic tissues that are, in some cases, quite compliant. When under load, spring-like tendons and aponeuroses (sheet-like connective tissue) deform within the whole MTU and alter the state of the muscle itself (Griffiths, 1991). When interacting with an external load or strain, a muscle's force output must act through these elastic tissues (Fig. 4A). This phenomenon introduces the possibility that active muscle fascicles (bundles of muscle cells) can change length and experience force very differently than the MTU in which they reside.

There is a large catalog of examples showing how elastic connective tissue in series with muscle can enhance mechanical performance of muscle during steady movement on land (Arellano et al., 2019; Daley et al., 2009; Holt, 2020; Konow et al., 2012; McGowan et al., 2007; Roberts and Azizi, 2011), in air (Konow et al., 2015) and – to a lesser degree – in water (Richards and Sawicki, 2012). The most straightforward example of the importance of series elasticity is that classical work loop experiments do not explain how strut-like muscle (i.e. isometric) behavior emerges within an MTU, because these experiments are performed in the absence of series elastic tissues (Roberts et al., 1997). However, very little is known about MTU interactions under unsteady conditions, especially with concomitant changes in neural stimulation.

To extend our understanding of MTU function to unsteady conditions, we can add the series elastic elements (or other structural elements) directly into the work loop preparation. For example,

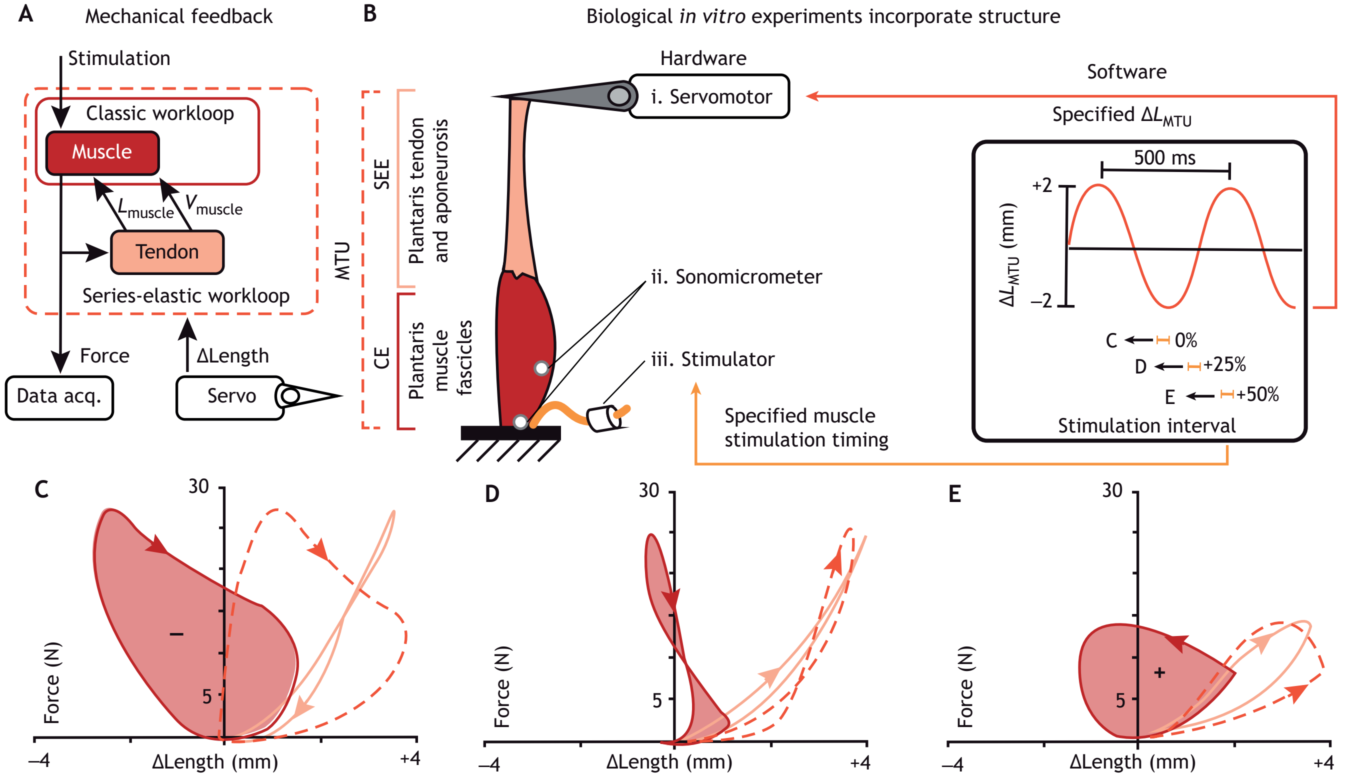


Fig. 4. Work loops that incorporate series elastic tissues reveal motor coordination for spring-like muscle–tendon function. (A) When a muscle produces force, stretch in the attached tendon and aponeurosis can modify the length (L_{muscle}) and velocity (V_{muscle}) of the muscle fascicles and, in turn, modify their capacity for ongoing force production. Data acq., data acquisition. (B) An *in vitro* MTU work loop using a cuff electrode attached to the sciatic nerve of an isolated bullfrog plantaris MTU. A servomotor prescribes overall change in MTU length (ΔL_{MTU}) and measures force, while a stimulator injects pulses at specific phases in the cycle. Sonomicrometry monitors the length of the muscle fascicles within the MTU, dissecting contributions of contractile elements (CE) and series elastic elements (SEE) to overall MTU dynamics. (C–E) MTU force versus displacement of the MTU (orange), CE (maroon) and SEE (peach) during work loop contractions with the phase of the interval of stimulation starting at (C) 0%, (D) 25% and (E) 50% of the MTU length-change cycle. As phase advances, the MTU shifts from performing net negative work (brake) to ∼net zero work (spring) to net positive work (motor), while the muscle (CE) shifts from brake to strut to motor, respectively. Data from Sawicki et al. (2015b).

Sawicki et al. (2015b) used a novel *in vitro* MTU work loop setup to directly investigate how stimulation onset timing (i.e. phase) influences MTU interaction dynamics during cyclical contractions (Fig. 4B). The main difference is that the strain is prescribed at the tendon, so that the muscle itself experiences a strain that is mediated by the series elasticity of the tendon and not set *a priori*. In addition, sonomicrometry crystals measure the length change of the muscle independently of the combined MTU, and forces are assumed to propagate through the combined structure.

Using this approach, it becomes evident that series elastic structures make it difficult for animals to directly control the trajectory of an MTU, joint or even the limb as a whole. However, what seems on the surface to be a motor control challenge may actually offer performance benefits. Similar to classical work loop experiments on muscle only, the function of the whole MTU can shift from net negative (Fig. 4C) through zero (Fig. 4D) to net positive (Fig. 4E) by advancing the stimulation phase from 0% (lengthening onset) to 50% (shortening onset). More importantly, if the timing of muscle stimulation is such that the peak force in the muscle coincides with peak stretch in the MTU, then the muscle and MTU will be nearly completely uncoupled (Fig. 4D). In this case, the muscle acts like an isometric strut (i.e. a biological clutch), enabling external energy to cycle directly into and out of the series elastic tissues. As a result, the MTU work loop behavior becomes nearly spring-like, with stiffness that matches that of its series elastic components (Fig. 4D).

Closing the feedback loop with biorobotic tools and simulations

The approach to build up the classical work loop paradigm by including series elastic tissues still lacks the closed-loop (see Glossary) interaction of a contracting MTU and the dynamic load it acts upon (Fig. 5A). During normal muscle function, the length (or strain) of a muscle is not prescribed, but results from the muscle producing force against some finite load. For example, during walking or running, the length of muscle or MTU comes from the work the muscle is doing to move the mass of the body relative to the contact point of the limb on the ground. Flight muscles act through the wing to do work against both inertial and aerodynamic loads. Dynamic loads mechanically feed back through the tendon to alter a muscle's work loop.

To accomplish closed-loop control of muscle–tendon length in a work loop experiment, experimenters can switch to using simulations of the mechanics or actual mechanical systems that take the muscle's force as an input and calculate the resulting length change (Fig. 5). The advantage of this approach is that the mechanics can be modeled but the experiment can utilize an actual muscle. This hybrid approach has advantages over pure simulations because current muscle models are not adequate, especially for unsteady conditions.

In a series of papers, Richards and Clemente pioneered this approach by coupling an isolated muscle work loop preparation to a robotic model of a frog foot moving through water (Fig. 5B; Richards, 2011; Richards and Clemente, 2012). Fluid loads will often scale with the square of the velocity and area of the actuator, so swimming frogs (as well as fliers) need to move their smaller limbs more quickly to increase propulsive forces compared with large organisms. When starting at longer muscle lengths, Richards and Clemente found that muscles acting against real hydrodynamic loads run up against limits imposed by the force–velocity curve (see Glossary) as opposed to those resulting from the force–length curve (see Glossary; Clemente and Richards, 2012). The real advantage of the robotic component of the system is that experimenters can easily and systematically vary system parameters to ask questions such as: how do speed and the performance of muscle change within species as an animal grows or across species of different size? By systematically varying the parameters of the speed, foot size and

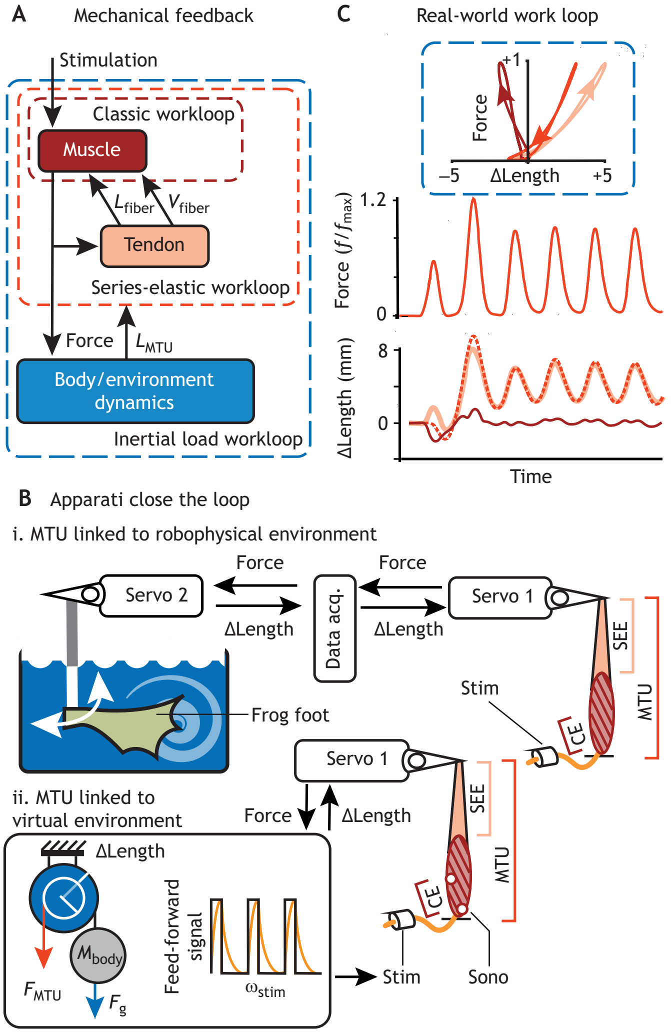


Fig. 5. Using biorobotic tools to create a mechanical feedback pathway between muscle–tendon unit (MTU) and real-world load. (A) MTU force allows the body to act against its surrounding environment, whereas the external load changes the length of the MTU depending on the movement that results (a mechanical feedback loop). (B) The set-up in Fig. 4 is extended so that the MTU interacts with real work loads. The servomotor attaches to a real-time robotics interface under high-bandwidth feedback control. The MTU is then coupled to (i) a robot operating in a physical real-world environment as in Richards and Clemente (2012) or (ii) a virtual environment where length is measured via sonomicrometry (Sono) and dynamic loads (M_{body}, mass of body; F_g, gravitational force) are simulated, as in Robertson and Sawicki (2015). In each time step, the MTU forces (F_{MTU}) act as the input to the physical or virtual load, and the resulting motions are fed back into the MTU as a change in muscle length. (C) Time series from a representative real-world work loop trial of an MTU interacting with a virtual load during hopping. When the driving frequency (ω_{stim}) is near the passive natural frequency, stimulation (Stim) onset occurs at ∼25% of the MTU length change phase and exhibits resonance, as demonstrated by a work loop with 'tuned' elastic behavior whereby the muscle contractile element (CE, maroon curve) behaves like a strut, producing high forces with small length changes. In contrast, the whole MTU (orange curve) exhibits spring-like stretch and recoil with stiffness approaching that of the series elastic elements (SEE, peach curve). Adapted from Robertson and Sawicki, 2015.

gear ratio of their hybrid biorobotic system with either *in vitro* or *in silico* plantaris longus muscle preparation, they found that different combinations of morphology could all converge on successful swimming strategies (Richards and Clemente, 2013). Instead of being locked into a single optimal set of parameters, which would be untenable as the animal changes sizes or conditions, the combination of morphology and speed allows the muscle to be broadly tuned for high-power output.

In a second example, Robertson and Sawicki extended the MTU work loop setup that incorporated series elastic structures (Fig. 4) into a ‘smart’ biorobotic interface that could emulate the mechanical feedback between a muscle, its body and environmental dynamics during unsteady locomotion over ground (Fig. 5A). They started with a previously developed simple hopping model comprising a body mass attached to a foot through a single joint (ankle) and driven by a single Hill-type compliant MTU (calf–Achilles tendon) (Robertson and Sawicki, 2014). They embedded the rest of the model (i.e. pulley+mass+gravity) in the software used to control an ergometer interacting with a living frog plantaris MTU *in vitro* (Fig. 5C). This model hops vertically when excited with a rhythmic neural input at a set frequency and captures most of the notable features observed during actual human hopping at steady-state (Robertson and Sawicki, 2014). The resulting biorobotic platform reads force from the MTU, applies it to a simple model of vertical hopping, computes the resulting change in MTU length and sends it back out to the MTU. This extends the work loop framework to include mechanical feedback by closing the loop between MTU force and MTU displacement in real time (Fig. 5A; Robertson and Sawicki, 2015; Robertson et al., 2017; Sawicki et al., 2015a).

In this biorobotic system, when a periodic force interacts with a mechanical system and a load, the work loop behavior depends on the relationship of the stimulation frequency, ω_{drive}, and the resonance frequency of the system. When activated with a set ω_{drive}, the system eventually settles into a steady cycle exhibiting net zero work by the MTU (i.e. total system energy is conserved; Fig. 5C). In a linear system without significant damping, the resonance frequency is the natural frequency at which the system passively oscillates. One important feature that emerges from the coupled dynamics between the MTU and the inertial/gravitational load is that matching the driving frequency, ω_{drive}, to the natural frequency of passive oscillation, ω_o, of a compliant MTU generates mechanical resonance and yields the largest spring-like behavior in cyclic contractions (Fig. 5C; Robertson and Sawicki, 2015). This work suggests that animals may choose a locomotor frequency that resonates with passive mechanical structures, but these structures have to consider the load, elasticity and muscle (Gau et al., 2021; Jankauski, 2020; Liao, 2004; Lynch et al., 2021; Robertson and Sawicki, 2015; Weis-Fogh, 1973).

Emulating the context in which a muscle acts by incorporating neural feedback or hybrid robotic systems

So far, we have demonstrated bottom-up techniques to take work loop experiments from isolated muscles operating in open loop (see Glossary) to isolated MTUs (Fig. 4) operating in closed loop (see Glossary) with dynamic loads representative of terrestrial and aquatic locomotion (Fig. 5). In this way, we get closer to recreating the *in vivo* loading conditions that an MTU would experience during real-world locomotion (Fig. 5A). The final step of the bottom-up approach is to further embody the muscle or MTU by incorporating realistic neural feedback and the dynamics of actual locomotion. The experimental capabilities for this step are in their infancy, but would involve taking the MTU embedded in a hybrid biorobotic system and coupling this to a locomoting animal or directly incorporating neural feedback through paired sensory neuron recordings and manipulations. Human biomechanics is only beginning to address muscle-level responses to explicit perturbations (e.g. falling in holes or pushes to the body) and the role of sensory feedback pathways in altering ongoing motor commands during perturbed movements (Dick et al., 2021; Golyski and Sawicki, 2022). These gaps between the current state of the bottom-up work loop paradigm and freely moving animals navigating unsteady environments motivate the next steps for integration toward systems with both biological and artificial parts (e.g. wearable robotic systems; Fig. 1B).

From an applied standpoint, the ultimate endgame for the bottom-up approach is to help inform fundamental principles for optimal design of legged machines, including wearable robotic systems that can augment human locomotion in variable environments (Fig. 1B). For example, recent closed-loop experiments using isolated muscle tendons on the benchtop have incorporated models of exoskeleton dynamics in parallel with body and environmental dynamics running in a virtual environment (Robertson et al., 2017). This approach enables detailed examination of how devices can interact with and augment biological muscle function. Indeed, incorporating dynamic properties of biological muscle–tendon systems into actuation systems on legged machines (e.g. autonomous robots and lower-limb joint prostheses and exoskeletons) could help speed the transition from tethered devices operating in stereotypical lab environments to autonomous devices operating in unstructured, natural environments with continuous perturbations (Badri-Spröwitz et al., 2022; Nishikawa and Huck, 2021; Rubenson and Sawicki, 2022; Shafer et al., 2021; Tahir et al., 2018).

Trade-offs of top-down and bottom-up approaches

The top-down and bottom-up articulation poses a heuristic dichotomy in the study of how muscle dynamics underpin unsteady movement. The top-down approach necessarily begins with informing the relevant parameter ranges of strain and activation and then prescribing these as open-loop, unsteady trajectories or recording them *in vivo* (Fig. 1A). This means that *in vivo* strain and activation are reproduced as open-loop replays of these trajectories *in situ* or using invasive means to measure force, work and other properties *in vivo*. Therefore, even though the top-down approach can systematically explore a diversity of conditions in a highly repeatable and tractable manner, it is limited either to the conditions recorded or requires the muscle to be removed from the natural feedback loops between force and length present in the organism.

In contrast, the bottom-up approach maintains, at least in part, the active coupling of force and length by preserving functional feedback loops. It gives unprecedented control for exploring the context for muscle’s dynamic behavior on the benchtop (Fig. 1B). By explicitly placing muscles within physiologically realistic mechanical and neural feedback loops, the bottom-up approach opens the door to studies that can systematically layer environmental dynamics, body morphology and sensory feedback on top of actual muscle dynamics. However, the bottom-up approach removes the ability to exactly match unsteady conditions to those experienced in free locomotion, making it difficult to synthesize findings back into the context of biologically relevant locomotion. Overall, both top-down and bottom-up approaches seek to untangle the causal determinants of muscle function in addition to characterizing the unsteady properties of nature’s most versatile actuator.

Conclusions and opportunities for synthesis

The challenge with the top-down and bottom-up approaches going forward lies in synthesizing results across experiments and scales. In a few systems outlined here, researchers have performed experiments across scales that allow for the interpretation of unsteady locomotor function and mechanisms, but this is still the exception rather than the rule. It also remains challenging to generalize the results beyond these systems, and no system has leveraged the strengths of both approaches. We need frameworks to facilitate systematic experimentation from top-down and bottom-up approaches, and to allow the results of these experiments to be related quantitatively to one another. Although the approaches outlined above provide two avenues for designing experiments, they could be enhanced in three ways: (1) connecting experiments to emerging models of muscle function at multiple scales, (2) leveraging new techniques that allow for simultaneous measurements across scales, and (3) utilizing the formalism of control theory and system identification to allow quantitative predictions from one experimental context to another based on opening and closing feedback loops.

Muscle modeling is rapidly moving beyond simple rate kinetics, Hill-type parameters, static material models and neuromechanical simulations with many highly simplified parameters. Computational power and our understanding of mechanochemistry are now sufficient to run detailed biophysical models of a half-sarcomere (Campbell et al., 2011a,b) and spatially explicit models of muscle that integrate myosin binding and three-dimensional myofilament lattice strain with other important molecular constituents such as titin (Powers et al., 2018; Tanner et al., 2008; Williams et al., 2010). These multiscale models have already shown some success in predicting physiological properties at least in steady-state regimes (Williams et al., 2013). Going forward, they may be able to predict muscle behavior under unsteady conditions because they capture the details of coupling between strain and activation. At the larger scales, rheological (the physics of deformation) models of muscle based on combining multiple linear material characterizations (see Glossary) have shown some recent success in modeling work loop conditions (Nguyen and Venkadesan, 2021 preprint; Nguyen et al., 2018). Detailed neuromechanical models that embody muscles in the connective elements and biomechanics of the organism are moving beyond Hill-type muscle models of their elements. These models become especially powerful when an optimality function can be defined and the model parameters are selected to minimize some cost validated by experiment (Knaus et al., 2022; Koelewijn et al., 2019; Pena et al., 2021; Van Wouwe et al., 2021). These new approaches provide some hope that we will be able to predict muscle function under unsteady conditions across the experimental steps in each of the top-down and bottom-up methodologies, but the models themselves currently are not integrated across scales.

Complementary to unsteady work loop experiments, there is a corresponding proliferation of new methods that provide unprecedented opportunities for capturing details of muscle function. For example, X-ray videography of lead markers, magnetic resonant imaging or sonomicrometry crystals embedded throughout a muscle can reveal important properties such as spatial heterogeneity in muscle strain (Ahn et al., 2003; Arellano et al., 2019; Azizi and Roberts, 2009; Camp et al., 2016; Kirkpatrick et al., 2022; Pappas et al., 2002). High-resolution optical techniques can resolve individual sarcomere dynamics (Rassier et al., 2003). Calcium imaging can monitor the *in vivo* activation of many muscles simultaneously (Lindsay et al., 2017). Optogenetic manipulation can stimulate muscles even in free flight, albeit not yet at the level of individual action potentials (Whitehead et al., 2022). X-ray microtomography can reveal the strain trajectories of many muscles during periodic contractions (Walker et al., 2014). Finally, X-ray diffraction through living muscle can give highly time-resolved (up to 5000 frames s^{-1}) information about structural interactions between molecules (Irving, 2007; Iwamoto, 2018). Many of these techniques could potentially be performed simultaneously or in parallel with physiological measurements from a work loop experiment, and such approaches may allow us to query multiple scales of muscle function at the same time. Doing so may facilitate achieving the three steps of each approach in more systems and might link unsteady function to underlying cellular and molecular mechanisms.

Although new modeling and experimental tools help, designing and quantitatively comparing experiments across different steps in the two approaches remains challenging. This is because the different steps have different mechanical and neural feedback pathways. In each of the approaches, some of these pathways are removed during the experiment (termed ‘opening’ the feedback loop). For example, an *ex vivo* muscle work loop preparation does not have intact neural feedback or mechanical feedback from a dynamic load. Other times, feedback loops are artificially ‘closed’ around the muscle, such as when a simulation measures muscle force and plays back a modeled change in length to the muscle preparation. See Roth et al. (2014) and Cowan et al. (2014) for more detail on opening and closing feedback loops in biological systems.

In experiments with different mechanical and neural feedback loops intact or artificially closed, the inputs (e.g. force, length change) and output (force) that the muscle or MTU experiences change. System identification and control theory provide a natural language and quantitative framework for combining experiments on the same muscle with different opened or closed feedback loops, because they provide mathematical tools to predict and relate the inputs and outputs from one such experiment to the next (Cowan et al., 2014; Madhav and Cowan, 2020; Roth et al., 2014; Sponberg, 2017; Thomas et al., 2019). These approaches are especially powerful when the input–output relationship is linear. Muscle force or work (output) is unlikely to have a linear relationship with activation, strain or velocity (inputs), except in small perturbation regimes. Hence, linear control theoretic approaches may face some limitations (Kawai and Brandt, 1980; Libby et al., 2020; Tytell et al., 2018). However, even if non-linearities are experimentally inescapable or indispensable, there are still tools and methods that can be used to qualitatively and quantitatively relate experiments with different opened and closed loops to one another, estimate the non-linear input–output relationship, and indicate how non-linearities are manifest in the organism (e.g. Roth et al., 2011, 2014; Tytell et al., 2018). Finally, control theoretic approaches are already becoming prevalent in the motor control and computational neuroscience communities (Cowan and Fortune, 2007; Cowan et al., 2014; Dahake et al., 2018; Frye and Dickinson, 2004; Kiemel et al., 2016 preprint; Roth et al., 2014; Stöckl et al., 2017; Ting and Chiel, 2017; Tytell et al., 2018). Similar approaches applied directly to characterizing muscle function promise to facilitate synthesis across experiments and models.

We still cannot predict, much less emulate, the versatility of muscle. Tackling this challenge will require us to move toward a synthesis of top-down and bottom-up approaches with the help of multiscale modeling and new experimental techniques in parallel with unsteady work loops. We can also embrace the systematic formalism of controls and dynamic systems theory not just to interpret but to guide experiments. Together, these approaches can help disentangle the complicated feedback pathways that shape muscle's function, especially under unsteady conditions. They provide a roadmap to help us understand, predict and potentially even improve upon nature's most dynamic actuator.

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Competing interests

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