Exploring the Role of Mechanical Force on Tendon Development in vivo Model: a Scoping Review

Yuna Usami
Graduate School of Saitama Prefectural University https://orcid.org/0000-0002-3757-8362
Hirotaka Iijima
Nagoya University
Takanori Kokubun (kokubun-takanori@spu.ac.jp)
Saitama Prefectural University https://orcid.org/0000-0001-6676-2356

Systematic Review

Keywords: Tendon development, mechanobiology, Scleraxis, animal model, movement, muscle contraction, scoping review

Posted Date: May 23rd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1681832/v1

Abstract

The tendon transmits the skeletal muscle-generated forces to bones and drives the joint motion. While extensive research has indicated the fundamental role of mechanical forces in tendon development during embryonic and postnatal phases, a comprehensive summary regarding the mechanical forces applied to tendons is lacking. This scoping review aimed to summarize the current knowledge regarding the effect of mechanical forces on tendon development. The electronic database search using PubMed was performed in October 2021 and yielded 586 articles, of which 16 articles met the prespecified inclusion criteria. We defined the mechanical force in terms of muscle existence, muscle contraction, or weight-bearing locomotion and identified methodological heterogeneity in applying mechanical force to tendons. Despite the lack of consensus regarding standard intervention methods, the majority of the mechanical interventions were used to regulate the expression or synthesis of well-known growth factors required for tendon development, especially the *Scx* expression. Our results provide a novel point of view to build future research about mechanobiology in tendon development.

Introduction

Tendons are connective tissue that transmits the forces generated by skeletal muscles to bones and drive joint movement. They are repeatedly exposed to strong mechanical forces, such as the tensional forces generated due to muscle contraction and endure mechanical forces throughout development. However, the current knowledge is not integrated into how mechanical forces modulate tendon development from embryonic to early postnatal development phase.

In tendon development, general biological processes; formation, differentiation, migration, and pattern formation have promoted understanding. Some transcriptional factors were identified in this process, but *Scleraxis* (*Scx*) is the most representative marker for tendon maturation. *Scx* is a basic helix-loop-helix (bHLH) transcription factor, Schweitzer et al. reported that *Scx* expression marks tendons maturation from the early progenitor stage to the formation of mature tendons (Schweitzer et al., 2001). *Scx* is used as a detective target to evaluate the tendon maturation during the early stage of tendon development. By observing this expression, we can gauge the progress of the tendon development. Throughout the early stage of development, *Scx* was detected as a highly specific marker for tendons and ligaments in chicks, mice, and zebrafish (J. W. Chen and J. L. Galloway, 2014; R Schweitzer et al., 2001).

In recent years, findings of biological processes put the spotlight on mechanical forces that can regulate and control key cellular processes and induce molecular responses during the development process. Many researchers reported the novel knowledge of tendon development using some species as a tendon development model. The chicks paralyzed by injecting d-tubocurarine did not show any changes in the tendon cells, collagen, and immature elastic fibers (C Beckham et al., 1977). Transgenic mice model (e.g., mdg or spd mice) showed undeveloped tendons, which were detected using ScxGFP (Huang et al., 2015). Similarly, paralyzed chick embryos showed reduced *Scx* expression, elastic modulus, and *lysyl oxidase* (*LOX*) activity (Havis et al., 2016; X S Pan et al., 2018). However, these research on tendon development were confounded by some factors such as species, age, intervention type, or tendon detection methods. Therefore, while the requirement of mechanical forces for tendon development has been researched extensively, consensus regarding the amount or type of mechanical forces to be applied to embryonic or postnatal animal tendons is lacking. Lack of consensus about the methods to modulate the mechanical stress and interpretation of these research in tendon development induces confusion about the role and effect of mechanical force on the molecular reactions in tendon development. A strict description of the mechanical force is required to provide an overview or map of the evidence in this field. Summarizing the available evidence is the logical first step toward establishing a consensus regarding the mechanical force used in the field of tendon mechanobiology.

This scoping review aimed to (1) summarize the characteristics of the current animal models used for determining the influence of mechanical force on tendons and (2) define the mechanical force in current literature. Furthermore, we assessed (3) the effect of mechanical force by determining how some types of mechanical forces affect tendon development. This review focuses on *in vivo* models because of the critical relationship between tendons and muscles or bones that is required for utilizing mechanical force.

Results

Manual database search yielded 586 articles (**Figure 1**). After the first screening stage of titles and abstracts, 84 articles were selected for full-text review. After the full-text screening, 68 articles were excluded, leaving 16 articles that met the inclusion criteria. They were excluded because they failed to assess papers, were not animal models (e.g., human sample), did not include interventions (e.g., observation of normal tendon development over time and gene modification model of the tendon), and did not have any *in vivo* model (e.g., *in vitro* and *ex vivo*), were not related to natural development (e.g., healing, engineering research), did not match the age (within four weeks from the day of birth), and the outcome did not include tendons (e.g., only muscle-tendon junction or enthesis) (**Figure 1**).

Experimental animal models were categorized into four species

The intervention methods and tendon development process vary with animal species, so we categorized by animal species. Table 1 shows the characteristics of the experimental model in the included studies. Four different species were used; chicks (n=13), mice (n=5), zebrafish (n=3), and rats (n=2). The age of the chick models was between stage (st)18-19; it was converted into the embryonic day E2-E3 (2 days from intervention at stage 4 and 5 (Hamburger and Hamilton, 1951)). Mice aged E12.5 to P0 and rats aged P0 to P10 were used as models. Zebrafish from 48 h post-fertilization (hpf) to 98 hpf were used as models. Most studies used embryos, and only three studies used postnatal animals.

This scoping review identified interventions that decrease and increase the mechanical force to understand the role of mechanobiology in tendon development. The interventions that reduced mechanical force involved 14 approaches; in contrast, only three ways were used to increase mechanical force. The intervention type for reducing mechanical force was categorized into five groups for various animal intervention methods: transgenic, surgery, drug treatment, locomotion, and added stimulation (**Table 2**). Five studies used multiple models. Three studies combined interventions that changed the mechanical force and techniques that inhibited specific signaling. *In situ* hybridization (section or whole mount) was frequently used to analyze tendons. Electronic microscopy, immunohistochemistry, transmission electron microscopy, and mechanical testing were used for assessing the tendons. The other methods are shown in Table S1. Some biological markers tested the effect of mechanical force. The most frequent marker was *Scx*, which is common in many studies. *Scx* mutant mice showed a dramatic defect in tendon differentiation. *Scx* is recognized as required for tendon differentiation and formation(Murchison et al., 2007). So, downregulated *Scx* expression in the intervention model means inhibiting tendon development. GFP fluorescence, *in situ* hybridization (ISH), and polymerase chain reaction (PCR) can also detect *Scx* expression.

The lack of consensus regarding standard intervention methods

We categorized types of intervention because that improved to catch the sight of defining mechanical force. Types of intervention were identified as ones that decreased or increased the mechanical force. The intervention type of decreased mechanical force was divided into four groups. The transgenic group included several muscle-deficient factor mutant models that included *Myf5*$^{-/-}$ *Myod1*$^{-/-}$ double mutants (Brent et al., 2005; J. W. Chen and J. L. Galloway, 2014), *Pax3* (splotch or splotch delayed) mutants (Schweitzer et al., 2001), muscular dysgenesis (*mdg*) mutant(Huang *et al.*, 2015), and voltage-dependent L-type calcium channel subtype bata-1 (*cacnb1)* mutant (A. Subramanian et al., 2018). Full-length mRNA encoding codon-optimized a-bungarotoxin (aBtx) was injected into the muscle (A. Subramanian *et al.*, 2018). Most studies used the mouse model, except for the *cacnb1* mutant and aBtx mRNA intervention, which were in zebrafish. *Myf5*$^{-/-}$ *Myod1*$^{-/-}$ double mutants do not contain any muscle progenitors, resulting in a muscle-less limb (Kassar-Duchossoy et al., 2004). *Pax3* is required for the establishment of muscle progenitor cells in the limb, and mutations in *Pax3* (Splotch) and *Pax3* (Splotch-delayed) cause a severe defect in limb muscle formation (E Bober et al., 1994). *mdg* is an autosomal recessive lethal mutation that results in the contraction of skeletal muscles (B A Adams and Beam, 1990). Full-length mRNA encoding codon-optimized aBtx was injected in zebrafish to prevent skeletal muscle contractions (Swinburne et al., 2015). The limb was paralyzed in the *cacnb1* homozygous mutant (Zhou et al., 2006).

Surgical procedures can remove muscle or neural tube in a region-specific manner. The surgery group experimented with coelomic wing graft (F. Edom-Vovard et al., 2002; Kardon, 1998), neural tube ablation (F. Edom-Vovard *et al.*, 2002), and dermomyotome removal (Ava E Brent et al., 2003) in chicks. Coelomic wing graft was performed in the lateral plate areas corresponding to the future wing buds, or migratory myogenic cells were isolated from chick embryos. This weakened the wing muscle. Neural tube ablation was performed on embryos before the exit of ventral root fibers to produce complete aneural wings. Dermomyotome removal is a method of surgically removing the dermomyotome before myotome formation, making it muscle-less.

In addition, interventions using four types of drugs were also performed. They were decamethonium bromide (DMB) (Havis *et al.*, 2016; J A Germiller et al., 1998; X S Pan *et al.*, 2018), pancuronium bromide (PB) (Havis *et al.*, 2016; X S Pan *et al.*, 2018) , d-tubocurarine (C Beckham *et al.*), and botulinum toxin (W.G.Hopkins, 1984) . Botulinum toxin was used in mice, while the other interventions were used for chicks. DMB prevented the effects of acetylcholine at the neuromuscular junction and depolarized the end-plate region, inducing rigid paralysis (Paton and Zaimis, 1951). PB blocked the response to acetylcholine and is characteristic of blocking drugs of the non-depolarizing type. Thus, chicks treated with doses of PB were paralyzed and flaccid (W R Buckett et al., 1968). D-tubocurarine is an acetylcholine receptor antagonist that inhibits neuromuscular activity (L T Landmesser and Szente, 1986; Paton and Zaimis, 1949). Botulinum toxin, an acetylcholine receptor antagonist, decreased the amount of acetylcholine interacting with receptors, thereby reducing muscle contraction and motility (Pittman and Oppenheim, 1978); PB, d-tubocurarine, and botulinum toxin induced flaccid paralysis.

In contrast, mechanical force was increased by injecting a group of drugs, which included only 4-aminopyridine (4-AP) (X S Pan *et al.*), a neuromuscular blocking drug that blocks the potassium channels in neurons. When applied to chicks, it stimulated the release of the neurotransmitter, acetylcholine (ACh) and enhanced its availability at the synaptic cleft (Heywood et al., 2005). Therefore, the 4-AP model induces high-frequency movement and hypermobility (X S Pan *et al.*).

The locomotion group focused on rodent locomotion (spinal cord (S. K.Theodossiou et al., 2021)) in the postnatal phase of rats. The developing locomotor behavior during the postnatal period was believed to increase mechanical loading for limbs. Rats with spinal cord transection did not show complete weight-bearing locomotion; hence, they were used as model systems to disrupt locomotor development in neonates. The postnatal rats showed changes in spontaneous posture and locomotion during the early postnatal week (H. E. Swann and M. R. Brumley, 2019). This locomotion development affects the limb mechanical loading (S. K. Theodossiou et al., 2019).

The last group includes the electrically stimulated model (A. Subramanian *et al.*) that induces muscle contractions in zebrafish. This intervention was applied to both normal and paralyzed muscles to increase mechanical force.

Lack of mechanical force inhibited tendon maturation

We summarized the model to investigate the role of mechanical force in tendon development throughout *Scx* expression. Tendon development of individual models is described below and summarized in **Table 3 and Table 4.** The most standard method to control the mechanical force of the tendon is to arrange the force depending on the muscles. Many researchers reported the way to modulate muscle function using a variable animal model. The defined mechanical force is roughly divided into three types. One of the methods is the Muscle-less model using a surgical extraction, *Pax3* mutant mice model. The others are muscle paralysis models that operate muscle contraction, such as DMD injection. The third is weight-bearing. This section showed how each type of mechanical force affects the expression of *Scx*.

At first, we show the relationship between muscle-less and *Scx* expression. *Myod1 Myf5*-deficient zebrafish expressed *Scx* 53-58 hpf, but not at 72 hpf (J. W. Chen and J. L. Galloway, 2014). *Myf5*$^{-/-}$*; Myod1*$^{-/-}$ double mutant muscle-less mouse embryos survived *Scx* expression in the limbs but not in the epaxial region at E13.5 (Brent *et al.*, 2005). In *Pax3* mutant embryos, which were muscle-less similar to the *Myf5*$^{-/-}$ *Myod1*$^{-/-}$ double mutant, *Scx* expression in limbs was not affected at E12.5 (R Schweitzer *et al.*, 2001). In the E16.5 *Pax3* mutant, Scx-GFP was not detected in the zeugopod tendon. However, it persisted in the autopod tendon segments at E18.5 (Huang *et al.*, 2015). At E16.5, Scx GFP was observed in the zeugopod of

the *mdg* mutant. Moreover, muscle-lessness can also be induced by surgical manipulation. Induction of *Scx* was not observed after surgically removing the dermomyotome before myotome formation (Ava E Brent *et al.*, 2003). Coelomic wing graft surgery indicated that tenascin reduced the proximal and intermediate tendon but rescued the distal limb (Kardon, 1998). Another study on coelomic wing graft surgery involving chick embryos at E2 showed *Scx* expression at the forearm at E6, but not at E10 (F. Edom-Vovard *et al.*, 2002).

The tested model the effect of muscle contraction, in chick E8 and E9, the neural tube ablated embryos showed downregulation of *tenascin* and *Scx* in tendons. Although *Scx* expression in the forearm at E10 (or E9) was limited in both models, the digit expressed *Scx* at E10 (F. Edom-Vovard *et al.*, 2002). The expression of factors related to tendon development, such as *Scx* and the *transforming growth factor-beta-2* (*Tgf-β2*), decreased at E6.5 in chicks paralyzed rigidly using DMB, while *Tenomodulin* (*Tnmd*) expression decreased at E7.5 (Havis *et al.*, 2016). Through E18, the tendons became uniformly smaller and correlated with reduced chick movement due to paralysis (J A Germiller *et al.*, 1998). In chicks with flaccid paralysis induced by PB, *Scx* expression decreased at E6.5 (Havis *et al.*, 2016) and *LOX* activity was reduced at HH43 (E17) (X S Pan *et al.*, 2018). The length of the muscles reduced in botulinum toxin-injected mouse, and the tendons were longer than the muscles (W.G.Hopkins, 1984). The fibrocartilaginous area and elastic vinculum were not formed in chick injected with d-tubocurarine. However, the tendon cells and collagen in immature elastic tendon fibers did not change (C Beckham *et al.*, 1977). The increased mechanical force in the 4-AP model increased the elastic modulus. Zebrafish stimulated by electronic stimuli to restore mechanical force reduced by aBtx-injection showed increase in axial tenocyte projection length compared to that observed in aBtx-injected only animals. Several factors, such as *Thrombospondin 4b* (*Tsp4b*), *TGFb-induced protein* (*Tgfbip*), and *connective tissue growth factor a* (*Ctgfa2*), were upregulated to control levels by electronic stimulation compared to that observed in aBtx-injected animals(A. Subramanian *et al.*, 2018).

The last is weight-bearing model. Theodossiou et al. reported the structural properties and cross-sectional area of the weight-bearing Achilles tendon at P10 were higher than those of the non-weight-bearing phases P1 and P5 in the rat model (S. K. Theodossiou *et al.*, 2019). But there is no research measuring *Scx* expression using the weight-bearing model.

Intervention for detecting molecular mechanism

In the above section, we focused on *Scx* expression. Moreover, we summarize several markers regulated by mechanical force along with *Scx*. One study confirmed the relationship between expression and molecular mechanism by reimplanting a source of *fibroblast growth factor 4* (*Fgf4*) in the aneural chick limbs and muscle-less chick wing (Havis *et al.*, 2016). *Fgf4* was not expressed in aneural and muscle-less wings; hence, reimplanting was performed to analyze the possible effects of *Fgf4* removal from aneural muscles on tendon markers. *Scx* and tenascin were downregulated in the aneural limbs and muscle-less wings. Consistent with this, both models showed that grafts of *Fgf4* cells rescued the expression of the tendon-associated molecules, *Scx* and *tenascin*. Another study tested whether Fgf rescued tendon gene expression without muscle contraction in DMD-injected chick. mFgf4/ RCAS-producing cells were grafted into forelimb buds. While immobilization following DMD induced a drastic decrease in *Scx*, *Tnmd*, and *Thrombospondin 2* (*Thbs2*) expression, the mFgf4-paralyzed-limbs significantly upregulated *Scx*, *ETS translocation variant 4* (*Etv4*) also known as *polyoma enhancer activator 3* (*Pea3*), and *sprout 2* (*Spry2*). The relative mRNA levels of *Tnmd*, *Tgf2*, *and Tgfβ3* did not change under this condition. These results suggested that the downregulation of several genes may be a molecular effect induced by the muscle-less state or inhibition of contraction. The muscle may require both mechanical and molecular aspects.

Discussion

This scoping review summarized the effects of mechanical stimulation on tendon development in animal models. We defined mechanical force in terms of muscle contraction, muscle existence, and weight-bearing (Fig. 2). However, we observed methodological heterogeneity in the application of mechanical force to tendons. Hence, the establishment of a consensus for mechanical intervention is required. We showed to categorize the “mechanical force” will contribute to an appropriate understanding of the effect of mechanical force based on muscle contraction or body movement *in vivo* to the

tendon development. This knowledge of the detailed role and effect of mechanical force helps to reveal the mechanism of tendon developmental mechanobiology.

What types of mechanical forces are defined and used in tendon development?

Mechanical force is defined generally in terms of muscle existence or muscle contraction. Muscle loss or muscle paralysis is considered a reduction of mechanical force. Few studies have shown that postnatal locomotion works as a mechanical force in rodent limbs. This means weight-bearing associated with the locomotion increases the mechanical force on the limbs compared to non-weight-bearing limbs. In contrast, the spinal cord transection was used as a model that couldn't locomote with weight-bearing for limbs.

These field studies mostly used chick models, in addition to mouse, zebrafish, and rat models. The chick embryo is amenable to drug and surgical treatment(X S Pan et al., 2018). As chick embryos are present in eggs, they are easy to manipulate. The mouse model is an established mammalian model organism. In addition, the mouse has been developed as a genetic model, and it is a widely-used tool for targeting specific genes(Huang et al., 2015). The zebrafish is also widely used as a genetically modified model(A. Subramanian et al., 2018). The rat model can be used to observe limb movements after birth; their bodies are bigger than those of mice and are hence easy to operate(S. K.Theodossiou *et al.*, 2021). Each animal has each strong point to identify the role of mechanical force as the experiment model. Although these features vary the way of intervention to operating mechanical force, each method has each limitation. In the genetic and drug model, muscle function can be controlled easily, although the side effects of the loss of gene or injected drug on the tendon cannot be excluded. The weight-bearing model cannot distinguish between loss of loading and only loss of muscle contraction due to spinal cord injury.

In this review, we targeted animals from the embryonic to postnatal stages. Many studies that we collected, targeted embryos; conversely, studies on postnatal stage were few. Further studies are required to consider the effect of the environment of each embryo on movement. For example, mice embryos exist with multiple litters in the womb, while the chick grows in a rigid eggshell. These may limit body movements depending on the spatial limit. On the contrary, postnatal animals can move without environmental limitations. We showed that muscle contraction and existence might play a key role as force generators to the tendon, and that future studies on the function of mechanical force in the postnatal phase will be important. Another important discovery is that mechanical force can be increased in three ways, that are muscle existence, contraction, on load. Decreased mechanical force command a majority in the reports. In future studies, the model of increased mechanical force should be developed. To compare decreased with increased mechanical force model, more supported the idea that tendon needs mechanical force for development. We can judge the role of mechanical force comprehensively by comparing the effects of enhanced and reduced intervention on tendon development.

What types of mechanical forces affect tendon development?

Many studies using muscle-less and inhibition of the muscle contraction models showed that the effect on tendon development was subtle immediately after inducing tendon cells, but that the levels of factors contributing to tendon development, such as *Scx* and tenascin, decreased or were absent in the late embryonic period. The role of other factors varies with studies, and it is too early to arrive at conclusions. The methods used for evaluating tendon development are confounded. This lack of common indicators has hindered advancement in the field. Tenascin is one of the most classical factors for detecting tendons; however, it is not a specific tendon marker. Identification of *Scx* as a tendon progenitor marker maked a significant breakthrough in research on tendon development. In the current study, *Scx* has been used to test the effect of mechanical force on tendon. No one showed upregulating *Scx* expression in the animal model of reduced mechanical force in early embryos (mice;~E12.5, chick; ~E6), *Scx* expression was not affected by the reduction in mechanical force. Few studies reported that loss of muscle progenitors inhibited *Scx* expression (Ava E Brent et al., 2003; Brent et al., 2005). Embryonic movement begins on E12.5 in mice (Shinoda, 1999), and at developmental day 2–6 in chick embryos (Wu et al., 2001). After the beginning of limb movement, most studies showed decrease in *Scx* expression, with few exceptions.

We suggest that investigating common *Scx* expression in multiple studies will be valuable, as no controlled definition of “tendon development” is lacking.

We obtained not only trends but also contradictory results. While muscle-less *Pax3* mice did not express Scx-GFP at E16.5, Scx-GFP expression persisted in *mdg* limbs, suggesting that the existence of muscles generated mechanical force as connections from muscle to bone; in addition, muscle contraction exerts a tensile force on tendons. However, the results obtained with the chick model differed. While at E6 ~ 6.5, DMD and PB paralysis models showed reduced *Scx* expression (Havis et al., 2016), wing graft surgery, a muscle loss condition, resulted in *Scx* expression (F. Edom-Vovard et al., 2002). These two studies used ISH to identify *Scx* expression. Although the intervention of these studies was the similar, the side effects or intensity of the changed mechanical force may differ. We found that the current literature in this field contains many conflicting reports. Future studies must consider the side effects of each intervention. For example, some transgenic models almost lack of myogenic determination factor such as Pax3. However, it is not clear how contribute Pax3 oneself tendon cell maturation. To resolve this problem, to use the same changing force in multiple intervention methods (muscle contraction, muscle existence, or loading) within a single study is needed. Bias due to detection methods can be excluded if the tendon reacts to mechanical force tested in a single publication. Although the molecular aspect may differ with the intervention methods, the mechanical force does not change between models. In this study, we included five studies involving models with different types of mechanical forces; however, the differences in the effects of mechanical stress on the tendon between models are less well understood. Furthermore, heterogeneity in species and intervention methods, such as genetics and effect of drugs, render intertrial comparison difficult.

Moreover, the sampled parts of tendon varied with the body part, such as trunk or limb, forearm or digit. Kardon et al. (Kardon, 1998)and Edom et al. (F. Edom-Vovard et al., 2002) reported that the proximal tendon in chicks degenerated when the muscles were induced less in the initial stage; subsequently, the distal tendons degenerated. Havis et al. (Havis et al., 2016) showed that the decrease in *Scx* expression in chick embryos injected with DMD and PB was more evident in the stylopod/zeugopod regions than in the digits. These results were observed not only in the forelimbs, but also in the hindlimbs. Huang et al. (Huang et al., 2015) identified tendons within the zeugopod of Sp^d mice at E16.5 with difficulty, whereas the autopod tendons were identified easily. These results showed that initiated parts of degeneration are common, that is a proximal part. These results indicate impairment of mechanical force due to muscle contraction or elongation of an existing bone. This might be because the tendon bears tensile force from both muscle and bone. However, the elastic modulus of the muscle-tendon complex should be considered while interpreting these results. Thus, if the tendon loads tensile force via bone elongation, muscles with lower elastic modulus will stretch more than the tendon. The time scale on development or sensitivity for force in each part might differ between proximal and distal tendons. However, why the results vary with the body part remain unclear.

Several studies have tested the biological effects of changing the mechanical force using an *Scx* expression. In addition, one study investigated the effects of *thrombospondin2* (*Thbs2*), an extracellular matrix protein that modulates collagen fibrillogenesis and angiogenesis (Bornstein et al., 2000) on tendon mechanobiology. In connective tissue development, as assessed using *Tnmd* expression (Qianman et al.), angiogenesis may potentially be involved in tissue maturation (Docheva et al., 2005; Sato et al., 2014). The tail tendon degenerated in TSP2-null mice and provided an impression of increased laxity in the tail (Kyriakides et al., 1998). There is no clear relationship between *Thbs2* and tendon development, although *Thbs2* may be involved in tendon maturation. Although some studies have combined the models to manipulate mechanical force by adding or removing several factors, whether the intervention effect is due to mechanical force or solution effect remains unclear. Therefore, the models introduced in this review induce both fluctuating mechanical force and biochemical effects, for example, because of the liquidity factor of the muscles. A model animal useful for elucidating the mechanism of mechanotransduction for tendon development will be required in the future.

Limitations and future studies

This review has some limitations. This review didn't consider any risk of bias assessment due to the nature of scoping reviews. It provides definitions of mechanical force used in tendon mechanobiology and knowledge regarding the contribution of mechanical force to tendon development in the context of compelling evidence. However, to inspect the function of mechanical force, we must conduct systematic reviews using studies on specific types of intervention and compare different models; however, currently, consensus regarding the definition of mechanical force defined in this review is lacking.

Conclusions

This scoping review provides insights regarding tendon development promoted by mechanical loading *in vivo*, as well as the lack of consensus regarding the effect of mechanical force applied on tendons. We defined mechanical force in terms of muscle existence, muscle contraction, or weight-bearing locomotion. Our results provide a novel point of view to build future research about mechanobiology in tendon development.

Declarations

ACKNOWLEDGMENTS

AUTHOR CONTRIBUTIONS

Y.U. designed and performed the majority of the data collection and analysis and wrote the manuscript. H.I. designed the experiments and reviewed & editing the manuscript. T.K. supervised and aided in the experimental design, carried out data analysis, and reviewed & editing the manuscript.

DECLARATION OF COMPETING INTERESTS

The authors declare no competing interests.

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Methods

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Takanori Koubun (kokubun-takanori@spu.ac.jp).

Materials Availability

This study did not generate new unique materials.

Data and Code Availability

This paper analyzes existing, publicly available data. These accession numbers for the datasets of the resource publications are listed in the table. This paper does not report the code. Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

METHOD DETAILS

The review followed the elaborate methodological framework according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for scoping reviews (PRISMA-ScR) guidelines (Tricco et al., 2018) and the five-stage framework outlined in Arksey and O'Malley (Arksey and O'Malley, 2005) (**Supplemental Appendix S1 and S2**). The detailed protocol for this scoping review was not published or registered.

The central research question was: "What is known in the existing literature about the role of mechanical forces in tendon development?" The specific research questions were as follows;

(1) What types of mechanical forces are defined and used in tendon development?

(2) What types of roles do mechanical forces in tendon development perform?

Inclusion criteria, literature search, and study selection

Inclusion criteria was set according to participants, concept, and context domains (PCC)(Micah D J Peters, 2015). The review considered studies that included (1) embryo and postnatal animals (within four weeks from the day of birth) (participants domain), (2) intervention to increase or decrease mechanical forces for the tendon (concept domain), and (3) tendon development as an outcome (context domain).

PubMed databases were used for electronic database search from the time of database inception to October 2021. The PubMed searches used the following search phrases: ((((("Animals" [MeSH]) AND ("Tendons" [MeSH] OR tendon[Title])) AND (((((((("embryology"[MeSH]) OR ("Morphogenesis" [MeSH])) OR ("Gene Expression Regulation, Developmental"[MeSH])) OR ("infant, newborn"[MeSH])) OR (embryo)) OR (postnatal)) OR (neonatal))) AND (((((((Biomechanical Phenomena[MeSH Terms]) OR (Movement[MeSH Terms])) OR (Stress, Mechanical[MeSH Terms])) OR (Paralysis[MeSH Terms])) OR (Locomotion[MeSH Terms])) OR (tendons/metabolism[MeSH Terms])) OR (Weight-Bearing[MeSH])))) NOT (review[Publication Type]). The last search for new manuscripts was performed in October 2021. References of the selected studies were screened for further relevant studies.

The inclusion criteria were as follows: (1) full-length original research article, (2) the target was in embryonic or postnatal stage (within four weeks from the day of birth), (3) induced fluctuation of mechanical force in the tendon, and (4) *in vivo* model. Studies written in languages other than English were excluded. According to the prespecified inclusion criteria, two reviewers (Y.U. and T.K.) independently screened titles, abstracts, and full texts of selected studies. Disagreements between reviewers were resolved via discussion till a consensus was reached. The reference list of the reviewed articles was also searched._

QUANTIFICATION AND STATISTICAL ANALYSIS

Data extraction

Basic study information regarding the author(s), year of publication, and the title was extracted. To answer the above research questions, we summarized the following information: animal species, sample age, type of mechanical intervention, comparator, duration of intervention, and biological findings (molecular biology data, histological findings, morphological

analyses, and mechanics testing). The number of studies was considered the total number. When multiple models were used in one study, the number of each intervention model, and not the number of papers, was considered. The biological data that the authors specified in sentences or figures were extracted. Small variations did not write clearly but showed only in figures were excluded.

Data synthesis

Data were synthesized in three sections, and we created an evidence map with a visual representation of outcome measures to identify knowledge gaps (Miake-Lye et al., 2016). We also identified methodology gaps in the literature during the process of collating and reporting the results using characteristics described in the data charting section. Given the focus on reporting methods and inclination of data rather than data of concrete figures or statistically significant, we didn't execute the statistical analysis.

Tables

Table 1. Summary of Studies

To answer the research questions, we summarized the following information: animal species, sample age, type of mechanical intervention, comparator, duration of intervention, and biological findings (molecular biology data, histological findings, morphological analyses, and mechanics testing).

	Study (Authors & Year)	Animal Species	Age	Tendon Sample Region	Intervention		Comparator
					Force-	Force	
#1	Beckham C et al.(1977)	chick	E18	limb	d-tubocurarine	–	Ringer solution, no injection
#2	Hopkins WG et al. (1984)	CD-1 mouse	P9	limb (gluteal muscles tendon)	botulinum toxin	–	PBS+Starved, no intervention
#3	Kardon G et al. (1998)	chick	st 28-32	limb	Coelomic graft surgery	–	Normal
#4	Germiller JA et al. (1998)	chick	E18	limb (achilles tendon)	decamethonium bromide (DMB)	–	Saline
#5	Schweitzer R et al. (2001)	mouse	E10	limb	splotch mutant	–	WT
#6	Edom-Vovard F et al. (2002)	chick	E6-E10 E7-E11	limb(forearm, digit)	Ceolomic Wing Graft Surgery Neural Tube Ablation	–	normal (no-intervension)
#7	Brent AE et al. (2003)	chick	stage 4 and 5+ 2days	sclerotome	surgically removed the dermomyotome (prior to myotome formation)	–	No intervention
#8	Brent AE et al. (2005)	mouse	At E10.5-11.0	trunk and limb	*Myf5*−/−; *Myod1*−/−	–	littermate controls (*Myf5*+/−; *Myod1*+/−)
#9	Eloy-Trinquet S et al. (2009)	mouse	E12.5	limb	*Pax3* mutant	–	WT
#10	Chen JW et al. (2014)	zebrafish	53-58 hpf, 72hpf	the craniofacial, fin, and myosepta	*Myod1* and *Myf5* Knockdown	–	WT
#11	Huang AH et al. (2015)	C57BL/6 mouse	E16.5-E18.5	limb (the zeugopod and autopod)	Spd, mdg	–	WT
#12	Havis E et al. (2016)	chick	E5.5 (24 h), E6.5 (48 h), and E7.5 (72 h)	limb (forelimb and digits)	Decamethonium bromide (DMB) pancuronium bromide (PB)	–	Hank's solution
#13	Pan XS et al. (2018)	Chick	HH45 (E19)	limb (Achilles Tandon)	DMB PB	4-AP (hypermotility)	saline
#14	Subramanian A et al. (2018)	zebrafish	48 hpf , 98hpf	axial	Full-length mRNA encoding codon-optimized a-bungarotoxin (aBtx) injected *cacnb1* mutant	Electrical stimulation	WT

#15	Theodossiou SK et al. (2019)	Sprague-Dawley rats	P1, 5, and 10	limb (achilles tendon, tail tendon)	–	locomotion and weight-bearing(P10)	non-weight-bearing(P1,P5)
#16	Theodossiou SK et al. (2021)	Sprague-Dawley rats	P10	limb (achilles tendon, tail tendon)	spinal cord-transected	–	sham

Table 2. Types of Intervention

The intervention type for reducing mechanical force was categorized into five groups for various animal intervention methods: transgenic, surgery, drug treatment, locomotion, and added stimulation.

Intervention methods	Effect	Force	Animal	Studies
Transgenic				
Myod1−/− Myf5−/−	muscle-less	↓	mouse	#8
Myod1 and *Myf5* Knockdown	muscle-less	↓	zebrafish	#10
Pax3-/-	muscle-less	↓	mouse	#9
Pax3(Splotch)	muscle-less	↓	mouse	#5
Pax3(Splotch delayed)	muscle-less	↓	mouse	#11
Muscular dysgenesis (mdg)	prevent muscle contraction	↓	mouse	#11
cacbn1 mutant	prevent muscle contraction	↓	zebrafish	#14
a-bungarotoxin (aBtx) mRNA	prevent muscle contraction	↓	zebrafish	#14
Surgery				
Ceolomic wing graft	muscle-less	↓	chick	#3, #6,
Neural tube ablation	prevent muscle contraction	↓	chick	#6
Dermomyotome removals	muscle-less	↓	chick	#7
Drug treatment				
decamethonium bromide (DMB)	rigid paralysis	↓	chick	#4, #12, #13
pancuronium bromide (PB)	flaccid paralysis	↓	chick	#12, #13
d-tubocurarine	flaccid paralysis	↓	chick	#1
botulinum toxin	flaccid paralysis	↓	mouse	#2
4-aminopyridine (4-AP)	hypermotility	↑	chick	#13
Locomotion				
spinal cord	reduce weight-bearing	↓	rat	#16
locomotor development	increase weight-bearing locomotor behavior	↑	rat	#15
Added stimulation				
Electrical stimulation	increased muscle contraction	↑	zebrafish	#14

Increased or decreased mechanical force ware shown in upward arrows or downward arrows.

Table 3. Types of Mechanical Forces

We defined the mechanical force in terms of muscle existence, muscle contraction, or weight-bearing locomotion and identified methodological heterogeneity in applying mechanical force to tendons.

Species		Muscle Contraction		Muscle Exist		On Load
mouse/rat	↓	Muscular dysgenesis (mdg)	↓	*Myod1*−/− *Myf5*−/−	↓	spinal cord
	↓	a-bungarotoxin (aBtx) mRNA	↓	*Pax3*-/-	↑	locomotor development
	↓	botulinum toxin	↓	*Pax3*(Splotch)		
			↓	*Pax3*(Splotch delayed)		
chick	↓	Neural tube ablation	↓	Ceolomic wing graft		
	↓	decamethonium bromide (DMB)	↓	Dermomyotome removals		
	↓	pancuronium bromide (PB)				
	↓	d-tubocurarine				
	↑	4-aminopyridine (4-AP)				
zebrafish	↓	*cacbn1* mutant	↓	*Myod1* and *Myf5* Knockdown		
	↑	Electrical stimulation				

Increased or decreased mechanical force were shown in upward arrows or downward arrows.

Table 4. Effects of Intervention

We summarized the model to investigate the role of mechanical force in tendon development throughout *Scx* expression.

Effect	Intervention	Animal	Duration	Outcomes	Study (Authors & Year)
decreased mechanical force					
Transegenic					
muscle-less	*Myod1*−/− *Myf5*−/−	mouse	E0~ At E10.5-11.0	E10.5: The limb/branchial arch Scx→ The somites Scx - The apical ectodermal ridge (Heinemeier et al.) Fgf4→ The anterior and posterior sclerotome Pea3→ E13.5: The epaxial region Scx − The limb Scx→ The axial tendin -	Brent AE et al. (2005)
	Myod1 and *Myf5* Knockdown	mouse	E0~ 72hpf	53-58 hpf : the craniofacial and fin scxa+, xirp2a- The myosepta scxa-, xirp2a- 72hpf: the head and fin scxa-, xirp2a-	Chen JW et al. (2014)
	Pax3-/-	mouse	E0 ~ E12.5	E12.5 Scx+	Eloy-Trinquet S et al. (2009)
	Pax3(Splotch)	mouse	E0~E10	Scx expression→	Schweitzer R et al. (2001)
	Pax3(Splotch delayed)	mouse	E0~E16.5, E18.5	E16.5 The zeugopod : ScxGFP- The autopod : Scx GFP+ The flexor tendons (near the wrist): Scx GFP-, The extenser tendons: Scx GFP+, several tendons were fused E18.5 The autopod: ScxGFP+, an aligned collagen matrix→, tendon size↓	Huang AH et al. (2015)
prevent muscle contraction	Muscular dysgenesis (mdg)	mouse	E0~E16.5	E16.5 The autopod: Scx GFP+, tendon size↓ The zeugopod: Scx GFP+, The extenser tendon fusion only near the wrist	Huang AH et al. (2015)
	cacbn1 mutant	zebrafish	E0 to 98hpf	98hpf: fail to compact and elongate	Subramanian A et al. (2018)
	a-bungarotoxin (aBtx) mRNA	zebrafish	E0 to 48hpf	48hpf: axial tenocyte projection length↓density↓ pSMAD3 signaling↓ tsp4b -, tgfbip -, ctgfa2 -, scxa→	Subramanian A et al. (2018)
Surgery					
muscle-less	Ceolomic wing graft	chick	st16-st27-35	st 28: tenascin → st 29-30: (dorsal proximal and intermediate) tenascin↓; do not individuate, but instead degenerate (the distal tendon) tenascin→ st31-32: (the distal tendon) tenascin↓; began to degenerate	Kardon G et al. (1998)
		chick	17–25 somites	The forearm: Scx E6 less segregated, E8↓E10-	Edom-Vovard F et al.

			(E2)~E12	The digit□Scx E10↓ [■□mFgf4-Expressing Cells] Scx E10↑, tenascin E10→ *compare to only Wing Graft Surgery	(2002)
	Dermomyotome removals	chick	stage 4 and 5+ 2days	stage 4 and 5+ 2days: Scx-	Brent AE et al. (2003)
prevent muscle contraction	Neural tube ablation	chick	15–23 somites (E2)~E11	(forearm/digit) E7. Fgf4→/→, Scx→/→, tenascin→/→, fgf8 n/d E7.5 Fgf4↓/, Scx↓/, tenascin n.d./, fgf8↓/ E8 Fgf4↓/↓, Scx↓/↓, tenascin↓/↓, fgf8↓/ E9 Fgf4-/-, Scx↓/↓, tenascin/↓, fgf8-/- E11 Fgf4-/-, Scx -/↓, tenascin n.d./n.d., fgf8 n.d./n.d. [■+mFgf4/RCAS-expressing cells] The forearm: Scx E10↑, tenascin E10↑ , Fgf8 E10→ *compare to only Neural tube ablation	Edom-Vovard F et al. (2002)
Drug Treatment					
rigid paralysis	decamethonium bromide (DMB)	chick	E6~E18	CSA↓	Germiller JA et al. (1998)
		chick	E4.5~E7.5	forelimbs(stylopod and zeugopod/digits *hole forelimbs; SMAD7) E5.5(24h) : Scx→ E6.5 (48h) : Scx↓/↓, COL1A2↓/↓, ETV4↓/→, SPRY2↓/↓, FGF4↓/, FGF8↓/, SMAD7↓, TGFB2↓/→ E7.5 (72h) : Scx↓/↓, COL1A2↓/→,ETV4↓/↓, SPRY2↓/→, FGF4↓/, FGF8↓/, SMAD7↓, TGFB2↓/→,TNMD-/, THBS2-/, The decrease of SCX expression was more obvious in stylopod/zeugopod regions compared with digits in ISH. Hindlimbs(stylopod and zeugopod/digits) E6.5(48h) : Scx↓/→, ETV4↓/→, SPRY2↓/→ E7.5(72h) : Scx↓/↓, ETV4↓/↓, SPRY2↓/→ [■+mFgf4/ RCAS- producing cells vs. DMB(forelimbs)] E7.5(72h) SCX↑, ETV4↑, SPRY2↑ COL1A2→, TNMD→, THBS2→, FGF8→, TGFB2→, TGFB3→,SMAD7→ *indicated by / showed that expression was difference for each part.	Havis E et al. (2016)
		chick	HH43 chick embryosto - after 48h	Elastic modules↓, LOX activity ↓, collagen content→	Pan XS et al. (2018)
flaccid paralysis	pancuronium bromide (PB)	chick	E8-E18	the proximal area : synovial cavity, fuzzy layer not apperded. the distal area : although a pulley did	Beckham C et al.(1977)

				not appear to be as well developed as that in the normal chicks. the vinculum like the normal chicks was not present. several small blood vessels were present. the tendon and their products (collagen and immature elastic fibers) were not changed	
		chick	E4.5~E7.5	E6.5(48h): Scx↓ E7.5(72h) Scx ↓ SCX expression was downregulated in stylopod/zeugopod regions of forelimbs and hindlimbs compared to control limbs.	Havis E et al. (2016)
		chick	HH43 chick embryosto - after 48h	LOX activity ↓	Pan XS et al. (2018)
	d-tubocurarine	chick	E8-E18	the proximal area : synovial cavity, fuzzy layer − the distal area : pulley−, the vinculum− several small blood vessels + the tendon cells, collagen and immature elastic fibers →	Beckham C et al.(1977)
	botulinum toxin	mouse	newborn-P9	length↑	Hopkins WG et al. (1984)
Locomotion					
reduce weight-bearing	spinal cord	rat	P1~P10	Achilles tendons linear region elastic modulus↑, cross-sectional↓ Maximum force, displacement at maximum force, linear region stiffness, toe region elastic modulus, maximum stress, strain at maximum stress, and transition strain→ SHG images→ Tail tendons linear region stiffness↑ cross-sectional area↑ Maximum force , displacement at maximum force, toe and linear region elastic modulus, maximum stress, strain at maximum stress, and transition strain→ SHG images→	Theodossiou SK et al. (2011)
Increased Mechanical Force					
Drug Treatment					
hypermotility	4-aminopyridine (4-AP)	chick	HH43 chick embryosto - after 48h	Elastic modules↑, LOX activity → , collagen content → ■+BAPN(inhibit LOX activity) vs 4-AP elastic module ↓	Pan XS et al. (2018)
Locomotion					
increase weight-bearing locomotor behavior	locomotor development	rat	P1,5,10	P10: Achilles tendon: maximum force↑, displacement at maximum force↑, stiffness↑, cross-sectional area↑(conpared to non-weight P1,P5) elastic modulus→, maximum stress→,	Theodossiou SK et al. (2019)

				strain at maximum stress→(conpared to non-weight P1,P5)	
Added Stimulation					
increased muscle contraction	Electrical stimulation	zebrafish	E0 to 48hpf	48hpf: axial tenocyte projection length, density→ [■+EMS vs aBtx] axial tenocyte projection length ↑(conpared to αBtx-injected) pSMAD3↑, scxa→, tsp4b↑, tgfbip↑, ctgfa2↑ (conpared to αBtx-injected)	Subramanian A et al. (2018)

Increased or decreased value were shown in upward arrows or downward arrows. Loss of expression was shown minus.

Figures

Figure 1

Flow Diagram Showing Selection of Articles Used in The Study

Manual database search yielded 586 articles. After the first screening stage of titles and abstracts, 84 articles were selected for full-text review.

Figure 2

Types of Intervention Methods

We defined mechanical force in terms of muscle contraction, muscle existence, and weight-bearing for each animal species.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalAppendixS1.pdf
- SupplementalAppendixS2.pdf
- SupplementalTableS1.pdf