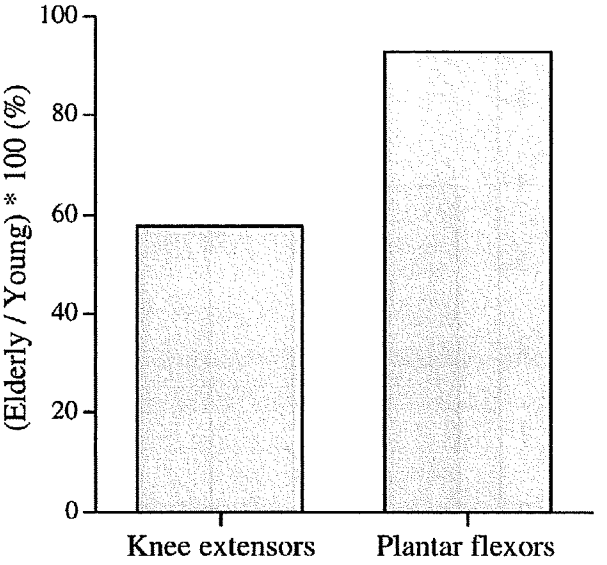

Figure 1. Comparison between knee extensors and plantar flexors concerning the percentage of the mean value of muscle thickness of the elderly group to that of the young group.

UST 5047-5; Aloka) was used to obtain longitudinal ultrasonic images of vastus lateralis and medial gastrocnemius muscles by the procedures described previously (18). The ultrasonic images were recorded on videotape at 30 Hz and were synchronized with recordings of a clock timer for subsequent analyses. The point at which one fascicle was attached to the aponeurosis was visualized on the ultrasonic images. This point moved proximally during isometric torque development up to a maximum [see Figure 1 of (18)]. The displacement of this point indicates the lengthening of the deep aponeurosis and the distal tendon (18). Strain was estimated from the tendon elongation value and the initial length of the tendon structures, which was estimated from the distance between the measurement site and the estimated insertion of the muscle over the skin (18,19).

The displacements of tendon and aponeurosis are attributed to both angular rotation and contractile tension, because any angular joint rotation occurs in the direction of knee extension and ankle plantar flexion during an "isometric" contraction (18,19). Thus, angular joint rotation needs to be accounted for to avoid an overestimation of tendon displacement during an isometric contraction. To monitor joint angular rotation, an electrical goniometer (Penny and Giles Biomechanics Ltd., Gwent, U.K.) was placed on the lateral aspect of each joint. To correct the measurements taken for the tendon and aponeurosis elongation, additional measurements were taken under passive conditions. The displacement of each site caused by rotating the knee and ankle from 110° to 70° was digitized in sonographs taken. Thus, for each participant the displacement of each site obtained from the ultrasound images could be corrected for that attributed to joint rotation alone (18). In the present study, only values corrected for angular rotation are reported.

Table 2. Absolute and Relative (to Limb Length) Muscle Thickness of Two Groups, Mean (SD)

	Young (N = 19)	Elderly (N = 17)
Knee extensors		
Muscle thickness, mm	54.3 (5.4)	29.8 (5.3)*
Muscle thickness/thigh length, mm · cm^{-1}	1.40 (0.14)	0.81 (0.15)*
Plantar flexors		
Muscle thickness, mm	74.9 (8.3)	62.7 (6.9)*
Muscle thickness/thigh length, mm · cm^{-1}	1.91 (0.22)	1.78 (0.19)

Note: $*p < .001$, significantly different from young.

Statistics

Descriptive data represent means ± standard deviation (*SD*). One-way analysis of variance (ANOVA) was used for the comparison between the two groups. The level of significance was set at $p < .05$.

RESULTS

The stature, body mass, and thigh and lower leg lengths of the elderly group were significantly smaller than those of the young group (Table 1). There was no significant difference in the numbers of steps per day between the two groups ($p = .617$).

Muscle thickness (absolute and relative) of the two groups is presented in Table 2. The elderly group had a significantly lower absolute muscle thickness than the young group in both sites (all $p < .001$). Relative muscle thickness of the elderly group was significantly lower than that of the young group in knee extensors ($p < .001$), although no significant difference was found between the two groups in plantar flexors ($p = .063$). The percentage of the mean value of relative muscle thickness of the elderly group to that of the young group was lower in knee extensors (57.8%) than in plantar flexors (93.2%) (Figure 1).

Muscle strength (absolute and relative) of the two groups is presented in Table 3. The elderly group had significantly lower absolute and relative muscle strength values than the young group in both sites (all $p < .001$). There was no difference in the relative strength between the two muscle groups of the elderly group compared to the young group (knee extensors = 62.5%, plantar flexors = 65.4%) (Figure 2).

Table 3. Absolute and Relative (to Body Mass) Muscle Strength of Two Groups, Mean (*SD*)

	Young (N = 19)	Elderly (N = 17)
Knee extensors		
Muscle strength, Nm	216.0 (58.3)	111.7 (34.5)*
Muscle strength/body mass, Nm · kg^{-1}	3.02 (0.70)	1.89 (0.58)*
Plantar flexors		
Muscle strength, Nm	115.8 (25.2)	63.5 (21.1)*
Muscle strength/body mass, Nm · kg^{-1}	1.62 (0.30)	1.06 (0.30)*

Note: $*p < .001$, significantly different from young.

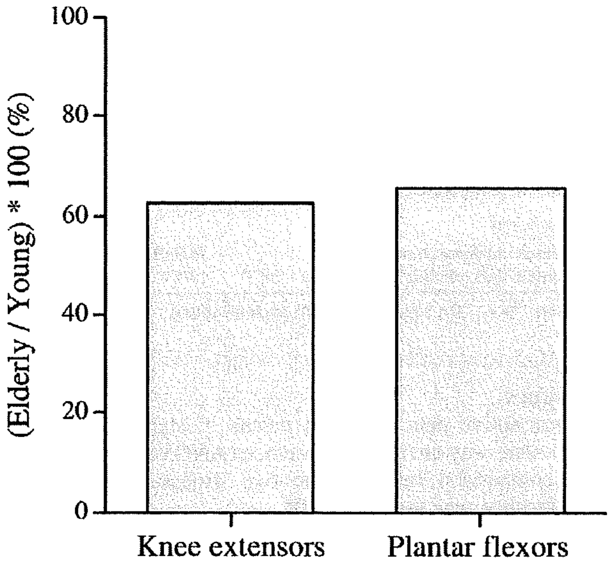

Figure 2. Comparison between knee extensors and plantar flexors concerning the percentage of the mean value of muscle strength of the elderly group to that of the young group.

The ratio of muscle strength to muscle thickness of the two groups is presented in Figure 3. Although there was no difference in this ratio between the young (3.96 ± 0.89 Nm mm^{-1}) and the elderly (3.88 ± 1.35 Nm mm^{-1}) groups in knee extensors (p = .831), this ratio of the elderly group (1.00 ± 0.28 Nm mm^{-1}) was significantly lower than that of the young group (1.55 ± 0.31 Nm mm^{-1}) ($p < .001$).

Maximal elongation and strain of tendon structures of the two groups are presented in Table 4. The elderly group had significantly lower maximal elongation and strain of tendon structures in both sites than the young group had. There was no difference in the percentage of the mean value of maximal tendon strain of the elderly group compared to that of the young group between knee extensors (83.8%) and plantar flexors (76.0%) (Figure 4).

Discussion

The major findings of this study were that the ratio of muscle strength to muscle thickness of the elderly group was significantly lower than that of the young group in plantar flexors, but not in knee extensors, and that the maximal strain of tendon structures of the elderly group was significantly lower than that of the young group in both sites.

It is well documented that the loss of muscle strength with aging is due to loss of muscle mass [e.g., (4)]. In particular, the knee extensors appear to be affected by muscle atrophy and consequent loss of strength (15,20). In the present study, the percentage values of the mean muscle thickness (58%) and strength (63%) of the elderly group and those of the young group were almost the same (Figures 1 and 2). Accordingly, there was no difference in the ratio of muscle strength to muscle thickness in knee extensors between the young and the elderly groups (Figure 3). Young and

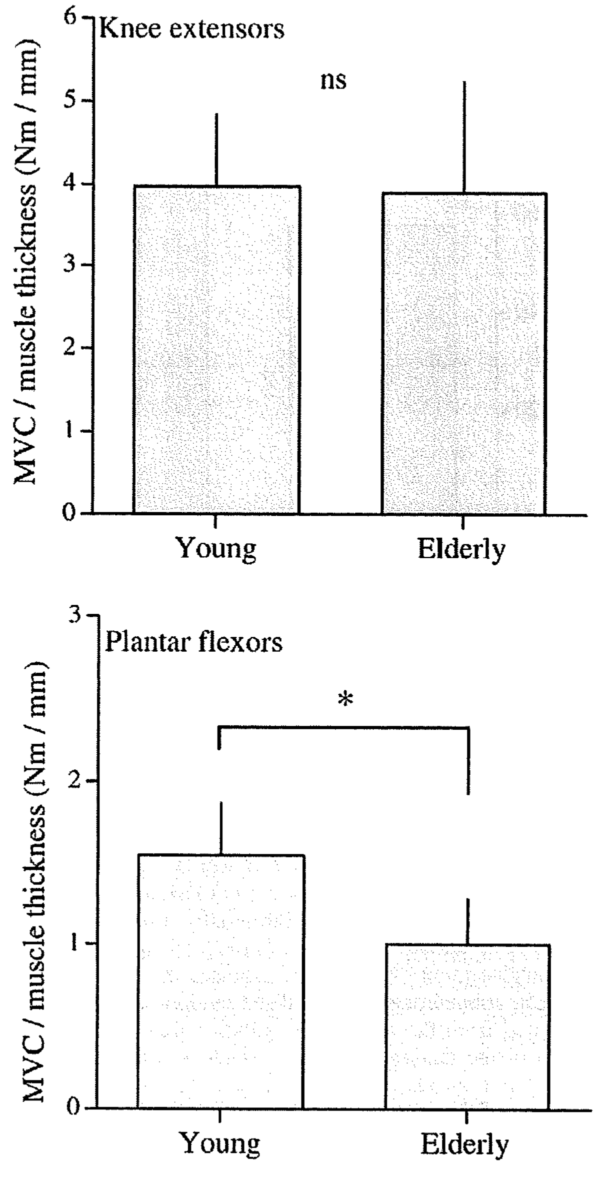

Figure 3. Comparison between young and elderly groups concerning the ratio of muscle strength to muscle thickness in knee extensors (*top*) and plantar flexors (*bottom*). *Significantly different between young and elderly groups. MVC = maximal voluntary isometric torque; ns = not significant.

coworkers (4) reported that the muscle strength and cross-sectional area of elderly participants were lower by 35% and 33%, respectively, than those of young participants. Furthermore, they showed that there was no difference in the ratio of muscle strength to the cross-sectional area of the quadriceps femoris muscles. Overend and colleagues (3) also demonstrated that the ratio of muscle strength to the cross-sectional area of the quadriceps femoris muscles was similar between young and the elderly men. Thus, the present results of muscle strength to muscle thickness in knee extensors agree with results in previous reports. Some previous researchers showed that there was no difference

Table 4. Maximal Elongation and Strain of Tendon Structures of Two Groups, Mean (SD)

	Young (N = 19)	Elderly (N = 17)
Knee extensors		
Maximal elongation, mm	30.7 (6.0)	24.6 (4.7)**
Maximal strain, %	14.2 (2.8)	11.9 (2.2)*
Plantar flexors		
Maximal elongation, mm	14.2 (3.0)	9.8 (2.7)***
Maximal strain, %	5.0 (1.3)	3.8 (1.0)***

Note: *$p < .05$; **$p < .01$; ***$p < .001$, significantly different from young.

between young and elderly groups in the activation level of the quadriceps femoris muscles assessed using twitch interpolation (21,22). Roos and coworkers (22) suggested that the substantial age-related weakness in knee extensors did not seem to be related to changes in neural drive. Taking the present results into account together with the findings quoted above (3,4,21,22), the age-related decline in knee extensor strength can be accounted for by a decrease in muscle mass but not by a decline in the neural activation level.

In plantar flexors, the muscle strength of the elderly group was significantly lower than that of the young group, whereas there was no difference in the relative muscle thickness (to limb length) between the two age groups (Tables 2 and 3). According to a recent finding (15), there was no significant difference in the relative muscle thickness of the medial gastrocnemius muscle between the young and the elderly groups. In the present study, the ratio of muscle strength to muscle thickness in the elderly group was significantly lower than that in the younger group (Figure 3). Some previous studies also demonstrated that, in plantar flexors, the ratio of muscle strength to the cross-sectional area of elderly participants was significantly lower than that of young participants (6,7). Furthermore, some previous researchers reported that the activation level (assessed using twitch interpolation) of plantar flexors was significantly lower in elderly participants than in young participants (6,23). These findings suggest that the decrease in plantar flexor strength with aging would be mainly attributed to a decline in the neural activation level of muscles.

However, we must draw attention to a limitation in the present study. The measurement of muscle thickness using ultrasonography cannot distinguish between muscle and intramuscular fat. Some previous studies showed that the amount of intramuscular fat in elderly persons was higher than that in young persons (3,24). Therefore, it is not to be denied that the muscle thickness of the elderly group would be overestimated. In other words, the difference in the age-related ratio of muscle strength to muscle thickness (Figure 3) could be caused by the difference in the quantity of intramuscular fat between knee extensors and plantar flexors. As far as we know, however, there is no definite information on the differences in the amount of intramuscular fat among muscle groups. Regardless, further investigations are needed to clarify this point.

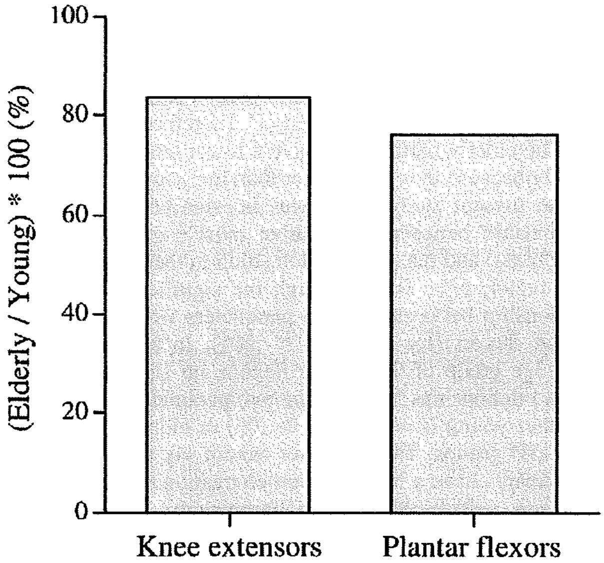

Figure 4. Comparison between knee extensors and plantar flexors concerning the percentage of the mean value of maximal tendon strain of the elderly group to that of the young group.

Another interesting finding of this study was that the maximal strain of tendon structures in the elderly group was significantly lower than that in younger participants at both sites. Recently, we observed that the maximal strain of tendon structures in knee extensors decreased significantly with aging (12). In contrast, other studies showed a different finding concerning the age-related changes in the human tendon properties (13,25). For example, Morse and colleagues (13) reported that the human gastrocnemius tendons of elderly persons were more compliant than were those of young adults. At the beginning of the study, therefore, it was expected that the age-related changes in the tendon properties would be different between knee extensors and plantar flexors. In the present study, however, no difference in the percentage of the mean values of maximal tendon strain of elderly participants to that of young participants was found between knee extensors (84%) and plantar flexors (76%) (Figure 4). According to previous findings obtained in in vitro studies using animals and human cadavers (9–11,26,27), the strength and elasticity of tendons, for example, failure load, Young's modulus, and ultimate strain, decreased with aging. These age-related changes in the mechanical properties of tendons would be caused by morphological and/or mechanical changes in collagen fibers [e.g., (28)]. Previous studies demonstrated that the diameter and crimp angle of collagen fibers decreased with aging (28–31). Furthermore, age-related increases in connective tissue and collagen cross-linking have been reported that might decrease the tendon elasticity during muscle contractions [e.g., (32)]. Considering these previous findings from in vitro studies, it seems reasonable to suppose that the extensibility of the tendon structures decreases with aging.

However, we should present the "stress-strain" relationship to compare the tendon properties of the different age groups accurately. To achieve this task, we must use some assumptions (e.g., moment arm length, relative contribution of muscles). In the present study, therefore, we used the maximal elongation and strain of tendon structures at MVC as an index of "tendon property." Therefore, it is possible that the elderly participants have a lower tendon elongation simply because they are weaker than the young participants. In the present study, there was no significant correlation relationship between the relative muscle strength (MVC/ body mass) and maximal tendon strain in the knee extensors ($r = 0.294$, $p > .05$), although the significant correlation relationship between the two parameters was found in the plantar flexors ($r = 0.582$, $p < .001$). In addition, within each age group of the plantar flexors, no significant correlation relationships between the two parameters were found in either young ($r = 0.203$, $p > .05$) or elderly ($r = 0.266$, $p > .05$) groups. Therefore, we cannot say that the elderly participants have a lower tendon elongation simply because they are weaker than young participants. In the present study, we intended to compare tendon elongation during "voluntary" contraction between the two age groups. Considering these points, it seems reasonable to suppose that the present result (age-related decline in the maximal strain of tendons) would indicate the age-related decline in tendon extensibility during "voluntary" contraction.

In the present study, the number of participants in each age group may have been relatively small. Concerning age-related differences in muscle thickness, however, our previous finding in about 300 men and women showed that the relative muscle thickness (to limb length) of the vastus lateralis muscle was significantly greater in young than in elderly participants, although there were no significant differences in the relative muscle thickness of the medial gastrocnemius muscle between young and elderly participants (15). In addition, the maximal tendon strain of elderly participants was lower than that of young participants according to our previous findings in 51 women (12). Therefore, the present results agreed with these previous findings (12,15). However, these conclusions are speculative and await additional data for clarification.

Conclusion

Our results indicated that the age-related weakening of knee extensors may be attributed to muscle atrophy, whereas in plantar flexors it may be related to a decline in the neural activation level. Furthermore, elderly persons have less extensible tendon structures in knee extensors and plantar flexors compared to young persons. According to the present results, it is desirable that the main aims of an exercise program for elderly individuals are increases in the muscle volume of knee extensors and the neural activation of plantar flexors. In addition, we should note that tendon structures in elderly persons are less able to cope with repetitive biomechanical stress due to a decrease in tendon extensibility.

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REFERENCES

1. Lindle RS, Metter EJ, Lynch NA, et al. Age and gender comparisons of muscle strength in 654 women and men aged 20–93 yr. *J Appl Physiol.* 1997;83:1581–1587.
2. Lynch NA, Metter EJ, Lindle RS, et al. Muscle quality. I. Age-associated differences between arm and leg muscle groups. *J Appl Physiol.* 1999;86:188–194.
3. Overend TJ, Cunningham DA, Kramer JF, Lefcoe MS, Paterson DH. Knee extensor and knee flexor strength: cross-sectional area ratios in young and elderly men. *J Gerontol Med Sci.* 1992;47A: M204–M210.
4. Young A, Stokes M, Crowe M. Size and strength of the quadriceps muscles of old and young women. *Eur J Clin Invest.* 1984;14: 282–287.
5. Jakobi JM, Rice CL. Voluntary muscle activation varies with age and muscle group. *J Appl Physiol.* 2002;93:457–462.
6. Morse CI, Thom JM, Davis MG, Fox KR, Birch KM, Narici MV. Reduced plantarflexor specific torque in the elderly is associated with a lower activation capacity. *Eur J Appl Physiol.* 2004;92: 219–226.
7. Davies CTM, Thomas DO, White MJ. Mechanical properties of young and elderly human muscle. *Acta Med Scand Suppl.* 1986;711: 219–226.
8. Candow DG, Chilibeck PD. Differences in size, strength, and power of upper and lower body muscle groups in young and older men. *J Gerontol Biol Sci Med Sci.* 2005;60A:148–156.
9. Blevins FT, Hecker AT, Bigler GT, Boland AL, Hayes WC. The effects of donor age and strain rate on the biomechanical properties of bone-patellar tendon-bone allografts. *Am J Sports Med.* 1994;22: 328–333.
10. Johnson GA, Tramaglini DM, Levine RE, Ohno K, Choi NY, Woo SLW. Tensile and viscoelastic properties of human patella tendon. *J Orthop Res.* 1994;12:796–803.
11. Lewis G, Shaw KM. Tensile properties of human tendo Achilles: effect of donor age and strain rate. *J Foot Ankle Surg.* 1997;36: 435–445.
12. Kubo K, Kanehisa H, Miyatani M, Tachi M, Fukunaga T. Effect of low-load resistance training on the tendon properties in middle aged and elderly women. *Acta Physiol Scand.* 2003;178:25–32.
13. Morse CI, Thom JM, Birch KM, Narici MV. Tendon elongation influences the amplitude of interpolated doublets in the assessment of activation in elderly men. *J Appl Physiol.* 2005;98:221–226.
14. Kallinen M, Markku A. Aging, physical activity and sports injuries. *Sports Med.* 1995;20:41–52.
15. Kubo K, Kanehisa H, Azuma K, et al. Muscle architectural characteristics in young and elderly men and women. *Int J Sports Med.* 2003; 24:125–130.
16. Miyatani M, Kanehisa H, Ito M, Kawakami Y, Fukunaga T. The accuracy of volume estimates using ultrasound muscle thickness measurements in different muscle groups. *Eur J Appl Physiol.* 2004; 91:264–272.
17. Reeves ND, Maganaris CN, Narici MV. Ultrasonographic assessment of human skeletal muscle size. *Eur J Appl Physiol.* 2004;91:116–118.
18. Kubo K, Kanehisa H, Fukunaga T. Comparison of elasticity of human tendon and aponeurosis in knee extensors and ankle plantar flexors in vivo. *J Appl Biomech.* 2005;21:129–142.
19. Magnusson SP, Aagaard P, Rosager S, Poulsen PD, Kjaer M. Load-displacement properties of the human triceps surae aponeurosis in vivo. *J Physiol.* 2001;531:277–288.
20. Larsson L, Grimby G, Karlsson J. Muscle strength and speed of movement in relation to age and muscle morphology. *J Appl Phyiol.* 1979;46:451–456.

21. Hurley MV, Rees J, Newham DJ. Quadriceps function, proprioceptive acuity and functional performance in healthy young, middle-aged and elderly subjects. *Age Ageing*. 1998;27:55–62.
22. Roos MB, Rice CL, Connelly DM, Vandervoort AA. Quadriceps muscle strength, contractile properties, and motor unit firing rates in young and old men. *Muscle Nerve*. 1999;22:1094–1103.
23. Scagloni G, Ferri A, Minetti AE, et al. Plantar flexor activation capacity and H reflex in older adults: adaptations to strength training. *J Appl Physiol*. 2002;92:2292–2302.
24. Rice CL, Cunningham DA, Paterson DH, Lefcoe MS. Arm and leg composition determined by computed tomography in young and elderly men. *Clin Physiol*. 1989;3:207–220.
25. Onambele GL, Narici MV, Maganaris CN. Calf muscle-tendon properties and postural balance in old age. *J Appl Physiol*. 2006;100: 2048–2056.
26. Vogel HG. Influence of maturation and age on mechanical and biochemical parameters of connective tissue of various organs in the rat. *Connect Tissue Res*. 1978;6:161–166.
27. Vogel HG. Influence of maturation and aging on mechanical and biochemical properties of connective tissue in tars. *Mech Ageing Dev*. 1980;14:283–292.
28. Parry DAD, Barnes GRG, Craig AS. A comparison of the size distribution of collagen fibrils in connective tissues as a function of age and possible relation between fibril size and distribution and mechanical properties. *Proc R Soc Lond B Biol Sci*. 1978;203: 305–321.
29. Diamant J, Keller A, Baer E, Litt M, Arridge RGC. Collagen: ultrastructure and its relation to mechanical properties as a function of ageing. *Proc R Soc Lond B Biol Sci*. 1972;180:293–315.
30. Dressler MR, Butler DL, Wenstrup R, Awad HA, Smitch F, Boivin GP. A potential mechanism for age-related declines in patellar tendon biomechanics. *J Orthop Res*. 2002;20:1315–1322.
31. Patterson-Kane JC, Firth EC, Goodship AE, Parry DAD. Age-related differences in collagen crimp patterns in the superficial digital flexor tendon core region of untrained horses. *Aust Vet J*. 1996;75:39–44.
32. Alnaqueeb MA, Goldspink G. Changes in fiber type, number and diameter in aging muscles. *J Anat*. 1986;152:31–45.

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Effects of Muscle Cooling on the Stiffness of the Human Gastrocnemius Muscle in vivo

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Key Words
Skeletal muscle · Temperature · Ultrasonography

Abstract
Background/Aims: The effects of muscle cooling on the stiffness of the human gastrocnemius muscle (GAS) were examined in vivo. ***Methods:*** The knee joint was passively extended from 90 to 0° (0° = full knee extended position) with a constant ankle angle of 10° dorsiflexed position (0° = the sole of the foot is approximately perpendicular to the anterior margin of the shaft of the tibia) in a control condition (room temperature of 18–23°C) and a cooling condition (muscle temperature decreased by 5.8 ± 1.7°C after cooling using a cold water bath at a temperature of 5–8°C for 60 min). The change in passive Achilles tendon force, muscle fascicle length of GAS and muscle temperature were measured (n = 6) during the motion. ***Results and Conclusion:*** GAS stiffness was significantly greater in the cooling condition (20 ± 8 N/mm) than the control condition (18 ± 8 N/mm). There was no cooling effect on the muscle slack length, beyond which passive muscle force arises. The maximum passive Achilles tendon force significantly increased by 19 ± 20% after cooling. These results suggested that cooling increased the passive muscle force due to the increase in the muscle stiffness rather than the shift of the muscle slack length.

Abbreviations used in this paper

CV	coefficient of variation
δ	angle between the ankle joint moment arm and sole of the foot
Fac	passive Achilles tendon force
Facmax	maximum value of Fac
Ff	force tangential to the ankle joint rotation arc
Fp	plantarflexion force
GAS	gastrocnemius muscle
Lf	fascicle length
Lfs	slack length of GAS
ΔLf	elongation of GAS
R^2	coefficient of determination
θs	slack knee joint angle for GAS

Introduction

In a resting skeletal muscle (that is, a muscle belly including sarcomeres, endosarcometric and exosarcometric cytoskeletons, and epi-, peri- and endomysium), passive force arises with stretching. This force contributes to joint moment in human movement [Muraoka et al.,

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2005b] and is an important factor for joint flexibility that is related to sports performance [Craib et al., 1996] and potentially for injuries during physical activities [Ekstrand and Gillquist, 1983; Witvrouw et al., 2003; Alter, 2004]. Ekstrand and Gillquist [1983] indicated that poor joint flexibility was a predisposing cause to injury in soccer. Witvrouw et al. [2003] showed that soccer players with an increased tightness of the hamstring or quadriceps muscles had a higher risk for musculoskeletal injuries. Thus, knowledge about the passive mechanical properties (that is, stiffness) of the muscle is essential for a better understanding of human movements and for the prevention of injuries. Previous studies using animals showed that the stiffness of muscle-tendon complex [Hill 1968; Noonan et al., 1993] and muscle fibers [Mutungi and Ranatunga, 1998] increased as tissue temperature decreased. The excessive stiffness of muscles, if any, would result in low flexibility of joints, which might cause injuries during physical activities [Ekstrand and Gillquist, 1983; Witvrouw et al., 2003; Alter, 2004]. Caution should be used when extrapolating the results from in vitro animal experiments to humans in vivo, because fixation and other treatment artifacts in in vitro experiments influence the stiffness of connective tissue [Smith et al., 1996], and the muscle stiffness of different species may have different response (that is, the difference in the extent of passive muscle force change) to muscle temperature. Therefore, it is necessary for the effects of muscle temperature on the stiffness to be examined in vivo in humans. So far, joint stiffness has been usually measured as an index of muscle stiffness in humans because of the difficulties in its direct measurement. Results concerning the effects of the decrease in muscle temperature (that is, muscle cooling) on joint stiffness were not consistent among studies. While Price and Lehman [1990] showed that muscle cooling increased ankle joint stiffness, Kubo et al. [2005] found no effect of cooling on ankle joint stiffness. It should be noted here that joint stiffness is influenced by the stiffness of many kinds of tissues crossing a joint (that is, muscle, tendinous tissues, skin, joint capsules, ligaments). Therefore, we have no primary information about the effects of cooling on human muscle stiffness in vivo. Recently, it has become possible to measure human muscle stiffness in vivo [Hoang et al., 2005; Muraoka et al., 2005a]. For example, Muraoka et al. [2005a] evaluated the stiffness of the human gastrocnemius muscle (GAS), which is a biarticular muscle for the knee and ankle joint, by measuring the muscle fascicle length (Lf) and passive plantarflexion force (Fp) during the passive knee extension with a constant ankle joint angle. The purpose of the present study was to examine the effects of muscle cooling on the stiffness of the human GAS in vivo. We hypothesized that the stiffness of GAS increased after cooling.

An abstract on this study was presented in the proceedings of the 27th Annual International Conference of the IEEE Engineering in Medicine and Biology Society [Muraoka et al., 2005c].

Materials and Methods

Subjects

The subjects of this study were 6 healthy males. The means ± SD for age, height, weight and lower leg length of subjects were 27 ± 4 years, 168 ± 6 cm, 58 ± 14 kg and 39 ± 1 cm, respectively. Lower leg length was defined as the distance between the popliteal crease and the center of the lateral malleolus. The subjects were fully informed about the purpose of the study and the procedures to be used. Written informed consent was obtained from all subjects. This study was approved by the Human Research Ethics Committee of the Faculty of Sport Sciences, Waseda University.

Experimental Procedure

The subjects were dressed with a shirt and shorts. Each subject was exposed to a room temperature of 18–23°C and the passive mechanical properties of GAS were measured (control condition; for details, see below). After this trial, the right lower leg was cooled by placing it into a cold water bath at a temperature of 5–8°C for 60 min before the passive mechanical properties of GAS were measured (cooling condition). During this cooling, the right lower leg was covered with a plastic bag to prevent the right lower leg from becoming wet, and the right foot was covered with towels to prevent excess cooling of the foot. Throughout the experiment, skin and muscle temperature of GAS was measured using a zero-heat flow thermometer (Coretemp CM-210; Terumo, Japan). Both probes for the measurement of skin and muscle temperature were placed on the skin surface of the medial head of GAS. The zero-heat flow thermometer can approximate muscle temperature by measuring the skin temperature at the thermally insulated region under the probe [Fox and Solman, 1971]. Thermal insulation is attained using a servomechanism that compensates for outward heat flow by applying controlled heating at the outer surface of the probe.

Measurement of Passive Fp and Calculation of Achilles Tendon Force

The experimental setup for the measurement of the passive mechanical properties of GAS used in the present study was almost the same as that used in our recent study [Muraoka et al., 2005a]. Each subject lay on his right side with the right hip joint flexed and held constant within the range of 30–50°. The right lower leg was placed on a cart and fixed by 2 straps to the cart. The ankle joint angle was fixed at 10° dorsiflexion (0° = the sole of the foot is approximately perpendicular to the anterior margin of the shaft of the tibia). After the knee joint angle was fixed at 90° for at least 30 s, the knee joint was passively extended to the full knee extended position (= 0°) by moving the cart manually while the subjects were completely relaxing their leg muscles. The test was repeated 3 times and the interval between the tests was more than 2 min. Fp was measured at the ball of the foot with a force transducer (type FP/100k; Shinkoh, Japan) and amplified (type CDV-700Am; Kyowa, Japan). To ensure the foot was placed on the same position in relation to the force transducer during the experiment, the position of the force transducer was marked on the foot. The force tangential to the ankle joint rotation arc (Ff) was calculated as follows:

$$Ff = Fp/\cos \delta,$$

where δ is the angle between the ankle joint moment arm, which is the line segment connecting the estimated center of the ankle joint and the ball of the foot, and the sole of the foot (fig. 1). δ is calculated from the ankle joint moment arm length and the distance between the estimated center of the ankle joint and the sole of the foot, which were both measured using a ruler. The plantarflexion moment was calculated by multiplying Ff and the ankle joint moment arm length (fig. 1). Then the Achilles tendon force (Fac) was calculated by dividing the plantarflexion moment by the Achilles tendon moment arm. The moment arm data were derived from Grieve et al. [1978], who reported the Achilles tendon moment arm as a function of the lower leg length and ankle joint angle. With a constant ankle joint angle, all of the soft tissues crossing the ankle joint except GAS develop constant Fp during a passive knee extension. Since GAS is a 2-joint muscle (that is, a plantarflexor and a knee flexor), the change in Fac during the passive knee extension with a constant ankle joint angle is equal to the force that applied to GAS [Muraoka et al., 2005a]. This force is also equal to the force that applied to the gastrocnemius tendinous tissues connected in series with GAS. The knee joint angle was measured using an electrical goniometer (SG150; Biometrics, UK) placed on the medial side of the right leg. The force and angle signals were stored on a personal computer at 1,000 Hz via an A/D converter (PowerLab/16s; ADInstruments, Australia). The force data at every 1° of knee joint angle were averaged in 3 tests, and the average values were used for all subsequent analysis with the exception of the analysis of the reproducibility of the tests. The mean knee joint angle velocity during knee extension in the control and cooling conditions was 3.2 ± 0.5°/s and 3.2 ± 0.4°/s, respectively. Both the capability of muscle force production [Ranatunga et al., 1987; Kubo et al., 2005] and the sensitivity of the muscle spindle to stretch [Ottoson, 1965; Michalski and Seguin, 1975] are affected by tissue temperatures, indicating that it could not be assumed that stretch-induced reflexive muscle force was the same before and after cooling. In addition, passive muscle force could be changed after muscle contraction [Whitehead et al., 2001]. Therefore, reflexive muscle activities must be avoided to accurately measure the change in passive muscle force after cooling. In the present study, we adopted a moderate dorsiflexed position (10° dorsiflexion) and slow knee joint angle velocity (approximately 3°/s). Previous studies suggested that reflexive muscle activities did not occur under these conditions [Vandervoort et al., 1992; Gajdosik et al., 1999]. In addition, we confirmed the absence of significant myoelectric activity of medial head of the GAS, soleus and tibialis anterior muscles using surface electromyography throughout the test. Surface electromyography was recorded using bipolar surface Ag/AgCl electrodes (5 mm in diameter) that were placed on each muscle belly with an interelectrode distance of 20 mm. The ground electrode was placed over the medial malleolus. The electrodes were connected to an amplifier (model Fla03; Furusawa Lab Appliance, Japan; input impedance >100 MΩ, CMRR >95 dB, bandwidth 5–500 Hz). Electromyography data were stored on a personal computer via an A/D converter at 1 kHz.

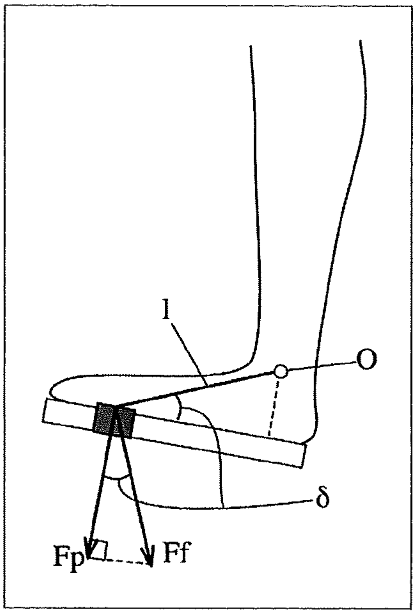

Fig. 1. Calculation of plantarflexion moment. Plantarflexion moment during passive knee joint motion with a constant ankle joint angle of 10° was calculated by multiplying Ff and the ankle joint moment arm length (l) that is the distance from the estimated center of the ankle joint (O) to the ball of the foot. Ff was calculated from the Fp at the ball of the foot as measured by the force transducer and the angle between the ankle joint moment arm and the sole of the foot (δ). Achilles tendon force was calculated by dividing plantarflexion moment by the Achilles tendon moment arm that was taken from the literature [Grieve et al., 1978].

Measurement of the Lf of the GAS

Lf was measured in the medial head of the GAS using an ultrasound apparatus (SSD-6500; Aloka, Japan; fig. 2). The probe of the ultrasound apparatus was longitudinally attached to the dermal surface over the mediolateral center of the medial head of the GAS by adhesive tape, which restrained the probe from sliding. To ensure the probe was placed on the same position in relation to the GAS during the experiment, the position of the probe was marked on the skin. The ultrasonography data were A/D converted (DVMC-DA2; Sony, Japan) and stored on a personal computer for the Lf measurement using the public domain NIH Image program (developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). Muramatsu et al. [2002] showed that there was no intramuscular variability of the Lf in the GAS. Therefore, in the present study,

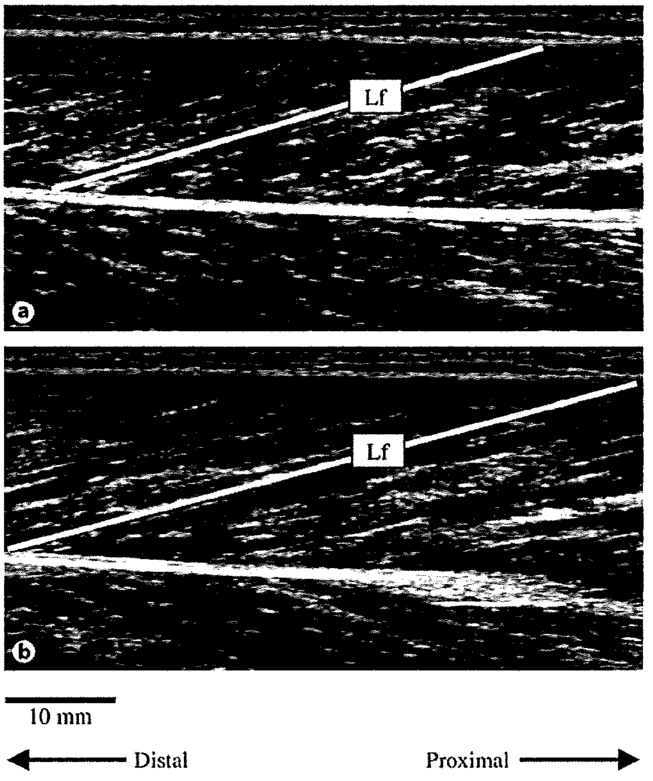

Fig. 2. Measurement of the Lf of the GAS. Typical ultrasound images of the medial head of the GAS at the knee joint angles of 60° (**a**) and 0° (**b**) in the control condition. White lines correspond to Lf.

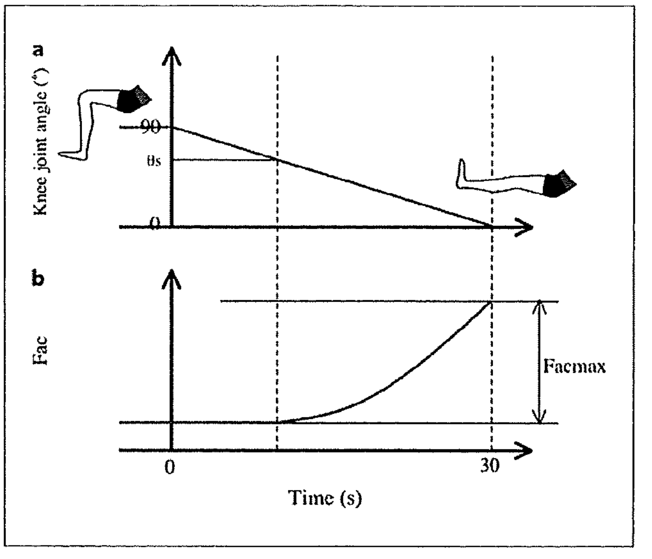

Fig. 3. Schematic of Fac and knee joint angle. **a** Knee joint is passively extended from 90° to full extended position for 30 s. **b** Fac begins to increase while extending the knee. θs is the slack knee joint angle for the GAS. Fac rises at the knee position extended more than θs.

Lf was measured from the fascicle that showed clear echoes in each ultrasonography image to measure Lf as accurately as possible. Thus, even in one trial, Lf was not always measured from the same fascicle. The ultrasonography, force and angle data were synchronized using a timer (VTF-55; FOR-A, Japan). The Lf data at every 1° of knee joint angle from 0 to 60° were averaged in 3 tests, and the average values were used for all subsequent analyses with the exception of the analysis of the reproducibility of the tests.

Calculation of the Slack Length and Stiffness of the GAS

The baseline of Fp was defined as the average Fp in the knee joint angle range of 60–65°, in which Fp was almost constant (SD of F was 0.16 N on average and ranged from 0.11 to 0.24 N). The baseline of Fp was produced by 2 straps that fixed the right foot to the force transducer, and the sum of the forces that applied to all monoarticular muscles crossing the ankle joint, ligaments, skin and other tissues surrounding the ankle joint. The first point to rise above the average Fac plus 3 times its SD for 5° while extending the knee joint was set as the onset of the Fac applied to GAS, and the knee joint angle at this onset was defined as the slack knee joint angle (θs) for GAS. The maximum value of Fac (Facmax) was measured at the end of the knee extension (fig. 3).

The slack length (Lfs) of the GAS fascicles, beyond which GAS begins to develop passive force, was the Lf at θs. The elongation (ΔLf) of the GAS fascicles was defined as the Lf at the end of the knee extension minus Lfs.

Statistics

Values are presented as means ± SD. The reproducibility of Lf and Facmax among the 3 tests was evaluated on the basis of the intraclass correlation coefficient and the coefficient of variation (CV). CV was calculated by dividing the average value of the measurements among the 3 tests by their SD values. A paired t test was used to test the differences between the control and cooling conditions in θs, Lfs, Facmax and the regression slope of the relationship between Lf and Fac. Statistical significance was set at $p < 0.05$.

Results

The intraclass correlation coefficient for Lf and Facmax was 0.95 ($p < 0.001$) and 0.92 ($p < 0.001$), respectively. The mean CV value of Lf among the 3 tests was 2.9% on average, which was within the range of previously reported values [Narici et al., 1996; Maganaris et al., 1998]. The mean CV value of Facmax among the 3 tests was 10.1% on average.

Skin and muscle temperature of GAS in the control condition was 30.1 ± 1.5°C and 34.0 ± 0.8°C, respec-

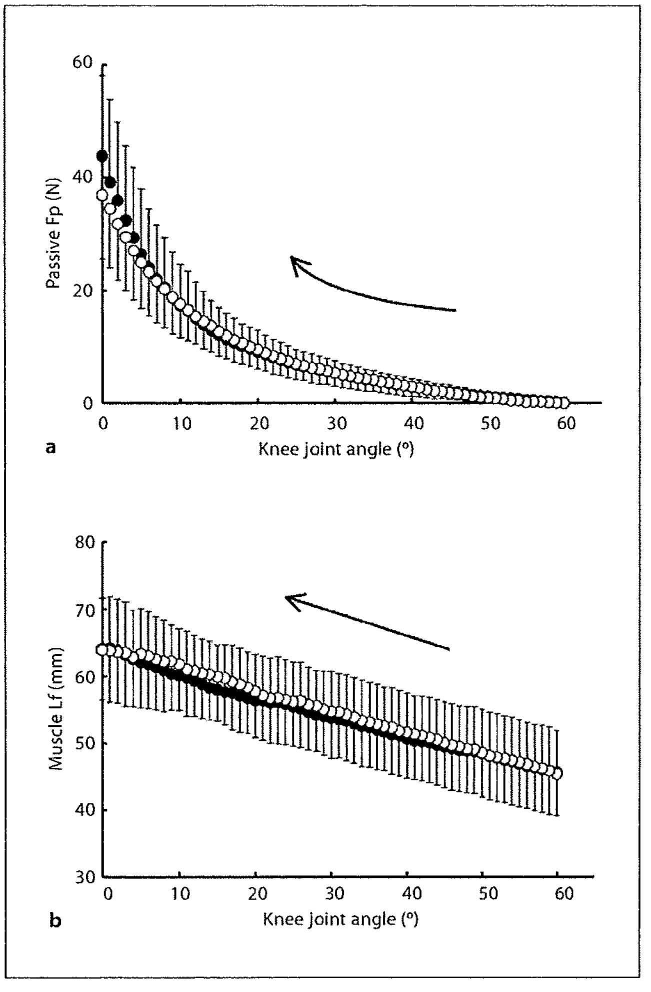

Fig. 4. Passive Fp and muscle Lf during knee extension. **a** Fac curvilinearly increases with the extension of the knee. **b** The Lf of the GAS increases almost linearly with the extension of the knee. Arrows indicate the direction of the data. Open circles correspond to the control condition, closed circles to the cooling condition. Values are means ± SE (n = 6).

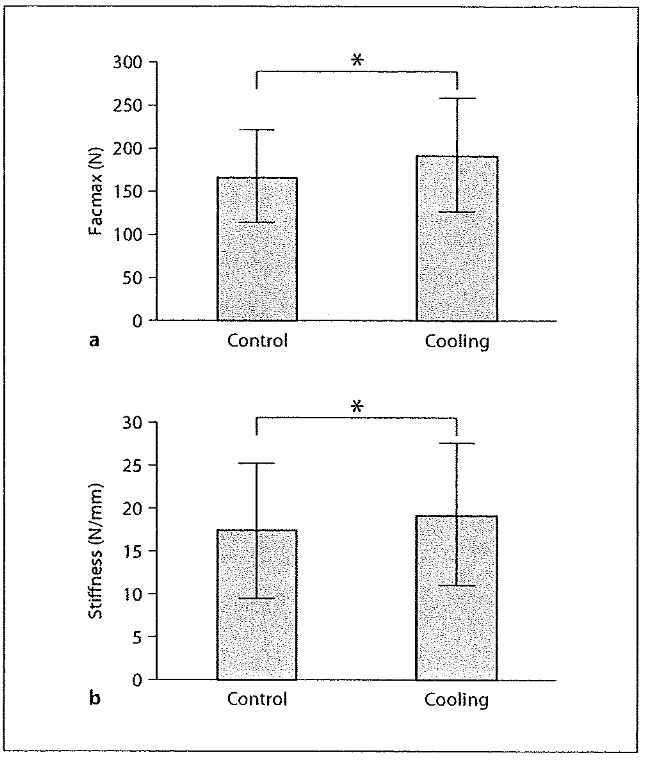

Fig. 5. a Facmax and **b** stiffness in the control and cooling condition. Values are means ± SD (n = 6). * Significant difference between the conditions ($p < 0.05$).

tively. Skin temperature of GAS in the cooling condition was 22.8 ± 2.5°C. Muscle temperature of GAS at the end of the 1st and 3rd measurement of the passive mechanical properties in the cooling condition was 28.3 ± 1.2°C and 28.1 ± 1.3°C, respectively, indicating that muscle temperature of GAS was kept to be almost constant during the tests after cooling. These values of muscle temperature were within the range of the muscle temperature directly measured using a needle thermistor probe from the leg or arm muscles with and without cold exposure [Oksa et al., 2002; Nosaka et al., 2004; Dewhurst et al., 2005].

Facmax significantly ($p = 0.028$) increased by 19 ± 20% after cooling (fig. 4a and 5a). Lf changed from 45 ± 6 mm (at 60°) to 64 ± 7 mm (at 0°) in the neutral condition and from 46 ± 5 mm (at 60°) to 64 ± 8 mm (at 0°) in the cooling condition (fig. 5b). There were no significant differences between the cooling and control conditions in either θs (53 ± 7 and 51 ± 9°, respectively) or Lfs (47 ± 4 and 49 ± 5 mm, respectively). The stiffness of GAS was calculated as the slope of the relationship between Lf and Fac in the Lf range from Lfs + 60% ΔLf to Lfs + 100% ΔLf (that is, 0% was Lfs and 100% was Lf at the end of the knee extension). The relationship between Lf and Fac was curvilinear in the Lf range from Lfs + 0% ΔLf to Lfs + 100% ΔLf. Thus, we determined the Lf range for the calculation of the stiffness (that is, Lfs + 60% ΔLf to Lfs + 100% ΔLf) by a visual inspection so that the relationship between Lf and Fac was almost linear in the range. As a result, the relationship was fitted well with a linear regression equation using a least squares method [coefficient of determination (R^2) = 0.84 ± 0.15; fig. 6]. Lfs + 60% ΔLf corresponded to 58 ± 6 mm in the control

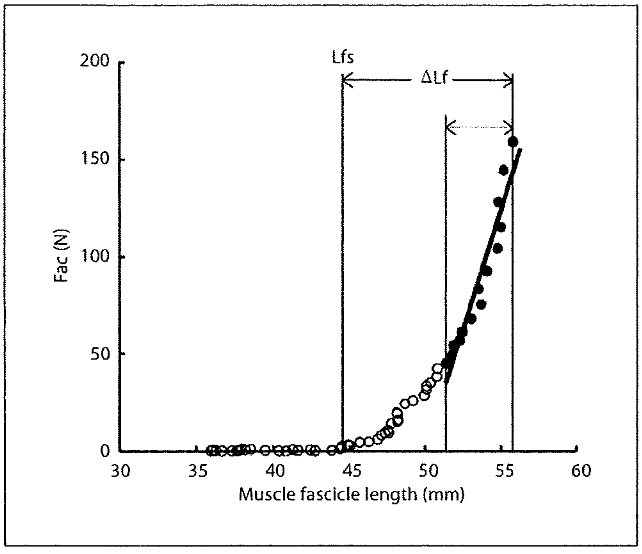

Fig. 6. Typical example of the relationship between Fac and muscle Lf in the control condition. The relationship between Fac and muscle Lf during the passive knee extension (n = 1). The relationship between Fac and Lf is fitted with a linear regression equation using a least squares method in the Lf range from Lfs + 60% ΔLf to Lfs + 100% ΔLf (the range indicated by the dashed arrow). ΔLf is the increase in Lf compared to Lfs during the knee extension. Closed circles represent the data used for the linear regression.

condition and 58 ± 6 mm in the cooling condition. The stiffness of GAS in the cooling condition (20 ± 8 N/mm) was significantly greater (p = 0.015) than that in the control condition (18 ± 8 N/mm; fig. 5b).

Discussion

The main finding of the present study was that the stiffness of GAS increased after cooling with no change in Lfs. Therefore, the hypothesis set out at the start of this study, which proposed that the stiffness of GAS increased by cooling, was supported by the results of the present study.

The passive force and the stiffness of GAS significantly increased after cooling, though there was large individual variability, which might be due to the individual variability of muscle volume that was suggested to be correlated to passive joint moment [Wiegner and Watts, 1986]. These results were consistent with previous results from animal experiments in vitro using muscle fiber bundles [Mutungi and Ranatunga, 1998] and whole muscle [Hill, 1968; Noonan et al., 1993] and from human experiments measuring ankle joint stiffness [Price and Lehman, 1990]. Since there was no cooling effect on θs and Lfs, the increase in the passive force was due to the increase in the stiffness of GAS (compare the decrease in Lfs with same stiffness results in an increase in the passive force). The stiffness was calculated from the relationship between Lf and Fac, which was obtained during the passive knee extension. Thus, the stiffness in the present study represented both the elastic and viscous characteristics of GAS. We could not show which characteristics were responsible for the increase in the stiffness after cooling. Hill [1968] showed in an animal experiment in vitro that muscle stiffness would increase with cooling as a result of a rise in elastic modulus. Price and Lehman [1990] showed in a human experiment in vivo that the viscous component of the ankle joint stiffness would increase after cooling. Further experiments are needed to investigate the relationship between muscle temperature and both the viscous and elastic characteristics of passive muscle. The moment arm of GAS at the knee increases as the knee extends [Grieve et al., 1978; Visser et al., 1990]. Therefore, the lengthening velocity of GAS muscle fascicle increases as the knee extends with a constant knee joint angle velocity, indicating that the viscous component of the passive muscle force might increase as the knee extended. However, the relationship between Lf and Fac was not highly curvilinear at long muscle length (fig. 6), which might be due to the slow knee joint angle velocity adopted in the present study. At the slow knee joint angle velocity, the elastic component of the passive muscle force might be much greater than its viscous component. Thus, it might be speculated that the increase in the stiffness after cooling in the present study was mainly due to the change in the elastic characteristics of GAS.

In humans, Price and Lehmann [1990] showed that ankle joint stiffness increased after 30 min of muscle cooling using ice-water mixture, while Kubo et al. [2005] showed that human ankle joint stiffness did not change by cooling lower leg muscles using cold water with a temperature of 5°C for 30 min. The difference between the results of the previous study [Kubo et al., 2005] and the present study might be due to the methodology adopted. Kubo et al. [2005] showed the passive ankle joint moment at 30° dorsiflexed position did not change by cooling. However, the plantar flexors should be active involuntarily at this extremely dorsiflexed position [Gajdosik et al., 1999]. Unfortunately, Kubo et al. [2005] did not show electromyographic activities of plantar flexors during passive ankle joint motion. Muscle cooling decreases both the sensitivity of the muscle spindle to stretch [Ottoson, 1965] at long muscle length [Michalski and Seguin, 1975] and the capability of muscle force production [Ranatunga et al., 1987; Kubo et al., 2005]. Thus, in the previous study [Kubo et al., 2005], the passive ankle joint moment after cooling might be underestimated due to the decrease in an involuntary active ankle joint moment, which may counteract the increase in muscle stiffness by cooling.

The level of the passive force depended on the form of conditioning. When a muscle is passively stretched, then contracts isometrically, and is returned to the original length, slack is introduced in muscle fibers and thus passive tension decreases [Whitehead et al., 2001]. In the present study, we did the test for the measurement of the passive mechanical properties of GAS in the control and cooling conditions. Before the first trial in the control condition, subjects lay with their leg muscles relaxed at least 15 min for the preparation of the subsequent tests (that is, determining the position of the probe of ultrasonography to obtain clear echoes of muscle fascicles, attaching the probes of ultrasonography and the thermometer on the skin surface over GAS, and attaching the goniometer to the medial side of the leg). During this period, except for a few occasions for the slow passive knee extension-flexion, the knee was flexed approximately 80–100° from the full knee extended position and the ankle was plantarflexed approximately 10–30°. After the test in the control condition, subjects walked a few steps, then sat on a chair, and the right lower leg was cooled by placing it into cold water bath. After this cooling, subjects stood up from the chair, then walked a few steps, and lay on their right side. We did the test within 5 min after the end of the cooling. Therefore, in our experiment, it seems reasonable to consider that the passive force of GAS did not decrease by the contraction at long muscle length. If slack was introduced in the GAS fascicles by the contraction in a few steps of walking, the slack was taken up when the GAS fascicles were stretched beyond the length at which the GAS fascicles contracted. In the stance phase of waking, the length of the GAS fascicles was almost constant (49–52 mm) because of the compliance of the tendinous tissues [Fukunaga et al., 2001]. The characteristics of subjects in the present study were not so different from those in their study (age, 27 vs. 25 years; height, 168 vs. 169 cm; weight, 59 vs. 69 kg). The length range of the GAS fascicles that was used for the calculation of the stiffness of GAS in the present study was 58–64 mm, indicating that the slack in the GAS muscle fascicles due to the contraction during a few steps of walking was, if any, taken up and did not affect the stiffness.

In the present study, we measured the Lf from the medial head of the GAS. The amount of connective tissue positively correlates with the passive force [Wiegner and Watts, 1986], and the muscle volume of the medial head of the GAS was found to be about 1.8 times greater than that of the lateral head of the GAS [Huijing, 1985; Fukunaga et al., 1996]. Therefore, Fac seems to be produced primarily by the medial head of the GAS. For the purpose of the present study, the passive mechanical properties of the GAS can be practically estimated by measuring the Lf from the medial head of the GAS, though the contribution of the lateral head of the GAS to Fac cannot be negligible.

In the present study, we measured the passive mechanical properties of GAS. GAS contains a considerably higher percentage of fast muscle fibers than soleus muscle [Johnson et al., 1973; Edgerton et al., 1975]. Muscle volume, which is suggested to be correlated to passive joint moment [Wiegner and Watts, 1986], of GAS is about 80% of that of soleus muscle [Fukunaga et al., 1996]. In terms of passive muscle force production, slow muscle fibers appear to be more important than fast muscle fibers because the former develop some 5-fold larger viscoelastic passive force compared to the latter [Mutungi and Ranatunga, 1996]. Mutungi and Ranatunga [1998] showed that the viscoelastic passive force in slow muscle fibers also increases as muscle temperature decreases, though temperature sensitivity of the viscoelastic force in slow muscle fibers was less than in fast muscle fibers. Thus, the present results concerning the effects of cooling on the muscle stiffness may be applied to soleus muscle, and it can be said that the passive resistance force of plantar flexor muscles increases as the muscle temperature decreases. Since approximately half of the passive joint stiffness was attributed to the passive muscle force [Johns and Wright, 1962; Barnett and Cobbold, 1969; Wiegner 1987], the flexibility of joints (that is, the compliance or the range of motion of joints) after cooling would decrease due to the increase in muscle stiffness. It may therefore be important not to decrease muscle temperature so as to prevent the decrease in joint flexibility that might be related to injuries during physical activities.

The slack in muscle increases electromechanical delay (EMD), which is the time lag between electrical activity and the mechanical response of the muscle [Laine Santa Maria, 1970; Muraoka et al., 2004]. Zhou et al. [1998] showed that EMD at a muscle temperature of 30°C was greater than at 36°C. Since the present study showed that the muscle temperature did not have an effect on the muscle Lfs, the previous result concerning the effect of muscle temperature on EMD could be due to the changes in neuromuscular [Rutkove, 2001] and muscle contractile [Ranatunga et al., 1987; Kubo et al., 2005] properties, rather than the shift of Lfs.

In summary, the present study examined the effects of muscle cooling on the stiffness of the human GAS in vivo. The results showed that the passive force and stiffness of the muscle increased due to the cooling, whereas Lfs of the muscle did not change. Therefore, it was suggested that muscle cooling increased the passive muscle force due to the increase in the muscle stiffness rather than the shift of the muscle Lfs.

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References

Alter, M.J. (2004) Injury Prevention; in Alter, M.J.: Science of Flexibility. Human Kinetics, Champaign, pp 11-13.

Barnett, C.H., A.F. Cobbold (1969) Muscle tension and joint mobility. Ann Rheum Dis *28:* 652-654.

Craib, M.W., V.A. Mitchell, K.B. Fields, T.R. Cooper, R. Hopewell, D.W. Morgan (1996) The association between flexibility and running economy in sub-elite male distance runners. Med Sci Sports Exerc *28:* 737-743.

Dewhurst, S., P.E. Riches, M.A. Nimmo, G. De Vito (2005) Temperature dependence of soleus H-reflex and M wave in young and older women. Eur J Appl Physiol *94:* 491-499.

Edgerton, V.R., J.L. Smith, D.R. Simpson (1975) Muscle fibre type populations of human leg muscles. Histochem J *7:* 259-266.

Ekstrand, J., J. Gillquist (1983) The avoidability of soccer injuries. Int J Sports Med *4:* 124-128.

Fox, R.H., A.J. Solman (1971) A new technique for monitoring the deep body temperature in man from the intact skin surface. J Physiol *212:* 8-10.

Fukunaga, T., K. Kubo, Y. Kawakami, S. Fukashiro, H. Kanehisa, C.N. Maganaris (2001) In vivo behaviour of human muscle tendon during walking. Proc Biol Sci *268:* 229-233.

Fukunaga, T., R.R. Roy, F.G. Shellock, J.A. Hodgson, V.R. Edgerton (1996) Specific tension of human plantar flexors and dorsiflexors. J Appl Physiol *80:* 158-165.

Gajdosik, R.L., D.W. Vander Linden, A.K. Williams (1999) Influence of age on length and passive elastic stiffness characteristics of the calf muscle tendon unit of women. Phys Ther *79:* 827-838.

Grieve, D., S. Pheasant, P. Cavanagh (1978) Prediction of gastrocnemius length from knee and ankle joint posture; in Asmussen, E., K. Jorgensen (eds): Biomechanics VI A, International Series on Biomechanics. Baltimore, University Park.

Hill, D.K. (1968) Tension due to interaction between the sliding filaments in resting striated muscle: the effect of stimulation. J Physiol *199:* 637-684.

Hoang, P.D., R.B. Gorman, G. Todd, S.C. Gandevia, R.D. Herbert (2005) A new method for 30 measuring passive length-tension properties of human gastrocnemius muscle in vivo. J Biomech *38:* 1333-1341.

Huijing, P.A. (1985) Architecture of the human gastrocnemius muscle and some functional consequences. Acta Anat (Basel) *123:* 101-107.

Johns, R.J., V. Wright (1962) Relative importance of various tissues in joint stiffness. J Appl Physiol *17:* 824-828.

Johnson, M.A., J. Polgar, D. Weightman, D. Appleton (1973) Data on the distribution of fibre types in thirty-six human muscles: an autopsy study. J Neurol Sci *18:* 111-129.

Kubo, K., H. Kanehisa, T. Fukunaga (2005) Effects of cold and hot water immersion on the mechanical properties of human muscle and tendon in vivo. Clin Biomech (Bristol, Avon) *20:* 291-300.

Laine Santa Maria, D. (1970) Pre-motor and motor reaction time differences associated with stretching of the hamstring muscles. J Mot Behav *2:* 163-173.

Maganaris, C.N., V. Baltzopoulos, A.J. Sargeant (1998) In vivo measurements of the triceps surae complex architecture in man: implications for muscle function. J Physiol *512:* 603-614.

Michalski, W.J., J.J. Seguin (1975) The effects of muscle cooling and stretch on muscle spindle secondary endings in the cat. J Physiol *253:* 341-356.

Muramatsu, T., T. Muraoka, Y. Kawakami, T. Fukunaga (2002) Intramuscular variability of the architecture in human medial gastrocnemius muscle in vivo and its functional implications. Adv Exerc Sports Physiol *8:* 17-21.

Muraoka, T., K. Chino, T. Muramatsu, T. Fukunaga, H. Kanehisa (2005a) In vivo passive mechanical properties of the human gastrocnemius muscle belly. J Biomech *38:* 1213-1219.

Muraoka, T., T. Muramatsu, T. Fukunaga, H. Kanehisa (2004) Influence of tendon slack on electromechanical delay in the human medial gastrocnemius in vivo. J Appl Physiol *96:* 540-544.

Muraoka, T., T. Muramatsu, D. Takeshita, H. Kanehisa, T. Fukunaga (2005b) Estimation of passive ankle joint moment during standing and walking. J Appl Biomech *21:* 72-84.

Muraoka T., K. Omuro, T. Wakahara, T. Fukunaga, K. Kanosue (2005c) Influence of muscle cooling on the passive mechanical properties of the human gastrocnemius muscle. Conf Proc IEEE Eng Med Biol Soc *1:* 19-21.

Mutungi, G., K.W. Ranatunga (1996) The viscous, viscoelastic and elastic characteristics of resting fast and slow mammalian (rat) muscle fibres. J Physiol *496:* 827-836.

Mutungi, G., K.W. Ranatunga (1998) Temperature-dependent changes in the viscoelasticity of intact resting mammalian (rat) fast- and slow twitch muscle fibres. J Physiol *508:* 253-265.

Narici, M.V., T. Binzoni, E. Hiltbrand, J. Fasel, F. Terrier, P. Cerretelli (1996) In vivo human gastrocnemius architecture with changing joint angle at rest and during graded isometric contraction. J Physiol *496:* 287-297.

Noonan, T.J., T.M. Best, A.V. Seaber, W.E.J. Garrett (1993) Thermal effects on skeletal muscle tensile behavior. Am J Sports Med *21:* 517-522.

Nosaka, K., K. Sakamoto, M. Newton, P. Sacco (2004) Influence of pre-exercise muscle temperature on responses to eccentric exercise. J Athl Train *39:* 132-137.

Oksa, J., M.B. Ducharme, H. Rintamaki (2002) Combined effect of repetitive work and cold on muscle function and fatigue. J Appl Physiol *92:* 354–361.
Ottoson, D. (1965) The effects of temperature on the isolated muscle spindle. J Physiol *180:* 636–648.
Price, R., J.F. Lehman (1990) Influence of muscle cooling on the viscoelastic response of the human ankle to sinusoidal displacements. Arch Phys Med Rehabil *71:* 745–748.
Ranatunga, K.W., B. Sharpe, B. Turnbull (1987) Contractions of a human skeletal muscle at different temperatures. J Physiol *390:* 383–395.
Rutkove, S.B. (2001) Effects of temperature on neuromuscular electrophysiology. Muscle Nerve *24:* 867–882.
Smith, C.W., I.S. Young, J.N. Kearney (1996) Mechanical properties of tendons: changes with sterilization and preservation. J Biomech Eng *118:* 56–61.
Vandervoort, A.A., B.M. Chesworth, D.A. Cunningham, D.H. Paterson, P.A. Rechnitzer, J.J. Koval (1992) Age and sex effects on mobility of the human ankle. J Gerontol *47:* M17–M21.
Visser, J.J., J.E. Hoogkamer, M.F. Bobbert, P.A. Huijing (1990) Length and moment arm of human leg muscles as a function of knee and hip-joint angles. Eur J Appl Physiol Occup Physiol *61:* 453–460.
Whitehead, N.P., J.E. Gregory, D.L. Morgan, U. Proske (2001) Passive mechanical properties of the medial gastrocnemius muscle of the cat. J Physiol *536:* 893–903.
Wiegner, A.W. (1987) Mechanism of thixotropic behavior at relaxed joints in the rat. J Appl Physiol *62:* 1615–1621.
Wiegner, A.W., R.L. Watts (1986) Elastic properties of muscles measured at the elbow in man. I. Normal controls. J Neurol Neurosurg Psychiatry *49:* 1171–1176.
Witvrouw, E., L. Danneels, P. Asselman, T. D'Have, D. Cambier (2003) Muscle flexibility as a risk factor for developing muscle injuries in male professional soccer players: a prospective study. Am J Sports Med *31:* 41–46.
Zhou, S., M.F. Carey, R.J. Snow, D.L. Lawson, W.E. Morrison (1998) Effects of muscle fatigue and temperature on electromechanical delay. Electromyogr Clin Neurophysiol *38:* 67–73.

Effects of Plyometric and Weight Training on Muscle–Tendon Complex and Jump Performance

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ABSTRACT

KUBO, K., M. MORIMOTO, T. KOMURO, H. YATA, N. TSUNODA, H. KANEHISA, and T. FUKUNAGA. Effects of Plyometric and Weight Training on Muscle–Tendon Complex and Jump Performance. *Med. Sci. Sports Exerc.*, Vol. 39, No. 10, pp. 1801–1810, 2007. **Purpose:** The purpose of this study was to investigate the effects of plyometric and weight training protocols on the mechanical properties of muscle–tendon complex and muscle activities and performances during jumping. **Methods:** Ten subjects completed 12 wk (4 d·wk^{-1}) of a unilateral training program for plantar flexors. They performed plyometric training on one side (PT; hopping and drop jump using 40% of 1RM) and weight training on the other side (WT; 80% of 1RM). Tendon stiffness was measured using ultrasonography during isometric plantar flexion. Three kinds of unilateral jump heights using only ankle joint (squat jump: SJ; countermovement jump: CMJ; drop jump: DJ) on sledge apparatus were measured. During jumping, electromyographic activities were recorded from plantar flexors and tibial anterior muscle. Joint stiffness was calculated as the change in joint torque divided by the change in ankle angle during eccentric phase of DJ. **Results:** Tendon stiffness increased significantly for WT, but not for PT. Conversely, joint stiffness increased significantly for PT, but not for WT. Whereas PT increased significantly jump heights of SJ, CMJ, and DJ, WT increased SJ only. The relative increases in jump heights were significantly greater for PT than for WT. However, there were no significant differences between PT and WT in the changes in the electromyographic activities of measured muscles during jumping. **Conclusion:** These results indicate that the jump performance gains after plyometric training are attributed to changes in the mechanical properties of muscle–tendon complex, rather than to the muscle activation strategies. **Key Words:** PLANTAR FLEXION, TENDON STIFFNESS, PRESTRETCH, HUMAN, ULTRASONOGRAPHY

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It is well known that plyometric training improves jumping and sprinting abilities and other ballistic movements (29,31). Previous studies suggested that the increase in jumping performance after plyometric training was attributed to neuromuscular adaptations, that is, the pattern of motor unit recruitment, muscle activities of agonists and antagonists (3,4,6,21,31,33). For example, Chimera et al. (3) show that the increased preparatory adductor activity and abductor-to-adductor coactivation presented preprogrammed motor strategies learned during plyometric training. They state that plyometric training might reduce the risk of injury by enhancing functional joint stability in the lower extremities. Kyrolainen et al. (21) also report that the preactivity of muscles increased after 4 months of plyometric training, and this change led to increased tendomuscular stiffness. Komi (10) suggests that higher stiffness levels of lower limb muscles during stretch-shortening cycle exercises led to a benefit in terms of the greater amount of stored and reused elastic energy. In fact, Toumi et al. (31) demonstrate that the knee joint stiffness during the eccentric phase of countermovement jump increased significantly for the jump training combined with weight training group, but not for weight training group. However, it is unclear whether these changes in tendomuscular stiffness were caused by the changes in the muscle activities and/or the mechanical properties of muscle–tendon complex itself.

Jumping and sprinting movements induce stretch-shortening cycles in the muscle–tendon complex in the lower limbs, in which lengthening and shortening actions of the muscle–tendon complex are repeated (10). During stretch-shortening cycle exercises, the elastic energy is stored in tendon structures in the lengthening phase and is reused in the shortening phase (10). Recent studies have investigated the relationship between tendon properties and

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jump performances *in vivo* (1,16). We found that tendon stiffness in knee extensors was inversely correlated with the relative difference in jump height between vertical jumps performed with and without countermovement—prestretch augmentation (16). In addition, the stiffness of the human tendon has been shown to increase after resistance training using heavy loads (12,18,27). Kubo et al. (18) report that after isometric squat training, the tendon stiffness in knee extensors increased, and, simultaneously, the prestretch augmentation during vertical jumping decreased. Considering these findings, it is hypothesized that plyometric training would change the tendon properties, making them suitable for stretch-shortening cycle exercises. In addition, the above-quoted findings (12,31) tempt us to assume that there is a difference in the effects on the mechanical properties of muscle–tendon complex and the neural adaptations between plyometric and weight training regimens. If so, the differences in these changes would lead to the differences in the effects on jump performances.

The purpose of this study was to investigate the effects of plyometric and weight training protocols on the mechanical properties of muscle–tendon complex and muscle activities and performances during jumping. These findings would be useful to elucidate the mechanisms of improved performances during the stretch-shortening cycle after plyometric training.

METHODS

Subjects. Ten healthy males (age: 22 ± 2 yr, height: 170 ± 3 cm, body mass: 63 ± 8 kg, mean ± SD) voluntarily participated in this study. They did not have an experience of regular exercise training. Therefore, the obtained results in this study would be different from those from trained subjects. They were fully informed of the procedures to be utilized as well as the purpose of this study. Written informed consent was obtained from all subjects. This study was approved by the office of the Department of Sports Sciences, University of Tokyo, and complied with their requirements for human experimentation.

Training. Subjects performed plyometric training on one side (PT) and weight training on the other side (WT). In each subject, the right and left legs were randomly allocated to the training protocols. They completed 12 wk (4 d·wk^{-1}) of plyometric and weight training protocols on the sledge apparatus (VR-4100, Cybex Corp.) with an inclination of 17° from the horizontal position. The subjects lay on the sliding table of this apparatus. The table was designed to slide with minimal friction with a constant load through a steel cable connected to adjustable weights. The measurement of one-repetition maximum was made every 4 wk to adjust the training load. At the end of the training session, the one-repetition maximum increased significantly by 55 ± 11% for PT and 58 ± 13% for WT, respectively (both $P < 0.001$). In a training session, a subject would train the PT protocol leg first, then the WT protocol leg, and in the next session, the order would be reversed.

For PT, the subjects performed the two kinds of training protocols, that is, hopping and drop jump training. During the hopping jump, the initial position was maximal plantar flexion. Then, the subjects developed the plantar flexion force to maximal dorsiflexion (eccentric muscle action), and rebounded to start plantar flexion until the toe finally lifted away from the footplate of this apparatus (concentric muscle action). The subjects repeated these movements without a pause. During the drop jump, the sliding table of this apparatus was moved to a height of 20 cm from the surface of the footplate of this apparatus to the sole of their foot with the assistance of an experimenter. They were dropped down from a height of 20 cm. After landing on the edge of the footplate of this apparatus, the ankle joint was dorsiflexed until the maximally dorsiflexed position (eccentric muscle action). Then, the subjects started plantar flexion and took off (concentric muscle action). The subjects repeated these movements without a pause. The subjects performed five sets of each exercise (hopping and drop jump) with a between-set rest interval of 30 s, which consisted of unilateral plantar flexion at 40% of the one-repetition maximum with 10 repetitions per set. According to the previous findings (8), maximal mechanical power has been thought to occur at a resistance of 30–45% of the one-repetition maximum.

For WT, the subjects were instructed to lift and lower the load at an approximately constant velocity, taking about 1 s for the concentric action and 3 s for the eccentric action. The exercise was performed over the range of motion from the fully dorsiflexed position to the fully plantar flexed position. The subjects performed five sets of exercise with a between-set rest interval of 1 min, which consisted of unilateral plantar flexion at 80% of the one-repetition maximum with 10 repetitions per set. It is generally known that high intensity (~80% of the one-repetition maximum) exercise is highly effective for gaining muscular size and strength (24).

Jump performance. Three kinds of unilateral maximal jumps using only the ankle joint (squat jump: SJ; countermovement jump: CMJ; drop jump: DJ) were performed on the sledge apparatus. The load used was 50% of the body mass for each subject. Because the body mass of the subjects did not change after training, the load for each subject was the same before and after training. A force plate (Kistler, 9281B, Switzerland) was mounted firmly onto the footplate of this apparatus. A wooden block was attached to the force plate, and the subjects placed the ball of their right foot on the block with the knee fully extended. The vertical component of the ground reaction force (F_z) was recorded from the force platform. Three retroreflective landmarks were placed over the following anatomical landmarks on the right side of the subjects: the fifth metatarsophalangeal joint, the lateral malleolus, and the lateral epicondyle of the knee. During jumping, subjects were filmed from the right (using the right ankle) or left

TABLE 1. Mechanical and morphological properties of muscle and tendon for plyometric and weight training protocols.

	Plyometric Training		Weight Training	
	Before	After	Before	After
Muscle volume (cm^3)	576.5 (49.7)	604.8 (51.8)*	579.4 (48.8)	610.8 (51.4)*
MVC (N·m)	116.0 (23.4)	131.4 (27.5)*	114.5 (24.6)	135.1 (26.0)*
Activation level (%)	90.7 (9.4)	95.2 (5.6)*	91.2 (8.0)	96.0 (6.0)*
Coactivation level (%)	12.8 (5.3)	12.6 (4.4)	13.5 (3.9)	14.4 (5.4)
Twitch torque (N·m)	16.0 (2.2)	16.4 (3.41)	16.6 (2.5)	16.8 (3.4)
Time to peak torque (ms)	153 (24)	139 (14)*	148 (25)	143 (10)
Rate of torque development ($N \cdot m \cdot s^{-1}$)	106.6 (22.3)	117.8 (16.8)	112.7 (12.3)	117.0 (23.3)
Maximal tendon elongation (mm)	13.7 (2.3)	15.0 (2.2)*	12.5 (2.0)	11.9 (2.6)
Tendon stiffness (N·mm)	129.0 (35.8)	154.0 (55.2)	127.9 (25.8)	165.9 (43.7)*
Elastic energy of tendon (J)	20.4 (7.0)	24.4 (7.9)*	19.9 (6.8)	19.0 (5.9)
Tendon CSA (mm^2)	57.2 (9.1)	59.1 (8.7)	59.0 (7.9)	58.3 (8.4)

Mean (SD).
* Significantly different from before training.

(using the left ankle) sides in the sagittal plane with a digital high-speed video camera at a sampling frequency of 200 Hz (HSV-500C[3], Nac, Japan).

Before the experiment the subjects were familiarized with the jumping actions. For all tests, they were instructed to jump to a maximal height. The test was repeated five times per subject, with at least 3 min between trials. For SJ, the subjects initially kept the ankle position maximally dorsiflexed, and supported the load in this position. Then, the subjects started ankle movement until the ankle was fully plantar flexed and the toe lifted away from the wooden block. For CMJ, the initial position was maximal plantar flexion. Then, the subjects developed the plantar flexion force to maximal dorsiflexion (eccentric muscle action), and rebounded to start plantar flexion until the toe finally lifted away from the footplate of this apparatus (concentric muscle action). For DJ, the sliding table of this apparatus was moved to a height of 20 cm from the surface of the footplate of this apparatus to the sole of their foot with the assistance of an experimenter. They were dropped down from a height of 20 cm. After landing on the edge of the wooden block with the ball of the right foot, the ankle joint was dorsiflexed until the maximally dorsiflexed position (eccentric muscle action). Then, the subjects started plantar flexion and took off (côncentric muscle action). We excluded the trials in which the knee joint was flexed slightly according to images taken by a high-speed video camera.

Using a public domain National Institutes of Health (NIH) image software package, the ankle joint angle and jump height were measured. Assuming that the displacement of the retroreflective landmark of the lateral malleolus was equal to that of the center of mass, the jump height was defined as the maximum displacement of the retroreflective landmark of the lateral malleolus from the resting position (ankle joint angle was 90°). Three individual jump height recordings excluding the largest and smallest values were averaged. The difference between the heights of CMJ or DJ and SJ, expressed as the percentage of that in SJ, was proposed as an index of prestretch augmentation (15).

The repeatability of the jump height measurements was investigated on two separate days in a preliminary study with six young males. There were no significant differences between the test and retest values of the SJ, CMJ, and DJ heights. The intraclass correlation coefficient (ICC) was 0.91 for SJ, 0.88 for CMJ, and 0.85 for DJ, respectively.

Joint stiffness. Ankle joint torque (TQ) during DJ was estimated from the following equation (9):

$$TQ = F_z L1 \cos (A_j - 90)$$

where F_z, L1, and A_j are the vertical component of the ground reaction force, the length from the estimated center of the ankle joint to the ball of the foot (measured for each subject), and the ankle joint angle, respectively (see Fig. 3 in Kawakami et al. (9)).

According to Kuitunen et al. (19), ankle joint stiffness was calculated as a change in joint torque divided by the change in the ankle joint angle during the eccentric phase. As mentioned above, three trial values for jumping heights were averaged.

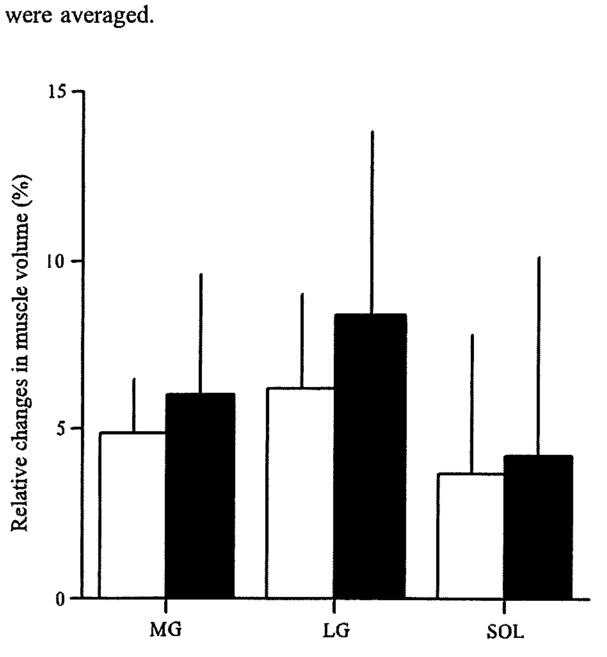

FIGURE 1—The relative changes in muscle volumes of medial gastrocnemius (MG), lateral gastrocnemius (LG), and soleus (SOL) muscles for plyometric (*open*) and weight (*closed*) training protocols. All muscle volumes of plantar flexors (MG, LG, SOL) increased significantly for both protocols.

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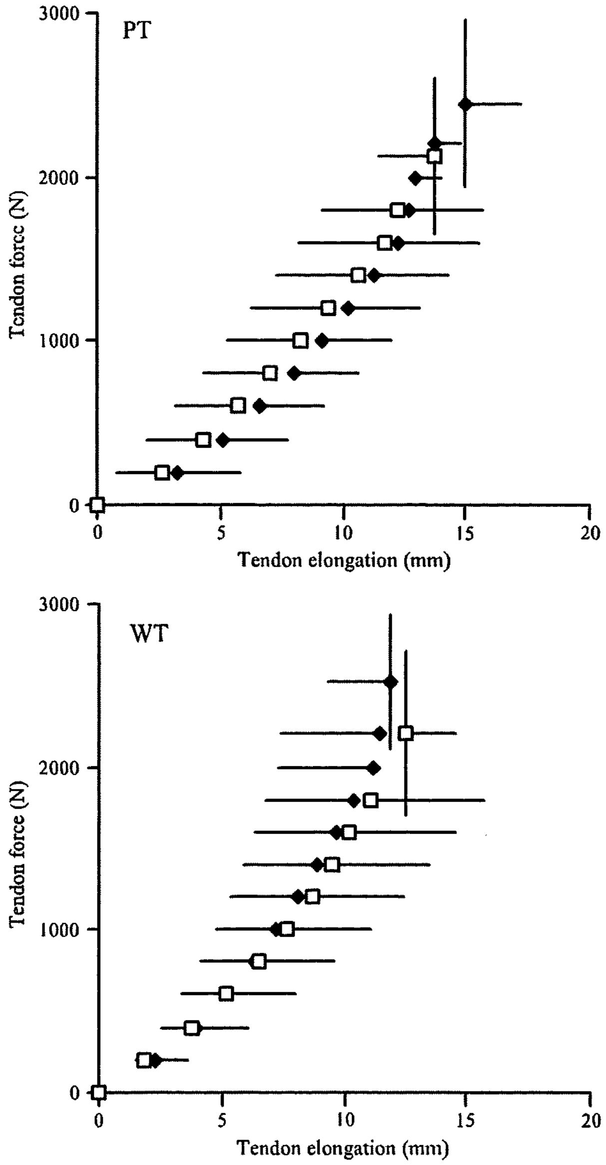

FIGURE 2—The tendon force and elongation relation before (*open*) and after (*closed*) training for 12 wk. Both protocols produced no significant differences in the tendon-elongation values at any force-production level after training.

The repeatability of the joint stiffness measurements was investigated on two separate days in a preliminary study with six young males. There were no significant differences between the test and retest values of joint stiffness. ICC was 0.86.

Cross-sectional area of muscle and tendon. Measurements of muscle and tendon cross-sectional areas (CSA) were carried out by magnetic resonance imaging scans (AIRIS II, HITACHI Medical Cop., Tokyo, Japan). T1-weighted spin-echo, axial-plane imaging was performed with the following parameters; TR 850 ms, TE 25 ms, matrix 256 × 256, field of view 250 mm, slice thickness 10 mm, and interslice gap 0 mm. The subjects were imaged in a supine position with the knee and ankle kept at 0° (full extension) and 90° (anatomical position), respectively. During the scanning, the subject lay supine with the base of the foot resting on a polystyrene block to maintain an ankle angle of 90°. The number of sections obtained for each subject was 42–47. The muscles investigated were as follows: medial gastrocnemius (MG), lateral gastrocnemius (LG), and soleus (SOL). From the axial image, outlines of each muscle were traced, and the traced images were transferred to a computer for CSA calculation using a public domain NIH image software package. The muscle volume was determined by multiplying the anatomical CSA of each image by the thickness (10 mm). In addition, the measurement of tendon CSA was taken at two positions (one above the calcaneous and the other at 10 mm proximal to the calcaneous). The average CSA at the two positions was calculated as the representative of tendon CSA.

The repeatability of muscle volume and tendon CSA measurements was investigated on two separate days in our previous study with six young males (13). There were no significant differences between the test and retest values of the muscle volume and tendon CSA. ICC was 0.92 for the muscle volume and 0.97 for the tendon CSA.

Muscle strength, resting twitch, and neural activation. The maximal voluntary isometric strength (MVC) of the plantar flexor muscles was determined using an electrical dynamometer (Myoret, Asics, Japan). The subject lay prone on a test bench and the waist and shoulders were secured by adjustable lap belts and held in position. The ankle joint was set at 90° with the knee joint at full extension, and the foot was securely strapped to a footplate connected to the lever arm of the dynamometer. Before the test, the subject performed a standardized warm-up and submaximal contractions to become accustomed to the test procedure.

The repeatability of the muscle strength measurements was investigated on two separate days in a preliminary study with eight young males. There were no significant differences between the test and retest values of joint stiffness. ICC was 0.96.

Resting twitch properties were assessed by supramaximal electrical stimulations. The stimulating lead electrodes were placed on the skin of the right popliteal fossa and oriented longitudinal to the estimated path of the tibial nerve with the anode distal. A high-voltage stimulator (SEN-3301, having a specially modified isolator SS-1963, Nihon-Koden, Japan) generated rectangular pulses (triple stimuli with a 500-μs duration for one stimulus and an interstimulus interval of 10 ms). Maximal twitch contractions were evoked in the resting muscle by progressively increasing the stimulation intensity until increases failed to elevate twitch torque further. The stimulus intensity that elicited peak twitch torque was used throughout the duration of the measurements. Peak torque, time to peak torque, and the rate of torque development were measured as the twitch properties.

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During MVC, evoked twitch contractions were imposed by supramaximal electrical stimulations to assess the activation level of muscles. The experimental procedures have been described in detail previously (17). In all subjects, the stimuli increased the force during MVC at the appropriate latency. Shortly (within 1–2 s) after MVC, when the potentiation effect of the contraction still persisted, the same stimulation was given to the muscle at rest (control twitch). The voluntary force at the instant of stimulation was used as the MVC force. The twitch force (difference between peak twitch force and MVC force) was measured, from which the level of muscle activation with voluntary effort (% activation) was assessed from the following equation (twitch interpolation technique; (17)): % activation = {1 − (twitch force during MVC/control twitch force)} × 100 (%), where control twitch represents the twitch imposed on the resting muscle after MVC.

Stiffness of Achilles tendon. The subject was instructed to develop a gradually increasing force from a relaxed state to MVC within 5 s. The task was repeated two times per subject with at least 3 min between trials. An ultrasonic apparatus (SSD-2000, Aloka, Tokyo, Japan) with an electronic linear array probe (7.5-MHz wave frequency with 80 mm scanning length; UST 5047-5, Aloka) was used to obtain longitudinal ultrasonic images of the medial gastrocnemius muscle. The probe was longitudinally attached to the dermal surface with adhesive tape, which prevented the probe from sliding. To evaluate the elongation of the Achilles tendon, the displacement of the distal myotendinous junction of the MG in the transition from a resting state to MVC was measured. In the present study, the Achilles tendon was defined as the distance from the Achilles tendon insertion on the calcaneus to the distal myotendinous junction of the medial gastrocnemius muscle. Ultrasonic images were recorded on videotape at 30 Hz, and synchronized with force recordings using a clock timer for subsequent analyses.

Tendon displacement is attributed to both angular rotation and contractile tension, since any angular joint rotation occurs in the direction of ankle plantar flexion during an "isometric" contraction (22). To monitor ankle joint angular rotation, an electrical goniometer (Penny and Giles) was placed on the lateral aspect of the ankle. To correct the measurements taken for the elongation of the Achilles tendon, additional measurements were made under passive conditions. Displacement of the myotendinous junction of the medial gastrocnemius muscle caused by rotating the ankle from 90 to 70° was digitized in sonographs taken as described above. Thus, for each subject, displacement of the myotendinous junction obtained from the ultrasound images could be corrected for that attributed to joint rotation alone (22). In the present study, only values corrected for angular rotation are reported.

The measured torque (TQ) during isometric plantar flexion was converted to tendon force (F_t) by the following equation:

$$F_t = \mathrm{TQ/MA}$$

where MA is the moment arm length of the triceps surae muscles at 90° of the ankle joint, which is estimated from the lower-leg length of each subject (15). In the present study, the tendon force and elongation values above 50% of MVC were fitted to a linear regression equation, the slope of which was adopted as stiffness (16). *Two trial values for tendon stiffness were averaged.* ICC of the two measurements of tendon stiffness for all subjects was 0.91.

The repeatability of the tendon stiffness measurements was investigated on two separate days in our previous study with eight young males (13). There were no significant differences between the test and retest values of tendon stiffness. ICC was 0.89.

TABLE 2. Ankle angle, angular velocity, and performance during jumping tests for plyometric and weight training protocols.

	Plyometric Training		Weight Training	
	Before	After	Before	After
Ankle angle at the lowest position (°)				
SJ	67.7 (4.7)	67.1 (7.3)	68.5 (4.5)	66.0 (9.9)
CMJ	68.1 (5.2)	68.2 (7.2)	68.3 (4.3)	67.7 (5.1)
DJ	68.9 (5.6)	69.4 (6.2)	69.3 (5.0)	68.9 (4.1)
Angular velocity during eccentric phase (°·s^{-1})				
CMJ	96.5 (26.4)	98.1 (27.4)	83.4 (8.5)	75.3 (15.6)
DJ	205.6 (33.1)	226.3 (48.1)	198.4 (33.5)	213.4 (46.4)
Angular velocity during concentric phase (°·s^{-1})				
SJ	81.4 (15.8)	115.4 (43.9)*	71.8 (11.2)	84.6 (18.9)
CMJ	122.7 (25.6)	141.4 (18.5)	126.2 (18.0)	137.0 (31.3)
DJ	153.8 (25.8)	158.1 (19.9)	144.3 (29.3)	155.3 (30.6)
Jump height (cm)				
SJ	20.7 (4.0)	26.6 (4.8)*	20.4 (3.0)	22.7 (2.9)*
CMJ	23.2 (4.6)	31.4 (5.0)*	23.6 (3.5)	24.4 (3.1)
DJ	23.8 (5.4)	33.8 (5.0)*	24.2 (4.3)	25.5 (3.7)
Prestretch augmentation (%)				
CMJ	13.0 (7.6)	19.1 (12.0)	12.8 (5.0)	8.3 (11.7)
DJ	14.7 (8.2)	29.2 (16.6)	17.9 (13.5)	13.1 (14.1)

Mean (SD).
* Significantly different from before training.

Electromyographic activity. The electromyographic activity (EMG) was recorded during the measurements of the maximal voluntary isometric strength, tendon properties, and jump performances. Bipolar surface electrodes (5 mm in diameter) were placed over the bellies of MG, LG, SOL, and tibialis anterior (TA) muscles with a constant interelectrode distance of 25 mm. The electrodes were connected to a preamplifier and differential amplifier with a bandwidth of 5 Hz to 500 Hz (model 1253A, NEC Medical Systems, Tokyo, Japan). The EMG signals were transmitted to a computer at a sampling rate of 1 kHz. The EMG was full-wave rectified and averaged for the duration of the contraction (mEMG). During the jumping tests, the mEMG values from MG, LG, SOL, and TA were calculated from the prelanding (defined as 100 ms preceding landing), eccentric and concentric phases, respectively, according to the ankle joint angle. In addition, the mean of mEMG in the MG, LG, and SOL was defined as the mEMG of plantar flexors. During the measurements of tendon properties, the mEMG of TA was measured to investigate the antagonist muscle activity of TA (coactivation level). To determine the maximal activation of TA, a maximal dorsiflexion isometric contraction was performed at the same angle (90° of ankle joint). We normalized the mEMG value of TA with respect to the mEMG value of TA at the same angle when acting as an agonist at maximal effort.

Statistics. Descriptive data included means ± SD. A two-way ANOVA with repeated-measures [2 (groups) × 2 (test times)] was used to analyze the data. The F ratios for main effects and interactions were considered significant at $P < 0.05$. Significant differences among means at $P < 0.05$ were detected using a Tukey *post hoc* test.

RESULTS

The muscle volumes of the plantar flexor muscles increased significantly 4.9 ± 2.3% for PT ($P = 0.003$) and 5.4 ± 2.8% for WT ($P = 0.002$), respectively (Table 1). No significant difference in the relative increase of muscle volume was found between PT and WT ($P = 0.379$). There were no significant differences in the relative increase in the muscle volume among MG, LG, and SOL (Fig. 1). Furthermore, no significant change in the Achilles tendon CSA was found between both the protocols (Table 1).

The MVC value increased significantly by 17.3 ± 21.7% for PT ($P = 0.017$) and 19.3 ± 13.6% for WT ($P = 0.003$), respectively (Table 1). There was no significant difference in the relative increase of the MVC value between the two protocols ($P = 0.818$). The activation level of the plantar flexor muscles assessed by superimposing electrical stimuli increased significantly by 5.6 ± 6.6% for PT ($P = 0.019$) and 5.8 ± 8.2% for WT ($P = 0.049$), respectively (Table 1). No significant change in the coactivation level was found after training for PT ($P = 0.771$) and WT ($P = 0.549$), respectively (Table 1). Although the twitch torque value and the rate of torque development did not change for both the protocols, the time to peak torque shortened significantly for PT ($P = 0.011$) but not for WT ($P = 0.499$) (Table 1).

Both protocols produced no significant differences in the tendon-elongation values at any force-production levels after training (Fig. 2). The maximal tendon elongation and elastic energy increased significantly for PT (tendon elongation $P = 0.031$, elastic energy $P = 0.044$), but not for WT (tendon elongation $P = 0.374$, elastic energy $P = 0.681$) (Table 1). The relative increases in the maximal tendon elongation and elastic energy were greater for PT than for WT (tendon elongation $P = 0.032$, elastic energy

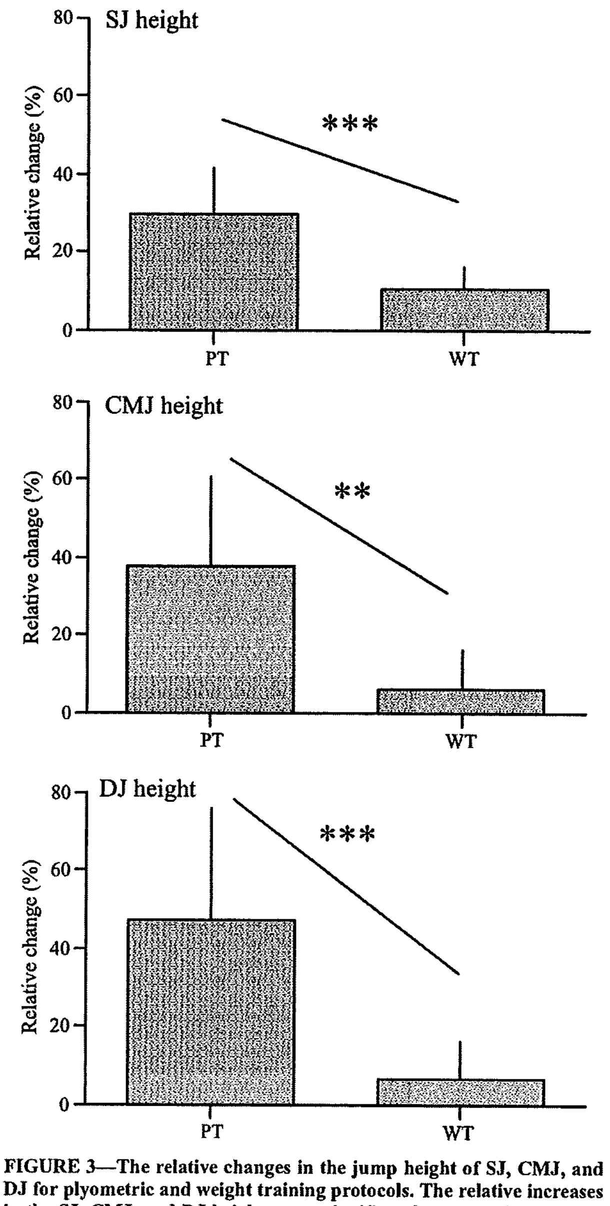

FIGURE 3—The relative changes in the jump height of SJ, CMJ, and DJ for plyometric and weight training protocols. The relative increases in the SJ, CMJ, and DJ heights were significantly greater for PT than for WT. ** $P < 0.01$; *** $P < 0.001$.

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