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Cryotherapy induces an increase in muscle stiffness

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Abstract

Although cold application (i.e., cryotherapy) may be useful to treat sports injuries and to prevent muscle damage, it is unclear whether it has adverse effects on muscle mechanical properties. This study aimed to determine the effect of air-pulsed cryotherapy on muscle stiffness estimated using ultrasound shear wave elastography. Myoelectrical activity, ankle passive torque, shear modulus (an index of stiffness) and muscle temperature of the *gastrocnemius medialis* were measured before, during an air-pulsed cryotherapy (-30°) treatment of 4 sets of 4 minutes with 1 min recovery in between, and during a 40-min post-cryotherapy period. Muscle temperature significantly decreased after the second set of treatment (10 min: 32.3 ± 2.5°C; $P < 0.001$), peaked at 29 min (27.9 ± 2.2°C; $P < 0.001$) and remained below baseline values at 60 minutes (29.5 ± 2.0°C; $P < 0.001$). Shear modulus increased by +11.5 ± 11.8% after the second set (10 min; $P = 0.011$), peaked at 30 min (+34.7 ± 42.6%; $P < 0.001$) and remained elevated until the end of the post-treatment period (+25.4 ± 17.1%; $P < 0.001$). These findings provide evidence that cryotherapy induces an increase in muscle stiffness. This acute change in muscle mechanical properties may lower the amount of stretch that the muscle tissue is able to sustain without subsequent injury. This should be considered when using cryotherapy in athletic practice.

Keywords: Muscle temperature, supersonic shear imaging, shear modulus, elastography, ultrasound, cooling

1. Introduction

Applying cold on soft tissues (i.e., cryotherapy) is popular in athletic practice for various reasons. In particular, cryotherapy is used to limit the rise in endogenous temperature during exercise in the heat (Ross *et al.*, 2013) and to treat acute sports injuries (strain,

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contusion, and muscle damage) (Bleakley *et al.*, 2004; Guilhem *et al.*, 2013). This practice may occur before, during and between games, pitch-side, or at half-time (Bleakley *et al.*, 2012; Pritchard and Saliba, 2014). Therefore, there is a growing trend where athletes go to or return to competitive activity immediately after the application of a cold treatment (Bleakley *et al.*, 2012; Pritchard and Saliba, 2014).

In this scenario, the benefit of cryotherapy on pain (Algafly and George, 2007), inflammation (Nadler *et al.*, 2004) and cell metabolism (Merrick *et al.*, 1999) must outweigh any short-term adverse effects on proprioception (Costello & Donnelly, 2010) and neuromuscular performance (Racinais & Oksa, 2010). In addition, several studies revealed a significant increase in passive joint torque with a larger maximal range of motion immediately after ice pack application or cold-water immersion (Price & Lehmann, 1990; Minton, 1993; Muraoka *et al.*, 2007). These results suggest that a higher mechanical stress is applied on a muscle-tendon unit whereas the capacity of the muscle to withstand active or passive stretch is reduced (i.e., increase in stiffness). It therefore seems reasonable to assume that the risk of muscle injury might increase for the same amount of fiber strain after cold treatment (Witvrouw *et al.*, 2003; McHugh and Cosgrave, 2010). This assumption was confirmed *ex vivo* (Noonan *et al.*, 1993; Scott *et al.*, 2016). However, it is important to note that the passive joint torque used to calculate passive stiffness *in vivo* results from a composite of various muscular (i.e., agonists and antagonists) and non-muscular structures, such as tendons, skin and articular structures (Riemann *et al.*, 2001; Herbert *et al.*, 2011). Therefore, this global measure does not provide a direct estimation of muscle stiffness. Although an *ex v*ivo study reported an increase in localized muscle stiffness (i.e., shear modulus) after cold application (Sapin-de Brosses *et al.*, 2010), this study was performed on bovine muscle samples over a range of non-physiological temperatures. Consequently, the temperature-dependent change in muscle stiffness needs to be confirmed in humans.

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Elastography techniques were recently used to quantify the elasticity of biological tissues by measuring the velocity of shear wave propagation by imaging techniques (Gennisson *et al.*, 2013; Brandenburg *et al.*, 2014). Supersonic shear imaging (SSI) is an ultrasound shear wave elastography technique that quantifies the shear modulus of a localized area of tissue (Bercoff *et al.*, 2004). This method provides an accurate measurement of muscle stiffness (Eby *et al.*, 2013), and a unique opportunity to quantify the effect of cooling on muscle stiffness. The purpose of the present study was to quantify the effect of cold air application on *gastrocnemius medialis* shear modulus estimated using SSI. In order to draw a direct relationship between muscle temperature and shear modulus, muscle temperature was measured throughout using a muscle probe placed next to the region of stiffness measurements.

2. Methods

2.1. Participants

Ten healthy volunteers [7 males and 3 females; age: 26.1 ± 2.8 years; height: 177.4 ± 6.1 cm; body mass: 73.5 ± 10.0 kg] participated in the study. Participants were informed regarding the nature, aims, and risks associated with the experimental procedures before they gave their written consent. All procedures were performed in accordance with the ethical standards of the national research committee (CPP Île de France X) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

2.2. Instrumentation

Muscle temperature. The temperature of the right *gastrocnemius medialis* was recorded manually with a Medical Precision Thermometer (DM852, Ellab A/S, Hvidovre, Danemark), with an accuracy of 0.1°C. A flexible temperature probe (MAC flexible probe, Ellab,

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Denmark) was inserted by a medical doctor (A.F.) 2 cm subcutaneous into the muscle through an indwelling flexible cannula (venflon 18GA Becton Dickinson, Sweden) (Costello *et al.*, 2012). The middle of the ultrasound probe location (previously determined) corresponded to the insertion point of the cannula (Fig. 1)

Passive torque. An isokinetic dynamometer (Con-Trex MJ, CMV AG, Switzerland) was used to measure ankle passive torque. Briefly, subjects were lying prone on the dynamometer and their right foot was attached securely to the footplate of the dynamometer. Then, the lateral and medial *malleolus* were aligned with the axis of the Con-Trex dynamometer. The knee was fully extended and the ankle was placed at 0° (foot perpendicular to the leg). Participants were instructed to stay relaxed during the overall protocol. Ankle joint angle and passive torque were digitized by a 12-bit analog to digital converter at 1000 Hz (DT9804, Data Translation, Marlboro, USA).

Shear modulus. An Aixplorer ultrasound scanner (version 6.0; Supersonic Imagine, Aix-en-Provence, France) coupled with a linear transducer array (4–15 MHz, Super Linear 15-4, Vermon, Tours, France) was used in SSI mode (musculo-skeletal preset) to measure *gastrocnemius medialis* shear modulus. The method used to obtain the shear wave speed (*V*s) is described in detail elsewhere (Bercoff *et al.*, 2004). Assuming a linear elastic behavior (Bercoff *et al.*, 2004; Catheline *et al.*, 2004), the muscle shear modulus (μ) was calculated as follows:

$$\mu = \rho V_s^2 \qquad (1)$$

where ρ is the density of muscle (1000 $kg.m^{-3}$). The shear modulus was calculated with the group velocity of the shear wave, assuming that the viscosity is negligible. This assumption is based on previous studies (Catheline *et al.,* 2004; Deffieux *et al.,* 2009) that showed no influence of the frequency on the shear wave velocity measured along the muscle fiber direction, indicating no significant viscous effects on the measurements performed using SSI.

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Maps of the shear modulus were obtained at 1 Hz with a spatial resolution of 1 × 1 mm. B-mode images were used to determine the *gastrocnemius medialis* fiber orientation. When several fascicles could be observed without interruption across the image (Blazevich, 2006), the probe location was traced on the skin and was kept the same for all the measurements within each participant (Fig. 1a). For each shear modulus measurement, the probe was positioned in the same plane as the fascicle direction. In that plane, it was shown that the effect of the probe direction in respect to the fascicle is small (Miyamoto *et al.,* 2015). Elastography measurements were performed at an ankle angle of 0°. This ankle angle was chosen such that the *gastrocnemius medialis* was moderately stretched while not being greatly affected by stretch relaxation (Freitas *et al.*, 2015). Participants were instructed to remain as relaxed as possible during each recording.

Electromyography. Cooling may induce an increase in the excitability of the motoneuron pool (Oksa *et al.*, 2000; Palmieri-Smith *et al.*, 2007). Therefore, *gastrocnemius medialis* myoelectrical activity was monitored during the experiment to ensure the absence of activation that might increase muscle stiffness. Two conductive adhesive hydrogel surface electromyography (EMG) electrodes (Blue sensor Q-00-S, A/S, Oelstykke, Denmark; 1 cm inter-electrode distance) were placed over the *gastrocnemius medialis*. Due to positioning of the ultrasound and temperature probes, the electrodes were placed at the most proximal site of the muscle mid-belly (Fig. 1a). Before applying the electrodes, skin was shaved and cleaned with alcohol to minimize impedance. Raw EMG signals were pre-amplified (Mazet Electronique Model, Electronique du Mazet, Mazet Saint-Voy, France; input impedance: 10 GΩ; common mode-rejection ratio: 100 dB; gain: 600; bandwidth: 6–500 Hz) and sampled at 1000 Hz through the same digital converter that was used for mechanical data.

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2.3. Protocol

Skin was prepared for EMG and for insertion of the cannula. Participants were then laid prone with the knee fully extended and firmly strapped to the ergometer to maintain the same body position throughout the protocol. A rest period of a total 20 minutes allowed participants to acclimatise to room temperature (22°C). Immediately after, ankle passive torque, myoelectrical activity, shear modulus and temperature of the right *gastrocnemius medialis* muscle were measured during 1-minute (Baseline; Fig. 1b). Then, the right *gastrocnemius medialis* was cooled down during 4 sets of 4 minutes with 1-minute recovery in between (Cryotherapy; Fig. 1b). All the parameters mentioned above were recorded during each recovery period. The time-course of each parameter was assessed for 10 s every minute during a 40-min period after cryotherapy (Post cryotherapy; Fig. 1b).

Cold application. Participants underwent 4 sets of cold air-pulsed (-30°C) applications of 4 minutes duration each, generated by a Cryo 6 skin cooling system (Zimmer Medizin Systems, Neu-Ulm, Germany). Cold-pulsed air was applied to the right *gastrocnemius medialis* at the maximal available airflow power (intensity = 9). The scanning consisted of vertical and horizontal motions, with a 5-cm distance kept between the tube nozzle and the skin (Guilhem *et al.*, 2013). One minute without cryotherapy was respected between each application to avoid any burns due to extreme cold.

2.4. Data processing

Data analysis was performed with custom-written scripts designed using MATLAB 10.0 (The Mathworks, Natick, MA, USA). SSI recordings were exported from software (Version 6.0, Supersonic Imagine, Aix en Provence, France) in “mp4” format and sequenced in “jpeg” with custom-written scripts. Image processing converted the colored map into shear elastic

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modulus values. For each image (1 Hz), the average value of shear elastic modulus was calculated over a region of interest (ROI) corresponding to the largest muscular region avoiding fascia.

EMG signals were filtered using a zero lag 5th order Butterworth band-pass filter (20-450 Hz) and then analysed with a 1-s root mean square (RMS) moving window to produce an EMG RMS envelope. Due to the pain induced by the transcutaneous probe temperature during voluntary contractions, EMG data was not normalized to EMG RMS obtained during maximal voluntary contraction. Therefore, EMG RMS was normalized to the average EMG RMS value obtained before treatment (baseline). Due to a technical problem during data export, EMG and torque data for two participants were lost.

All variables were averaged over 1 minute before and during the cryotherapy (0, 5, 10, 15, 20 min; Fig. 1b). For the 40 minutes follow-up after the cryotherapy, each parameter was measured over 10 seconds every minute (Fig. 1b).

2.5. Statistical analysis

Analyses were performed with Statistica (v 7.1, StatSoft, Tulsa, OK, USA). As Kolmogorov-Smirnov testing revealed a non-normal distribution for muscle temperature and shear modulus data, these data were log-transformed. Analyses of variance (ANOVA) with repeated measurements (time as within-subject factor) were used to test the effects of treatment on muscle temperature, shear modulus, passive torque and EMG RMS. When required, *post hoc* analyses were performed using the Newman–Keuls test. The effect size was also calculated using Cohen's *d* (Cohen, 1988) considering 0.2, 0.5 and 0.8 as a small, medium and large effect, respectively. The significance level was set at $P < 0.05$.

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3. Results

A significant effect of time was found on muscle temperature ($P < 0.001$). More precisely, muscle temperature was significantly decreased after the second set of treatment (10 min: 32.3 ± 2.5°C; $P < 0.001$; $d = 1.33$). This decreased temperature peaked at 29 min (i.e., 9 minutes after the end of the cryotherapy treatment; 27.9 ± 2.2°C; $P < 0.001$; $d = 3.92$) and persisted at 60 minutes (29.5 ± 2.0°C; $P < 0.001$; $d = 3.33$) (Fig. 2a). This decreased temperature did not affect EMG amplitude of the *gastrocnemius medialis* (main effect of Time: $P = 0.871$).

Figure 2b depicts the time-course of *gastrocnemius medialis* shear modulus before, during and after cold treatment. A significant effect of time was found on muscle shear modulus ($P < 0.001$). *Gastrocnemius medialis* shear modulus increased by +11.5 ± 11.8% immediately after the second set of treatment (10 min; $P = 0.011$; $d = 0.65$). This increase peaked at 30 min (i.e., 10 minutes after the end of the cryotherapy treatment; +34.7 ± 42.6%; $P < 0.001$; $d = 0.75$) and persisted until the end of the post-treatment period (+25.4 ± 17.1%; $P < 0.001$; $d = 0.69$). No significant change of passive torque was found (main effect of time: $P = 0.878$).

4. Discussion

We aimed to determine the effect of cold air application on *gastrocnemius medialis* muscle shear modulus. We found a concomitant decrease in muscle temperature and increase in shear modulus throughout and after the 20 minutes of air-pulsed cryotherapy. This acute change in muscle mechanical properties after cryotherapy may lower the amount of stretch that the muscle tissue is able to sustain without subsequent injury. This finding should be considered when using cryotherapy in athletic practice, especially just before returning to activities that expose muscle tissue to exercise-induced damage.

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The localized cryotherapy treatment used in the present study was effective in decreasing muscle temperature (-6.1 ± 1.8°C at the end of the treatment). This is in line with previous studies showing a decrease in muscle temperature (measured ~2 cm subcutaneous) between -2 and - 8°C immediately after cryotherapy (Jutte *et al.*, 2001; Dykstra *et al.*, 2009; Costello *et al.*, 2012; Rupp *et al.*, 2012). The variability in muscle temperature decrease after cryotherapy in the literature is mainly explained by the heterogeneity of the duration of the treatment [*e.g.*, between 3 min (Costello *et al.*, 2012) and 40 min (Rupp *et al.*, 2012)] and the cryotherapy method used (*e.g.*, cubed ice, crushed-ice, cold-water immersion, and whole body cryotherapy). The present study showed that the decrease in muscle temperature peaked 9 minutes after the end of cold treatment (-7.1 ± 1.0°C), and remained below baseline values 40 minutes post-cryotherapy (-5.5 ± 1.6°C). A similar time-course of muscle temperature was observed after localized cryotherapy was applied to lower limb muscles (Jutte *et al.*, 2001; Dykstra *et al.*, 2009).

A significant increase in passive stiffness measured using global methods has been reported immediately after cryotherapy (Price & Lehmann, 1990; Minton, 1993; Muraoka *et al.*, 2007). For instance, Muraoka et al. (2007), found that the passive stiffness of plantar flexor muscles increased by + 11% after 60 minutes of cold water immersion at 5–8°C. In the present study, there was no significant effect of cooling on passive torque, as previously shown by others (Kubo *et al.*, 2005). The discrepancy between our results and those obtained by Muraoka et al. (2007) might be explained by: 1) difference in protocol duration (60 min in Muraoka et al. (2007) *vs.* 20 min in our study) and 2) difference in the cryotherapy technique used (5-8°C water immersion in Muraoka et al. (2007) *vs.* localized cold air-pulsed cryotherapy in our study). Although cold air-pulsed cryotherapy affects mainly skin and underlying tissues properties, cold water immersion is likely to alter the properties of various structures crossing

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the joint (all agonists and antagonists muscles, tendons, articular structures, and skin) (Bleakley & Costello, 2013). As passive torque results from a combination of these muscular and non-muscular structures (Riemann *et al.*, 2001; Herbert *et al.*, 2011), it is likely that cold water immersion largely affects this parameter.

In the present study, air-pulsed cryotherapy induced an increase in local *gastrocnemius medialis* muscle stiffness (i.e., shear modulus) while passive ankle torque was unchanged. Muscle cooling induced an increase in shear modulus (+26.7 ± 26.1%) for all subjects at the end of cold treatment, which remained throughout 40 min post-cryotherapy (Fig. 2b). Some hypotheses can be proposed to explain this finding. Cooling may induce an increased excitability of the motoneuron pool (Oksa *et al.*, 2000; Palmieri-Smith *et al.*, 2007). However, it was not observed in our experiment as suggested by the absence of change in myoelectrical activity throughout the protocol. We therefore believe that the increase in shear modulus did not originate from muscle activation. Instead, the increased shear modulus is likely explained by global viscoelastic changes induced by temperature. This could be located in muscle proteins as demonstrated by *in vitro* studies (Chen & Humphrey, 1998; Leikina *et al.*, 2002; Tornberg, 2005). For example, Tornberg (2005) showed that an increase in muscle temperature from 25 to 37°C induces myosin and collagen unfolding (i.e., protein denaturation). Therefore, the increased resting shear modulus reported in the present study can be likely attributed to changes in both active (stable actin-myosin bridges) and passive elastic components within the extracellular matrix. A viscoelastic model may provide a deeper understanding of the relationship between muscle temperature and mechanical properties (Green *et al.,* 2012).

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Interestingly, a lower passive muscle deformation before rupture has been found in cold muscle compared to warm muscle (Noonan *et al.*, 1993; Scott *et al.*, 2016), especially for muscle temperature below 32°C (Scott *et al.*, 2016). This was combined with a higher stiffness (Scott *et al.*, 2016). The current study confirms an increase in muscle stiffness due to cold application in human. An increase in muscle shear modulus should theoretically lead to a decrease in energy-absorption capacity. Therefore, it would limit the capacity of the muscle-tendon unit to sustain external strain, leaving muscle fibers more prone to damage (Hicks *et al*., 2013; Morales-Artacho *et al*., 2016). Moreover, the fact that maximal range of motion (Witvrouw *et al.,* 2003) and muscle-tendon stiffness (Watsford *et al.,* 2010) are related to muscle strain injury incidence highlights the putative involvement of muscle shear modulus in the occurrence of such injury. Consequently, for an immediate or 40 minutes after cryotherapy return to play, cryotherapy should either be avoided in the muscles (i.e., bi-articular muscles) and locations (i.e., myotendinous junctions) more susceptible to strain injury, or immediately followed by a progressive and specific warm-up of the cooled muscles.

This experiment requires consideration of several methodological aspects. First, the measurement of shear modulus is sensitive to the angle between the muscle fascicles and the ultrasound transducer, and therefore to the pennation angle (Gennisson *et al*., 2010; Eby *et al*., 2013; Koo *et al*., 2015). Thus, we conducted an additional analysis to assess the changes in pennation angle at 29 min (i.e., time at the peak decrease in muscle temperature). This analysis showed no significant ($P = 0.51$) change in pennation angle compared to PRE (-0.2 ± 0.8°C; range: -1.4 to +1.0°C). Therefore, the orientation of muscle fascicles did not influence the changes in shear modulus observed after cryotherapy. Second, the impact of changes in muscle temperature on muscle stiffness cannot be accurately related. The insertion of the temperature probe into the muscle *via* the cannula was not monitored by ultrasound. Thus, the

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muscle area and depth where the temperature and stiffness were measured was not perfectly matched. To circumvent this limitation, the cannula was inserted close to the middle of the ultrasound probe (Fig. 1). Finally, muscle temperature did not return to baseline values after 40 minutes post-cryotherapy (29.5 ± 2.0°C). In the absence of a washout condition, we were not able to determine whether the observed temperature-dependent change in muscle stiffness was reversible. Irreversibility of change in stiffness has been observed *ex vivo* during heating at non-physiological temperatures (> 49°C) (Sapin-de Brosses *et al.*, 2010), this finding has yet to be demonstrated *in vivo.* The hysteresis effect observed during a cooling-heating cycle may explain the slight change in muscle shear modulus during the post cryotherapy period, while the muscle temperature increased more rapidly (*cf.* fig. 2). Future research is required to better understand the relationship between muscle stiffness and temperature during heating-cooling and cooling-heating cycles over physiological ranges (~25-40°C).

5. Perspectives

Cryotherapy is often used before, during and between games, pitch-side, or at half-time, either to limit the rise in endogenous temperature during exercise in the heat (Ross *et al.*, 2013) or to treat acute sports injuries (strain, contusion, and muscle damage) (Bleakley *et al.*, 2004; Guilhem *et al.*, 2013). The current study showed that twenty minutes of air-pulsed cryotherapy induces an immediate decrease in muscle temperature associated with an increase in muscle stiffness. Both muscle stiffness and temperature were above baseline values 40 minutes after the cryotherapy. Consequently, for an immediate or 40 minutes after cryotherapy return to play, cryotherapy should either be avoided in the muscles and locations more susceptible to strain injury, or immediately followed by a progressive warm-up of the cooled muscles. It is important to note that a cold and wet environment can decrease muscle temperature between 20 and 30°C (Pugh, 1967). In that case, a longer and specific warm-up

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before starting or resuming activity (e.g., after half-time) may prevent the increase in muscle stiffness.

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Figure legends

Figure 1. Experimental design. Participants were lying prone with their right foot attached to the footplate of the isokinetic dynamometer (a). An ultrasound probe was placed over the right *gastrocnemius medialis*. A muscle temperature probe was located above the middle of the ultrasound probe. EMG electrodes were placed over a proximal portion of the muscle mid-belly. Muscle EMG activity, ankle passive torque, shear modulus and muscle temperature were measured over 1-minute (baseline) (b). Then, the *gastrocnemius medialis* was cooled during 4 sets of 4 minutes with a 1-minute recovery in between (cryotherapy). All the parameters were recorded during each recovery period. Finally, the time-course of each

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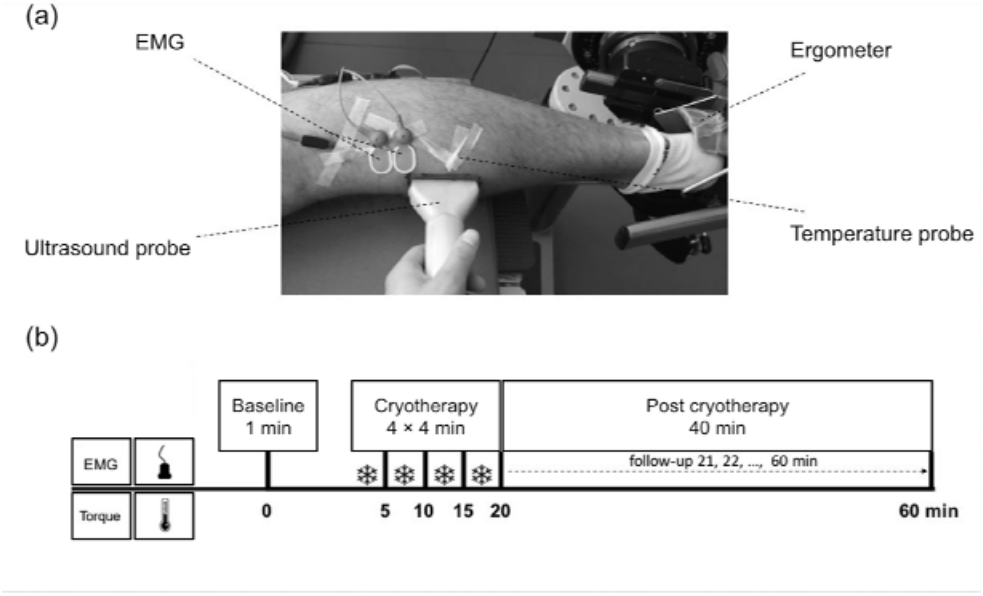
parameter was assessed for 10 s every minute during a 40-min post-treatment period (post cryotherapy)

Figure 2. Time-course of muscle temperature (a) and shear modulus (b). Muscle temperature (°C) and shear modulus (kPa) changes over time. Values are presented as mean ± SD. *P < 0.05: a significant difference when compared with the baseline value (0 min).



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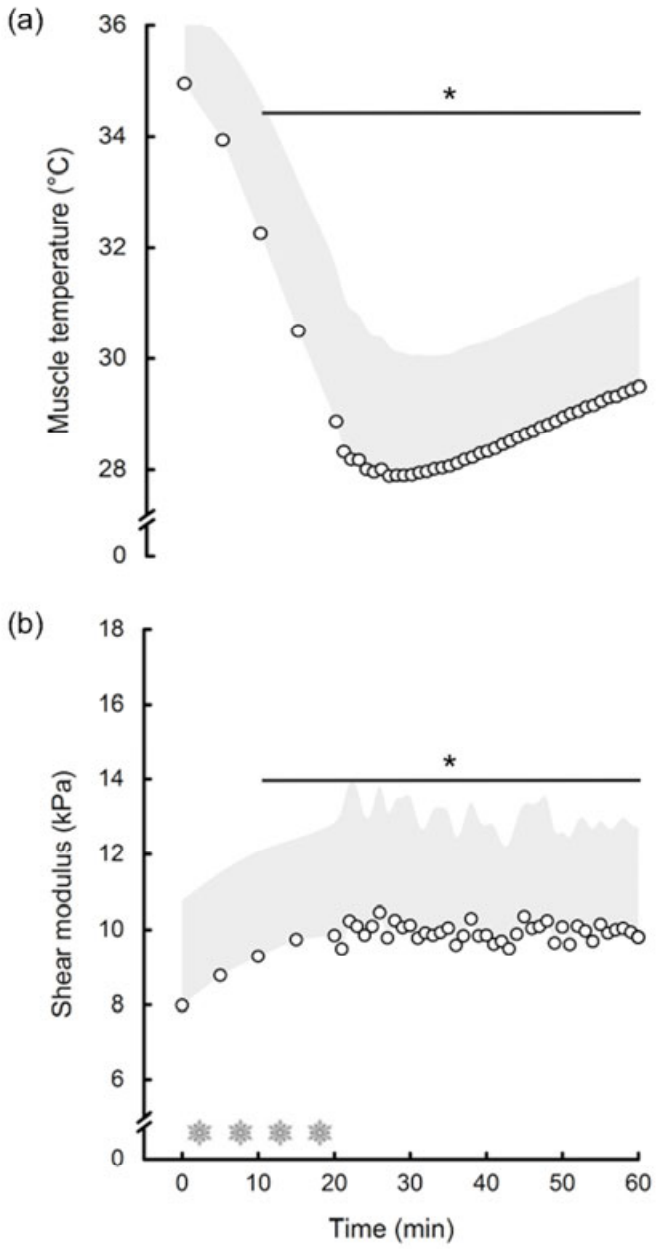

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