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ORIGINAL ARTICLE

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Force deficits after stretches of activated rat muscle-tendon complex with reduced collagen cross-linking

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Abstract The forces produced during stretches of passive and activated muscles, and isometric force deficits after stretching of activated muscles were examined in rat plantor flexor muscle-tendon complexes with reduced collagen cross-links (pyridinoline). Female Sprague-Dawley rats ($n=6$, age 87 days) were injected twice daily for 43 days with β-aminopropionitrile (BAPN, 333 mg/kg/day i.p.), an inhibitor of lysyl oxidase, which is responsible for the production of collagen cross-links. The relative weights of the plantar flexor muscles were similar for BAPN and saline-injected (control, C) rats ($n=6$). Pyridinoline was lower in the tendon (22.9%), and in the plantaris (17.1%), and soleus (7.4%) muscles ($P<0.05$), with no changes observed in collagen content (hydroxyproline), as determined by high-pressure liquid chromatography. At an ankle position of 90°, groups had similar forces at 5, 10, 20, 40, 60 and 80 Hz before stretching. Forces at 40° with stretches of the passive muscles (five times from 90° to 40°) were lower for all stretches in BAPN-injected rats ($P<0.05$). Isometric force deficits resulting from stretches of activated muscles (80 Hz, 20 times from 90° to 40°, rest intervals 3 min) followed similar courses for BAPN-injected and C rats, and were 51.1 (2.4)% (C) and 54.7 (4.6)% (BAPN) before the last stretch. After 1 h of rest, isometric force deficits were 26% and 29% larger at 10 Hz and 5 Hz, respectively, in BAPN-treated rats ($P<0.05$). The reduction in BAPN-injected collagen cross-linking of the skeletal muscle-tendon complex reduced the forces produced during stretches without muscle stimulation (i.e. passive stretch), and stretching of activated muscles produced larger isometric force deficits only at low stimulation frequencies.

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Introduction

Force production in contracting skeletal muscles generates torques about joints and results in movement. During movements, active forces are produced by skeletal muscles while changing length (i.e. shortening contractions and lengthening contractions – stretching of activated muscles). Skeletal muscles produce larger forces during stretches when activated than during isometric contractions (i.e. force production at a constant muscle length), and repeated unaccustomed stretches of activated muscles are known to produce isometric force deficits (i.e. stretch-induced muscle injury) in human muscles (Davies and White 1981; Brown et al. 1997) and rodent muscles (Warren et al. 1993; Balnave and Allen 1995; Lowe et al. 1995; Hesselink et al. 1996; Ingalls et al. 1998; Willems and Stauber 2000). These force deficits have been associated with the high forces produced during stretching of activated muscles (McCully and Faulkner 1986; Warren et al. 1993). Recovery of the force deficits within the 1st day after stretching of activated muscles is small (Lowe et al. 1995; Warren et al. 1999) and complete recovery can take up to 28 days (Ingalls et al. 1998).

The collagens surrounding muscle fibres and whole muscles can provide extracellular support. In the connective tissue of a skeletal muscle-tendon complex, the extracellular structural protein, collagen, exists as collagen fibrils. During the maturation of collagen fibrils, cross-linking occurs between collagen molecules, increasing the tensile strength of the mature collagen fibrils (Danielsen 1981) and making them less vulnerable to biochemical degradation. Mature collagen cross-linking is influenced by the activity of the enzyme lysyl oxidase. In the bone of female rats, treatment with β-aminopropionitrile (BAPN), a compound known to inhibit lysyl oxidase activity (Pinnell and Martin 1968), results in a

reduction in concentration of the collagen cross-link, pyridinoline (HP; Oxlund et al. 1995). This treatment caused decreased elastic stiffness and decreased bending strength (Oxlund et al. 1995). Cross-linking of collagen molecules influences some of the mechanical properties of skeletal muscles. For example, it has been shown that an increased amount of collagen cross-linking in avian dystrophic muscles increases passive stiffness (Feit et al. 1989). Collagen fibrils are likely to play a role in transmitting the intracellularly produced force of activated muscles to the skeletal system via the tendon (Street 1983; Patel and Lieber 1997; Huijing et al. 1998).

In the present study, female Sprague-Dawley rats were treated chronically with BAPN to reduce the amount of HP (and hence HP cross-links) in the plantar flexor muscle-tendon complex. We examined the effects of this reduction in collagen cross-links on: (1) the force resisting ankle dorsiflexion with stretches of the passive muscle-tendon complex, (2) the development of an isometric force deficit during repeated stretches of activated muscles, and (3) the isometric force deficits at different stimulation frequencies after 1 h of rest following the repeated stretches of activated muscles. The isometric force deficits after 1 h of rest following the repeated stretches of activated muscles were taken as indirect evidence of stretch-induced muscle injury (e.g. Balnave and Allen 1995).

Methods

Female Sprague-Dawley rats were used in this study. Rats were provided with water and laboratory chow ad libitum and were housed (two per cage) in animal facilities maintained at 21°C with a 12 h:12 h light:dark cycle. All experimental procedures were approved by and followed the guidelines of the West Virginia University Animal Care and Use Committee (WVU-ACUC no. 9809-02).

Rats ($n=6$; age 87 days) were injected subcutaneously with 333 mg/kg BAPN (Sigma Chemical, St Louis, Mo., USA). They were given two injections per day (09.00 a.m. and 05.00 p.m.) for a period of 43 days. Age-matched, saline-injected rats served as controls ($n=6$). Volumes between 0.4 ml and 0.9 ml were injected in a single injection. Injections were given in the nape of the neck, with sites changing during the treatment period. Body weights were measured every morning.

Rats were anaesthetised with sodium pentobarbital (75 mg/kg i.p.) and supplementary doses (15 mg/kg i.p.) were administered as required to suppress the hindlimb withdrawal reflex when squeezing the footpad. Details on the dissection procedure for nerve cuff placement, animal positioning, dynamometer and force recording are described in detail elsewhere (Cutlip et al. 1997; Willems and Stauber 1999). Briefly, the rat's foot was positioned on an aluminium plate that was connected to a dynamometer. The knee was held at 90°. The dynamometer consists of a DC permanent magnet servomotor (Model 1410C) and a Unidex 100 single-axis motion controller (Aerotech, Pittsburgh, Pa., USA). A Z-11/5 kg load cell is positioned below the aluminium plate (HBM, Marlboro, Mass., USA). Force of the plantar flexor muscles was induced by electrical stimulation of the tibial nerve and was recorded under the sole of the foot (Willems and Stauber 1999). The timing of stimulation and rotational movement of the aluminium plate were computer-controlled. Isometric contractions of 600 ms were performed to determine the stimulus parameters for high-intensity activation of the plantar flexor muscles [0.2 ms pulse duration; 80 Hz; 5.0 (0.3) V]. For each preparation, the voltage was kept constant throughout the experiment. For all preparations, a stimulation frequency of 80 Hz provided over 90% of the maximal force value obtained with 120 Hz and was used for contractions with stretching of activated muscles. The time course of the experimental protocol for measurements of functional properties of the plantar flexor muscle-tendon complex is presented in Fig. 1, and consisted of force/frequency measurements, stretching of passive muscle-tendon complex and stretching of activated muscles.

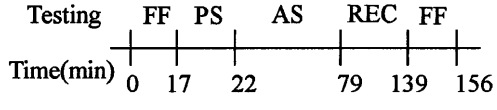

Fig. 1 Time sequence of functional testing of the plantar flexor muscle-tendon complex. Force/frequency (*FF*) measurements were performed at 5, 10, 20, 40, 60, and 80 Hz. Five stretches of passive muscles (*PS*), and 20 stretches of activated muscles (*AS*) imposed on isometric contractions were performed at 80 Hz. After the stretches of activated muscles, there was a period of 1 h of recovery (*REC*) followed by FF

Stretching of passive skeletal muscle-tendon complexes

The passive force resisting ankle dorsiflexion without muscle activation (i.e. stretching of the relaxed plantar flexor muscle-tendon complex) was measured during five intermittent rotations from an ankle position of 90° to 40° at an angular acceleration of 3000°/s^2, and deceleration (Fig. 2). The return movement, plantar flexion from 40° to 90°, also without muscle activation, was performed 1.0 s after the end of the dorsiflexion (not shown in Fig. 2). The time between dorsiflexion movements was 1 min. After the series of stretches of the passive muscle-tendon complex, ankle dorsiflexions with muscle activation (i.e. stretching of activated muscles) were performed.

Stretching of activated skeletal muscles

In each group, 20 stretches of activated muscles were initiated 500 ms after the onset of stimulation from an ankle position of 90° (isometric pre-stretch force) by movement to 40° with similar acceleration and deceleration phases as described for the stretches of the passive muscle-tendon complex. The return movement of the foot was performed after the end of stimulation. The total stimulation time of each contraction was 1.1 s, with rest periods of 3 min to minimise fatigue. From each group, one experiment had to be terminated due to stimulation problems during the series of stretches of activated muscles, marked by a substantial loss of force and the absence of an isometric plateau during the tetanus pre-

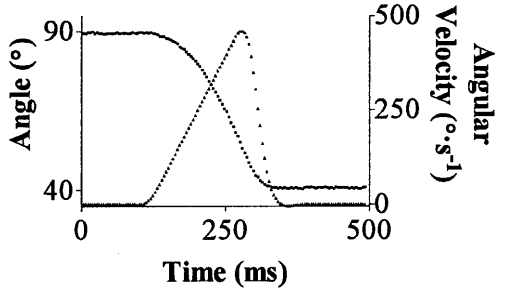

Fig. 2 Ankle angle (*line* starting at the top left of the graph) and angular velocity (*line* starting at the bottom left of the graph) with time during dorsiflexion with stretches of passive and activated rat muscles. Stretches were performed in an ankle range of motion of 50° at an angular acceleration of 3000°/s^2, and deceleration (knee was held at 90°). The duration of movement was about 230 ms

ceding the stretch. However, samples for collagen and collagen cross-links measurements were still taken (see below).

Force/frequency measurements

Force/frequency measurements were performed at an ankle position of 90° using isometric contractions. Plantar flexor muscles were stimulated at 5 Hz (1500 ms), 10 Hz (1500 ms), 20 Hz (1500 ms), 40 Hz (900 ms), 60 Hz (600 ms) and 80 Hz (600 ms), performed twice at each frequency. Typical examples of force traces of plantar flexor muscles for a BAPN-injected rat obtained at stimulation frequencies of 5 Hz, 20 Hz, 40 Hz and 80 Hz are illustrated in Fig. 3. Force/frequency measurements were obtained before (pre-test) and after 1 h of rest (post-test) following the stretching of activated muscles. The isometric force deficits observed following a recovery of 1 h were taken as indirect evidence of stretch-induced muscle injury (e.g. Balnave and Allen 1995).

Collagen and collagen cross-links

After functional testing of the plantar flexor muscle-tendon complex, the rats were euthanised by exsanguination and the Achilles tendon and mid-belly muscle samples from individual plantar flexor muscles (soleus, plantaris and gastrocnemius medialis) were removed. Sampling, handling of the tissue and details on the methods used for measuring the content of collagen (hydroxyproline, HYP) and the mature, non-reducible cross-link HP have been described elsewhere (Miller et al. 1999). It has been found that HP cross-links in the lateral and medial gastrocnemius of male rats of 4–5 months of age are similar (Palokangas et al. 1992). Therefore, HP in the medial gastrocnemius was taken as representative for the entire gastrocnemius muscle. Levels of HYP and HP were determined by high-performance liquid chromatography and quantified relative to known amounts of collagen and HP, respectively. Collagen content is expressed as μg/mg dry weight, and the amount of HP is expressed as mol HP/mol collagen.

Data analysis

Mann-Whitney tests were utilised to test for differences between the groups for: (1) the relative muscle weight of the soleus, plantaris and gastrocnemius muscles, (2) collagen content in the tendon and in the soleus, plantaris and gastrocnemius medialis muscles, (3) HP levels in the tendon and in the soleus, plantaris and gastrocnemius medialis muscles, and (4) relative force at the end of the first stretch (i.e. at 40°, SF40°) of activated muscles. Two-way analysis of variance (ANOVA; GraphPad Prism version 3.00, GraphPad Software, San Diego, Calif., USA) was used to test between groups for: (1) relative isometric forces at the different stimulation frequencies, (2) ankle position and force during stretch number five of the passive muscle-tendon complex, (3) the peak forces of stretch numbers one to five of the passive muscle-tendon complex, (4) the decline in relative isometric force at 90° (IF90°) during the protocol of stretches of activated muscles, (5) the decline in relative stretch force at 40° during the protocol of stretching of activated muscles, and (6) the isometric force deficits at the different stimulation frequencies. Post hoc testing for differences was performed using a Bonferroni test. Values are presented as means (SEM). The level of statistical significance was accepted at $P < 0.05$.

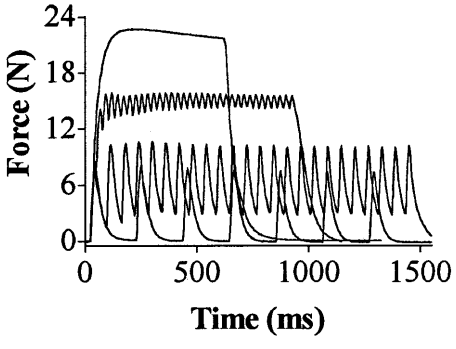

Fig. 3 Examples of force/frequency measurements of a rat plantar flexor muscle-tendon complex. Forces were measured with the knee and ankle at 90°. Plantar flexor muscles were stimulated at 5 Hz (1500 ms), 10 Hz (1500 ms), 20 Hz (1500 ms), 40 Hz (900 ms), 60 Hz (600 ms) and 80 Hz (600 ms). Examples of 10 Hz and 60 Hz are not shown for clarity of the figure

Table 1 Muscle mass and force data of plantar flexor muscles of saline-injected (*Control*) and β-aminopropionitrile-injected (*BAPN*) female Sprague Dawley rats. Values are presented as mean (SEM), with $n = 5$ per group. (*SOL* Soleus muscle, *PL* plantaris muscle, *GAST* gastrocnemius muscle, *Mm* muscle mass, *Bm* body mass, *SF40°* stretch force at an ankle position of 40°, *%Δ* percentage change)

Variable	Control	BAPN	%Δ
SOL Mm/Bm (g/100 g)	0.51 (0.01)	0.48 (0.01)	–6
PL Mm/Bm (g/100 g)	1.37 (0.05)	1.33 (0.03)	–3
GAST Mm/Bm (g/100 g)	6.61 (0.29)	6.38 (0.14)	–3
SF40°/Mm (N/g)	13.4 (0.6)	12.5 (1.1)	–8

Results

Treatment of rats with BAPN to reduce the number of HP cross-links resulted in a slightly slower growth rate (i.e. decrease in body weight gain) than in the saline-injected control rats (the slopes of regression lines were significantly different, $P = 0.002$; Fig. 4). Using the equations for the calculated regression lines, the weight gains at day 43 were 8.9% (control) and 6.8% (BAPN). There were no differences between BAPN-injected and control rats with regard to relative mass of the soleus, plantaris, and gastrocnemius muscles (Table 1).

Collagen and collagen cross-links of the Achilles tendon and plantar flexor muscles

Collagen content in the Achilles tendon and individual plantar flexor muscles was similar in both BAPN-injected and control rats (Table 2). However, in

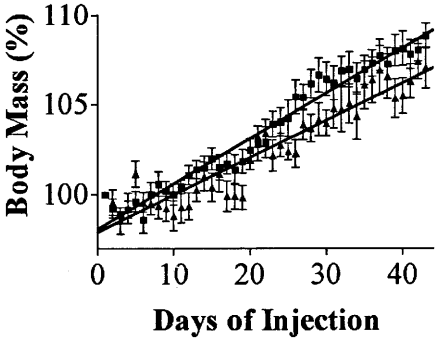

Fig. 4 The relationship between body mass and number of days of injection. Body mass is expressed as a percentage of the body mass on day 1 of injections. ■ Saline-injected rats, ▲ BAPN-injected rats

BAPN-injected rats, the amount of HP was significantly decreased in the tendon (22.9%), plantaris muscle (17.1%) and soleus muscle (7.4%) (Table 2). In the gastrocnemius medialis muscle of BAPN-injected rats, the amount of HP was 4.4% lower than in the control rats, but this decrease did not reach statistical significance ($P = 0.2$).

Stretching of the passive muscle-tendon complex

The relationship between ankle position and the force produced for the fifth ankle dorsiflexion with stretching of the passive plantar flexor muscles is illustrated in Fig. 5A. The relationship between force increase and ankle position was non-linear and was smaller for BAPN-injected rats between ankle positions of 47° and 40° (two-way ANOVA). For all five ankle rotations, force at the end of each rotation (i.e. at 40°) was significantly lower for BAPN-injected rats than for the controls (two-way ANOVA; Fig. 5B).

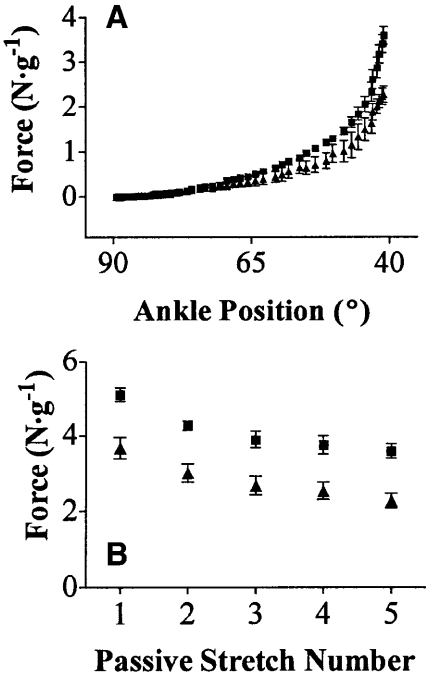

Fig. 5 Forces as a function of ankle angle (**A**) and forces at an ankle position of 40° for stretches 1–5 (**B**) of a passive rat plantar flexor muscle-tendon complex. ■ Saline-injected rats, ▲ BAPN-injected rats. Values are mean (SEM) ($n = 5$)

Mechanics of the activated muscle-tendon complex

No differences between BAPN-injected rats and controls were observed for the isometric force at 5, 10, 20, 40, 60 and 80 Hz before the first stretch (Fig. 6) and for the stretch force of the first stretch at an ankle position of 40° (Table 1). During a protocol of 20 stretches of the activated plantar flexor muscles, isometric force deficits that developed at an ankle position of 90° and the decline in stretch force at an ankle position of 40° followed the same course for BAPN-injected rats and controls (Fig. 7A, B). Before the last stretch, the isometric force deficits were 51.1 (2.4)% for control and 54.7 (4.6)% for BAPN-injected rats.

The relationship between the isometric force deficits that were present after 1 h of rest following the protocol of stretches of activated muscles as a function of stimulation frequency is illustrated in Fig. 8. After 1 h of rest, there were no differences between BAPN-injected and control rats with respect to deficits in isometric force at stimulation frequencies of 20, 40, 60 and 80 Hz (Fig. 8). However, deficits in isometric force at 5 Hz and 10 Hz were 29% and 26% larger, respectively, for

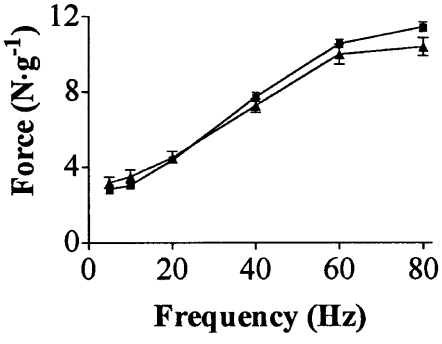

Fig. 6 Force as a function of stimulation frequency for the rat plantar flexor muscle-tendon complex. Forces were measured with the knee and ankle at 90° before the first stretch. ■ Saline-injected rats, ▲ BAPN-injected rats. Values are mean (SEM) ($n = 5$)

Table 2 Content of collagen and pyridinoline (*HP*) in Achilles tendon and plantar flexor muscles of saline-injected (Control) and BAPN-injected female Sprague-Dawley rats, as determined using high-pressure liquid chromotography analysis. Values are means (SEM); $n = 6$ per group. (*dw* dry weight)

Parameter	Control	BAPN	Control	BAPN	Control	BAPN	Control	BAPN
	Achilles tendon		GAST		PL		SOL	
Collagen, μg/mg dw	944.3 (7.7)	940.0 (14.7)	21.7 (1.4)	18.0 (0.8)	24.1 (1.4)	23.6 (2.5)	36.9 (1.7)	36.9 (1.5)
Mol HP/mol collagen	0.083 (0.011)	0.064 (0.012)*	0.300 (0.032)	0.287 (0.035)	0.280 (0.029)	0.232 (0.047)*	0.325 (0.024)	0.302 (0.019)*

*Significant difference between saline- and BAPN-injected rats ($P < 0.05$)

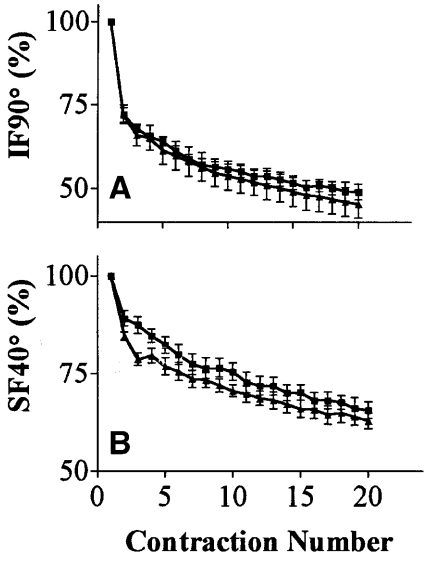

Fig. 7 Relative isometric force (**A**) and relative stretch force (**B**) as a function of stretch number of the activated rat plantar flexor muscle-tendon complex. Isometric force at an ankle position of 90° (*IF90°*) was normalised to the isometric force during the first stretch in the series. Stretch force at an ankle position of 40° (*SF40°*) was normalised to the stretch force at 40° during the first stretch in the series. ■ Saline-injected rats, ▲ BAPN-injected rats. Values are mean (SEM) ($n = 5$)

BAPN-injected compared to control rats (two-way ANOVA, $P < 0.05$).

Discussion

In the present study the effects of an alteration in collagen cross-linking on the functional properties of a skeletal muscle-tendon complex in an intact rat during stretches of passive and activated muscles were examined. Collagen cross-links are formed during the maturation of collagen molecules, a process regulated by the enzyme, lysyl oxidase (Siegel et al. 1978; Rucker et al. 1998). Fibre type differences are known to exist in the collagen content of skeletal muscles (Kovanen et al. 1984a), but cross-link and turnover data are scant (Palokangas et al. 1992). The inhibition of lysyl oxidase by chronic treatment with BAPN seems to have a systemic effect: reductions in collagen cross-linking in cartilage (Ahsan et al. 1999), skin (Marsh and Gallin 1994), bone (Oxlund et al. 1995), and cardiac muscle (Kato et al. 1995) have been reported. In femoral mid-diaphyseal cortical bone, for example, a 30-day treatment with 333 mg/kg BAPN in 47-day-old female Wistar rats resulted in a 45% reduction of the collagen cross-link HP (Oxlund et al. 1995). After a 43-day treatment with 333 mg/kg BAPN in 87-day-old female Sprague-Dawley rats, concentrations of HP in the Achilles tendon and plantaris and soleus muscles were reduced (Table 2). The reductions in HP (mol/mol collagen) were substantially smaller in the soleus muscle (7.4%), which is composed mainly of slow-twitch muscle fibres, compared with the plantaris muscle (17.1%), which is composed mainly of fast-twitch muscle fibres; however, no significant changes in the amount of HP were observed in the gastrocnemius medialis muscle, which is also composed mainly of fast-twitch muscle fibres. The mechanical loading of slow- and fast-twitch fibres in hindlimb muscles might have influenced the differences in collagen cross-linking observed in the soleus, plantaris and gastrocnemius muscles of BAPN-treated rats.

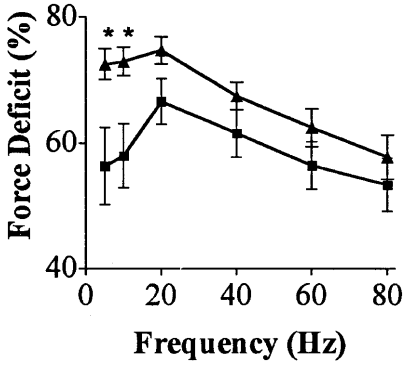

Fig. 8 Relationship between the isometric force deficit as a function of stimulation frequency following 1 h of rest after the stretches of the activated rat plantar flexor muscle-tendon complex. ■ Saline-injected rats, ▲ BAPN-injected rats. Values are mean (SEM) ($n = 5$)

Force/frequency measurements were normal in BAPN-treated rats, suggesting that the reduction in HP did not alter the functional contractile properties of the plantar flexor muscle-tendon complex. However, the reduction in HP resulted in substantial decreases in force during stretching of passive muscles. Similarly, Kovanen et al. (1984b) reported decreases in stress during length changes of the soleus and rectus femoris muscles of rats that were fed BAPN.

The effects of a reduction in HP (and hence HP cross-links) on the isometric force deficits that develop as a result of repeated unaccustomed stretches of activated muscles were tested in this study. Isometric force deficits after unaccustomed exercise with stretches of activated muscles (e.g. Davies and White 1981) have been taken as indirect evidence for stretch-induced muscle injury (e.g. McCully and Faulkner 1985). Several studies have shown that it is the high forces produced during the stretches of activated muscles that cause the isometric force deficits (McCully and Faulkner 1986; Warren et al. 1993). In the present study, the reduction in HP did not alter the force production during repeated stretches of activated muscles, and similar isometric force deficits developed over time. Because of the similarity in peak stretch forces for normal muscles and muscles with reduced HP, the similarity in the isometric force deficits that developed during the protocol of stretching of activated muscles was not surprising. Several mechanisms contribute to the acute isometric force deficits observed after a series of unaccustomed stretches of activated

skeletal muscles. According to Ingalls et al. (1998), failure of excitation-contraction coupling caused 75% of the isometric force deficits, because substantially smaller caffeine-induced isometric force deficits than nerve-stimulated isometric force deficits were found. Excitation-contraction coupling ends with the release of Ca^{2+} from the sarcoplasmic reticulum. A loss of Ca^{2+} homeostasis (Fridén and Lieber 1996) and loss of sarcoplasmic reticulum membrane integrity (Yasuda et al. 1997) were observed after unaccustomed stretching of activated skeletal muscles, providing support for the failure of excitation-contraction coupling as the main cause of isometric force deficits. Other mechanisms such as overstretched sarcomeres (Talbot and Morgan 1996; Balnave et al. 1997), membrane disruption (McNeil and Khakee 1992; Warren et al. 1995), and disruption of the force transmission structures (Lieber et al. 1996) should then contribute to the remaining 25% of these isometric force deficits.

Following stretching of activated skeletal muscles in protocols with additional muscle fatigue, force deficits are larger at low frequencies of stimulation and take more time to recover than force deficits at high frequencies of stimulation (Edwards et al. 1977; Davies and White 1981; Newham et al. 1983; Sargeant and Dolan 1987; Brown et al. 1997). The absence of frequency-dependent force deficits in control muscles 1 h following stretching of activated muscles provides evidence that our stimulation protocol (rest periods between contractions of 3 min) minimised muscle fatigue. However, in skeletal muscles with reduced HP, larger force deficits were present at stimulation frequencies of 5 Hz and 10 Hz, but not at frequencies over 20 Hz.

The reduction in force during low-frequency stimulation following stretches of activated muscles in BAPN-treated rats could be caused by a reduction in Ca^{2+} release from the sarcoplasmic reticulum (Westerblad et al. 1993), which would decrease the amount of Ca^{2+} available for binding to troponin. Consequently, fewer cross-bridges would produce force. However, frequency-dependent force deficits were not present in control muscles before the stretches, which excludes differences in Ca^{2+} release as an explanation for the difference in the isometric force deficits. In addition, if excitation-contraction coupling is the weak link for stretch-induced muscle injury, then nerve stimulation of muscles with deficiencies in proteins that provide intracellular stability, such as desmin, would produce similar isometric force deficits. In fact, it has been shown that the skeletal muscles of desmin-knockout mice are not more vulnerable to muscle injury (Sam et al. 2000), which supports the idea that excitation-contraction coupling is the major failure point following repeated stretching of activated muscles.

Excitation-contraction coupling would not be expected to be altered by inhibition of lysyl oxidase unless some intracellular protein(s) were altered. Recently, lysyl oxidase has been localized in the nuclei of fibroblasts (Li et al. 1997) and is thought to play a role in the condensation of nuclear material, thus altering the regulation of specific proteins. Interestingly, an electron-microscope analysis of a muscle biopsy specimen from a patient with decreased activity of lysyl oxidase showed an abnormal arrangement of sarcomeres (Wakai et al. 1993). If an abnormal arrangement of sarcomeres in plantar flexor muscles in BAPN-treated rats did exist, it did not result in different force/frequency measurements before the stretching of activated muscles. It is possible that stretching of the activated skeletal muscle-tendon complex with reduced collagen cross-links might have resulted in an increase in series compliance. It was shown by Hill (1951) that an increase in series compliance lowers the maximum twitch tension. In our study, an increase in series compliance after stretching of the activated skeletal muscle-tendon complex with reduced collagen cross-links could have lowered the ability of the complex to generate force at lower stimulation frequencies. It is concluded that a reduction in the amount of HP (and hence HP cross-links) in the connective tissue of a stretch-injured skeletal-muscle-tendon complex does not alter the production of muscle force at high frequencies of stimulation.

In summary, the plantar flexor muscle-tendon complex in intact rats with reduced amounts of HP produced lower resistive forces during ankle rotations with stretches of passive muscles. Force production during isometric contractions and stretching of activated muscles was not altered in skeletal muscles with reduced amounts of HP (and hence HP cross-links). However, stretching of activated skeletal muscles of rats treated with BAPN resulted in larger isometric force deficits at low frequencies of stimulation.

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