Effects of Cooling on Repeated Muscle Contractions and Tendon Structures in Human

Su-Dong Chae, MS; Myeong-Soo Jung, PhD; Akira Hori, PhD

Department of Health and Sport Science, Graduate School of Nippon Sport Science University; 1Department of Physical Education, Chosun University; 2Department of Health and Sport Science, Graduate School of Nippon Sport Science University

Purpose: This study compared the effects of non-cold and cold conditions on the viscoelastic properties of tendon structures in vivo. Methods: Seven male subjects performed plantar flexion exercise with maximal isokinetic voluntary contraction, which consisted of muscle contraction for 6 sec and relaxation for 60 secs, 10 times for 1 set. Totally 10 sets were repeated. Before and after each task, the elongation of the tendon and aponeurosis of the medial gastrocnemius muscle (MG) was directly measured by ultrasonography. (The relationship between the estimated tendon force and tendon elongation.) Tendon cross-sectional area and ankle joint moment arm were obtained from magnetic resonance imaging (MRI). The tendon force was calculated from the joint moments and the tendon moment arm and stress was obtained by dividing force by cross-sectional areas (CSA). The strain was measured from the displacements normalized to tendon length. Results: After cooling, the tendon force was larger in cold than non-cold. The value of the tendon stiffness of MVC were significantly higher under the cold condition than under the non-cold condition. The maximal strain and stress of 7.4±0.7% and 36.4±1.8 MPa in non-cold and 7.8±8.5%, 31.8±1.1 MPa in cold (P<0.05). Conclusion: This study shows for the first time that the muscle endurance in cooling increases the stiffness and Young's modulus of human tendons. The improvement in muscle endurance with cooling was directly related to muscle and tendon. (J Kor Soc Phys Ther 2006;18(6):1-11)

Key Words: Tendon, Cooling, Ultrasonography, Muscle endurance

1. Introduction

It was generally reported that the cooling causes decrease of muscle force(Bigland-Ritchie et al, 1992; Davies et al, 1983; Edward et al, 1972; Faulkner et al, 1992; Howard et al, 1994; Sargeant, 1987). The maximum force decreases significantly with cooling below 25°C for human muscle in vivo (Edward et al., 1972). On the other hand, the muscular endurance appears to improve with cooling (Bundschuh et al., 1982; Clarke et al., 1978). The major thrust of the present paper is to investigate the fundamental nature of strength and endurance of local musculature as influenced by exposure to various cold temperatures. It has been observed that when a limb is cooled in water at temperatures below 25°C, the muscle strength decreases (Edward et al., 1972). On the other hand, the muscular endurance appears to improve with cooling (Bundschuh et al., 1982; Clarke et al., 1978). The decline in strength as the muscle cools has been attributed to the failure of contraction of superficial muscle fibers. An increase in muscle temperature is thought to accelerate muscle metabolism and result in the accumulation of waste

The muscle activation results in force transmission to bone via tendons to produce joint movement. However, tendons are not inextensible, but have non-linear spring-like characteristics. These properties allow for the tendon to be stretched for energy storage, which is subsequently converted into kinetic energy upon release (Alexander et al, 1975). The Achilles tendon is subjected to considerable loads during human locomotion. In fact, the Achilles tendon may reach tensile forces of up to 2600 N during walking (Finni et al, 1998; Giddings et al, 2000) and up to 5330 N during running (Giddings et al, 2000). Nonetheless, the effects of the treatments of cooling in human locomotion and rehabilitation on the muscle and Achilles tendon were unclear.

Recent reports have shown that the ultrasonography can be used to determine the cold and heat of human tendon-aponeurosis structures in vivo (Kubo et al, 2005). (However, a report is not unclear about stress and Young's modulus influence of give to the viscoelastic of a tendon structures.) Furthermore, we have little knowledge on the influences of muscle endurance on tendon structures in cooling. The purposes of this study were to determine the muscle endurance-related changes in tendon properties and to investigate the effects of non-cold and cold on the tendon-aponeurosis structures.

II. Methods

1. Subjects

The subjects of this study were eight healthy men (age 24.3±3.7 yrs, height 170.3±8.2 cm, weight 71.2±4.6 kg). All subjects were volunteers; they were informed of the potential risks and benefits of testing protocols and gave their written consent to participate.

2. Experimental protocol

Before the experimental session, each subject performed times of maximal isometric contractions to enable us to for gathering an average value of maximal voluntary contraction (MVC). The subject was instructed to gradually increase force (10% MVC step) from a relaxed state to MVC within 3 sec. At this time, it was measured by an ultrasonographic probe was attached and that an electrodes was attached to monitor EMG. A thermistor was attached to measure the skin temperature. After the plantar flexion exercising 10-time × 10-set, (in order to obtain the average value of the maximal voluntary contraction (MVC)) the two greatest isometric contractions was performed like movement before test. In this study, data obtained by serial measurement provided control values. Data for each subject was obtained under cold and non-cold conditions, respectively, and measurements of various parameters of the left or right leg, chosen randomly, were taken. The subjects were subjected the same control measurements for skin cooling conditions one week later.

3. Skin temperature measurement

Cooling was conducted on the surface skin of whole calf muscle by applying three ice packs for 20 min. After the 20 minutes, ice packs were removed and plantar flexion isometric exercise was performed. The skin temperature was measured using skin thermistor 1) attached to the skin with

1) LT-8, Gram, Japan
adhesive tape at four sites: the medial gastrocnemius (MG), lateral gastrocnemius (LG), soleus (SOL), and tibialis anterior (TA). These temperatures were recorded at 10 sec intervals using a data logger. The average skin surface temperature was derived from the values during the rest period and those during the plantar flexion exercise. The calculation method of the averaged data was recorded at intervals of 2 sec intervals over 5 min increments.

4. Measurement of plantar flexion joint moment

A biodex system\(^2\) was used to fix the ankle joint and to measure the plantar flexion torque. Each subject was seated on a test bench of a Biodex System 3 with a backrest and secured by straps around the waist, chest, and right and left footplate. The center of rotation of the Biodex was visually aligned with the center of the rotation of the ankle joint. The foot was fixed at a neutral anatomic position, where the sole of the foot was at 0 degrees to the tibia. Prior to the test, the subject performed a standardized warm-up and sub maximal contractions to become accustomed to the test procedure. The subject was instructed to gradually increase force from a relaxed state to maximal voluntary contraction (MVC) within 3–4 sec. Strong verbal encouragements was used to motivate the subjects during the test. The signal from the load cell was stored on a computer\(^3\).

5. Measurement of tendon displacement

A real-time ultrasonic apparatus\(^4\) was used to obtain a longitudinal ultrasonic image of medial gastrocnemius muscle (MG) at the level of 30% of the lower leg length. The width and depth resolutions of ultrasonography with this probe are 0.67 and 0.4 mm, respectively. The investigator visually confirmed the echoes reflected from the aponeuroses and interspaces among fascicles in MG on the ultrasonic images. They were displayed on a real-time basis on a monitor and recorded on videotape that was synchronized with a clock timer for subsequent analyses. Any movement of the line cast by the external marker on the ultrasound image indicated movement of the transducer with respect to the scanned structure, and this trial would be omitted from any further analysis. The measurements of displacement were analysed at 10% intervals of maximal torque, using digitising software\(^5\).

6. Tendon cross sectional area and stress

The measurements of tendon cross-sectional areas and distance were carried out on an MRI system\(^6\) with a spine-array surface coil and a field of view (FOV) of 360 mm in all registered planes. The images were T1-weighted (Spinnekesequences with TE = 25 ms and TR = 850 ms) to distinguish between the anatomical boundaries. The number of images varied from 17 to 19 with a slice thickness of 10 mm, obtained at 10-mm intervals. All the images were scanned with a matrix 256×256, giving a pixel size of 0.9 mm. Artifactual errors as breathing and small movements were reduced using spatial presaturation pulses. The cross-sectional area of the Achilles tendon was measured 3 cm proximal to the Achilles tendon insertion onto the calcaneus, which corresponds to the narrowest portion of the Achilles tendon (Voigt et al, 1994). The measurement was performed 2 times for each

\(^2\) Biodex System 3, Sakai, Tokyo, Japan.
\(^3\) PowerbookG3, Apple, Tokyo, Japan.
\(^4\) SSD-1000, Aloka, Japan.
\(^5\) Scion image version 1.61, National Institutes of Health, USA.
\(^6\) Airis, HitachiMedical Corp, Tokyo, Japan.
subject, and the mean was used as the cross-sectional area. The mean coefficient of variation for repeated measures across subjects was 4.2%.

7. Tendon length and strain

The tendon length was obtained from MRIs (T1-weighted TR/TE: 640/25; FOV 320; matrix 256×256, slice thickness 10 mm). The tendon path and distal point of insertion were visualized in the sagittal plane scans, and the tendon origin was visualized in the axial plane scans (Fukunaga et al, 1992). The whole tendon length was digitized by following its curved path from origin insertion.

8. Calculation of moment arm

Sagittal plane MRIs (T1-weighted TR/TE: 640/25; FOV 320; matrix 256×256, slice thickness 10 mm) were obtained with the ankle in the neutral position (90°) to estimate with the Achilles tendon moment arm using a modified Reuleaux method (Voigt et al, 1994). The measurement of the moment arm from the MRIs was 2 times for each subject and the mean was used as an estimate for the moment arm. The mean coefficient of variation for repeated measures across subjects was 1.7%.

9. Tendon force

The tendon force was calculated from the moment equilibrium equation around the ankle joint, i.e. by dividing the externally measured joint moment by the calculated moment arm.

10. Stiffness and young's modulus

The tendon stiffness (N/mm-1) was calculated from the force-elongation relationship in the final 10% of the force range, (i.e. from 90 to 100%). Young's modulus (GPa) over the respective stress intervals was calculated by multiplying the stiffness value estimated by the ratio of tendon length to tendon CSA.

11. Measurement of the EMG

The electromyogram (EMG) activity was recorded during the isometric contraction and under cold and non-cold conditions, respectively. Single differential electrodes (10 mm in width) were placed over the bellies of the medial gastrocnemius (MG), lateral gastrocnemius (LG), soleus (SOL), and tibialis anterior (TA). The EMG7) signals were transmitted to a PC® and sampled at a rate of 1 kHz. The EMG was full-wave rectified and integrated for the duration of the contraction and under cold and non-cold conditions to obtain an integrated EMG (iEMG) value.

12. Statistics

One-way analysis of variance (ANOVA) with repeated measures was used to detect the significant effects of force level (%MVC) on the tendon force and elongation at every 10% MVC. In the event of significant values of F in the ANOVA, Tukey's post hoc test of critical difference was used to locate significance between the different means. The level of significance was set at p<0.05.

III. Results

There were no significant differences between cold and non-cold in MVC during plantar flexion; 162.4±48.3 N m in non-cold vs. 142.2±35.7 N m.

7) Delsys, USA.
8) PowerbookG3, Apple, Tokyo, Japan.
in cold.

Figure 1 indicates the relation between the tendon force and stiffness in MG. In the case of the stiffness after plantar flexion, under cold and non-cold conditions, the stiffness values at MVC were significantly higher under the cold condition than under the non-cold condition (p<0.05). On the other hand, before plantar flexion there was no significant difference between significantly non-cold and cold in stiffness at any force level, and the maximum stiffness of after plantar flexion was similar in both the conditions.

The average stress-strain characteristics for under non-cold and cold conditions are shown in Figure 2. In the case of the stress after plantar flexion, for cold conditions, the displacement and the cross-sectional area of the achilles tendon yielded an estimated maximal strain and stress of 7.4±7 % and 36.4±1.8 MPa, respectively, with a resulting Young's modulus of 1624±128 MPa. For non-cold conditions, the respective strain, stress and Young's modulus were 7.8±8.5 %, 31.8±1.1 MPa and 1164±225 MPa. In the case of the stress after plantar flexion, the difference more significant than non-cold at MVC of the lower intensity (10%~20% and 50%~60%) of cold was accepted (p<0.05).

Table 1 shows the relationships between the tendon force and elongation in MG. In the case of the tendon force after plantar flexion, the difference was more significant in non-cold than in cold. However, there were no significant difference in elongation between non-cold and cold at any force production level, and the maximum elongation was similar in both the conditions; 20.9±1.9 mm in non-cold and 19.7±6.8 mm in cold. In addition, the Young's modulus of tendon-aponeurosis complex changed after cooling.

Table 2 shows the integral electromyograms (iEMG) activity of the MG, LG, SOL, and TA muscles under the cold and non-cold conditions. The mean of iEMG activity of the MG, LG, and SOL changed under the cold condition: the MG, LG, and SOL increased (p<0.001), but TA remained unchanged (p<0.25)

![Figure 1](image)

**Figure 1.** The mechanical stiffness for the non-cold and cold conditions. The mechanical stiffness increased significantly in the after cooling. Values are means and SD. **p<0.01**
Figure 2. Relationships between stress and strain during the plantar flexion exercise in the before (A) and after (B) cooling. Values are means and SD. *p<0.05, **p<0.01

Table 1. Measured variables non-cold and cold conditions. (Mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
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<tbody>
<tr>
<td></td>
<td>Non-cold</td>
<td>Cold</td>
</tr>
<tr>
<td>Tendon force (N)</td>
<td>3497.5 (.8)</td>
<td>3338.8 (6.2)</td>
</tr>
<tr>
<td>Elongation (mm)</td>
<td>24.04 (1.4)</td>
<td>22.7 (8.2)</td>
</tr>
<tr>
<td>Young's modulus (GPa)</td>
<td>1226.4 (3.5)</td>
<td>1342.3 (1.2)</td>
</tr>
</tbody>
</table>

** Significantly different from non-cold (p<0.05 and p<0.01, respectively)
Table 2. Measured variables non-cold and cold conditions. (Mean±SD)

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
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<tbody>
<tr>
<td></td>
<td>Non-cold</td>
<td>Cold</td>
</tr>
<tr>
<td>MG</td>
<td>3.0(1.9)</td>
<td>4.1(8.9)***</td>
</tr>
<tr>
<td>LG</td>
<td>2.5(4.4)</td>
<td>3.4(2.9)***</td>
</tr>
<tr>
<td>SOL</td>
<td>1.2(8.6)</td>
<td>1.9(7.6)***</td>
</tr>
<tr>
<td>TA</td>
<td>0.2(9.4)</td>
<td>0.5(0.2)</td>
</tr>
</tbody>
</table>

*** Significantly different from non-cold (p<0.001)

IV. Discussion

The finding that non-cold and cold muscle strength were not significantly affected by decreasing local temperature is in disagreement with several studies which have reported that cold temperature lowered maximum muscle strength (Clarke et al, 1962; Johnson et al, 1977). Clarke et al (1958) investigated maximum strength throughout a temperature range of 10°C to 42°C, he reported that local immersion up to thirty minutes had no real effect on maximum grip strength above temperatures 18°C. On the other hand, several studies have previously demonstrated lowering ambient temperature, or cooling with ice packs improves exercise performance. Clarke et al (1958) found that the optimal forearm muscle temperature for improved muscle endurance was 27°C. Edwards et al (1972) reported an improvement in isometric endurance of the knee extensors with leg cooling. The results of the present study extended these observations to include isokinetic endurance of the knee extensors. However, the extent of the improvement in endurance appears to depend upon the amount of cooling of the skin surface. All but one of the eight subjects improved in endurance with cooling.

It has been previously demonstrated that the improvement in endurance with cooling was due to a lower metabolic rate and a slower accumulation of metabolites (Clarke et al, 1958; Parkin et al, 1999; Ray et al, 1997). Parkin et al (1999) suggested that the reason for the improved performance with cooling is likely to be related to carbohydrate availability because we observed a lower glycogenolytic rate. The change in the muscle function by cooling will influence the tendon characteristics, because muscle fibers are attached to the tendon and aponeurosis. In study, the stiffness increased significantly after cooling although no changes in the elongation were found after both the conditions. In the previous findings, isokinetic endurance increased with cooling, it appears that either the increase in muscle viscosity was not very great or that improvement in blood flow to the muscle may have accelerated the removal of metabolites (Clarke et al, 1958). As for the reason which endurance increases after cooling, not only change of a muscle function but change of the characteristic of a tendon was related.

In addition to the effects of skin surface temperature on the muscle force, Asmussen et
al (1976) reported that muscle stiffness increases with decreases in skin surface temperature. Some previous studies also showed that the maximal rate force development decreased in soleus muscle following a cooling (Segal et al, 1986). In this study, the rate of muscle force development decreased significantly together with elongation. These changes in various muscle functions could be attributed to the changes in the tendon properties with their temperatures, because muscle fibers are attached to tendon and aponeurosis. The tendon stiffness increased significantly after cooling although changes in the tendon properties were found after cooling. Taking the present result into account with the previous findings, the reasons for the stiffness of a tendon increases with cooling is the decrease in viscosity and fluids in the tendinous tissue.

In vivo measures of stiffness for the tibialis anterior aponeurosis and tendon have been reported to be 32–161 N m\(^{-1}\) with a Young's modulus of 530–1200 MPa (Magnusson et al, 2001). The stiffness and Young's modulus for the combined human triceps surae aponeurosis and tendon were recently reported to be 467 N m\(^{-1}\) and 1474 MPa (Magnusson et al, 2001).

In the present study, the tendon strain and elongations did not change after cooling for plantar flexion exercise although the tendon stiffness and tendon stress increased significantly. Some previous study have demonstrated the mechanical properties of muscles and tendons by cooling and heating (Kubo et al, 2005). Kubo et al (2005) reported that the mechanical properties (elongation, strain, and stiffness) did not changed muscles and tendons when the temperature was raised from 5 to 40°C. In the present study however, we measured the tendon property during before and after plantar flexion exercise, and compared the tendon properties of non-cold and cold conditions. A limitation associated with the available literature on muscle temperature and performance is related to the methodology used and more specifically the mode of cooling utilized. For example, ice massage was administered for 15 minutes (Lowdon et al, 1975), ice bag application for 20 minutes (Haymes et al, 1983), and cold water (Sargeant, 1987). This differences in the mode and length of the cold treatment may result in different amounts of tissue cooling, quite possibly bringing about different results when comparing performance variables. This difference is important because of the fact that extra stress of increased tension that is placed on the medial gastrocnemius muscle in a cold environment during contraction can be detrimental to the integrity of the tendon if the stress-strain relation were to be increased to the yield point.

The stress of the tendon increased over low force levels after cooling (after plantar flexion), but maximal tendon stress did not change following cooling. The tendon stress in humans, calculated from in vivo measurements performed on young adults, has been reported to be ~25 MPa in the tibialis anterior tendon (Maganaris et al, 1999) and ~42 MPa in the triceps surae tendon structures (Magnusson et al, 2001). In vitro tests have demonstrated the tendon stress at failure to be between 86 and 100 MPa (Bennett et al, 1986; Wren et al, 2001). The safety factor, (i.e. the stress during strenuous activity divided by the fracture stress), is about 8 for the majority of tendons (Ker et al, 1988), but it may be as low as 1~2 (Biewener et al, 2000). In the present study, the safety factor was 1.7 (37MPa) for the cold and 1.1 (36MPa) for the non-cold conditions, assuming a failure stress of 100 MPa. Thus, it is conceivable that the Achilles tendon approaches the safety limit during typical eccentric activities that results in greater tendon strain and stress. Interestingly, it has been suggested that increased aponeurosis stiffness results in slower sarcomere shortening for a given
fixed and contraction, which in turn may augment muscle force, and possibly contribute to the risk of muscle tissue injury (Lieber et al, 2000), indicating that the duration of cooling may be important for the ability of tendon tissue to avoid overuse injuries. In the present study, the effect of repeated plantar flexion contraction with cooling appeared to yield a tendon properties change. It remains unknown if the combined effect of cooling and repeated contractions related tendon properties serve to lower the stress on the tendon and thereby the injury risk.

V. Conclusion

The results suggested that the cooling on repeated muscle contractions resulted in an increase in the stiffness and Young's modulus of tendon structures. The improvement in muscle endurance with cooling was directly related to muscle and tendon.

References

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냉각이 반복된 근수축과 사람의 건 구조에 미치는 영향

채수동, 정병수¹, 아카라 호리²

일본체육대학교 대학원 운동과학과, ¹조선대학교 체육대학 체육학과, ²일본체육대학교 대학원 운동과학과

<국문초록>

연구목적: 이 연구는 피부표면 냉각이 생체내의 건구조 점탄성 특성에 미치는 영향을 비교하였다. 방법: 7명의 남성 실험 대상자는 촉지활용 운동을 10회×10set, 60초 간격으로 최대 수의적 수축을 6초간 각각 실시하였다. 각 측정 전후에 내측 비복근(MG)의 건과 건막의 신장은 초음파 검사법에 의하여 직접 측정되었다(건 장력과 건 신장의 관계로 평가 되었다). 건 횡단면적과 즉 환절 모멘트 압은 자기공명영상법(MRI)으로부터 얻어졌다. 건 장력은 관절 모멘트와 건 모멘트 압으로 계산되었다. 또한 스트레스는 횡단면적영역(CSA)을 힘으로 나눈으로써 얻어졌다. 스트레인은 건의 길이로 표준화된 치환으로부터 측정되었다. 결과: 냉각 후에 건 장력은, 비냉각 보다 냉각한 측이 유의하게 높았다. 스트레네스는 비냉각 조건 보다 냉각 조건하에서 높은 유의수준을 나타내었다. 최대의 스트레인과 스트레스는 냉각조건에서 7.4±0.7와 36.4±1.8 MPa를 나타냈고, 비냉각 조건하가 7.8±8.5와 31.8±1.1 MPa (p<0.05)를 나타냈다. 결론: 이 연구의 결과는 피부표면 냉각으로 인해 인간의 근지구력이 건 스트레네스와 탄성률을 증가시키는 것을 시사하는 연구라 하겠다. 피부 표면 냉각으로 인한 근지구력의 개선이 근과 건에 직접적인 영향을 미친을 나타내주고 있다.

핵심단어 : 건, 냉각, 초음파 검사법, 근지구력