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Muscle-Tendon Crosstalk During Muscle Wasting

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Abstract

In organisms from flies to mammals, the initial formation of a functional tendon is completely dependent on chemical signals from muscle (myokines). However, how myokines affect the maturation, maintenance, and regeneration of tendons as a function of age is completely unstudied. Here we discuss the role of three myokines – fibroblast growth factors (FGF), myostatin, and the secreted protein acidic and rich in cysteine (SPARC) – in tendon development and hypothesize a role for these factors in the progressive changes in tendon structure and function as a result of muscle wasting (disuse, aging and disease). Because of the close relationship between mechanical loading and muscle and tendon regulation, disentangling muscle-tendon crosstalk from simple mechanical loading is experimentally quite difficult. Therefore, we propose an experimental framework that hopefully will be useful in demonstrating muscle-tendon crosstalk *in vivo*. Though understudied, the promise of a better understanding of muscle-tendon crosstalk is the development of new interventions that will improve tendon development, regeneration, and function throughout the lifespan.

Introduction

Muscle wasting occurs as a result of disuse, aging and disease. Muscle disuse can result from traumatic injury (such as nerve crush or spinal cord injury), forced inactivity (bed rest, immobilization, or pandemic-induced inactivity), or voluntary changes in activity (such as athletes during the offseason or a change in lifestyle or job). The loss of muscle mass resulting from disuse, aging, and disease is associated with decreased muscle function (24, 71, 75). Interestingly though, similar amounts of muscle wasting can result in large differences in muscle function, for example in young and old individuals (2). Specifically, adult and old animals lose approximately the same amount (~30%) of muscle over a two week period of hindlimb unloading; however, the old animals lose 50% of force production, whereas the adult animals only lose only 10% of their strength (2). Further, during normal aging muscle strength is lost at a rate 3-times faster than muscle mass (25). These data suggest that strength loss is dependent on more than just a loss of contractile proteins from muscle and suggest that force transfer through the matrix and tendon to the bone may play a role in strength loss.

A significant amount of research has been devoted to elucidating the cellular mechanisms underlying muscle atrophy. Mechanical loading prevents the cell signaling associated with muscle atrophy (34, 37) and more importantly short bouts of neuromuscular activity are sufficient to prevent muscle wasting (13). Interestingly though, whereas neuromuscular electrical

stimulation prevents muscle atrophy, it is not enough to prevent the loss of strength that occurs with immobilization (13). Like muscle, strength training increases and detraining decreases tendon size and mechanical properties (42). However, whether the change in tendon size and function are strictly the result of the altered mechanical environment, or whether there is molecular crosstalk between muscle and tendon has yet to be determined. Muscle-tendon crosstalk has been elegantly demonstrated during development of the of the muscle-tendon unit (8, 16, 64) and we will use this excellent work to describe how muscle-tendon crosstalk could function within the adult. However, few studies have determined whether the developmental signals between muscle and tendon are modulated during muscle wasting in the adult.

We found only a handful of studies where muscle derived or circulating factors were shown to increase tendon-specific markers or tendon strength. Because of the complexities of isolating mechanical and chemical signals *in vivo*, both studies used cell culture for the initial experiments. In the first, Ghebes and colleagues used a transwell system to show that co-culturing human hamstring tendon cells with muscle cells increased the expression of tendon specific markers like scleraxis and tenomodulin, whereas osteoblast or chondrocyte co-culture had no such effect (23). Further, longer term co-culture of tendon cells with muscle cells increased the amount of collagen the tendon cells produced, suggesting that a factor secreted from muscle

had an anabolic effect on tendon. In the second study, young men provided a blood sample before and 15 minutes after a heavy strength training session, the serum was isolated, and the effect of the different sera was determined using an engineered human ligament model (74). Engineered ligaments grown in the post-exercise serum contained more collagen and were mechanically stronger than those grown in the resting serum, again suggesting that a circulating factor released from active muscle could improve tendon size and strength. Given that both studies suggest that a circulating factor can influence tendon size and strength, the next question is what is that factor and how does it work? In principle, the muscle-derived factor could be a protein that is produced in muscle cells under load that diffuses or circulates to the associated tendon cells. As we will see below, this is what happens during development. Alternatively, the muscle-derived factor could be exosomal, potentially a microRNA (miR) or long non-coding RNA that alters mRNA quantity or translation in the tendon cell. Either way, the protein or exosomal factor would help coordinate the functional status of both the muscle and tendon.

In the current review we will discuss muscle-tendon crosstalk in development, potential mediators of muscle-tendon crosstalk in the adult, and what would be required experimentally to identify the factors beyond mechanical load that drive muscle-tendon crosstalk.

Muscle Tendon Crosstalk in Development

Much of the early work in the development of the muscle-tendon unit was performed in *Drosophila*. This work was done in *Drosophila* since each muscle connects to a specialized epidermal attachment cell called a tendon cell. Before the discovery of scleraxis (*Scx)*, a molecular marker of tendon precursor cells in mammals (63), there was no way to determine whether changes in muscle altered tendon formation in rodent models. In *Drosophila*¸ the fate of the tendon and muscle cells could more easily be tracked, leading to early progress in the molecular understanding of the development of muscle-tendon unit.

Stripe **in Muscle/Tendon Development**

The earliest example of muscle-tendon crosstalk during development involves the *stripe* genes In *Drosophila, Stripe* is a tissue-specific gene found in tendon precursor cells that is required for tendon cell differentiation. The *stripe* gene is necessary to initiate the differentiation program of epidermal muscle attachment (tendon) cells and in the absence of *stripe* these cells do not differentiate (3). Expression of *stripe* transforms epidermal cells into tendon cells, and these cells initiate myotube adhesion to their target cells through the *stripe*-regulated production of thrombospondin, a tendon cell produced extracellular matrix (ECM) protein (66). The *stripe* gene has been shown to produce two splice variants, *stripeA* and *stripeB* (19). *StripeB* defines the tendon precursor cells, and its levels are held constant

throughout development. By contrast, *stripeA* expression increases in the muscle-bound tendon cell following adhesion of myotubes to a tendon precursor cell. The increase in *stripeA* leads to tendon cell differentiation (70). The upregulation of *stripeA* in tendon cells is established by the expression and secretion of the paracrine myokine *vein* by muscle cells. *Vein* produced in the muscle cell is secreted and binds to the epidermal growth factor receptor (EGFR) on the surface of the attached tendon cell (Figure 1). Binding of *vein* to the EGFR activates ERK1/2 signaling (49) and this promotes the high level and sustained expression of *stripeA* in the differentiated tendon cell (78). Thus, autonomous *stripeB* expression in early development defines the tendon precursor cell and initiates myotube attachment. Once contact is made, *vein* is released from the muscle cell and drives the expression of *stripeA* that is necessary for differentiation and maturation of the interface.

FGF Signaling in Muscle/Tendon Development

Whereas in Drosophila *vein* is released from muscle and drives tendon development, in mammals the functional homologs are the fibroblasts growth factors (FGFs). During development, FGF4 and/or 8 are secreted from the myotome and are necessary for the formation of *Scx* expressing tendon progenitors. When FGF4 or 8 are secreted from the myotome they act in a paracrine fashion on cells within the sclerotome where they bind to FGF receptors. As with *vein* in *Drosophila*, FGF receptor activation leads to the

phosphorylation and activation of the mitogen activated protein kinases (ERK1/2) and subsequent activation of the ETS-domain transcription factors *Pea3* and *Erm*, resulting in *Scx* expression (5). Using FGF coated beads, Brent and Tabin demonstrated that they could establish ectopic expression of Scx in a *Pea3*-dependent manner. Consistent with the role of muscle-derived FGF in tendon formation, the expression of *Pea3* and *Sprouty*, another FGF target gene, is diminished in tendons of *muscleless* mice, demonstrating that *Pea3 and Sprouty* gene expression is dependent on the presence of muscle (18). The FGF signal coming from muscle is also proportional to the contractility of the muscle. In the absence of muscle contraction, the expression of tendon related FGF signaling components are decreased (31). In support of the role of contraction on FGF secretion from muscle and the development of tendon, in the absence of muscle contraction, the addition of mFgf4-expressing retroviruses to immobile limbs can rescue ERK1/2 signaling and *Scx* expression (31). These data suggest that during development muscle contraction drives FGF secretion, and that FGF derived from contracting muscles binds to FGF receptors on tendon precursor cells resulting in the phosphorylation and activation of ERK1/2. The activation of ERK1/2 increases the expression of *Pes3, Erm,* and *Sprouty*, which drive the scleraxis expression required for the development of the associated tendon.

As with the *Drosophila stripe* genes, their mammalian homologues*,* Egr1 and Egr2, are also important in the development and maintenance of tendons *in*

vivo. Forced expression of Egr1 or 2 is sufficient to drive *Scx* and *COL Ia1* expression in both mesodermal and ectodermal tissues (46). Further, ablation of Egr1 decreases the expression of *Scx* and other tendon-associated genes by ~50%, suggesting that Egr1 and 2 are necessary and sufficient for tendon cell differentiation in mammals. In support of this hypothesis, Egr1 knockout mice produce tendons that have a quarter the tensile strength of WT tendons and fail to repair after injury (27). Unlike their *Drosophila* homologue *stripeA*, which is upregulated by vein, Egr1 and 2 are upregulated during tendon development by FGFs. Implanting a bead coated in FGF4 into a developing limb results in the rapid (<4 hrs) upregulation of Egr1 and 2 ahead of increased expression of tendon-enriched genes like *Scx* (6 hrs) and *COL Ia1* (24 hrs) (46). Interestingly, the regulation of Egr1 by muscle contraction is maintained in adult animals. Injection of botox to inactivate the gastrocnemius muscle of adult mice results in a rapid 60% decrease in Egr1 expression and a concomitant decrease in Scx and Col1a2 mRNA (21). Transient overexpression of Egr1 in the tendon before botox injection was able to increase the expression of Scx, Col1a1, and tenomodulin. These data suggest that Egr1 remains important in the regulation of tendon function in adult animals; however, whether Egr1 is activated directly by the mechanical load, or a secreted factor such as FGF remains to be determined.

Taken together, these data suggest that, in mammals, actively contracting muscle cells secrete FGF proteins that bind to FGF receptors on tendon cells, activate ERK1/2 signaling, and during development this signal can increase the production of *Pea, Sprouty,* Egr1 and 2. In the adult, the same ERK signal increases Egr1 and this periodic high level of Egr expression is necessary to maintain functional tendons.

FGF and Muscle Atrophy: Potential Crosstalk

Beyond their role in development, fibroblast growth factors have also been studied in the regulation of muscle mass and regeneration from injury in adults. In model organisms, FGFs play a critical role in the renewal of muscle satellite cells and therefore the maintenance of adult muscle mass (59). FGF is released following mechanical loading (10), suggesting that decreased activity could decrease secreted FGF levels concomitant with decreased tendon function. In support of this hypothesis, FGFR1 levels were found to be increased in atrophying muscles due to hindlimb suspension, and further overexpression of FGFR1 in immobilized muscle prevented muscle atrophy (15). In a model of unloading in humans, release of FGF from muscle decreased in proportion to the reduction in muscle fiber cross-sectional area (9). Further, using the exercise transcriptomic profiling tool MetaMEx (60), we find that the key FGF protein FGF8 is transcriptionally repressed by 80% following inactivity (Figure 2). Consistent with a drop in FGF8 levels, the FGF8 target protein polyoma enhancer activator 3 (PEA3), also known as ETS

translocation variant 4 (ETV4), decreases almost the same amount. These data suggest that, in inactive people there is a loss of FGF8 signaling that may play a role in the loss of tendon function; however, it remains unclear whether decreased FGF8 signaling in adult muscle is sufficient to inhibit *Pea*/Egr1 expression resulting in a decrease in the size and strength of the tendon.

Myostatin

Another myokine that is associated with muscle size and tendon function is myostatin. During development, inhibition of myostatin activity leads to increased muscle fiber size and total fiber number (45) and increases bone mass (30). In muscle, myostatin signals through the binding and activation of the ActRIIB and ALK4/ALK5 receptor complex. The activated receptor complex phosphorylates Smad2 and/or Smad3 (62). Phosphorylated Smad2 or 3 then binds with Smad4 and translocates into the nucleus to alter gene expression (12) resulting in decreased muscle mass (29, 61, 77). Physiologically, myostatin activity can be reduced through decreased expression of myostatin (33, 57), increased c-ski protein (11, 47, 58), or increased Notch activity (7). All of these are associated with an increase in muscle mass. For example, resistance exercise results in the cleavage of Notch protein at the membrane resulting in the release of the notch intracellular domain, and a decrease in myostatin activity that leads to increased expression of Akirin1 (mighty) (50). Since mighty is a direct target

of myostatin (51), this gene is an important indicator of myostatin activity. In support of the role of myostatin activity and mighty in muscle growth, the production of mighty 6 hours after resistance exercise is directly proportional to the increase in muscle mass as a result of 6 weeks of training. Together, these data suggest that increased loading results in reduced myostatin activity in the loaded skeletal muscle, which is associated with muscle hypertrophy.

In contrast, following three days of unloading, myostatin mRNA and protein levels increase significantly in humans (28). Consistent with the increase in myostatin following a short period of inactivity, MetaMEx analysis of mighty mRNA shows that akirin1 goes down ~40% following 2-5 days of inactivity (Figure 3; Pillon et al. 2020). Similarly, in hind limb suspension models, myostatin increases with unloading (6) and decreases with subsequent reloading (72). Taken in total, myostatin activity is inversely related to muscle mass such that myostatin, and its downstream targets, is upregulated during muscle wasting.

Given the fact that myostatin activity increases during muscle atrophy and that myostatin is a paracrine factor whose protein has local/global affects, it is an important factor to consider when addressing potential muscle-tendon crosstalk. Myostatin is a member of the transforming growth factor (TGF) β family. This family of more than 30 cytokines includes the growth and

differentiating factors (GDF), activins, and bone morphogenetic proteins (BMP). These proteins regulate many bodily functions including fibrosis, making them likely regulators of tendon function. In support of this hypothesis, tendons are smaller, stiffer, and hypocellular in myostatin deficient mice when compared to control mice (53). Further, treatment of fibroblasts *in vitro* with myostatin induces proliferation (53) and can drive tenogenic differentiation of C2C12 myoblasts and rat bone marrow-derived mesenchymal stem cells (44, 68). These data suggest that myostatin regulates progenitor cell differentiation into fibroblasts and their subsequent proliferation, which could be important in tendon maintenance and regeneration following injury. Further support for the role of muscle derived myostatin comes from the fact that the expression of the myostatin inhibitor follistatin in tendon decreases following injury and is further decreased with loading even though myostatin expression itself is unchanged in the tendon (17). In support of these results, Heinemeier and colleagues have shown that myostatin expression decreases with 4 days of loading in muscle, whereas tendon expression is unaffected by loading or unloading (32, 33). Moreover, myostatin is able to stimulate the differentiation of bone marrow-derived stem cells into tenocytes in a regenerating tissue (43). Using myostatin coated sutures to repair an injured rabbit Achilles tendon did not improve tendon mechanics 4 or 7 days following the repair (55). However, Muraoka and colleagues did find that the myostatin coated suture increased Collα1 and Collα2 mRNA more than 10-fold at both 4 and 7 days (55), suggesting

that the early increase in collagen production has yet to be translated into mechanical properties. Together, these data suggest that myostatin effects tendon maintenance and/or regeneration and that myostatin represents a potential mechanism of muscle-tendon crosstalk during muscle wasting. In such a model, myostatin production would increase with muscle wasting, move to the tendon, and increase collagen production and tendon stiffness. However, whether this occurs or not has yet to be demonstrated experimentally.

SPARC

The secreted protein acidic and rich in cysteine (SPARC) is a multifunctional extracellular matrix (ECM) protein that is associated with development, tissue remodeling, and the response to injury (67). SPARC is expressed during muscle development and regeneration, as well as in satellite cells and myoblasts suggesting a potentially important role in muscle (39). SPARC enhances myotube formation in differentiating C2C12 cells and increases ECM protein production during proliferation and after myotube formation (52). By contrast, inhibition of SPARC decreases ECM production throughout the myogenic process (52). Important for its potential as a myokine, muscle-derived SPARC is released during exercise (48). Given that SPARC is present in muscle, and plays a role in muscle development and regeneration, SPARC may be important in the regulation of muscle mass. In support of this hypothesis, SPARC knockdown mice have smaller myofiber diameters than

WT mice (56). This suggests SPARC is required to maintain muscle mass even when muscle is being loaded normally. Additionally, SPARC expression is significantly decreased following 14 days of unloading and increases following either 1 or 7 days of reloading, suggesting that SPARC is regulated by loading (40). In contrast, SPARC protein levels were unchanged in muscle following 12 weeks of ladder climbing and then increased within the muscle following 7 days of immobilization (65). Interestingly, even though SPARC protein increased in muscle, the circulating level of SPARC was lower in the immobilized animals, suggesting that other tissues may be receiving less SPARC when muscle is wasting. More research is necessary to determine the precise role of SPARC with regards to muscle mass regulation; however, current work suggests that SPARC is important for the maintenance of muscle mass and function (38).

As in muscle, lower SPARC is associated with smaller, less functional tendons. In mice, SPARC protein decreases with age and this is associated with a decrease in the expression of tendon-specific genes (22). To better determine the cause-and-effect relationship between SPARC and tendon function, Gehwolf and colleagues generated a whole body SPARC knockout (22). In the resulting animals, Achilles tendon diameter was smaller and cellularity was increased compared to WT controls (22). The knockout mice also had smaller collagen fibril diameters, increased tendon stiffness and a decreased maximum tensile force (22), suggesting that in the absence of

SPARC tendons became small and brittle. A follow-up study on the SPARC knockout mice showed that SPARC was essential for the response to mechanical loading in tendon. Instead of tendons becoming thicker and stronger following exercise training, Achilles tendons in the SPARC knockout mice spontaneously ruptured, suggesting that in the absence of SPARC tendons were more prone to load-induced injury. By contrast, inhibiting muscle contraction using Botox resulted in an increase in tendon-specific gene expression, and patellar tendon thickness and a decrease in cell number. Interestingly, gene expression and structure of the SPARC knockout and WT tendons looked similar with the addition of Botox, suggesting that the phenotype of a tendon attached to an inactive muscle resembles that of a SPARC knockout (73). Wang and colleagues also identified a heterozygous missense mutation in SPARC that results in impaired collagen I production and may increase the risk of tendon injury in humans (73). Even though these data are suggestive of muscle-tendon crosstalk through SPARC, to determine whether SPARC functions as a myokine that regulates tendon function, the structure/function of tendons from mice with a muscle-specific knockout or overexpression of SPARC needs to be determined.

Muscle Exosomes and Regulation of Matrix Production

Even though protein signals can drive muscle adaptations, West and colleagues, whose work showed that engineered ligaments grown in post-exercise serum made more collagen and were mechanically stronger than

those grown in serum from resting individuals, were unable to find proteins within the serum that could explain the difference in collagen production or tissue mechanics (74). This suggests that muscle-tendon crosstalk could be mediated by a different molecular mechanism. One possible mechanism is through exosomal transmission of a non-protein factor. Exercise increases the production of exosomes, nano-sized extracellular vesicles that are released from cells, by muscle (76) and exosomes can alter the production of matrix (20). Fry and colleagues have shown that satellite cell-derived exosomes can decrease the production of collagen by fibro/adipogenic precursor (FAP) cells. Removing dicer reversed the effect of the satellite cell-derived exosomes, suggesting that the inhibition of collagen production by FAPs was the result of a microRNA in the exosome. The authors went on to identify the miRNA as miR-206 and showed that removal of miR-206 resulted in an increase in the production of collagen by FAPs. Lastly, combining load and removal of satellite cells (to remove miR-206) resulted in an increase in collagen within the matrix of the muscle (20). Together, these data suggest that muscle-derived exosomes can modify collagen production within the matrix; however, whether these exosomes can affect tendon cell collagen production and mechanics has yet to be demonstrated.

If exosomal transmission of miRNAs underlies muscle-tendon crosstalk, the next question is which miRNA could mediate the effect of muscle wasting on tendon physiology? A 2018 systematic review of the effect of miRNAs on

tenocytes and tendon-related gene expression identified 12 miRNAs that met their criteria (14). Of these, the miR-29 family (a, b, or b-3p) is the best described. miR-29 was first described by van Rooij and colleagues as being down-regulated in response to a myocardial infarction and targeting mRNAs that encode proteins involved in fibrosis, including multiple collagens, fibrillins, and elastin (69). Using an anti-miR to decrease miR-29b induced the expression of collagen, whereas over expression resulted in a dose-dependent decrease in collagens Ia1, Ia2, and IIIa1. Interestingly, Hu and colleagues found a 5- to 13-fold increase in members of the miR-29 family in sarcopenic muscle (35), and metamex data indicates that miR-29a and miR-29b1 go up with inactivity (Figure 3B,C). If this increase in miR-29 in old or inactive muscle resulted in a similar effect on exosomal levels of miR-29, and these exosomes reached the tendon, the expected result would be a decrease in collagen mRNA within the tendon with age, as has been described (1, 41).

The understanding of how miRNAs could signal tissue crosstalk through exosomes is in its infancy. The understanding of how miRNAs affect tendon collagen expression and mechanical function is also poorly understood. Both areas need far more research moving forward so that we can advance our understanding of muscle-tendon crosstalk.

Demonstrating Muscle-Tendon Crosstalk

Only a small number of studies have actively looked at both tendon and muscle during muscle growth or wasting (1, 4, 32, 79). Furthermore, we are not aware of any studies that have specifically explored muscle-tendon crosstalk via cellular signals originating from adult muscle in response to anabolic/catabolic stimuli. This is a particularly important area of research since the identification of chemical signals originating in muscle that can modulate tendon structure/function would have a dramatic effect on recovery following injury or the maintenance of strength in the face of muscle wasting.

With this in mind, it is important to lay out how one could experimentally demonstrate muscle-tendon crosstalk *in vivo*. By definition, a myokine that is important in muscle-tendon crosstalk needs to be produced in muscle and should be sufficient and required for tendon adaptations to changes in muscle mass. Experimentally, this could take several forms.

1. The most effective way to remove the mechanical component completely is to use an *in vitro* system. For these experiments, proteins or exosomes isolated from muscles that are undergoing a growth stimulus (after resistance exercise or overload hypertrophy) should increase collagen synthesis and improve mechanics of cultured tendon cells or engineered tendons/ligaments. By contrast, proteins or exosomes isolated from muscles that are undergoing an atrophy stimulus (unloading, immobilization, aging) should have the opposite

effect. The constituents of exosomes could then be sequenced, and the individual components tested in the same model. Here, a specific miRNA sponge could be transfected into the tendon cells *in vitro* and the ability of the exosomes to improve collagen synthesis and mechanics measured directly.

2. To test a positive factor (such as FGF or SPARC) *in vivo*, overexpression in muscles of mice using viral transduction (26) or inducible overexpression (36) should increase collagen synthesis and improve tendon mechanics even in the absence of load. For example, overexpressing a positive factor in the gastrocnemius during hind limb unloading would still result in more collagen and a stronger Achilles tendon than a control limb that has been unloaded.
3. Similarly, to test a negative regulator (such as myostatin), that increases in muscle as the muscle gets smaller, overexpression of the myostatin inhibitor follistatin in the gastrocnemius muscle should decrease collagen and mechanics of the Achilles as has been described (54). Because over expressing follistatin would increase muscle mass, performing this experiment when the animal was unloaded would highlight the effect of muscle mass in determining tendon mass. In this example, if muscle mass drove tendon mass, unloading an animal where follistatin was over expressed would result in a bigger muscle and tendon. If load and crosstalk were more important, the muscle

would be bigger, but the tendon would have lower collagen content and mechanics.

Using these techniques, the obvious questions raised by the discussion above are:

1. Is increased expression of FGF in muscle in response to load necessary for increased *Pea3/Egr1* expression and mechanics in tendon?
2. Can FGF release from muscle overcome the negative effect of unloading on tendon gene expression and mechanics?
3. Can blocking myostatin activity during unloading decrease tendon function even while increasing muscle mass?
4. Is SPARC expression in muscle sufficient to improve tendon structure/function even in the absence of loading?
5. Does ablation of SPARC specifically from muscle prevent load-induced improvements in tendon gene expression and mechanical function?
6. What miRNAs are found within exosomes of loaded and unloaded muscles that might affect tendon function?
7. Does miR-29 released from muscle alter collagen expression in tendons?

Hopefully, this review will increase the interest in investigating shared cell signaling pathways between muscle and tendon and that the resulting novel work will provide better insight into muscle-tendon crosstalk. A better

understanding of this communication will lead to improved interventions to maintain tendon health in instances of muscle atrophy due to injury or inactivity. In the long term, such an intervention could reduce the occurrence of tendon injury and improve clinical outcomes following periods of forced inactivity or immobilization.

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Author Contributions

A.A. and K.B both wrote and revised the manuscript.

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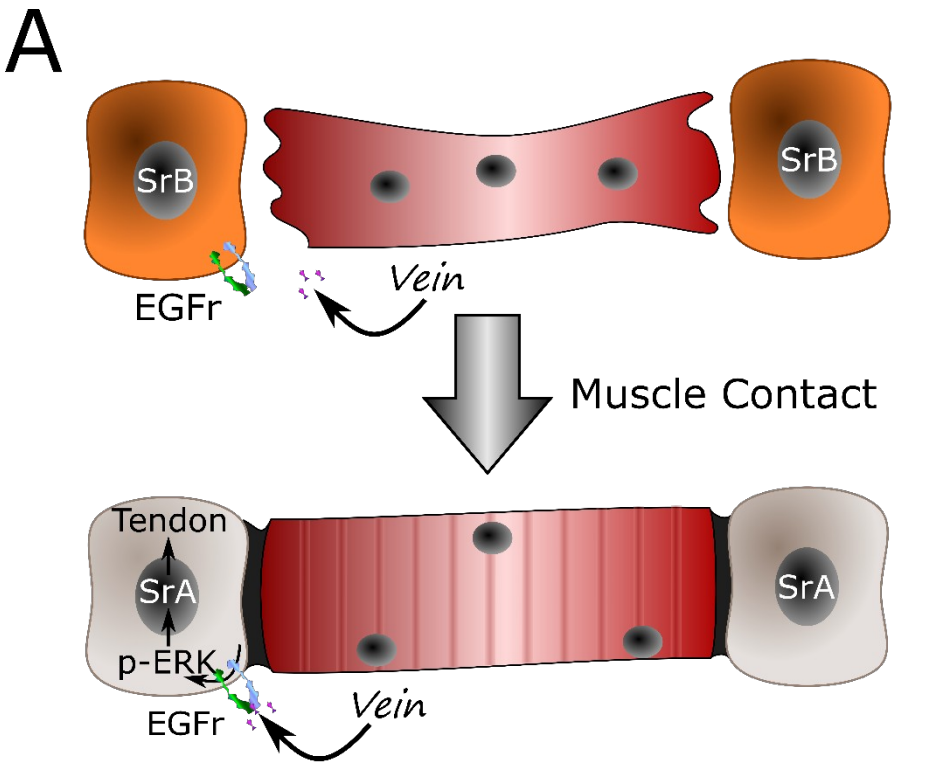
Figure Legends

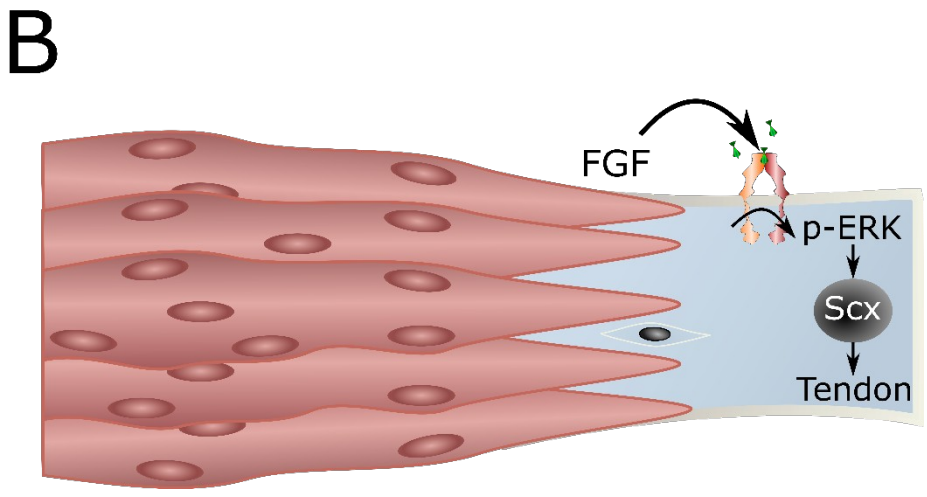
Figure 1. Cartoon comparing muscle-tendon crosstalk in the development of the myotendinous junction in (A) *Drosophila* and (B) mammals. In *Drosophila*, prior to muscle contact epidermal cells express stripe (SrB) and muscle produced vein does not reach the epidermal cell. As muscle comes into contact with the epidermal cell, *vein* binds to the EGF receptor activating signaling through ERK1/2 resulting in the expression of stripe A (SrA) and the differentiation of the epidermal cell into a tendon cell. In mammals (B), developing myotubes secrete FGFs, which binds to FGF receptors on tendon cells once again activating ERK1/2 and this time increasing the production of scleraxis (Scx) and driving tendon cell differentiation.

Figure 2. Analysis of the expression of (A) FGF8 and (B) Pea3 (ETV4) in human inactivity studies using the MetaMEx expression tool (60). Both FGF8 and Pea3 are decreased as a result of inactivity or unloading by a log10 fold change (logFC) of ~0.3, equating to a percent change of ~80%.

Figure 3. Analysis of the expression of (A) akirin1 (*mighty*) in short-term (2 to 5 days) human inactivity studies using the MetaMEx expression tool (60). Mighty decreased as a result of short-term inactivity or unloading by a log10 fold change (logFC) of 0.2, equating to a percent change of 40%. (B) miR-29a and (C) miR-29b1 levels following inactivity in human studies using the

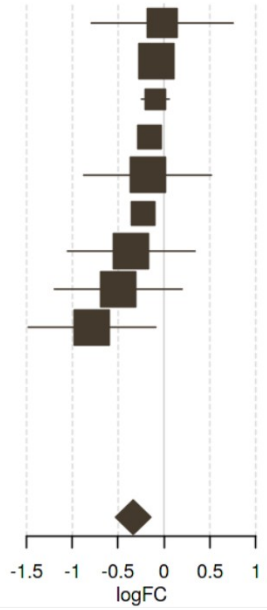
MetaMEx expression tool. Note that both miR-29a and b1 increase, whereas miR-29b2 and c are unchanged by inactivity (not shown).


A
SrB
SrB
EGFr
Vein
Muscle Contact
Tendon
SrA
SrA
p-ERK
EGFr
Vein


B
FGF
p-ERK
Scx
Tendon

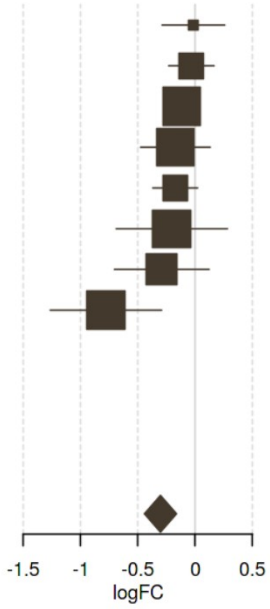
A

FGF8 in inactivity studies	logFC	FDR	n
GSE24215_BED_VAL_M_YNG_ACT_LEA_HLY_D10	-0.02	9.9e-01	10
GSE148152_BED_VAL_M_YNG_ACT_LEA_HLY_D84	-0.08	1.2e-01	12
GSE21496_LIM_VAL_M_YNG_SED_LEA_HLY_D02	-0.09	9.8e-01	7
GSE14798_BED_VAL_F_YNG_SED_LEA_HLY_D60	-0.16	4.9e-02	8
GSE14901_LIM_VAL_M_YNG_SED_LEA_HLY_D02	-0.18	9.4e-01	12
GSE14798_BED_SOL_F_YNG_SED_LEA_HLY_D60	-0.23	3.1e-03	8
GSE14901_LIM_VAL_M_YNG_SED_LEA_HLY_D14	-0.36	7.4e-01	12
GSE14901_LIM_VAL_F_YNG_SED_LEA_HLY_D02	-0.50	7.5e-01	12
GSE14901_LIM_VAL_F_YNG_SED_LEA_HLY_D14	-0.79	3.5e-01	12
GSE113165_BED_VAL_F_ELD_ACT_OWE_HLY_D05	NA	NA	
GSE113165_BED_VAL_F_YNG_ACT_LEA_HLY_D05	NA	NA	
GSE113165_BED_VAL_M_ELD_ACT_OWE_HLY_D05	NA	NA	
GSE113165_BED_VAL_M_YNG_ACT_LEA_HLY_D05	NA	NA	
Meta-analysis score	**-0.34**	**5.9e-04**	**93**



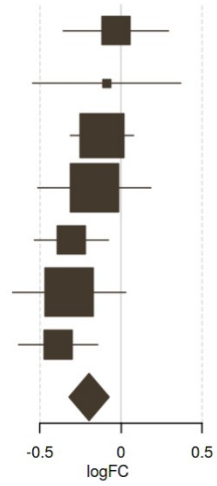
B

ETV4 in inactivity studies	logFC	FDR	n
GSE33886_LIM_VAL_M_YNG_SED_LEA_HLY_D21	-0.01	1.0e+00	3
GSE14798_BED_SOL_F_YNG_SED_LEA_HLY_D60	-0.03	8.8e-01	8
GSE148152_BED_VAL_M_YNG_ACT_LEA_HLY_D84	-0.12	7.9e-02	12
GSE104999_BED_VAL_M_YNG_ACT_LEA_HLY_D21	-0.17	7.1e-01	12
GSE14798_BED_VAL_F_YNG_SED_LEA_HLY_D60	-0.17	3.1e-01	8
GSE14901_LIM_VAL_M_YNG_SED_LEA_HLY_D14	-0.20	8.0e-01	12
GSE24215_BED_VAL_M_YNG_ACT_LEA_HLY_D10	-0.29	6.5e-01	10
GSE14901_LIM_VAL_F_YNG_SED_LEA_HLY_D14	-0.78	1.0e-01	12
GSE113165_BED_VAL_F_ELD_ACT_OWE_HLY_D05	NA	NA	
GSE113165_BED_VAL_F_YNG_ACT_LEA_HLY_D05	NA	NA	
GSE113165_BED_VAL_M_ELD_ACT_OWE_HLY_D05	NA	NA	
GSE113165_BED_VAL_M_YNG_ACT_LEA_HLY_D05	NA	NA	
Meta-analysis score	**-0.30**	**2.3e-05**	**77**



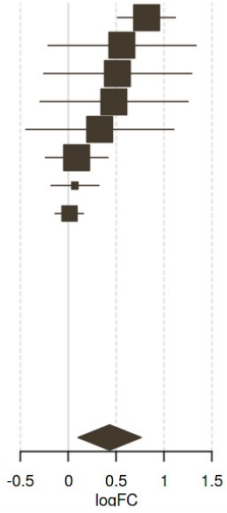
A

AKIRIN1 in inactivity studies	logFC	FDR	n
GSE21496_LIM_VAL_M_YNG_SED_LEA_HLY_D02	-0.03	9.9e-01	7
GSE113165_BED_VAL_M_YNG_ACT_LEA_HLY_D05	-0.09	9.5e-01	2
GSE113165_BED_VAL_M_ELD_ACT_OWE_HLY_D05	-0.12	9.1e-01	11
GSE14901_LIM_VAL_F_YNG_SED_LEA_HLY_D02	-0.16	8.6e-01	12
GSE113165_BED_VAL_F_ELD_ACT_OWE_HLY_D05	-0.31	2.3e-01	7
GSE14901_LIM_VAL_M_YNG_SED_LEA_HLY_D02	-0.32	6.3e-01	12
GSE113165_BED_VAL_F_YNG_ACT_LEA_HLY_D05	-0.39	9.8e-02	7
Meta-analysis score	**-0.20**	**1.8e-03**	**58**



B

MIR29A in inactivity studies	logFC	FDR	n
GSE148152_BED_VAL_M_YNG_ACT_LEA_HLY_D84	0.82	4.5e-04	12
GSE14901_LIM_VAL_F_YNG_SED_LEA_HLY_D14	0.56	6.3e-01	12
GSE14901_LIM_VAL_F_YNG_SED_LEA_HLY_D02	0.52	7.8e-01	12
GSE14901_LIM_VAL_M_YNG_SED_LEA_HLY_D14	0.48	6.7e-01	12
GSE14901_LIM_VAL_M_YNG_SED_LEA_HLY_D02	0.33	8.9e-01	12
GSE104999_BED_VAL_M_YNG_ACT_LEA_HLY_D21	0.09	8.9e-01	12
GSE33886_LIM_VAL_M_YNG_SED_LEA_HLY_D21	0.07	9.7e-01	3
GSE21496_LIM_VAL_M_YNG_SED_LEA_HLY_D02	0.01	1.0e+00	7
GSE113165_BED_VAL_F_ELD_ACT_OWE_HLY_D05	NA	NA	
GSE113165_BED_VAL_F_YNG_ACT_LEA_HLY_D05	NA	NA	
GSE113165_BED_VAL_M_ELD_ACT_OWE_HLY_D05	NA	NA	
GSE113165_BED_VAL_M_YNG_ACT_LEA_HLY_D05	NA	NA	
GSE14798_BED_SOL_F_YNG_SED_LEA_HLY_D60	NA	NA	
GSE14798_BED_VAL_F_YNG_SED_LEA_HLY_D60	NA	NA	
GSE24215_BED_VAL_M_YNG_ACT_LEA_HLY_D10	NA	NA	
Meta-analysis score	**0.43**	**1.0e-02**	**82**



C

MIR29B1 in inactivity studies	logFC	FDR	n
GSE14901_LIM_VAL_F_YNG_SED_LEA_HLY_D14	0.56	6.3e-01	12
GSE14901_LIM_VAL_F_YNG_SED_LEA_HLY_D02	0.52	7.8e-01	12
GSE14901_LIM_VAL_M_YNG_SED_LEA_HLY_D14	0.48	6.7e-01	12
GSE148152_BED_VAL_M_YNG_ACT_LEA_HLY_D84	0.38	1.6e-03	12
GSE14901_LIM_VAL_M_YNG_SED_LEA_HLY_D02	0.33	8.9e-01	12
GSE104999_BED_VAL_M_YNG_ACT_LEA_HLY_D21	0.13	7.6e-01	12
GSE21496_LIM_VAL_M_YNG_SED_LEA_HLY_D02	0.01	1.0e+00	7
GSE33886_LIM_VAL_M_YNG_SED_LEA_HLY_D21	-0.05	9.7e-01	3
GSE113165_BED_VAL_F_ELD_ACT_OWE_HLY_D05	NA	NA	
GSE113165_BED_VAL_F_YNG_ACT_LEA_HLY_D05	NA	NA	
GSE113165_BED_VAL_M_ELD_ACT_OWE_HLY_D05	NA	NA	
GSE113165_BED_VAL_M_YNG_ACT_LEA_HLY_D05	NA	NA	
GSE14798_BED_SOL_F_YNG_SED_LEA_HLY_D60	NA	NA	
GSE14798_BED_VAL_F_YNG_SED_LEA_HLY_D60	NA	NA	
GSE24215_BED_VAL_M_YNG_ACT_LEA_HLY_D10	NA	NA	
Meta-analysis score	**0.37**	**9.9e-03**	**82**