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COMMENTARY

(How) do animals know how much they weigh?

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ABSTRACT

Animal species varying in size and musculoskeletal design all support and move their body weight. This implies the existence of evolutionarily conserved feedback between sensors that produce quantitative signals encoding body weight and proximate determinants of musculoskeletal designs. Although studies at the level of whole organisms and tissue morphology and function clearly indicate that musculoskeletal designs are constrained by body weight variation, the corollary to this – i.e. that the molecular-level composition of musculoskeletal designs is sensitive to body weight variation – has been the subject of only minimal investigation. The main objective of this Commentary is to briefly summarize the former area of study but, in particular, to highlight the latter hypothesis and the relevance of understanding the mechanisms that control musculoskeletal function at the molecular level. Thus, I present a non-exhaustive overview of the evidence – drawn from different fields of study and different levels of biological organization – for the existence of body weight sensing mechanism(s).

KEY WORDS: Alternative splicing, Body weight sensing, Mechanotransduction, Muscle plasticity, Scaling, Tensegrity

Introduction

The design of most animal locomotor systems appears to be constrained mainly by body weight and associated considerations of durability. The effect of gravity, the one truly constant environmental variable, on body mass (giving body weight, or body mass×$\boldsymbol{g}$) must be resisted and overcome to achieve movement. This is particularly true for terrestrial and flying animals, but may even apply to aquatic animals (that experience variable degrees of buoyancy), which must move volumes of water equivalent to their body mass (Bejan and Marden, 2006) in order to swim. Indeed, Marden and Allen demonstrated that the maximum force output of most running, flying and swimming animal motors is proportional to motor mass (Marden, 2005; Marden and Allen, 2002) and, therefore, to body mass, for many animals. This generality of design requirements and performance outcomes suggests that there are (phylogenetically) conserved mechanisms that have tuned (and continue to tune) both the gross morphology and the associated molecular level design of animal musculoskeletal systems to body weight variation in a quantitatively precise fashion.

In this Commentary, I aim to present evidence for the less commonly discussed idea that the musculoskeletal system is also sensitive to body weight variation at the molecular level. In doing so, I will discuss gene regulatory mechanisms regulating muscle sarcomere molecular variation (such as alternative splicing) and highlight potential mechanosensitive mechanisms that can affect muscle properties at the molecular level. It is unclear whether there are any precise and absolute signals of body weight that are detected by some central body sensor(s); the mechanisms I discuss here can detect and/or produce signals that are proportional to body weight changes transmitted across musculoskeletal designs. I will address mainly animal systems here, but would like to point out that strong parallels exist between mechanobiology in animals and plants (for recent plant biomechanics reviews, see Moulia, 2013; Niklas et al., 2006). A more thorough mechanistic understanding of body-weight-sensitive molecular-level changes to the musculoskeletal system will greatly improve our understanding of the basic biology of musculoskeletal design and function during growth and across animal size ranges, and of disease (and aging) phenotypes that impair locomotion.

Body weight, musculoskeletal design and locomotion

The proportionality of animal motor force output to body mass is central to the principles of dynamic similarity and stress similarity (see Box 1) that are pervasive in comparative biomechanics and were first applied to animal locomotion by Robert McNeill Alexander (Alexander, 1976; Alexander and Jayes, 1983; see also Alexander, 2003) and Andrew Biewener (Biewener, 1989; see also Biewener, 2005), respectively. The dynamic similarity principle (and the associated Froude number) allows comparisons of the locomotion mechanics of roughly geometrically similar animals of varying body size and movement speeds (Alexander, 2003), and it defines most terrestrial animal gaits well (Biewener, 2003). Similarly transformative, the assumptions of body weight proportionality of musculoskeletal force output and similarity of peak musculoskeletal stresses among terrestrial mammals led to the realization that the postural changes [i.e. changes in effective mechanical advantage (EMA); Biewener, 1989] that are observed with size in terrestrial mammals serve as a means to compensate for unequal scaling of body mass and cross-sectional area of muscles and bones. That is, to maintain appropriate safety factors (the ratio of fracture stress to peak operating stress) in their musculoskeletal designs, larger animals adopt a more erect posture to reduce the length of the moment arms of ground reaction forces relative to those of smaller animals (which have a more crouched posture), hence reducing the peak stresses experienced by their muscles, tendons and bones (see Box 1). An exception to this general trend seems to be macropod marsupials (McGowan et al., 2008). A similar mechanical leverage solution to the variations in EMA observed in mammals has been found to operate in flight motors from dragonflies varying an order of magnitude in body weight (Schilder and Marden, 2004), but whether other non-terrestrial animals (e.g. flying, swimming and burrowing insects and vertebrates) employ analogous variable EMA strategies across size ranges remains poorly understood.

During ontogeny, large size differences also occur (although less so than across some phylogenetic lineages), yet the postural changes are typically absent or much smaller than those observed across phylogenies, and stress similarity is not generally maintained during

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Box 1. Allometry and similarity concepts in biomechanics

Allometry, geometric and dynamic similarity

Animals of different sizes are considered geometrically similar when they can be made identical to one another (i.e. can be scaled isometrically) by multiplying all body dimensions by the same factor. Allometry is the study of relationships between body dimension traits (but also physiological and behavioral traits), and 'allometric scaling' refers to deviations from geometric similarity (isometry). Similarly, dynamic similarity is used to compare locomotion by animals of different size. Animal movements are dynamically similar if they can be made identical by multiplying all lengths by factor *x*, all times by factor *y* and all forces by factor *z*. The Froude number and the Reynolds number (not discussed in this Commentary) refer to dimensionless quantitative expressions that need to be similar for motions to be considered dynamically similar when gravitational and viscous forces are dominant, respectively.

Stress similarity

A related concept, that of stress similarity, predicts that animal musculoskeletal systems that vary in size, are built from similar materials and support body weight should vary in their morphology such that they experience equal stresses. The main operator of stress similarity is the effective mechanical advantage (EMA) – the ratio of muscle force and ground reaction force moment arms around a joint fulcrum. The EMA can be used in a static fashion to compare animal musculoskeletal postures, or in a dynamic fashion to analyze or compare stresses during locomotion. For terrestrial mammals, EMA scales as ~body mass$^{0.26}$, i.e. larger animals have adopted more erect postures, thus decreasing the moment arm of the ground reaction force, to maintain stress similarity. Indeed, muscle and bone stresses tend to be proportional to body mass$^{-0.06}$ and body mass$^{0.10}$ (i.e. approximately independent of body mass), respectively (Alexander, 2003), at least for terrestrial mammals. For interested readers, Alexander (2003) provides excellent and highly accessible treatises on the topics discussed above.

ontogeny in vertebrates, i.e. juveniles tend to suffer relatively high mechanical stresses in their musculoskeletal designs (Allen et al., 2010; Dial and Jackson, 2011; Main and Biewener, 2006; Smith and Wilson, 2013). Stress similarity or considerations of durability may be less important to a juvenile animal than maximizing burst performance, because of life history and environmental demands (e.g. predation, competition for resources) at an early age (Herrel and Gibb, 2006). Thus, natural selection may have favored juveniles of some species to be over-designed very early in life, and maturational growth may therefore reflect the most optimal path to an adult musculoskeletal design that adheres to stress similarity (i.e. steadily trading off burst performance with durability as animals mature). Therefore, even if ontogeny may allow for temporary deviations from adult design durability rules, generally speaking, gravitational load in the form of body weight appears to be a crucial determinant of the development and movement of all locomotor designs.

The remainder of this Commentary is primarily focused on skeletal muscle, but I would like to point the reader to an extensive literature on the application of concepts mentioned above to studies of body size effects on the architectural and functional properties of bone (for example, see Alexander, 1977; Alexander et al., 1979; Biewener, 1982; McMahon, 1975; Selker and Carter, 1989).

Molecular-level sensitivity of the musculoskeletal system to body weight variation

Because body weight affects the design of the musculoskeletal system, the corollary is that body weight should also be a crucial determinant of the designs of the major building blocks of vertebrate locomotor systems – muscle and bone. In accordance with this, whole muscle and bone properties tend to scale with body mass (Alexander, 1985; James et al., 1998; Medler, 2002; Schiaffino and Reggiani, 2011; Van Wassenbergh et al., 2007). It is also clear that variation in skeletal muscle composition at the molecular level affects muscle properties [e.g. force, maximum shortening velocity (V_{max}), etc.; James et al., 1998; Perry, 1998; Schiaffino and Reggiani, 2011], and it therefore is reasonable to assume that muscle molecular composition should scale with body mass. The majority of work in this area has been focused on myosin heavy chain (MyHC) gene and protein expression patterns. The expression pattern of different skeletal muscle fiber MyHC protein isoforms (encoded by individual genes, and used as the key marker for designations of muscle fiber type, i.e. slow, intermediate or fast) tends to correlate with animal size; the relative abundance of fast fibers decreases (i.e. showing a transition from MyHC IIB to MyHC IIX/A to MyHC I) as body mass increases (Pellegrino et al., 2003; Marx et al., 2006; Schiaffino and Reggiani, 2011). Another example in this area is work by Coughlin and colleagues (Coughlin et al., 2001; Campion et al., 2012) on several species of centrarchid fish. Observed decreases in the V_{max} of locomotor muscles with body size during fish development correlate with changes in MyHC protein isoform expression. During early mammalian growth, similar highly predictable transitions between MyHC proteins (from the embryonic to the neonatal and finally to the adult form) can be observed in skeletal muscle (Schiaffino and Reggiani, 2011; again, all three proteins are encoded by different genes). Unfortunately, much less is known regarding the functional characteristics of the products of the mammalian MyHC genes that are expressed in early development. To the best of my knowledge, most of the MyHC molecular level work has focused on qualitative shifts in expression in response to changes in functional demand during life history, as well as in response to experimental muscle loading regimes (e.g. unloading, stretching of muscle; Goldspink et al., 1992; Pette and Staron, 2000). Whether MyHC can serve as a quantitative marker of skeletal muscle plasticity remains to be determined.

The use of gene regulatory mechanisms (i.e. using multiple genes encoding variants of proteins or single genes encoding multiple, functionally different proteins) that allow muscle to vary its mechanical properties by changing the relative abundance of proteins involved may allow tissues (and musculoskeletal designs in particular) to circumvent or mitigate size-related geometric constraints (i.e. volume/area scaling) and enhance musculoskeletal performance to accommodate variation in body weight. Moreover, such changes in muscle properties are likely to be especially useful to animals with life histories that demand plasticity in performance due to sudden body weight variation (e.g. weight gain associated with pregnancy or pre-migratory and pre-hibernation behavior). It is likely that natural selection will favor musculoskeletal designs that are capable of accommodating such variable functional demands, and so these gene regulatory mechanisms are likely to be important determinants of animal fitness. There are therefore good reasons to deepen our understanding of why and how musculoskeletal designs are shaped by gravitational loads at a molecular scale. However, as pointed out by Scott Medler in 2002 (and this situation has not changed much since then; Medler, 2002), with the exception of some of the work on MyHC (mentioned above), there is a general paucity of data relating the molecular composition of muscle to the variable functional demands posed by body weight variation. Moreover, even fewer studies have empirically tested whether sarcomere molecular designs are directly and quantitatively sensitive to naturally occurring body weight variation.

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An evolutionarily conserved, quantitative marker of muscle responses to body weight variation

What sort of molecular-level changes might we expect to result from changes in body weight? One potential mechanism linking body weight variation with muscular function is alternative splicing (Box 2) of muscle-specific genes. As an extension of earlier work (e.g. Marden et al., 1999, 2001) on the relationship between flight muscle performance and alternative splicing of the sarcomere gene troponin T (e.g. Marden et al., 1999, 2001), Marden and colleagues (2008) addressed the question of whether muscle is sensitive to body weight at a molecular level, and demonstrated that alternative splicing of troponin T in fall armyworm moth flight muscle responds rapidly (i.e. within 5 days) and in a quantitative and precise manner to experimental manipulations of body weight (Fig. 1A). Experimental weight loading of adult moths affected the alternative splicing of flight muscle troponin T in a manner that was nearly identical to the effect of equal increases in actual body weight. Alternative splicing of troponin T in response to altered muscle use (i.e. unloading; Stevens et al., 2002; Yu et al., 2006) had been demonstrated previously, but the precise quantitative relationship between body weight and alternative splicing of troponin T was novel.

Box 2. Alternative splicing

A ubiquitous gene regulatory mechanism

Alternative splicing is a ubiquitous mechanism that produces multiple mature mRNAs (splice variants) from a single genetic locus through selective inclusion or exclusion of exons and/or introns during pre-mRNA transcription (Andreadis et al., 1987). Alternative splicing serves critical roles in metazoans and, in most cases, it results in expression of protein isoforms with different biological properties, including altered protein–protein interactions, subcellular localization and/or catalytic activity. Thus, alternative splicing allows modulation of tissue function without altering the overall molecular stoichiometry – e.g. in response to loading, alternative splicing allows the distribution of troponin T protein isoform in skeletal muscle to change while overall troponin T expression relative to that of other sarcomere proteins remains the same. This mechanism may be especially important in the context of highly organized skeletal muscle sarcomeres in providing a way to vary muscle properties by changing the protein isoform composition involved rather than through additional muscle mass investments.

Organismal complexity and the proportion of alternatively spliced genes appear to be correlated; current estimates indicate that between ~60% and 95% of genes are alternatively spliced in *Drosophila* (Graveley et al., 2011) and humans (Pan et al., 2008), respectively. Inappropriate alternative splicing events are associated with many human disease conditions, such as cancer, spinal muscular atrophy, Alzheimer's disease and myotonic dystrophy.

Regulation of alternative splicing

Splicing reactions are catalyzed by the spliceosome (Black, 2003), a large dynamic protein complex consisting of five small nuclear ribonucleoprotein particles and numerous auxiliary proteins. Spliceosome localization and subsequent exon and/or intron exclusion or inclusion are, to a large degree, determined by specific exonic and intronic RNA sequence elements. Depending on their location and effect, these elements are classified as exonic splicing enhancers (ESEs) or silencers (ESSs), and as intronic splicing enhancers or silencers. In general, these RNA sequence elements function by binding trans-acting splicing factors (RNA-binding proteins) that activate or suppress splice site recognition or spliceosome assembly by various mechanisms. These splicing factors are typically divided into two groups: the serine-arginine-rich proteins and heterogeneous nuclear ribonucleoproteins (e.g. Licatalosi and Darnell, 2010). Alternative exon splicing is generally determined by the ratio of ESE- to ESS-bound members from the two groups.

Alternative splicing is a gene regulatory mechanism that generates multiple different transcripts from the coding region of a single gene; it is thus a highly efficient means of enhancing the functional diversity of the proteome. Alternative splicing of troponin T affects muscle performance by modulating the Ca^{2+} sensitivity of muscle contraction (Brotto, 2005; Gomes et al., 2004; Ogut et al., 1999). Extrapolating variation in Ca^{2+} sensitivity in muscle fiber preparations to whole muscle and animal function is non-trivial, but generally speaking, higher Ca^{2+} sensitivity should cause muscle to contract sooner and remain active for longer during each neurally induced Ca^{2+} wave, thereby modulating both mechanical performance and energy (i.e. ATP) consumption. Thus, by varying the relative abundance of troponin T mRNA splice variants, animals can make continuous, fine-scale adjustments to both muscle performance and energy consumption rate (Marden et al., 2008).

Recently, my collaborators and I have obtained nearly identical findings for rat gastrocnemius muscle and fruit fly leg muscles. Rats varying in body mass from ~100 to ~300 g show strong linear correlations between body mass and alternative splicing of troponin T in the gastrocnemius muscle during ontogeny. Importantly, in response to experimental body weight manipulation by means of a weighted harness (Schilder et al., 2011) (Fig. 1B), rat gastrocnemius adjusts the alternative splicing of troponin T in a precise fashion, dependent on laden body weight. Weighting fruit flies in this manner is problematic, so, instead, we use a custom-made centrifuge device to expose fruit flies to higher ***g***-forces. *Drosophila* lines that differ in body mass (~0.5–0.8 mg) and undergo a 24 h hypergravity treatment show alternative splicing of troponin T in leg muscle that is strongly correlated with body mass and, therefore, with the actual body weight loads that the legs of these flies experienced during experiments (R.J.S. and M. Raynor, unpublished data). Importantly, in each of the different animal systems examined, the troponin T splice variants showing the strongest correlations (see Fig. 1A,B,D) with natural and experimentally applied body weight variation are those known to convey higher Ca^{2+} sensitivity and force output to muscle fiber preparations. That is, in response to increased body weight, we observe increased abundance of relatively short troponin T splice variants in insect systems (Marden et al., 1999), and of those missing exon 4 and containing exon 16 (Briggs and Schachat, 1996; Gallon et al., 2006) in rodent systems (Fig. 1D). As with the centrarchid fish example mentioned above, increased muscle Ca^{2+} sensitivity associated with body size increase also fits with the observation that V_{max} generally is proportional to body mass$^{-0.12}$ (i.e. smaller animals tend to have faster muscles; Medler, 2002). That is, higher Ca^{2+} sensitivity conveyed by troponin T splicing changes should reduce the rate of cross-bridge turnover during contraction and, hence, the contraction velocities that are possible. Together, these results indicate that the pattern of alternative splicing of troponin T in load-bearing muscle is a highly sensitive marker for load-dependent skeletal muscle plasticity, and that these splicing events represent the output of an evolutionarily conserved mechanism that allows load-bearing muscle to precisely adjust its molecular design – and, as such, performance – to variable body weight.

Interestingly, this mechanism appears to be impaired in mammalian obesity (Schilder et al., 2011) (Fig. 1C) and in naturally occurring metabolic disease in insects (Schilder and Marden, 2006; R.J.S., unpublished observations), both of which are associated with reduced muscle performance and mobility. Similarly, aging in humans impairs the ability of muscle to tune

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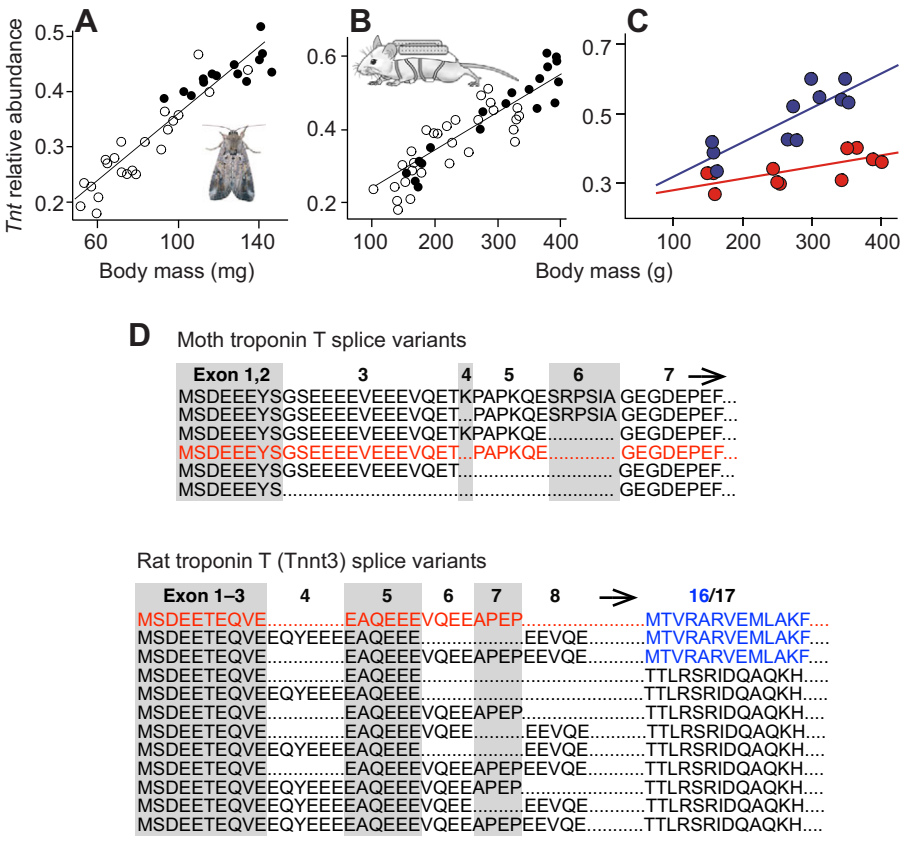

Fig. 1. The relative abundance of troponin T splice variants is responsive to variation in body weight. Weight-loaded (filled circles) moth (A) and rat (B) muscles adjust the abundance of the major troponin T (*Tnt*) splice variant (highlighted in red in D) to match a natural growth-related (open circles) weight-dependent trajectory. Attached weight loads increased body weight by ~30% and ~120% for rats and moths, respectively. (C) This adjustment is impaired in obesity-prone Zucker rats (red circles) compared with non-obese Zucker rats (blue circles). (D) Amino acid sequences of the 5′ end of moth and rat troponin T showing all expressed splice variants. In addition, the rat troponin T gene contains a set of mutually exclusive exons at the 3′ end [exons 16 (blue) and 17 (black)], allowing for many more potential splicing combinations to be produced.

troponin T alternative splicing to body weight in a manner that correlates quantitatively with age-associated reductions in mass-specific muscle performance (Coble and Schilder et al., 2015). Given that >90% of human genes (including many related to sarcomere function) undergo alternative splicing, it is likely that troponin T is just one of many genes expressed in the musculoskeletal system that are responsive to body weight.

Mechanosensing

By what mechanism might variations in body weight result in molecular-level changes in the musculoskeletal system, such as alternative splicing? In my opinion, there are several research areas addressing how mechanical signals may control animal movement and body weight support that point to avenues of research to answer this question. These potential mechanisms are not mutually exclusive; in fact, they are likely to function in combination. Note again that these mechanisms do not concern the detection or monitoring of an absolute, systemic body weight signal by the body, a possibility that has not yet been examined. The research areas detailed here concern local detection of signals that are likely to be proportionally equivalent to body weight change as it is transmitted across the entire support structure. Below, I attempt to connect the concepts in whole-organism biomechanics discussed above to recent insights from cellular biomechanics and bioengineering, and to recent progress in the study of mechanosensitive biochemical signaling pathways controlling musculoskeletal design at the molecular level. I also discuss the possible roles of the musculoskeletal mechanosensory organs.

Tensegrity and mechanotransduction

Over the last 30–40 years, significant developments within the realm of mechanotransduction (i.e. the area of research concerned with how cells sense mechanical forces and convert them into biochemical and gene expression changes; e.g. Burkholder, 2007; Paluch et al., 2015) have led to the adoption and application of the ‘tensegrity’ (tensional integrity) principle to biological structures and systems. The tensegrity principle views animal bodies as interconnected networks of compression- and tension-resistant and tension-generating structures (Box 3). At rest, these networks are under ‘pre-stress’ because of a basic (isometric) tension generated between the structures (Ingber, 1997) to maintain body support. External mechanical forces (e.g. through body movement or other forms of loading) can deform these structures, which then rearrange, recoil (see also Alexander, 1988) and/or remodel.

This principle also applies to cellular-level dynamics (Ingber, 1997). Cells are thought to be under similar pre-stress because of the highly organized architecture of their internal cytoskeletons. Cells in tissues are connected to each other by means of extracellular matrix (ECM) materials that directly connect to the insides of individual cells through membrane-spanning integrins, which tend to be concentrated in areas called focal adhesions. Any mechanical forces associated with body movement or loading can thus be transmitted through these connections to cells, which can then absorb and recoil from the forces. The idea is that when mechanical stresses become excessive or sustained, cellular structures can remodel themselves (Ingber, 2008) to achieve new (local) pre-stress set points and molecular designs. This can happen quickly, because the ECM and cytoskeleton molecules can self-assemble and disassemble in response to such stresses. Additional remodeling responses of cells are made possible by the fact that many proteins involved in signal transduction are directly associated with the internal cytoskeleton, integrins and ECM. Mechanical stresses can activate these signal transduction pathways and achieve biochemical and gene expression changes. Thus, tensegrity architectures can be

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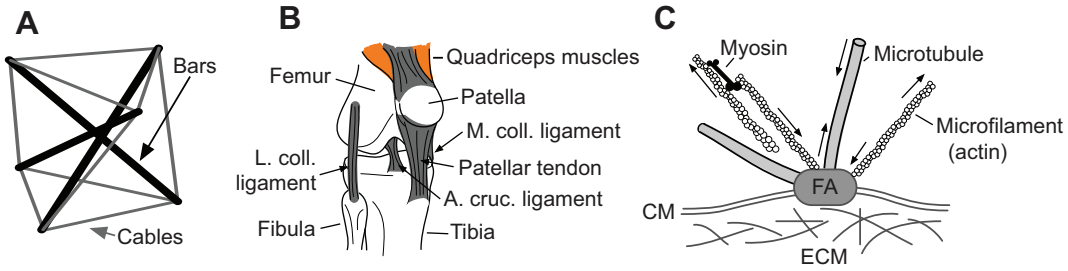
Box 3. Tensegrity structures in architectural and animal systems

Classic tensegrity



Originally an engineering principle described by Fuller (1961), tensegrity structures can be described as interconnected networks of tensed (or pre-stressed) elements that are stabilized by additional elements that resist compression. That is, the pre-stressed elements provide isometric tension to position their joints with compression-resistant elements. Consequently, the freedom of movement of the entire structure is constrained in response to mechanical stress along minimal paths (Ingber, 2003). To illustrate, in panel A, the cables provide pre-tension and bars provide compression resistance. One can readily imagine how muscle–tendon–bone interactions in vertebrate joints (panel B) and across the entire body can similarly determine default joint shape and allow responses to mechanical stress inputs (i.e. movement) in one direction, but limit them in another; muscle–tendon units would generate pre-stress, with bones giving compression resistance. Therefore, it should be noted that tensegrity not only describes static structures. Instead it predicts the dynamics and, by extension to the cellular level (see below and 'Tensegrity and mechanotransduction' section above), plasticity of tensegrity systems.

Cellular tensegrity

Ingber (1993) and, over the years, many of his colleagues extended the tensegrity concept to the cellular level and showed that it can help to explain how cell shape, movement and cytoskeletal mechanics are controlled, as well as how cells sense and respond to mechanical forces. Cellular tensegrity (panel C) assumes that cells are the pre-stressed tensegrity structure. In this view, cytoskeletal microfilaments and intermediate filaments generate (using actin–myosin interactions) and bear tension forces that are balanced by interconnected, compression-resistant microtubule struts, cell-to-cell adhesions and ECM [to which the cytoskeleton is connected through focal adhesions (FA)]. Cellular pre-stress can also be generated passively by means of adhesion to motile adjacent cells and by osmotic forces acting on the cell membrane (CM) to which the micro- and intermediate filaments connect. This view of organisms as consisting of interconnected tissues built from interconnected cells with highly structured and plastic internal organization provides a physical (and molecular) basis for how external and internal mechanical forces may be translated into physiological responses at multiple levels of biological organization.

seen to act as mechanosensors, whose values (or tension or compression states) and cellular context determine the level of adjustments made to cellular (and, consequently, tissue level) design and function. How animal designs respond to body weight variation in this sense is the sum of how their individual cells respond to locally acting mechanical forces in a quantitative fashion.

To my knowledge, there is currently no formal way to model or predict complex musculoskeletal designs and their behavior or evolution based on tensegrity 'rules', but it should be possible to develop such models. At a minimum, tensegrity should be a very attractive principle to whole-organism and musculoskeletal biomechanists, because it implies that changes in tissue biochemistry and gene expression that determine, for example, the strength and elasticity of biomechanical designs are first and foremost dictated by mechanical forces and their transmission by (molecular) lever systems.

Muscle plasticity

Muscles are organs that can perhaps be considered the epitome of tensegrity, as they consist of fused cells packed full of specialized cytoskeletal structures called sarcomeres that produce forces and movement when constituent flexible proteins slide along each other between relatively stiff protein struts (e.g. Z-discs). Muscle cells display a relatively high potential for remodeling or plasticity, which, as in other cells, is achieved through their extracellular connection to the ECM by integrins (Burkholder, 2007). Over the last few decades, much has been learned about the role of mechanotransduction in skeletal muscle plasticity. Forces imposed on and generated by muscle during contraction *in vivo* are transmitted to the ECM, from where biochemical signals activate intracellular signaling cascades (Jaalouk and Lammerding, 2009). Among these, integrin-linked kinase and focal adhesion kinase (FAK) can signal through mitogen-activated protein kinase [MAPK; e.g. p38 MAPK, ERK (Martineau and Gardiner, 2001) and Akt/mTOR/S6K signaling cascades (Clemente et al., 2012; Klossner et al., 2009)]. These are strong candidate pathways for the control of plasticity in muscle mass and fiber type (Bassel-Duby and Olson, 2006; Durieux et al., 2009; Hanke et al., 2010), and are sensitive to nutritional signals and stress and growth factors such as insulin-like growth factor-1 (IGF-1). IGF-1 also has Akt/mTOR/S6K-independent, positive effects on skeletal muscle mass through replenishment of the muscle stem cell pool mediated by mechano growth factor (MGF; reviewed in Goldspink, 2005), an autocrine growth factor produced by alternative splicing of the IGF-1 pre-mRNA in response to mechanical stimulation (e.g. exercise).

In heart muscle, Akt/mTOR signaling also regulates alternative splicing of the giant protein titin, which is thought to act as a sarcomere-based mechanical stretch sensor (Granzier et al., 2007); this alternative splicing regulates passive muscle stiffness (Ottenheijm et al., 2009). Moreover, my recent work with collaborators in this area indicates that inhibition of Akt signaling prevents mechanically stimulated alternative splicing of troponin T following cyclic stretching of skeletal muscle myotubes growing on flexible collagen-coated membranes (Schilder et al., 2012). This suggests once more that cell-autonomous mechanisms may ultimately control the body-weight-dependent alternative splicing patterns we observe.

In addition to these intracellular mechanosensitive mediators of muscle plasticity, mechanical loading of muscle also results in the release of systemically acting molecules (myokines) by muscle, including myostatin and several interleukins that affect muscle growth (reviewed in Pedersen and Febbraio, 2012). Myostatin is an

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interesting myokine with respect to our observations of body-weight-sensitive alternative splicing of troponin T, because the transforming growth factor (TGF)-β superfamily of growth factors – of which myostatin is a member – is also known to regulate alternative splicing events. Thus, there is at least a potential for systemic factors to function as quantitative signals of the body weight (load) perceived by skeletal muscles, even if this has not been examined in any detail.

Overall, there appears to be crosstalk between the mechanosensitive pathways controlling muscle mass, fiber type and sarcomere gene alternative splicing. This is not surprising, but it does complicate the clean dissection of mechanisms responsible for body-weight-dependent adjustments to muscle molecular design.

Neural input

Much of the data discussed above focuses on cell-autonomous mechanisms controlling musculoskeletal responses to body weight variation, but there is evidence that neural factors may also contribute in important ways. Neural input is likely to be an important determinant of skeletal muscle composition, as cross-innervation experiments and neural interventions have shown that changes in muscle fiber type (and therefore protein composition, e.g. troponin I isoform changes; Calvo et al., 1996) can be achieved by varying the rates and timing of depolarization of muscle (Buller et al., 1960; Pette and Staron, 1990; Pette and Vrbová, 1985). Presumably, these effects occur as a result of the induction of specific transcription factor activity in response to these neural patterns. Similarly, it has been demonstrated that Ca^{2+} released following load-induced neural activity can activate the calcineurin–HDAC–NFAT–MEF2 pathway, which controls the gene expression patterns of MyHC and other sarcomere genes (Metzger, 2002; Pandorf et al., 2009; Wu et al., 2000). The question is, can neural signals convey quantitatively accurate information regarding body weight?

In a study aimed at determining how mechanical stress due to intra-peritoneal implantation of inert masses may control body weight plasticity in deer mice (*Peromyscus maniculatus*), Adams et al. (2001) suggested that quantitative neural feedback mediated by muscle spindles and Golgi tendon organs could be responsible for the new body weight set points that they observed in these mice after removal of the implants. Adams et al. (2001) provided only indirect evidence to support their suggestion, and, to my knowledge, no studies have examined the possibility of such additional functionality for these sensors. However, for invertebrates, there is a body of literature that provides evidence for a role for these types of sensors in body-weight-dependent adjustments to posture and locomotion and, possibly, given the above-mentioned effects of innervation patterns on muscle fiber types, to skeletal muscle composition.

Insect campaniform sensillae are sensory organs that are analogous in function to vertebrate Golgi tendon organs (Fig. 2). In insect legs, these sensors detect cuticular strains caused by changes in weight loads on the legs (Pringle, 1938), but they are found on almost every other body part as well (Snodgrass, 1935). Sasha Zill's laboratory in particular has studied these sensors extensively with regard to their role in body weight sensing (reviewed in Zill et al., 2004; see also Zill et al., 1981) and, relevant to the issue at hand, work by Zill and colleagues has demonstrated that, in cockroaches, these sensors produce neural discharges with frequencies proportional to the amount of total body weight experienced (Noah et al., 2004). Because campaniform sensillae can modulate the activity of motoneurons in a reflex manner (Zill et al., 1981), and motoneuron activity affects muscle protein composition (see above), these organs may be perfectly suited to provide body-weight-proportional neural feedback to muscle. Campaniform sensillae are mainly studied with respect to short-term (body-weight-dependent) postural and locomotion control (e.g. Zill et al., 2004), but one could readily conceive of experiments that examine whether they can facilitate muscle plasticity in the face of chronic leg loading that may occur during the life history of many insects. Similar to campaniform sensillae, vertebrate Golgi tendon organs (Fig. 2) can directly affect motoneuron activity patterns via interneurons in the spine, so there is the potential for Golgi tendon organs to affect muscle fiber composition through similar means as demonstrated for vertebrate cross-innervation experiments (see above). The outcomes of experiments in insect systems could have significant implications for additional roles of the analogous vertebrate sensors.

Conclusions

It will be interesting to see whether we can start answering the question of how animals 'know' how much they weigh more generally than at the level of alternative splicing of troponin T by adding to the number of empirical datasets demonstrating that musculoskeletal (including muscle, bone and tendon) traits are plastic in proportion to quantitative variation in experienced loads. At the same time, it is my hope that at least some readers will agree that the questions of how animals know how much they weigh and how they make the appropriate adjustments to their musculoskeletal designs need to be studied across levels of biological organization.

Research at different levels of biological organization clearly indicates that body-weight-sensitive sensors and pathways exist, but our understanding of how their signals are integrated and translated into useful modifications to an organism's support structure and locomotor design is still quite rudimentary. The tensegrity paradigm

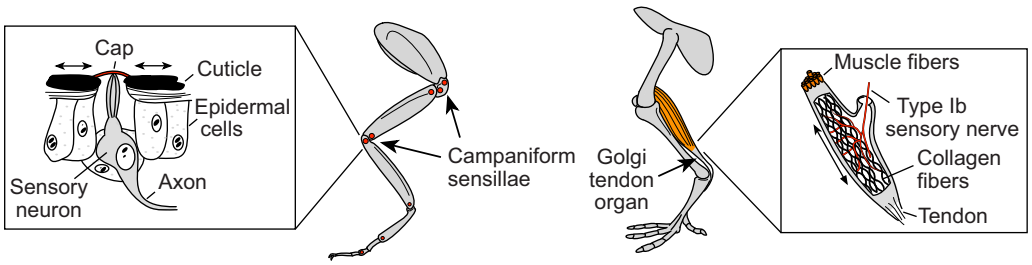

Fig. 2. Body-weight-sensitive force sensors in insects and vertebrates. Left, changes in externally or internally applied loads on the body of insects (indicated by double-headed arrows) result in cuticular strain and are detected as shape changes in the 'cap' of campaniform sensillae. In addition to the central nervous system (CNS), their associated axons can interact in a reflex manner with motoneurons controlling muscle force output and locomotion. Right, in vertebrates, muscle force production is detected in Golgi tendon organs (located at the intersection of muscle fibers and tendon) through compression of type Ib sensory nerve endings by muscle-contraction-induced stretching (indicated by double-headed arrow) of collagen fibers. As with campaniform sensillae, type Ib sensory nerves synapse with interneurons connecting to motoneurons and the CNS.

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provides us with an integrative platform to enhance this understanding, given its ultimate dependency on mechanical force input, a unit familiar to all who work in the relevant research areas. It will be very interesting to see whether and how tensegrity can incorporate or be integrated with the wealth of information generated by the fields of tissue- and organismal-level biomechanics and physiology. Even at the cellular level, many questions come to mind. For example, are cells, tissues and organs adjusting to mechanical stress by trial and error, or are there inherent mechanical stress set points that control decisions on cellular remodeling? How are such set points set and monitored, and (how) do disease and environmental factors affect them? Do the durability principles (e.g. stress similarity, safety factors) that are so pervasive in whole-animal biomechanics exist, and do they apply when we move from cellular to organ and whole-organism levels? Can we translate the EMA principle to study mechanical leverage at the cellular level as well? Answering such questions will require more intense collaboration between fields of organismal, tissue and cellular biomechanics.

In particular, at the tissue and cellular levels, research relating mechanical signals to design has mainly focused on traditional model species and mechanisms that control biomedically relevant traits in biomedically relevant contexts. But it is also evident that studying natural non-traditional systems in a comparative fashion can provide us with insights that laboratory cultures may not. Although a paradigm shift from solely gene-focused thinking to acknowledging the role of mechanical forces as master regulators of organismal traits is evident, we need to be aware of the incredible wealth of genomics and transcriptomics data that are rapidly becoming available for many non-model species (e.g. Misof et al., 2014). Such datasets can allow researchers to bypass the (classically time-consuming) need to obtain relevant and detailed sequence information for genes of interest. Thus, an understanding of the size and weight sensitivity of musculoskeletal design (e.g. at the level of alternative splicing or other gene regulatory control of sarcomere, bone and tendon genes) across phylogenies has become feasible within a reasonable time frame, given appropriate collaborations. Similarly, knowledge about the function of mechanosensitive intracellular pathways is more valuable when combined with data showing how the amino acid coding sequences (and hence, for example, protein kinase activation potential) of the signaling pathway components may evolve. Vice versa, genetic associations with quantitative traits such as body weight and muscle mass mean little without a biochemical, physiological and evolutionary context. Collaborative and integrative studies of how animal designs accommodate body weight variation, across levels of biological organization in different species as well as across evolutionary time scales, will allow us to deepen our understanding of the evolution, plasticity and longevity of animal designs in the face of variable diet, lifespan and environmental stressors. These are important issues that will be relevant to both basic biology and biomedical research.

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