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Morphological changes of the triceps surae muscle–tendon unit during passive extension: an *in vivo* rabbit model

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Abstract

Objective. To elucidate the morphological and biomechanical manifestation of the triceps surae muscle–tendon unit during passive extension.

Design. The instantaneous changes within the load-deformation curve of muscle–tendon unit were analyzed by an *in vivo* rabbit model.

Background. Although muscle strains occur more frequently than complete failures, the failure mechanism of these sub-failure injuries is rarely investigated. Monitoring of the instantaneous changes in the load-deformation curve allows correlation with the morphological changes that occur during passive extension.

Methods. After anesthesia, the triceps surae muscle of rabbit was dissected and then stretched to failure by a MTS Bionix 858 machine. The morphological changes in failure patterns were recorded by photographs.

Results. The morphological and biomechanical manifestations of the triceps surae muscle–tendon unit was divided into five different portions: first, the viscoelastic portion with minimal morphologic change; second, the portion of micro-failure with local ecchymosis; third and fourth, the portions of macrofailure with sequential rupture of the muscle fibers; and fifth, the portion of rupture and separation of muscle parenchyma.

Conclusions. A threshold for stretch-induced injury does exist. The threshold of the initiation of micro-failure in this model was 16.5% of the strain, which corresponded to 16.6% of the maximal sustainable force.

Relevance

Elucidation of the existence of micro-failure in skeletal muscle gives a good reference point to determine the functional capabilities and limitation of muscular tissue. With this, the deleterious effect of muscle stretching can be avoided by limiting muscle stretching to below this limit.

Keywords: Micro-failure; Macrofailure; Triceps surae muscle; Passive extension

1. Introduction

The tensile test is the most widely used experimental method to characterize the mechanical properties of materials. From a complete test record, one can obtain important information concerning the material's elastic properties, character and extent of plastic deformation, yield and tensile strengths, and toughness. By using various modes of loading, the material properties of bone, cartilage, tendon and ligaments have been studied [1–3]. The structures of these materials can be described by their representative load-deformation curve. The conventional load-deformation curve (Fig. 1) includes a linear region (known as linear elastic region) followed by a non-linear region where 'yielding' occurs. After yielding, there is an internal rearrangement of the structure and damage accumulation until

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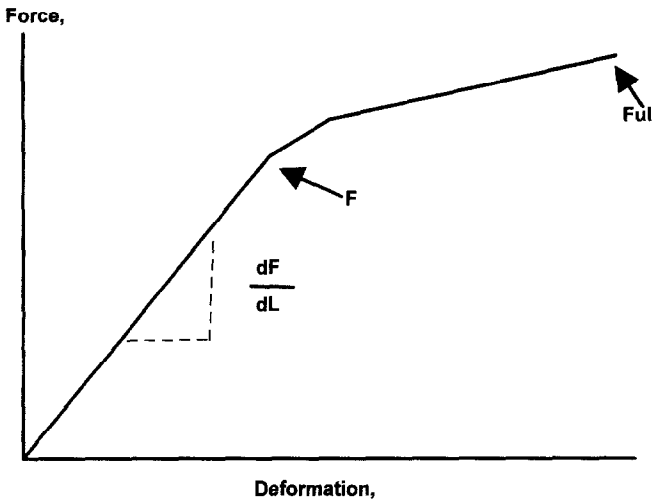

Fig. 1. Plot of force-deformation curve defines structural behavior. Ful: ultimate failure load; Fy: yield load; dF/dL: stiffness.

finally, failure results in the loss of the load-bearing capacity [4]. Recently, Garrett [5] showed that it is safe to cyclically stretch the skeletal muscle below 50% of peak force. When the force produced in the muscle is beyond 70% of maximal sustainable force, many muscles demonstrated macroscopic evidence of disruption, i.e. microscopic failure of muscle may appear before 70% of the maximal sustainable force. In preliminary work at our institute, the muscle–tendon unit behaved viscoplastically during dynamic loading. The dynamic responses of muscular tissues were both strain and strain-rate dependent [6]. Later, we demonstrated that a threshold for stretch-induced injury does exist. When the muscle–tendon units were cyclically stretched beyond the threshold, the biomechanical parameters of the muscle–tendon units changed significantly [7]. When muscle–tendon units are ramp-up passively extended, a similar threshold of micro-failure must exist. The purpose of this study was to correlate morphological and biomechanical manifestations in load-deformation curves. Our hypothesis was that the micro-failure of the muscle–tendon unit during passive tensile testing can occur at a point far before the yield point.

2. Methods

Twelve adults New Zealand white rabbits [mean body weight 2.5 kg (SD, 0.2 kg)] were used. The set-up of biomechanical tests was the same as previous reported [8]. Briefly, after general anesthesia with intramuscular administration of Ketamine (50 mg/kg), the hind limb was prepared. A skin incision on the lateral aspect of the hind limb was made. The Achilles tendon was isolated with special care to maintain the neurovascular supply and to keep the tendon insertion intact. A dial caliper (accurate to 0.05 mm) was used to measure the distance between the origin of the triceps surae at the femur and the insertion at the calcaneus when the knee and ankle joints were flexed at 90°. This distance was defined as the resting length (Lo) of muscle–tendon unit.

The anesthetized rabbit was then placed in a frame attached to the MTS machine (MTS BionixTM 858 Test System, USA). The hind limb was immobilized with a K-wire transfixation through the proximal tibia. The knee joint was kept at 90° flexion with a transverse bar of the fixation frame. The distal tendon's insertion was freed by osteotomization at the calcaneal tuberosity and clamped to the MTS load cell (MTS 458.20, Microconsole, Axial, USA). A 3-N preload was placed on the muscle, and then the muscle length was again measured to restore its resting length. During the test, the muscle was stretched at a constant speed (5 mm/min) until a macroscopic tear or full division of the ruptured muscle fragments occurred. The muscles were kept moist at physiologic temperatures with warm (25°C) normal saline irrigation. Additional dosage of anesthetics was given as needed. This study received prior approval of the National Taiwan University Medical College's Animal Research Committee. After completion of the experiments, the rabbits were killed.

A Testlink™ system Software (PCLAB™ Data Translation, Data Translation Inc., Marlboro, MA, USA) was used to simultaneously record the force and length changes when the muscle was contracted. The load changes were continuously monitored during the test. The morphologic changes of the muscle–tendon unit were closely observed and recorded photographically at intervals of 10 N. The sampling rate was at 20 Hz, with a PC computer. The deformation (L) was defined as the change in length of muscle–tendon unit at various loads and before distraction ($dL = L - Lo$). The measurement of strain was calculated by dividing the deformation with the initial resting length before distraction (dL/Lo).

The load-deformation curve of muscle–tendon unit was divided into five portions (Fig. 2). The first was the region before the foot region, the second portion was the region of increasing slope, the third was at the portion of maximal sustainable force, the forth was the portion of second peak, and the fifth portion was the final descent of load-deformation curve. Repetitive serrate changes within the curve of each muscle–tendon unit were measured and analyzed. At the middle of each portion, five serrations from different portion of the load-deformation curve were plotted

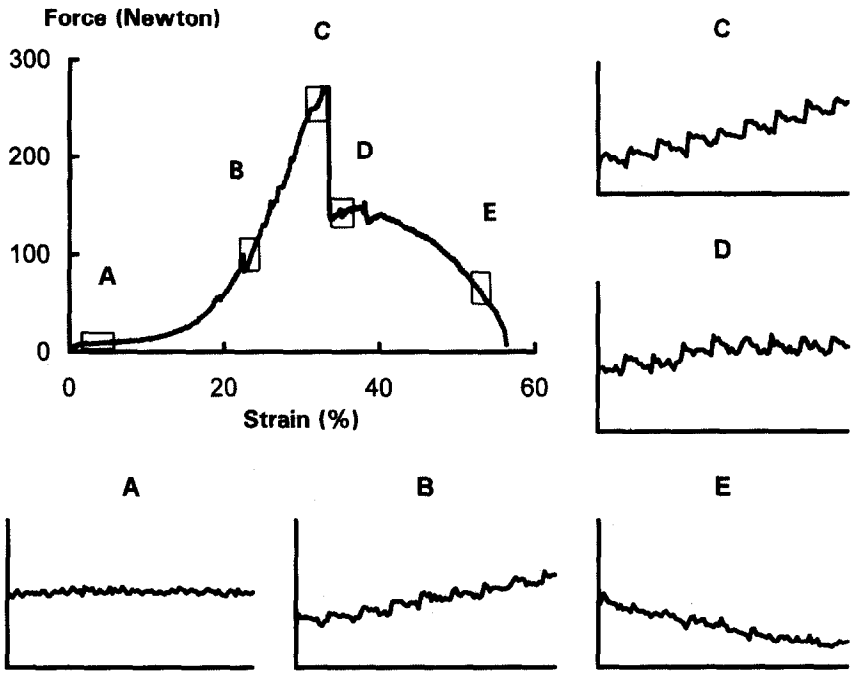

Fig. 2. Load-deformation curve of tensile test in a representative triceps surae muscle–tendon unit. Representative 100 data are depicted within A to E, the scale of X-axis and Y-axis of each bracket were 0.5% strain and 20 Newton. (A: 7.1–7.6%, 0–20 N; B: 20.6–21.1%, 60–80 N; C: 30.1–30.6%; 225–245 N; D: 35.6–36.1%, 135–155 N; E: 53.2–53.7%; 50–70 N). The maximal amplitude of serration within each bracket were A: 0.9 N, B: 2.06 N, C: 3.28 N, D: 2.67 N, E: 1.53 N.

and analyzed. The magnitude of load changes within various portions of the curve was measured by means of initial ascent of these serrations. The significance in the magnitude of load changes within various portions of the curve was tested by one-way ANOVA. The threshold of the serration appearance was chosen by observation from each curve that showed obvious serration.

3. Results

3.1. Shape of the load-deformation curve

The shape of the load-deformation curve of triceps surae muscle–tendon unit (Fig. 2) was the same as has been previously reported [7]. There was an initial linear region with a lower slope followed by a non-linear region with a higher slope until maximal load and plateau was achieved. After the point of maximal sustainable force, there was a steep drop followed by a curve that corresponded to a gradual increase and decrease of force.

3.2. The morphologic changes during passive extension

The correlation between the progressive failure of the muscle–tendon units and their biomechanical manifestation in load-deformation curves was clearly demonstrated by the recorded photographs. In the initial foot portion of load-deformation curve, there was a minimal morphologic change as recorded by photographs (Fig. 3A). In the portion of initial increasing slope of the load-deformation curve, the morphological manifestation was lengthening, thinning in the diameter of the muscle–tendon unit and local ecchymosis at the distal muscle–tendon junction (Fig. 3B). At peak load, the photographs recorded at these portions showed sequential rupture of individual muscle fiber (Fig. 3C and Fig. 3D). After the plateau portion, the recorded photograph showed gross disruption of the muscle–tendon unit (Fig. 3E).

3.3. The instantaneous changes within the curves

Stress relaxation behavior of the triceps surae muscle during passive extension is illustrated in Fig. 2. In the initial foot portion of load-deformation curve (Fig. 2A), only negligible changes in oscillations were noted. After the initial foot portion of the curve, there were obvious repetitive serrations of the load-deformation curve found throughout the residual portion of the curve (Figs 2B, 2C, 2D and 2E). Of these serrations, the initial increased amount of load in the portion of initial increasing slope (Fig. 2B) was greater than the decreased load amount in the descending portion. This resulted in an increased slope. In the portion of peak

load (Figs 2C and 2D), the maximal amount of load changes within each serration was noted. There were equal amount of changes in the ascent and descent of the serrations. After the plateau portion (Fig. 2E), there were more load changes in the descent portion than that in the ascent.

3.4. Analysis of changes in serrations

For various areas of the triceps surae muscle–tendon curve, significant differences existed in magnitude of load changes within serrations (Table 1). The load of threshold was 44.6 Newton, which represented 16.6%

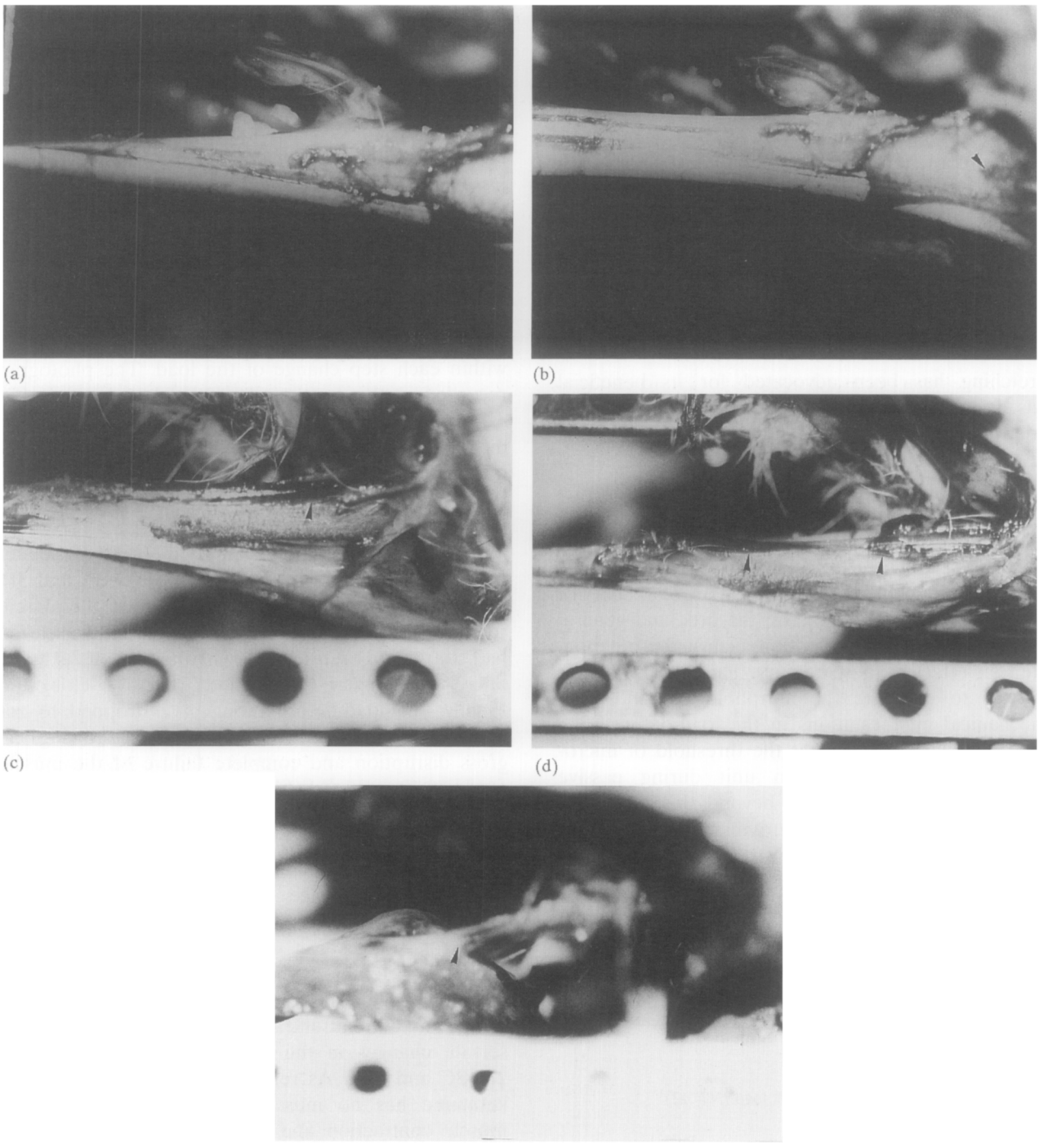

Fig. 3. Photographic manifestation in progressive failure of the triceps surae muscle–tendon unit tested by tension to failure. Photos correspond to similarly numbered points on the curve depicted in Fig. 2. (A): minimal morphologic changes. (B): lengthening, thinning in the diameter of the muscle–tendon unit with focal ecchymosis (►) at the distal muscle–tendon junction. (C and D): sequential disruption of the individual muscle fibers (►). (E): gross disruption (►) of the muscle–tendon unit.

Table 1
Analysis of the magnitude of load change within serration of various portions of the load-deformation curve ($N = 12$)

Portions*	Load change in Newtons (SD)
Portion I	1.48 (0.26)
Portion II	2.29 (0.38)
Portion III	3.68 (1.22)
Portion IV	3.59 (1.42)
Portion V	2.12 (0.61)

*$P < 0.0001$: analyzed by one-way ANOVA.

maximal sustainable force of the corresponding muscle–tendon unit. The strain of threshold was 16.5%, which represented 52.9% strain at the maximal sustainable force of the corresponding muscle–tendon unit (Table 2).

4. Discussion

Stretching has been advocated for its beneficial effects. In sports medicine, stretching has been recommended to prevent injury [9–11]. In rehabilitation, stretching is used as a method to regain range of motion and to improve overall function [12]. Eccentric muscle contraction can result in muscle soreness, and muscle injury had also been reported [13]. Because of the paucity of study of stretching, the extensive clinical interest in stretching and the wide-spread use of stretching techniques in athletes, the arts, and general fitness programs are a prevention against the potential deleterious effect of muscle injury. The purpose of this study was to correlate the morphological and their biomechanical manifestations in the load-deformation curves. Our hypothesis is that the threshold of micro-failure of the muscle–tendon unit during passive tensile testing does exist, and beyond this threshold, potential deleterious muscle injury may occur.

The triceps surae muscle is structurally inhomogeneous and mechanically non-linear, and is an anisotropic tissue. Under tensile deformation with a constant rate of elongation, the biomechanical properties of muscular tissue were analyzed. The curve starts with the foot portion (Fig. 2A). In this portion, the tissue deforms easily without the need of excessive force. The curve pattern and behavior are more like the properties of elastic materials, as there is a minimal morphologic change recorded by photograph (Fig. 3A). This behavior can be explained by the serial spring-like elastic component of the tendon and the second parallel elastic component of the perimysium and sarcolemma [14]. Several investigators have suggested that the cross-bridges of myosin filaments also have a spring-like properties and contribute to the elastic properties of muscle [15,16]. The distensibility and elasticity of the elastic components tends to keep the muscle in readiness for contraction and may help prevent the passive over-stretch of the contractile elements when these elements are relaxed, thereby lessening the danger of muscle injury [17].

Table 2
Summary data of the threshold and peak load ($N = 12$)

	Threshold	Peak load
Load (Newton)	44.55 (SD: 5.81)	275.04 (SD: 37.16)
Range (Newton)	28.38–50.27	217.36–334.93
Strain (%)	16.45 (SD: 1.47)	30.98 (SD: 1.84)
Range (%)	14.62–20.50	28.43–34.31

When the muscle–tendon unit *in vivo* was subjected to loading that exceeded the foot portion, stress relaxation behavior of the triceps square muscle during passive extension appeared, which was manifested as repetitive serration of the curve (Fig. 2B). The instantaneous load gradually decreased to a lower level within each step change of the load. The photograph recorded in this portion shows local ecchymosis (Fig. 3B). The ecchymosis, lengthening and thinning of the diameter of the muscle–tendon unit may represent micro-plastic deformation (i.e. micro-trauma) within the material. In the portions of peak load, there was almost equal ascent and descent of serration (Figs 2C and 2D). The photographs recorded here show sequential ruptures of the individual fiber (Figs 3C and 3D). This means that when the displacement of the muscle–tendon unit continued to peak load, the muscle still had continuity, even though it had undergone extensive micro- and macro-failure and extensive elongation. Finally, the load-deformation curve dropped to zero when the displacement was further increased (Fig. 2E): gross disruption and complete failure of the muscle–tendon unit occurred (Fig. 3E).

Muscle is a highly specialized tissue that has an elaborately organized structure, i.e. the cross-bridge-myofilament array. A possible source of the behavior may be the presence of cross-bridges that remain unbroken during long stretches. It has been proposed that cross-bridges can exist in an attached state but produce no force [18]. When the muscle is stretched, these 'latent' cross-bridges are strained, and the cross-bridges may break and then reunite, to manifested as serrate changes in the load-deformation curve (Figs 2B, 2C and 2D). As reported by Wingard *et al.* [19], ketamine has no muscle-relaxant effect. The reflex muscle contraction and hardening response was well preserved in this study. The serrate changes of the curve illustrate the simultaneous competition between the hardening of the muscular tissue, by increasing the rate of cross-bridge reunion, and the softening of muscular tissue, by breaking the cross-bridges. In the

second area of the load-deformation curve (Fig. 2B), the strain-hardening process is much more influential than the softening process. This resulted in a positive slope for the serration of the load-deformation curve. At peak load (Figs 2C and 2D), the strain-hardening process had an equal influence with the softening process. This resulted in equal ascent and descent in the serration of the load-deformation curve. In the area portion (Fig. 2E), this relationship was reversed as the breaking of cross-bridges progressed, and the softening and degradation of muscular tissue overwhelmed the ability of the muscular tissue to harden. Macro-failure progressed and the final rupture of the muscular tissue occurred.

Under continuous measurement of load in this tensile test, there were strain-dependent changes in serrations within the load-deformation curve of the muscle–tendon unit. The maximal change of serration occurred at the maximal degree of tissue destruction, i.e. in the two peaks of the load-deformation curve (Figs 2C and 2D). The difference in the changes of serration between these portions is significant (Table 1). As the 3rd and 4th portion of load-deformation curve corresponds to the sequential rupture of individual muscle fiber recorded photographically (Figs 3C and 3D), the serration of the curve may be a biomechanical manifestation of destructive behavior. We consider the minor magnitudes of serrations in the second portion of the curve to represent intrinsic microscopic destruction of the muscular tissue (Fig. 2B). The existence of micro-failures in the ligaments was elucidated by Kennedy *et al.* [20]. They demonstrated that if the ligaments were stretched to ultimate failure, the ligaments were intact macroscopically, but electron microscopy revealed widespread disruption of the collagen fibril. After further application of stress, actual macroscopic disruption occurred. Tidball *et al.* [21] showed that when the non-stimulated muscle–tendon units were strained to failure, there was myofibrillar damages within the sarcomere. These reports further support the idea of micro-failures, as elucidated in this study.

In 1992, Mair and Dalton reported that there was muscular injury within the physiologic range [22]; however, the limit after which the muscular injury occurred was not mentioned. Garrett [5] showed that if force was produced in the muscle beyond 70% of maximal sustainable force macroscopic evidence of disruption occurred. As their results showed macroscopic disruption of the muscle fibers, we suggest that micro-failure must have existed before this point. Liber *et al.* [23] reported that muscle damage can be induced by eccentric contractions of 25% strain. In our model, the threshold of the initiation of micro-failure was 16.6% of the maximal sustainable force, or 16.5% of strain. These were much lower than the data reported by Garrett [5] and Liber *et al.* [23]. In 1984, Fish *et al.* [24] reported similar serration changes of the length–tension curve in isolated skeletal myocytes. In their study, the threshold for the appearance of serration was about 20% strain, which is very close to the threshold in our study (Table 2).

In most biological tissue, muscle is thought to act viscoelastically. Therefore, muscle is considered to have both elastic and viscous properties. In this study, we demonstrated that the growth of micro-failures are embedded in the region of the curve after the toe portion. The threshold of these micro-failures is much before the previously assumed yield point of this biological tissue. During the preliminary study, excursion of the Achilles tendon was measured between 17.8 and 22.6% strain (Sun *et al.*, unpublished data). On the basis of this observation, we can hypothesize that sub-failure injuries of muscle–tendon unit may attain a sufficient magnitude to cause significant adverse effects on the muscle–tendon unit, especially at extreme physical ranges of motion. If a stretch-strengthening exercise program is considered, it should be performed below this region to avoid potential deleterious effect of micro-failure.

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