Effect of Static Stretching with Superficial Cooling on Muscle Stiffness

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Introduction
The application of thermal agents such as heat, ultrasound, and ice is popular treatment in rehabilitation and sports settings. Changes in tissue temperature due to thermal agents are believed to cause changes in metabolism, nerve transmission, hemodynamics, and mechanical properties [5, 6]. In particular, tissue cooling by the application of ice (i.e., cryotherapy) is expected to have an analgesic effect, inhibiting inflammation and tissue metabolism. Therefore, cooling by ice or other forms is the preferred method of injury management for muscle strain, damage, and bruising [5]. In addition, cooling has been reported to be useful for recovery after exercise [7, 29]. Thus, cooling is assumed to be one of the most effective treatments in rehabilitation and sports settings.

It is commonly believed that decreased flexibility (i.e., range of motion: ROM) is a harmful effect of cooling. In fact, previous studies investigating the effect of cooling on passive properties and muscle stiffness have reported that passive torque and muscle stiffness increased after cooling [4, 21, 25]. However, previous studies reported that ROM increased after cooling [18, 23]. Therefore, there is a discrepancy between the effect of cooling on ROM and muscle stiffness or passive torque. An increase in ROM may be caused not only by decreased passive torque and muscle stiffness but also changes in stretch tolerance because of changes in senso-

ABSTRACT
This study aimed to clarify the acute effect of static stretching (SS) with superficial cooling on dorsiflexion range of motion (DF ROM) and muscle stiffness. Sixteen healthy males participated in the cooling condition and a control condition in a random order. The DF ROM and the shear elastic modulus of medial gastrocnemius (MG) in the dominant leg were measured during passive dorsiflexion. All measurements were performed prior to (PRE) and immediately after 20 min of cooling or rested for 20 min (POST), followed by 2 min SS (POST SS). In cooling condition, DF ROM at POST and POST SS were significantly higher than that at PRE and DF ROM at POST SS was significantly higher than that at POST. In addition, the shear elastic modulus at POST was significantly higher than that at PRE and the shear elastic modulus at POST SS was significantly lower than those at PRE and POST. However, there were no significant differences in the percentage changes between PRE and POST SS between the cooling and control conditions. Our results showed that effects of SS with superficial cooling on increases in ROM and decrease in muscle stiffness were no more beneficial than those of SS alone.
ry mechanisms for pain and discomfort [20, 31]. Furthermore, the previous study reported the change in stretch tolerance after cooling because of the analgesic effect of cooling [24]. Therefore, it is assumed that the increase in ROM after cooling could be involved in the change in stretch tolerance and not in the decrease in muscle stiffness.

In addition, previous studies have investigated the effect of stretching with superficial cooling, hypothesizing that the analgesic effect of cooling would increase the effect of stretching more than stretching alone. The results showed that there were no significant differences in terms of ROM gains between stretching with superficial cooling and stretching alone [13, 28], whereas greater ROM gains in stretching with superficial cooling were shown than those in stretching alone [8, 15]. Thus, the effectiveness of stretching with superficial cooling relative to stretching alone has not yet been established. However, to the best of our knowledge, there have been no studies showing harmful effects of stretching with superficial cooling on ROM.

Meanwhile, as described above, passive torque and muscle stiffness increased after cooling [4, 21, 25]. Increased muscle stiffness and passive torque showed higher mechanical stress during passive stretching and reduced capacity of the muscle to withstand active or passive stretching [25]. Thus, increased muscle stiffness may increase the risk of muscle strain and damage [30]. Considering both of these points, it seems reasonable to assume that stretching with superficial cooling may not be effective in reducing muscle stiffness, despite there being no studies that have investigated this. Moreover, previous studies have reported that an increase in ROM may be caused not only by decreased passive torque and muscle stiffness but also changes in stretch tolerance [20, 31]. Therefore, it is necessary to investigate the effect of static stretching (SS) with superficial cooling not only on ROM but also on muscle stiffness and stretch tolerance. However, whether changes in muscle stiffness and stretch tolerance after SS with superficial cooling lead to ROM gains remains unclear. Therefore, this study aimed to clarify the acute effect of SS with superficial cooling on ROM, stretch tolerance, and muscle stiffness. The hypothesis of this study was that changes in stretch tolerance caused by superficial cooling would lead to increased stretching intensity, resulting in large ROM gains and decreased muscle stiffness.

Material and Methods

Subjects

Sixteen healthy males volunteered to participate in the study (mean ± SD: aged 20.9 ± 0.3 years; height, 171.5 ± 5.6 cm; weight, 66.2 ± 5.3 kg). We excluded subjects with a history of neuromuscular disease and/or musculoskeletal injury involving the lower extremities. All subjects were fully informed of the procedures and purpose of the study, and all provided written informed consent. The study was approved by the Ethics Committee of Niigata University of Health and Welfare, Niigata, Japan (17677). This study was also performed in accordance with the ethical standards of the International Journal of Sports Medicine [11].

Experimental protocol

A randomized repeated-measures experimental design was used to investigate the acute effects of SS with superficial cooling on DF ROM, passive torque at DF ROM, and muscle stiffness of medial gastrocnemius (MG) in the dominant leg (the preferred leg for kicking a ball). All subjects participated in the cooling condition and a control condition in a random order for at least 2 days, with no longer than a 1-week interval between both conditions. As shown in Fig. 1, in the cooling condition, all measurements were performed prior to (PRE) and immediately after 20 min of cooling (POST), followed by 2 min SS (POST SS). In the control condition, subjects rested in the same position as the cooling condition for 20 min (POST) after the PRE measurement; SS intervention was then performed for 2 min (POST SS). The subjects were familiarized with the procedure and instructed to remain relaxed throughout the measurement period.

Assessment of dorsiflexion range of motion and passive torque

The subjects were instructed to lie in the prone position on a Biodex System 4.0 (Biodex Medical Systems Inc., Shirley, NY, USA) with their hips securely held in place with an adjustable lap belt. The knee of the dominant leg was kept in full extension, and the foot of the same leg was securely attached to the dynamometer footplate with adjustable lap belts. The footplate of the dynamometer was manually moved by an examiner slowly to avoid stretch reflex starting from an ankle angle of 30° plantarflexion to the dorsiflexion angle just before the subjects started to feel discomfort or pain. The subjects were instructed to verbally inform the examiner when they started to feel discomfort or pain, and the angle just before this point was defined as dorsiflexion range of motion (DF ROM) [2, 3]. In addition, passive torque at DF ROM was defined using the index of sensory perception, and an increase in passive torque at DF ROM was defined as a change in stretch tolerance (sensory perception) for passive stretching [19, 20].

Assessment of shear elastic modulus

In this study, we adopted the shear elastic modulus measured by ultrasonic shear wave elastography (SWE) as an index of muscle stiffness. The shear elastic modulus of MG was measured using ultrasonic SWE (Apio 500, Toshiba Medical Systems, Tochigi, Japan) with a 5–14 MHz linear probe at 10° dorsiflexion, similar to the conditions during the DF ROM and passive torque measurements. Based on the method used in previous studies [1, 3], the shear elastic modulus of MG was measured at the proximal 30% of the lower leg length (from the popliteal crease to the lateral malleolus). An ultrasound transducer was positioned on the measurement points parallel to the direction of the muscle fibers, which were confirmed by tracing several fascicles without interruption across the B-mode image.
In the present study, the quadrangular region of interest (ROI) was set to cover the whole muscle. The obtained elastographic images were analyzed using image analysis software (MSI Analyzer version 5.0; Institute of Rehabilitation Science, Tokuyukai Medical Corporation, Japan). The quadrangular ROI was set to be as large as possible within the color-coded area of the elastographic images while accounting for the artifact from aponeurosis (▶Fig. 2). The average value of Young’s modulus in the quadrangular ROI was automatically calculated in this software. On the basis of previous studies [12, 27], the shear elastic modulus was calculated by dividing the obtained Young’s modulus by three. The elastographic image of MG was measured twice, and the average value of the shear elastic modulus was used for further analysis [2, 3, 22].

Cooling condition

The subjects received a bag containing ice cubes in the prone position with the knee extended, similar to the conditions during the DR ROM and shear elastic modulus measurements in the cooling condition. The ice bag was secured to the whole MG for 20 min, in accordance with previous studies [9, 15].

Static stretching

The SS intervention was performed using a dynamometer in the prone position with the knee extended, similar to the conditions during the DR ROM and shear elastic modulus measurements. The footplate of the dynamometer was manually moved by an examiner, starting from 30° plantarflexion to DF ROM, and the ankle joint was held at DF ROM for 2 min, which has been reported to decrease the shear elastic modulus [22].

Assessment of skin temperature

Skin temperature of MG in the cooling condition was measured at PRE, POST, and POST SS using an infrared thermometer (73010, Shinwa Rules Co., Ltd, Niigata, Japan). Skin temperature was 32.4°C ± 1.5°C at PRE, whereas skin temperature was 16.0°C ± 4.2°C at POST and 23.9°C ± 2.5°C at POST SS. Statistical analysis showed that the POST and POST SS skin temperatures were significantly lower than that of PRE, which confirmed that MG was cooled during the measurements of DF ROM, passive torque, and shear elastic modulus of MG.

Inter-day reliability of measurements of shear elastic modulus

To assess the inter-day reliability of the measurements of shear elastic modulus, these measurements were done twice on two different days by the same investigator with a 2-day to 1-week interval between the two sessions for ten participants (mean ± SD: age, 20.9 ± 0.3 years; height, 170.3 ± 4.7 cm; weight, 63.4 ± 2.6 kg).

A priori sample size calculation

We calculated the sample size needed for a repeated two-way analysis of variance (ANOVA) (alpha error = 0.05; power = 0.80; effect size = 0.4 [large]); the requisite number of participants for this study was 12 in each condition. We chose a large effect size on the basis of a previous study [2].

Statistical analysis

SPSS (version 24.0; SPSS Japan Inc., Tokyo, Japan) was used for statistical analyses. The inter-day reliability of the measurement of shear elastic modulus was assessed using the intraclass correlation coefficient (ICC [1, 1]). For all variables, a repeated two-way ANOVA using two factors [condition (cooling vs. control condition) and test time (PRE vs. POST vs. POST SS)] was used to analyze the interaction and main effect. When a significant interaction was observed, the Bonferroni multiple comparison test was used to determine the differences among the PRE, POST, and POST SS variables in both the cooling and control conditions. In addition, the paired t-test was used to compare the percentage change from PRE to POST SS between the cooling and control conditions to investigate the combinational effect of SS with superficial cooling. The percentage change was calculated using the shear elastic modulus in PRE and POST SS in each condition, using the following equation:

\[
\text{Percentage change} = \frac{\text{PRE value} - \text{POST SS value}}{\text{PRE value}} \times 100
\]

The statistical significance was set at an alpha level of 0.05.

Results

Inter-day reliability of measurement of shear elastic modulus

The ICC (1, 1) for the shear elastic modulus measurement was 0.919 (trial 1: 18.9 ± 11.4 kPa; trial 2: 19.4 ± 9.1 kPa).

Changes in DF ROM and passive torque at DF ROM

DF ROM and passive torque at DF ROM in both the cooling and control conditions are presented in ▶Table 1. The two-way ANOVA indicated a significant interaction (p < 0.01) and main effect for test time (p < 0.01) for DF ROM. In the cooling condition, a post hoc test revealed that DF ROM at POST and POST SS were significantly higher than that at PRE and that DF ROM at POST SS was significantly
higher than that at POST. In addition, in the control condition, the post hoc test revealed that DF ROM at POST SS was significantly higher than those at PRE and POST. Moreover, the two-way ANOVA indicated a significant interaction (p < 0.01) and main effects for condition and test time (condition: p = 0.012; test time: p < 0.01, respectively) for passive torque at DF ROM. In the cooling condition, the post hoc test revealed that DF ROM at POST and POST SS was significantly higher than that at PRE, whereas there was no significant difference between POST and POST SS. In addition, in the control condition, the post hoc test revealed that DF ROM at POST SS was significantly higher than those at PRE and POST.

Changes in shear elastic modulus of MG
The shear elastic modulus of MG in both the cooling and control conditions is presented in Table 1. The two-way ANOVA indicated a significant interaction (p = 0.014) and main effect for test time (p < 0.01).

In the cooling condition, the post hoc test revealed that the shear elastic modulus of MG at POST was significantly higher than that at PRE and the shear elastic modulus at POST SS was significantly lower than those at PRE and POST. In addition, in the control condition, the post hoc test revealed that the shear elastic modulus at POST SS was significantly lower than those at PRE and POST.

Comparison of the percentage changes between the cooling and control conditions
The percentage changes between PRE and POST SS are presented in Table 2. For all variables, there were no significant differences between the cooling and control conditions.

Discussion
In the present study, we investigated the effect of SS with superficial cooling, not only on ROM but also on muscle stiffness and stretch tolerance, and investigated the effectiveness of stretching with superficial cooling. There were three major findings. First, DF ROM, passive torque at DF ROM, and muscle stiffness increased after 20 min of superficial cooling. Second, DF ROM and passive torque at DF ROM significantly increased and muscle stiffness decreased after SS with superficial cooling. Third, there were no significant differences in the percentage changes between PRE and POST SS for all variables between the cooling and control conditions. To the best of our knowledge, this is the first report to investigate the effect of SS with superficial cooling, not only on ROM but also on muscle stiffness and stretch tolerance.

In the present study, DF ROM significantly increased after superficial cooling, which is consistent with the results of previous studies [18, 24]. The increase in ROM has been assumed to be involved in changes in stretch tolerance because of the analgesic effect of cooling [8, 24]. In the present study, passive torque at DF ROM, as an index of stretch tolerance, significantly increased after superficial cooling, which is consistent with the results of previous studies [8, 24]. In addition, muscle stiffness significantly increased after superficial cooling, which is also consistent with the results of a previous study [25]. Increased muscle stiffness and passive torque showed higher mechanical stress applied by passive stretching, and the capacity of the muscle to withstand active or passive stretching reduced [25]. Thus, increased muscle stiffness may increase the risk of muscle strain and damage [30]. Moreover, many previous studies have observed that muscle strength and performance declined after cooling [14, 26]. Considering our results and those of previous studies, it is assumed that increased muscle stiffness and decreased performance after cooling may lead to a high risk of injury. Therefore, athletes should not return to play immediately after cooling. However, the cooling duration used in the present study was 20 min, which may be longer than that commonly used in clinical and sports settings. Thus, future research is needed to investigate the effect of superficial cooling on muscle stiffness and performance with the cooling duration used in clinical and sports settings.

In the cooling condition, DF ROM increased and muscle stiffness significantly decreased immediately after SS with superficial cooling, whereas there was no significant change in passive torque at DF ROM. Meanwhile, DF ROM and passive torque at DF ROM increased and muscle stiffness significantly decreased immediately after SS in the control condition. These results indicate that the increase in DF ROM was contributed to by both the decrease in muscle stiffness and the change in stretch tolerance after SS intervention only. However, the increase in DF ROM in the cooling condition was contributed to not by the change in stretch tolerance but by

| Table 1 | Changes in DF ROM, passive torque at DF ROM, and shear elastic modulus of MG. |
|---|---|---|---|---|---|---|
| | Cooling condition | Control condition | Interaction effect |
| | PRE | POST | POST SS | PRE | POST | POST SS | p value | partial η² |
| DF ROM (degree) | 36.2 ± 4.8 | 40.4 ± 5.1 ** | 43.0 ± 6.2 *** ## | 37.0 ± 4.2 | 36.6 ± 3.9 | 41.8 ± 4.7 *** ## | p < 0.01 | 0.506 |
| Passive torque at DF ROM (Nm) | 24.5 ± 4.7 | 30.1 ± 7.4 *** | 32.1 ± 8.9 *** | 24.8 ± 4.3 | 23.4 ± 4.6 | 29.4 ± 7.7 *** ## | p < 0.01 | 0.409 |
| Shear elastic modulus of MG (kPa) | 15.9 ± 7.2 | 21.3 ± 8.3 *** | 10.1 ± 5.5 *** ## | 17.0 ± 9.5 | 18.0 ± 9.8 | 11.1 ± 5.6 *** ## | p = 0.014 | 0.247 |

注: *: P < 0.05, **: P < 0.01; significant difference between PRE and POST; #: P < 0.01; significant difference between POST and POST SS; SS = static stretching; ROM = range of motion; MG = medial gastrocnemius

| Table 2 | Comparison of the percent changes between cooling condition and control condition. |
|---|---|---|---|---|---|---|
| The percent change between PRE and POST SS (%) | Cooling condition | Control condition | P value |
| DF ROM | 18.9 ± 6.9 | 13.3 ± 10.0 | P = 0.077 |
| passive torque at DF ROM | 30.3 ± 21.8 | 18.4 ± 18.4 | P = 0.152 |
| shear elastic modulus of MG | -35.5 ± 17.5 | -29.1 ± 26.4 | P = 0.391 |

SS: static stretching, ROM: range of motion, MG: medial gastrocnemius
the decrease in muscle stiffness. In addition, there were no significant differences in the percentage changes between PRE and POST SS for all variables between the cooling and control conditions. Therefore, the results did not support our hypothesis that the magnitude of changes in DF ROM and muscle stiffness in the cooling condition would be larger than those in the control condition. However, the results were consistent with those of previous studies in that there were no significant differences in ROM between stretching with superficial cooling and stretching alone [13, 28]. Thus, as described above, muscle stiffness increased immediately after cooling, whereas there was no significant percentage change in muscle stiffness between the cooling and control conditions. It was assumed that these results are related to the analgesic effect of cooling. A previous study reported that the higher the applied stretching intensity, the greater the decrease in muscle stiffness [10]. In the present study, because stretch tolerance, shown by the significant increase in the passive torque at DF ROM, changed after cooling (cooling condition: 30.1 ± 7.4 Nm vs. control condition: 23.4 ± 4.6 Nm, p < 0.01), SS intervention was higher in the cooling condition than in the control condition. Although muscle stiffness temporarily increased after cooling, there was a large decrease in muscle stiffness following SS intervention by higher stretching intensity. Therefore, it was assumed that the magnitude of the decrease in muscle stiffness in the cooling condition was similar to that in the control condition.

A previous study reported that subjects with tight hamstrings show lower stretch tolerance compared to those with normal hamstrings [16]. In addition, a study has reported that stretch tolerance decreases in muscles that suffer from eccentric exercise-induced damage [17]. Our results showed that SS intervention with higher stretching intensity could be performed because cooling changed the stretch tolerance, whereas there was an adverse effect of increased muscle stiffness. Therefore, it could be expected that ROM and muscle stiffness in subjects with tight hamstrings, and subjects who suffer from delayed muscle pain, could be more effectively improved by stretching with superficial cooling than by SS intervention alone. Therefore, it is necessary to investigate the effect of SS with superficial cooling in subjects with decreased stretch tolerance due to tight hamstrings or muscle damage.

In the present study, we measured skin and not muscle temperature, which is a limitation. Future studies are needed to investigate the relationship between changes in muscle temperature and muscle stiffness after SS with superficial cooling.

Conclusion
In this study, we investigated the acute effect of SS with superficial cooling on ROM, stretch tolerance, and muscle stiffness in the present study. Our results showed that muscle stiffness decreased immediately after SS with superficial cooling, whereas there was no significant difference in terms of the magnitude of the decrease in muscle stiffness between the cooling and control conditions. Therefore, we concluded that the effects of SS with superficial cooling on increases in ROM and decreases in muscle stiffness were no more beneficial than those of SS alone.

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Conflict of Interest
The authors declare that they have no conflict of interest.

References


