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## Review

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# Is muscle involved in the mechanical adaptability of echinoderm mutable collagenous tissue?

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### Summary

The mutable collagenous tissue (MCT) of echinoderms has the capacity to change its mechanical properties in a time scale of less than 1 s to a few minutes under the influence of the nervous system. Although accumulating evidence indicates that the mechanical adaptability of MCT is due primarily to the modulation of interactions between components of the extracellular matrix, the presence of muscle in a few mutable collagenous structures has led some workers to suggest that contractile cells may play an important role in the phenomenon of variable tensility and to call for a re-evaluation of the

whole MCT concept. This contribution summarises present information on MCT and appraises the argument implicating muscle in its unique mechanical behaviour. It is concluded that there is no evidence that the variability of the passive mechanical properties of any mutable collagenous structure is due to muscle.

Key words: Echinodermata, mutable collagenous tissue, connective tissue, extracellular matrix, muscle, juxtaligamental cell, mechanical properties, variable tensility.

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### Introduction

In the summary of a recent paper, Elphick and Melarange (2001) opine that "... the concept of 'mutable connective tissue' in echinoderms may therefore need to be re-evaluated to incorporate the involvement of muscle, as proposed recently for the spine ligament in sea urchins". Although the focus of their article is the neural control of muscle relaxation in echinoderms, it includes a digression into the field of mutable collagenous tissue that arrives at the above conclusion on the basis of a limited selection of published observations. Since this is the second time in recent years that a role for muscle in the variable tensility of mutable collagenous tissue has been proposed (see del Castillo et al., 1995), there is a need to review critically the evidence for this proposition. My aims in the following contribution are to summarise present information on mutable collagenous structures, to examine the case for muscle being involved in their mechanical behaviour and to demonstrate that, whilst the presence of muscle in a minority of these structures has interesting implications for their functioning, these are independent of, and irrelevant to, the mechanical adaptability of their extracellular matrix.

### What is the current concept of 'mutable connective tissue'?

Mutable collagenous tissue (MCT) shows rapid (time course less than 1 s to a few minutes) changes in passive mechanical

properties (tensile strength, stiffness, viscosity, etc.) that are under nervous control. [I would advocate the expression mutable *collagenous* tissue to emphasise the uniqueness of its properties in comparison with those of 'conventional' collagenous tissues and because there are other kinds of mutable connective tissue such as the plasticisable chitin of some insects (Reynolds, 1980)]. Without exception, all confirmed mutable collagenous structures, i.e. those in which the capacity for variable tensility has been demonstrated experimentally by mechanical tests on isolated tissue preparations, are permeated by, or in contact with, the processes of neurosecretory-like juxtaligamental cells that contain large electron-dense granules. These cells are absent from the few definitely non-mutable collagenous structures that have been examined. It has been observed that juxtaligamental cells of crinoids, echinoids and ophiuroids come into close contact with conventional axons, sometimes at chemical synapse-like junctions (Wilkie, 1996a). Current information on the supramolecular organisation of MCT extracellular matrix, the molecular basis of its variable tensility and the possible effector role of the juxtaligamental cells is derived largely from the intensive efforts of J. A. Trotter and collaborators. The consensus that has emerged from the work of this and other groups, mainly on the echinoid capsular spine ligament and holothurian dermis, is that mutable collagenous structures consist of discontinuous collagen fibrils organised into bundles (fibres) by an elastomeric network of fibrillin microfibrils and

interconnected by a stress-transfer matrix consisting partly of stiparin, a glycoprotein that binds to and aggregates the fibril. [A notable exception to this pattern of organisation is provided by the autotomy tendons of ophiuroid intervertebral muscles. These are extensions of the basal laminae of the muscle cells and consist of non-fibrillar collagen (Wilkie and Emson, 1987)].

It is clear from transmission electron microscope observations that changes in the mechanical properties of MCT result from alterations in interfibrillar cohesion rather than in the collagen fibrils themselves. Other molecules that have been isolated from MCT can influence its interfibrillar cohesion. These include a stiparin inhibitor, which binds to stiparin and prevents its interaction with collagen, a 'stiffener' and a 'plasticiser'. There is evidence that the stiffener and plasticiser are stored in the juxtaligamental cells and that the mechanical properties of MCT are modulated by the release of these factors from the juxtaligamental cells by  $\text{Ca}^{2+}$ -dependent cellular mechanisms (see Szulgit and Shadwick, 1994, 2000; Trotter and Koob, 1995; Trotter et al., 1996, 1999; Thurmond and Trotter, 1996; Koob et al., 1999; Wilkie et al., 1999; Trotter, 2000). The current concept of MCT thus attributes its mechanical adaptability entirely to the modulation of interactions between components of the extracellular matrix. Elphick and Melarange (2001), in contrast, believe that enough evidence is available to justify the suggestion that this is not the whole story and that the mechanical adaptability of at least some mutable collagenous structures is due partly or, in one case, wholly to contractile cells.

### Mutable collagenous structures are functionally diverse

Before assessing the significance of muscle in the functioning of MCT, I need to demonstrate that mutable collagenous structures do not show a uniform pattern of passive tensile changes. In fact, they show three patterns that, for the purpose of this review I shall call types A, B and C. In intact animals, type A structures undergo only reversible stiffening and destiffening; type B tissues undergo reversible stiffening and destiffening, but they can also show irreversible destabilisation (always associated with autotomy mechanisms) (see Wilkie, 2001); type C tissues are normally stiff and show only irreversible destabilisation (again, always associated with autotomy). This classification can be summarised as follows: type A, stiff $\leftrightarrow$ compliant; type B, stiff $\leftrightarrow$ compliant $\rightarrow$ friable; type C, stiff $\rightarrow$ friable.

The word 'friable' is used here in an attempt to depict the complete loss of tensile strength and tendency to disintegrate of MCT in this physiological state. It should also be noted that there may not be a clear distinction between types A and B, since some type A structures may be able, in unusual circumstances, to destiffen enough to bring about detachment of body parts (Wilkie, 2001) and since experimental (but physiologically relevant) treatments can elicit extreme weakening of some type A structures *in vitro*. The order is also chosen deliberately to suggest an evolutionary sequence, with type A the most primitive and type C the most advanced, although the justification for this will be argued elsewhere.

In addition to changes in passive mechanical properties, some crinoid ligaments can generate an active contractile force (Birenheide and Motokawa, 1996b, 1998; Birenheide et al., 2000). As no myocytes or other possible cellular source of tension development have been detected in these ligaments (Birenheide and Motokawa, 1996b, 1998; Birenheide et al., 2000; Holland and Grimmer, 1981a), it appears that force production must be added to the functional repertoire of the MCT extracellular matrix.

Table 1 shows the pattern of passive tensile changes exhibited by all confirmed mutable collagenous structures and also indicates the occurrence of muscle cells in these structures. The following discussion assesses the possible role of muscle first in irreversible and then in reversible changes in mechanical properties.

### Irreversible destabilisation

This is the most extreme manifestation of variable tensility. Most spectacular of all is the 'melting' of the body wall of certain aspidochirote holothurians in response to pressure and other stimuli (Junqueira et al., 1980). [It has been proposed, on the basis of preliminary data, that the mechanism responsible for this phenomenon is unrelated to that underpinning other types of variable tensility (Hill and Rahemtulla, 1998; Hill, 2001).] However, irreversible destabilisation is demonstrated mainly by collagenous structures that cross autotomy planes and is expressed in mechanical tests as a rapid and profound drop in tensile strength, stiffness or viscosity and in ultrathin sections as a disorganisation of the collagen fibres and mutual separation of their constituent fibrils. Ultrastructural investigations have failed to detect myocytes or evidence for a contractile apparatus in other cell types, in eight of the 10 type B or C structures that have been shown to have this capacity, and contractile cells would therefore appear to have no role in their destabilisation (whatever that role could be). The exceptions are starfish aboral dermis and the introvert dermis of dendrochirote holothurians. Starfish aboral dermis contains calcite ossicles interconnected by collagen fibres and by small bundles of myocytes. At autotomy, but only within the basal autotomy plane of the arm of asteriid starfish, the collagen fibres of the dermis disaggregate and the muscles undergo an endogenous rupturing process. The small size of the muscles in relation to the extracellular components and the fact that their orientation would not enable them to exert tension on the collagen fibres (see Wilkie et al., 1990) preclude the possibility that dermal disruption could be due in some way to their activity. For the same reasons, it is not credible that holothurian introvert dermis is disaggregated by the few muscle fibres that are dispersed within it and occupy only 1–4% of its cross-sectional area (Byrne, 2001).

### Reversible stiffening and destiffening

Most mutable collagenous structures investigated have the capacity to switch reversibly between a stiffened state, which fixes posture, and a destiffened or compliant state, which

Table 1. *Mutable collagenous structures*

Class	Type A Stiff↔compliant	Type B Stiff↔compliant→friable	Type C Stiff→friable
Crinoidea	Synarthrial ligaments of arm <sup>1,a</sup> ; symplexal and/or PTG ligaments of stalk <sup>2</sup> ; cirral ligaments <sup>3a</sup>	NIY	Syzygial ligaments of arm <sup>4</sup> ; synostosal ligaments of stalk <sup>2</sup>
Asteroidea	Aboral dermis <sup>5</sup> (M) and longitudinal interambulacral ligaments <sup>6</sup> outwith autotomy region; spine ligaments <sup>7</sup> (M)	Aboral dermis <sup>5</sup> (M) and longitudinal interambulacral ligaments <sup>6</sup> within autotomy region	NIY
Ophiuroidea	Proximal oral arm plate ligaments <sup>8</sup> ; oral shield–oral plate ligaments <sup>9</sup>	Intervertebral ligaments <sup>10</sup> ; distal oral arm plate ligaments <sup>8</sup> ; disc dermis <sup>11</sup>	Autotomy tendons of intervertebral muscles <sup>12</sup>
Echinoidea	Tooth ligaments <sup>13</sup> ; peristomial membrane <sup>14</sup> (±); compass depressors <sup>15,c</sup> ; capsular spine ligaments <sup>16</sup> (M); central spine ligaments <sup>17</sup> (±)	NIY <sup>b</sup>	NIY
Holothuroidea	Main body wall dermis <sup>18</sup>	Introvert dermis <sup>19</sup> (M)	PL tendons <sup>19</sup>

The table lists only those structures whose capacity for variable tensility has been supported by published experimental evidence derived from mechanical tests on isolated tissue preparations. Superscript numerals refer to key references.

<sup>1</sup>Birenheide and Motokawa, 1994, 1998. <sup>2</sup>Grimmer et al., 1985; Wilkie et al., 1994b. <sup>3</sup>Holland and Grimmer, 1981a; Birenheide et al., 2000. <sup>4</sup>Holland and Grimmer, 1981b; Wilkie et al., 1999. <sup>5</sup>Wilkie et al., 1990. <sup>6</sup>Wilkie et al., 1995a. <sup>7</sup>Motokawa, 1982a, 1986. <sup>8</sup>Wilkie, 1992. <sup>9</sup>Candia Carnevali et al., 1994. <sup>10</sup>Wilkie, 1988. <sup>11</sup>Wilkie et al., 1984. <sup>12</sup>Wilkie and Emson, 1987. <sup>13</sup>Birenheide and Motokawa, 1996a; Birenheide et al., 1996. <sup>14</sup>Wilkie et al., 1993; Wilkie et al., 1994a. <sup>15</sup>Wilkie et al., 1992. <sup>16</sup>Smith et al., 1981. <sup>17</sup>Motokawa, 1983. <sup>18</sup>Motokawa, 1981, 1982b. <sup>19</sup>Byrne, 1985, 2001.

M, myocytes detected within structure; NIY, none identified yet; PL, pharyngeal retractor muscle-longitudinal body wall muscle; PTG, peripheral through-going; (±) variable tensility not demonstrable in all species tested.

<sup>a</sup>Actively contractile in some species.

<sup>b</sup>The ligaments that cross the autotomy planes of globiferous pedicellariae consist very probably of mutable collagenous tissue, but this has still to be confirmed experimentally in isolated preparations (Hilgers and Splechtina, 1982).

<sup>c</sup>The capacity for variable tensility is strongly expressed in the compass depressor ligaments of certain echinoids and, in these, the myocytes are present as only an outer myoepithelial layer. The compass depressors of other echinoids show weak mutability and, in these, the myocytes are intraligamental and interspersed between the collagen fibres (Wilkie et al., 1998).

allows posture to be altered by muscles. It is these reversible changes in stiffness that are the particular target of the suggestion of Elphick and Melarange (2001) that muscles might be involved in the variable tensility of MCT to an extent that requires a re-evaluation of the MCT concept itself. Their case can be summarised as follows: (i) light and electron microscopy has revealed the presence of muscle cells in some mutable collagenous structures; (ii) these muscles probably influence overall stiffness, and it is likely that the muscle cells in one of these structures are entirely responsible for its variable tensility; and (iii) the responses of mutable collagenous structures to certain pharmacological agents are comparable with those of muscles. I will address these three points in turn.

(i) Table 1 shows that muscle cells have been found in only four (21 %) of the 19 separate structures in which the capacity to stiffen and destiffen reversibly has been demonstrated. It thus seems reasonable to conclude that muscle plays no role in the mechanical adaptability of most mutable collagenous structures since it is highly unlikely that, over a period of 25

years, a succession of electron microscopists has overlooked myocytes, or other cells containing a contractile apparatus, that are numerous enough, or can generate sufficiently powerful contractions, to be responsible for the wide variation in stiffness shown by the structures containing them.

(ii) Elphick and Melarange (2001) refer explicitly to two of the four structures recorded as containing myocytes in Table 1. They consider that the contractile state of the small muscles connecting ossicles in the aboral dermis of asteroids 'probably influences the stiffness of the body wall'. However, this supposition is not supported by the available evidence. In its stiffened state, the whole body wall of the spinulosid starfish *Echinaster spinulosus* has a breaking strength of approximately 40 MPa (O'Neill, 1989). The strongest known muscle is the anterior byssus retractor muscle (ABRM) of *Mytilus edulis*. Its breaking strength while generating a maximal isometric force of 1.4 MPa is approximately 10 MPa (Sugi et al., 1999). Thus, the breaking strength of whole starfish body wall, less than 1 % of the volume of which is

occupied by interossicular muscles (O'Neill, 1989), is four times that of the ABRM. The interossicular muscles cannot be responsible for the stiffened state of *E. spinulosus* body wall. Even in starfish that never become as rigid as spinulosids, such as forcipulatids, the variable tensility of the body wall cannot be attributed to these muscles: the breaking strength of *Coscinasterias calamaria* body wall, which probably contains approximately the same amount of muscle as that of *E. spinulosus*, is 3.3 MPa (O'Neill and Withers, 1995), and Wilkie et al. (1990) estimated that, were they to be responsible for the acetylcholine-induced stiffening of the body wall of *Asterias rubens*, the muscular components would have to generate an active stress of 4.4 MPa, i.e. three times the maximal tension produced by the bivalve ABRM (Sugi et al., 1999).

The second muscle-containing collagenous structure discussed by Elphick and Melarange (2001) is the capsular ligament, or 'catch apparatus', of the echinoid spine joint. They observe correctly that studies of this ligament led to the initial concept of neurally controlled 'connective tissue catch', but then state that "... the problem with this model is that there is no plausible molecular mechanism by which release of neurotransmitters by nerves could influence the mechanical state of collagen fibrils". This comment contains two inaccuracies. First, it has long been accepted that the variable tensility of MCT depends on changes in interfibrillar cohesion, not in the collagen fibrils themselves. Second, no-one, as far as I am aware, has ever suggested that neurotransmitters affect directly the mechanical properties of the extracellular matrix of this or any other mutable collagenous structure. The tensility of many isolated MCT preparations is affected by exogenous neurotransmitters that have always been assumed to be acting on *cellular* targets – either juxtaligamental cells or neural elements that modulate the activity of these cells – that remain in a functioning state in the excised tissue (Motokawa, 1987; Wilkie et al., 1990, 1995a; Birenheide et al., 1996, 2000). For example, since there are juxtaligamental perikarya intermingling with conventional axons both within the capsular ligament and on its outer surface, isolated preparations of this ligament are bound to include intact juxtaligamental cells and perhaps also neurons (Takahashi, 1967a; Smith et al., 1981; Hidaka and Takahashi, 1983; Peters, 1985).

In support of their case, Elphick and Melarange (2001) cite the hypothesis of del Castillo and co-workers (del Castillo et al., 1995; del Castillo and Smith, 1996; Pérez-Acevedo et al., 1998) according to which the destiffened state of the capsular ligament results from the sliding of ligament loops round calcite bars in the ossicle insertion regions, and the stiffened or 'catch' condition is achieved by contraction of the intraligamental myocytes, which pulls the ligament loops against the calcite bars and prevents their slippage, a model that dispenses with the concept of mutable collagenous tissue altogether. This hypothesis is untenable for a number of reasons (detailed by Wilkie, 1996b), the main one being that it depends on the myocytes being in series with the collagen

fibrils and being the only mechanically important linkage between them, even in the maximally stiffened state. This would mean that when the ligament supports a tensile load all of this load would be sustained by the myocytes. Hidaka and Takahashi (1983) found that the acetylcholine-stiffened capsular ligament of *Anthocidaris crassispina* had a tensile strength of 18–38 MPa. The myocytes occupy approximately 3% of the cross-sectional area of this ligament. Were they in series with the collagen fibres, their tensile strength would need to be at least 600 MPa, 60 times that of the mollusc ABRM, which is highly improbable. Furthermore, this hypothesis ignores other evidence that the capsular ligament consists of MCT. It is, for example, stiffened dramatically by agents that cause cell disruption, such as distilled water or the non-ionic detergent Triton X-100 (Shadwick and Pollock, 1988; Szulgit and Shadwick, 1994), effects that could not be mediated by myocytes and that occur in other mutable collagenous structures that lack myocytes (Trotter and Koob, 1995; Wilkie et al., 1995b; Trotter and Chino, 1997).

(iii) Elphick and Melarange (2001) believe that pharmacological data from two separate collagenous structures provide a further indication of the involvement of muscle in the variable tensility of MCT. Takahashi (1967b) first reported that, when the echinoid capsular ligament is stretching under a constant load, acetylcholine arrests and adrenaline accelerates its extension. Because acetylcholine is the major excitatory neuromuscular transmitter in echinoderms, Elphick and Melarange (2001) suggest that this, in itself, implies the involvement of muscle. It is also relevant to mention here that, by recording separately active force development and passive stiffness, del Castillo and co-workers discovered that there are similarities in the pharmacological characteristics of the *contractile* response of the capsular ligament, which is undoubtedly due to the myocytes, and its *stiffening* response (Morales et al., 1989, 1993; Vidal et al., 1993). This was taken to indicate that contraction and 'catch' are different aspects of one phenomenon – shortening of the myocytes – and led to the hypothesis discussed above. However, it has already been argued that the myocytes cannot be responsible for the changes in the passive mechanical properties of the capsular ligament that are induced by acetylcholine. Moreover, exogenous acetylcholine affects the mechanical properties of echinoderm collagenous structures that lack myocytes: it increases the stiffness of the central spine ligament of an echinoid (Motokawa, 1983), destiffens the cirral ligament of a crinoid (Birenheide et al., 2000) and has a biphasic stiffening/destiffening effect on the dermis of a holothurian (Motokawa, 1987).

Clearly, then, there is no correlation between acetylcholine-induced changes in passive mechanical properties and the presence of muscle cells. The effects of acetylcholine on the stiffness of all these structures, capsular ligament included, can be explained only in terms of changes in the tensility of the extracellular matrix, and the only possible cellular effectors bringing about these changes are the juxtaligamental cells. That the activities of these cells are controlled at least

partly by cholinergic pathways is indicated by both pharmacological data (cited above) and ultrastructural evidence for functional contacts between cholinergic nerve fibres and juxtaligamental perikarya (Cobb, 1985; Welsch et al., 1995).

Elphick and Melarange (2001) also refer to two neuropeptides that have been isolated from the body wall of the aspidochirote holothurian *Stichopus japonicus*: NGIWAYamide causes contraction of the longitudinal body wall muscle of this animal and increases the stiffness of isolated preparations of its dermis, whereas holokinin 1 inhibits electrically invoked contractions of the muscle and destiffens the dermis (Iwakoshi et al., 1995; Birenheide et al., 1998; Inoue et al., 1999). Elphick and Melarange suggest that the responses of the dermis are due to muscle cells. Indeed, they assert that “It seems *most likely* that this effect of [holokinin 1] on body wall dermis is mediated by *constituent muscle cells...*” [my emphases]. This ignores the fact that ultrastructural investigations have failed to locate muscle cells in the dermis of *S. japonicus* (Motokawa, 1982b) or other aspidochirotetes (Junqueira et al., 1980; Trotter and Chino, 1997), and Stott et al. (1974) found that a high-ionic-strength extract of the dermis of yet another aspidochirote showed no ATP-sensitivity, indicating that there is no contractile mechanism based on an actin/myosin interaction.

It needs to be reiterated that echinoderms possess two major mechano-effector systems consisting of muscles, which actively contract and relax, and extracellular matrix/juxtaligamental cell complexes, which undergo changes in passive stiffness (and sometimes develop force). These systems can form composites, as in the echinoid capsular ligament, or separate organs, such as aspidochirote body wall muscles and dermis. Whatever their mutual relationship, the two systems receive independent innervation, as has been demonstrated indisputably in crinoids (Birenheide et al., 2000), ophiuroids (Wilkie, 1979) and holothurians (Inoue et al., 1999) and for which there is evidence in the echinoid capsular ligament (Peters, 1985). The pharmacological results cited above inform us only that there are features common to the control pathways regulating the two mechano-effector systems in echinoids and holothurians, i.e. they employ the same neurotransmitters or neuromodulators, and/or they include, at unknown locations, cellular receptors with similar properties.

#### **What is the role of muscle in the mutable collagenous structures that contain it?**

There may not be one answer to this question, since the organisation and anatomical relationships of the muscle cells are diverse: in asteroid body wall, small bundles of myocytes form discrete muscles that interconnect the intradermal ossicles (O’Neill, 1989; Wilkie et al., 1990); in the echinoid capsular ligament and asteroid spine ligament, individual myocytes are separated by, and are topologically parallel to (but not mechanically in parallel with), collagen fibres (Smith et al., 1981); and in the introvert dermis of dendrochirote

holothurians the scattered bundles of myocytes are orientated variously in relation to the collagen fibres (Byrne, 2001). O’Neill (1989) speculated that the intradermal muscles of the asteroid *Echinaster spinulosus* realign the body wall ossicles after stress relaxation or large deformations. As suggested originally by Smith et al. (1981), this may also be the function of the myocytes in the echinoid capsular ligament, since it undergoes considerable elongation when there is extreme flexion at the spine joint (which involves dislocation of the joint) (Takahashi, 1967c), and those in dendrochirote introvert dermis, which also exhibits great extensibility during normal activities. Although all these structures contain microfibrils that are believed to provide an elastic restoring force (Thurmond and Trotter, 1996), contraction of the myocytes may contribute to reshortening after large deformations.

Intraligamental myocytes may, in particular circumstances, work synergistically with adjacent conventional muscles. For example, one function of the myocytes in the echinoid capsular ligament may be to assist the spine muscle in re-erecting the spine.

Finally, it is not impossible that intraligamental myocytes influence the passive mechanical properties of mutable collagenous structures when the extracellular matrix is itself in a compliant state, in the way that smooth muscle cells affect the wall stiffness of mammalian blood vessels, although this might be an incidental effect rather than a biological function of the myocytes in MCT. At present, however, there is no evidence for this, and none will be forthcoming until techniques are devised to incapacitate selectively either the intraligamental muscle or the molecular mechanisms responsible for the variable tensility of the extracellular matrix.

#### **Concluding remarks**

The most spectacular manifestation of MCT variable tensility – the irreversible destabilisation that occurs during autotomy and holothurian dermis ‘melting’ – cannot possibly be attributed to the activities of muscle cells. Muscle cells have not been detected in most confirmed mutable collagenous structures that show reversible stiffening and destiffening. Investigations of the few mutable collagenous structures in which muscle has been detected have demonstrated that the latter cannot account for the variability of their passive mechanical properties. For example, to be responsible for the maximally stiffened state of these structures, intraligamental muscle fibres would have to develop a tensile strength many times greater than that of the strongest muscle known heretofore. It is possible, however, that the muscle fibres affect the passive mechanical properties of these structures when the extracellular matrix is in its low-stiffness state. Pharmacological data provide no evidence for the involvement of muscle in the variable tensility of MCT, although they reveal features common to the control pathways regulating contractile and collagenous components. There are no grounds for reformulating the current concept of mutable collagenous tissue to include a role for intraligamental muscle.

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