The acute effect of cold pack applied for different periods on the biomechanical properties of the rectus femoris muscle of healthy individuals: a randomized experimental study

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Abstract

Background

Cold packs are silica gel packs that are the most commonly used modalities in sports clinics. In the present study, we investigated the acute effect of different periods of cold pack application, on the biomechanical properties of the rectus femoris muscle of healthy individuals.

Methods

This study is a randomized experimental study. Sixty individuals (18–23 age) were randomly divided into four groups. The cold packs were applied for 10 minutes (Group 1), 12 minutes (Group 2), 15 minutes (Group 3), and 20 minutes (Group 4). The temperature of the skin (thermal camera) and the biomechanical properties evaluations of the muscle (Myoton Pro) were measured (before and immediately after, 5, 10, 15, 20 and 30 minutes after the application).

Results

After the application, while Group 1 and 2 showed an increase in muscle tone and stiffness and a decrease in elasticity (p < 0.05), they began to approach their pre-application state by the fifth and fifteenth minutes, respectively (p > 0.05). In Group 3 and 4, the muscle stiffness was increased at all time points (p < 0.05).

Conclusions

The results of this study show that the rectus femoris muscle of healthy people becomes stiffer and less elastic as a result of cooling with a cold pack applied for different time periods. The duration that affects the biomechanical properties of the muscle least and provides optimum cooling is the 10-minute cold pack application.

Trial registration:

Ethical approval The Research Ethics Committee of Acibadem University and Acibadem Healthcare Group has approved the study (reference no. ATADEK-2019/17/19) and retrospectively registered clinical trial number (NCT04277481, February 20, 2020). All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki Declaration. Verbal and written consent was obtained from all participants.

Background
Cryotherapy is used in various clinical and sports environments to reduce edema, nerve conduction velocity, and tissue metabolism, as well as to facilitate recovery of exercise-related muscle damage [1]. Various cryotherapy modalities such as whole body cryotherapy, cold spray, cryotherapy clamps, frozen peas, cold-water immersion, ice, and cold packs are used in sports clinics [2]. Cold packs are silica gel packs that are the most commonly used modalities in sports clinics. Special silicate gel impregnated with water in a soft rubber envelope is stored in coolers whose temperature is maintained between -12.2 °C and 9.4 °C [3]. Cold packs have different sizes and shapes. Since they do not lose their softness when cooled, they adjust well to the contours of the body. By placing a towel between the skin and the package, a homogeneous cooling is approached and hygiene is provided, and it becomes easier to tolerate the feeling of extreme cold felt in the first contact with the cold pack. A cold pack can be applied for a relatively long time without losing its coldness due to its low conductivity. A significant increase in package temperature was observed from the 15th minute of application. It is stated that it decreases the skin temperature by 7 to 10 °C and keeps it at 20 °C–30 °C for 3 hours depending on the room temperature. They can be used repeatedly by cooling [4].

After cold application, there is a rapid increase in superficial tissue temperature initially, while a slow recovery is observed in deep tissue with a gradual decrease in temperature. In addition, studies have shown that local cold application causes an increase in resistance against movement [5, 7]. This can make it difficult to do necessary activities after cold application because the cold can cause the agonist muscle to expend more power to reach the desired performance and endurance and may cause an increase in the frequency of injury due to the deterioration of muscle flexibility [8]. According to in vitro animal studies, muscle tissue shows high resistance to stretching after it has cooled. In human studies, there is only one study evaluating the direct mechanical responses of the muscles [5,9-11]. In this study carried out by Mustalampi et al.[12], parameters were measured indicating the biomechanical properties of the muscle immediately after the application of cold pack to the quadriceps muscle for 20 minutes and 15 minutes after. According to the results of the study, the muscle became stiffer and less elastic in terms of biomechanical properties. After 15 minutes, these changes had not completely recovered. Furthermore, they did not measure these parameters again more than 15 minutes after the removal of the cold pack to see if there was additional recovery.

Twelve to fifteen minutes of intense cold application is sufficient to reduce pain and muscle spasm [13]. It has also been shown that applying cold for more than thirty minutes can lead to frost bite and nerve problems [14,15]. However, in practical applications, sufficient cold application time for pain, muscle spasm, and edema varies between 15-30 minutes [13, 15-17]. In a review made by Auley [18] regarding cryotherapy treatments, it was shown that ice application was used for 5 to 85 minutes in many studies. They said that cold application does not have a specified frequency and duration, but repetitive 10 minutes of cold application is effective. It has also been noted that there is evidence of a slight decrease in temperature from 10 minutes to 20 minutes, while the temperature decreases the most in the first 10 minutes.
It is suggested that the decrease in muscle temperature in long-term applications (more than 15 minutes) results in a decrease in muscle tone, but in short-term applications, cooling of the skin probably increases the tone of the muscle by reflexing alpha motor neurons [19, 20]. Despite this information, there are limited studies in the literature regarding the effect of increasing or decreasing durations of cold on muscle tone and stiffness. For this reason, it is necessary to investigate the duration of cold pack application that reduces the skin temperature before increasing the relative muscle tone and stiffness according to the desired treatment method. In the light of this information, we aim to investigate the acute effect of cold pack therapy, which is used most in clinics, for different periods (10-12-15-20 minutes) in healthy individuals, on skin temperature and biomechanical properties of rectus femoris muscle.

Methods

Study design and participants

This study is a randomized experimental and parallel group study in accordance with the CONSORT statement 2010. Local ethics committee approval (2019/17/19) and clinical trial number (NCT04277481) of this study were obtained. 60 volunteer individuals (30 females, 30 males, aged 18–23 years) with subdermal fold thickness of the quadriceps muscle between 5 mm and 15 mm [21] were included in the study. The volunteers included in the study consisted of individuals who responded to the study advertisement posted via social media. Individuals who did not smoke or use any drugs and had no history of cardiovascular or peripheral vascular disease, diabetes mellitus, neuromuscular pathology, peripheral neuropathy, lower extremity pain, or previous lower extremity surgery were included in the present study. Individuals who had a history of insensitivity to local heat or cold were excluded from the study.

The GPower V.3.1.7 (Kiel University, Kiel, Germany) program was used to determine the appropriate sample size. In order to obtain the power ratio of the study as 80% at 95% confidence limit (with 0.05 error margin), the effect size was determined as 0.51 and the sample size was calculated. Using this method, the sample size was determined as 60. The participants were randomized by block randomization method and divided into four groups with the aid of Random Allocation SoftwareÒ computer program by the first author. Cold pack (35*29 cm) application, achieved by wrapping a towel on the rectus femoris part of the quadriceps, was performed in the following order:

i. 10 minutes for the first group (Group 1, n = 15),

ii. 12 minutes for the second group (Group 2, n = 15),

iii. 15 minutes for the third group (Group 3, n = 15),

iii. 20 minutes for the fourth group (Group 4, n = 15)

Towel cloth application was used to prevent the individual from developing a cold burn [22].
To help balance systemic blood flow, participants stopped exercising 12 hours before the start of the test and caffeine/alcohol consumption 1 hour before. All subjects were evaluated between 12:00 and 13:00. Individuals were asked to be in the room twenty minutes before evaluations. This time interval was chosen to adapt participants' skin surface temperature to the temperature of the room and to control circadian changes in body temperature [23]. Primary outcomes of temperature and biomechanical properties were measured before cold application, immediately after cold application, and 5 minutes, 10 minutes, 15 minutes, 20 minutes, and 30 minutes thereafter. Individuals who stated that they could not bear cold during the study were finished. The study was completed between February and March 2020.

**Determination of the dominant limb**

In determining the dominant extremities of the individuals, the foot they used when jumping forward and preferred to hit the ball rolling towards their front was taken into consideration [24].

**Measurement of the skinfold thickness**

Measurements, taken while the individual was lying on their back, were made starting from the mark placed in the middle of the distance between the anterior superior of the spina and the upper edge of the patella. SaehanÒ (Saehan Electronic, Gyeonggi-do, South Korea) skinfold caliper was used to measure the skinfold thickness. The measurement was made while the individual was in the standing position and body weight was transferred to the non-measured foot. The knee on the limb measured was slightly flexed (15-30 degrees) and the foot was in loose contact with the ground. For the measurement, the skin and subcutaneous adipose were removed from the muscle by the thumb and forefinger of the physiotherapist. The arms of the Skinfold instrument were placed on the skinfold, and the caliber was read in millimeters. The measurement was repeated 3 times; the average was taken and recorded in mm. When a difference greater than 2 mm was found between the measurements, a fourth measurement was performed [25].

**Measurement of skin temperature**

The skin temperature was measured with the help of a non-contact thermal camera. This technique is a safe and non-invasive method [26, 27]. Non-contact thermal camera technology is a complex way of receiving electromagnetic radiation and converting it into electrical signals. These signals are finally displayed in grayscale or in colors that represent temperature values. The use of non-contact thermal camera has recently become a popular method for evaluating skin temperature following cryotherapy in individuals such as cold air cryotherapy, cold-water immersion, or ice cube or cold pack applications [2].

For thermal imaging, a 120-9-90 pixel resolution FLIR E5 thermal cameraÒ (FLIR Systems AB, Sweden) was used, and Color Palette iron was chosen to display photographs. The video was taken at a speed of 8 photos/sec at a distance of 1 m. Skin temperature of the area to be treated (rectus femoris muscle) was measured with a P45 thermographic camera with high thermal sensitivity (Flir System, ThermaCAM, Sweden) while the individual was standing upright in an anatomical position. Using FLIR Quick-Report
1.2 software, skin temperature was determined from the heat data obtained from this region. In the calculation of skin temperature, the emissivity value of human skin was accepted as 0.98 [28].

**Evaluation of the passive mechanical properties of the muscle**

Muscle tone, stiffness and elasticity were measured with MyotonProÒ (MYO, Tallin, Estonia). MYO is a recently manufactured and reliable device that measures the passive mechanical properties of superficial muscles. MYO provides a unique, reliable, accurate, and precise method for objective and noninvasive digital palpation of superficial skeletal muscles. Moreover, it provides measurement not only of muscles, but also tendons, ligaments, skin, and other soft tissues. Therefore, it can be used as a daily diagnostic and monitoring device in medical practice for soft tissue evaluation. The MYO can measure subcutaneous tissue up to a 2 cm depth [29, 30]. MYO has a good test repeat reliability (ICC: 0.84-0.85) in measurement of the passive mechanical properties of the quadriceps rectus femoris fragment [31].

Measurements were carried out by the same physiotherapist while the individual was in a resting position, without active muscle contraction. While the individual was in a long sitting position and the knee was in semiflexion, a measurement was made from the middle region of the rectus femoris muscle (from the middle of the line extending between the anterior superior iliac spine and the upper edge of the patella) [32, 33]. MYO measurement was done at right angles to the muscle area in question. In addition, there was an angle no greater than 30 degrees between the probe and the vertical axis. MYO provides information on muscle tone [frequency of oscillation (Hz)], dynamic stiffness (N/m), and decrement (elasticity). The higher the decrement parameter, the less the elasticity [30, 34].

**Statistical analysis**

Statistical analyses were performed using SPSS 21.0 program (SPSS Inc., Chicago, IL). Normality was evaluated using the Shapiro-Wilk test. Descriptive statistics are given as mean (± standard deviation) or frequency in the evaluation of the study data. Repeated measures ANOVA was used to evaluate variables, and paired sample t-test was used to evaluate binary comparisons. One-way ANOVA and Tukey’s post hoc test were used to compare the groups. Pearson Correlation Analysis was used to evaluate the correlation between the skin temperature and muscle mechanical properties. Significance criteria was accepted as p <0.05.

**Results**

Each individual in the groups was evaluated and their analysis was included in the all results (n=60). The demographic data are shown in Table 1. There was no statistical significance in comparing demographic information of groups (p>0.05, Table 1). Since the minimum and maximum repeat measurements of ANOVA results in skin temperature evaluations were significant (p<0.001), bilateral evaluations were initiated. The minimum and maximum skin temperatures were significantly lower in all groups compared to before application (p<0.05, Table 2). There was no significant difference when looking at the difference between the groups (p=0.091–0.830, Table 2).
Since the statistical measurement (Repeated measure ANOVA) results of all parameters related to the passive mechanical properties of the muscle were significant ($P < 0.01$), dual evaluations were initiated. Frequency measurements evaluating muscle tone increased in Group 1 after application ($P=0.021$) and decreased at 5 minutes compared to before application ($P=0.467$). While it increased again at the 10th minute ($P=0.018$), it approached the initial value at other times ($P>0.05$, Table 3). In Group 2, while frequency measurements increased before application, after application ($P<0.001$), 5 ($P=0.030$) and 10 minutes ($P=0.029$), it approached its original value in the 15th minute ($P=0.077$). Then, while it increased again in the 20th minute ($P=0.046$), it approached the initial value again in the 30th minute ($P=0.400$, Table 3). In Group 3, an increase in frequency was observed only after application ($P<0.001$); at other times, there was no increase ($P>0.05$, Table 3). There was no difference in frequency in Group 4 ($P>0.05$, Table 3). There was no significant difference in the frequency comparison between the groups ($P=0.215–0.548$).

Muscle stiffness increased in Group 1 after application ($P<0.001$), and decreased at 5 minutes compared to before application ($P=0.127$). Then it increased again in the 10th minute ($P=0.04$) and continued to be low at other times ($P>0.05$, Table 3). In Group 2, while the stiffness increased before application, after application, and at 5 and 10 minutes ($P<0.001$, all), it approached its original value in 15 minutes ($P=0.096$). Then, while it increased again in the 20th minute ($P=0.026$), it continued to decrease in the 30th minute ($P=0.096$, Table 3). Stiffness was significantly higher at all time points in Groups 3 and 4 ($P<0.05$, Table 3). There was no significant comparison between the groups ($P=0.093–0.253$).

Decrement measurements evaluating muscle elasticity increased in Group 1 after application ($P=0.04$) compared to before application and decreased in the 5th minute ($P=0.181$). Then it increased again in the 10th minute ($P=0.04$) and continued to decrease at other times ($P>0.05$, Table 4). In Group 2, after application, decrement increased compared to before application ($P=0.03$), while it approached its original value in the 5th minute ($P=0.577$). Afterwards, it increased again at 10 minutes ($P=0.03$) and continued to decrease at other time points ($P>0.05$, Table 4). In Group 3, it increased immediately after application ($P=0.004$) and also 5 minutes after application ($P=0.03$), then approached its previous value at 10th minute ($P=0.190$). Then, while it increased again in the 15th minute ($P=0.04$), it continued at a low rate in the 20th and 30th minutes ($P>0.05$, Table 4). In Group 4, except for the 30th minute ($P=0.29$), the decrement was significantly higher at all time points ($P<0.05$, Table 4). There was no significance in comparison to the groups ($P=0.060–0.265$). There was no statistically significant relationship between skin temperature and muscle biomechanical properties ($P>0.05$, data not shown).

**Discussion**

In this study, we investigated the acute effects of the cold pack, which is the most frequently used cryotherapy method in the sports clinic, over different lengths of time on the biomechanical properties of the rectus femoris muscle in healthy individuals. After application, a decrease in skin temperature was seen in all cold pack times we applied, and this decrease continued even in the thirtieth minute after the cold pack was removed. In Group 1, where 10-minute cold pack application was applied, muscle tone,
increase in stiffness and decrease in elasticity were observed, while muscle tissue began to approach its pre-application state in the fifth minute. In Group 2, where 12-minute cold pack application was performed, while there was an increase in the muscle tone and stiffness, the elasticity decreased and the muscle tissue began to approach its pre-application state only after the fifteenth minute. In Group 3, where cold pack was applied for fifteen minutes, the muscle tone increased only after the application, while at other time points, it was similar to the status before application. In the same group, muscle stiffness increased at all time points whereas elasticity decreased after application and started to return to normal levels after 10 minutes. In Group 4 (20-minute cold pack application), while muscle tone did not change at any time point after application, the stiffness increased and elasticity (except at the thirtieth minute) decreased in all time intervals. Interestingly, the changes that occurred in many parameters in all groups returned to baseline levels after the application, deteriorated again after five minutes, and then approached baseline levels.

Costello et al., [2] in their review published in 2012, summarized the studies evaluating the effect of various cryotherapy methods on skin temperatures by using thermal cameras. They observed that although the most commonly used cryotherapy method in the clinic is cold pack application, there are few studies on this subject; the studies are generally comparisons of cryotherapy methods. They stated that the studies using the thermal camera and measuring the effects of application periods did not exist at that time. As far as we know, our study is the first in the literature to evaluate and compare the application of the cold pack at different times with a thermal camera. In our study, the skin temperature was decreased by 7.3–8.3 °C with 10 minutes of cold application, by 6.2–6.4 °C with 12 minutes of application, by 4.7–5.8 °C with 15 minutes of application, and by 7.0-7.3 °C with 20 minutes of application. The skin temperature did not return to its pre-application state in any group, even at 30 minutes. Although there was no significant difference between the groups, it was noticed that the best cooling was observed with 10 minutes cold application. When Ring et al.[35] in 2004, applied 15 minutes cold to the L4 level, they observed an average temperature decrease of 7 °C. However, Kennet and colleagues [22] showed that applying 20 minutes of cold pack to the right ankle provided 12 °C of cooling. In a review published in 2001, Auley [18] reported that with 15 minutes of cold pack application on the skin, temperatures measured by telemetriography decreased an average of 7.3 °C; the most appropriate cold application time was 10 minutes, as in our study. They also stated that there was some evidence of a lower temperature drop between the 10th and 20th minutes, against the drop in the first 10 minutes. However, it should be remembered that the temperature drop is also related with the contact area between the surfaces, the temperature difference, and the tissue conductivity.

MYO provides a unique, reliable, accurate, and precise method for objective and noninvasive digital palpation of superficial skeletal muscles. MYO gives a short mechanical impulse to the tissue and records the mechanical response of the muscle through an acceleration probe. The frequency (muscle tone), decrement (elasticity), and stiffness of the oscillation are calculated from the acceleration wave obtained. Abnormally high muscle tone and high intramuscular pressure causes the muscle to become tired more quickly and delay muscle recovery by limiting blood flow [30, 36]. The oscillation stiffness parameter characterizes the muscle's ability to resist the force that shapes it. Increased stiffness
(increased swing stiffness) means more energy is needed to change the shape of the muscle [33, 36]. Increased stiffness can cause damage to the stretched muscle group (antagonist). Elasticity refers not to the ability of a muscle to stretch (elongation), but to the ability to return to its original shape after a force that creates deformation in the muscle [37–39]. The higher the decrement parameter, the less the elasticity. In our study, after 10, 12, and 15 minutes of cold pack application, the muscle tone increased in the 5th, 15th, and 5th minutes post-application, respectively; it returned to its pre-application state. The effect of 20 minutes of cold pack application on muscle tone was not observed, in contrast to Mustalamphi et al. [12]. This is similar to studies suggesting that a drop in muscle temperature in long-term applications (more than 15 minutes) results in a reduction in muscle tone [19, 20].

In the stiffness parameter, an increase was observed in each group after the application, starting from the 5th minute in the 10-minute cold pack application, the 15th minute after in the 12-minute application, and in the other groups, the hardness was high in all time evaluations. This result was similar to the work of Mustalamphi et al. [12], who investigated the mechanical properties of the muscle after 20-minute cold pack application. The elasticity decreased with 10, 12, 15, and 20 minutes of cold pack application (increase in decrement) and returned to its normal levels at the 5th, 15th, 10th, and 30th minutes, respectively.

The results of this study in healthy individuals are parallel to most of the animal studies of muscle tissue whose mechanical properties vary due to local cooling. Noonan et al.\textsuperscript{11} found that increased biomechanical stiffness occurred in chilled rabbit skeletal muscles. Mutungi and Ranatunga (1998) [10] showed that the stretching of rat muscle fiber bundles increased at low experimental temperatures. Some studies looking at indirect muscle parameters had similar findings. Price and Lehmann (1990) [7] found that 30 minutes of cold increased ankle joint stiffness. Similarly, Muraoka et al. (2008) [6] determined the same results for the legs after 60 minutes of cold water bath. Lakie et al. (1986) [5] showed that cooling the forearm in cold water causes a stiffer wrist for joint movements. Unlike other studies, Kubo, Kanehisa, and Fukunaga (2005) [40] found that cooling the leg for 30 minutes with cold water did not change the ankle joint stiffness or the extension of the joint motion of the joint fascicle, tendon, and aponeurosis.

**Conclusions**

In the current study, patients remained in a resting position after cold administration and evaluations were made in this way. Therefore, this study does not give us information about how the biomechanical properties of the muscle will change when the activity is performed. This is one of the limitations of our study. This issue needs to be further explored in future studies. In addition, since we evaluated the muscle without injury, we could not look at the effects of cold pack application after injury. This constitutes another limitation of our study. The results of this study showed us that in healthy people, the rectus femoris muscle becomes harder and less elastic as a result of cooling with cold pack application for different time periods. It also determined that the time that minimizes the biomechanical properties of the muscle and provides optimum cooling is 10 minutes of cooling. If continue cold application is desired, we
anticipate that repeated cooling should be done for 10 minutes. However, careful warm-up is recommended before high performance after the muscle is cooled, and care must be taken.

**Abbreviations**

MYO; MyotonPro; SPSS: Statistical Package for Social Sciences

**Declarations**

**Ethics approval and consent to participate**

Ethical approval The Research Ethics Committee of Acıbadem University and Acıbadem Healthcare Group has approved the study (reference no. ATADEK-2019/17/19) and retrospectively registered clinical trial number (NCT04277481, February 20, 2020). All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki Declaration. Verbal and written consent was obtained from all participants.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The dataset used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare have no competing interests to declare

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None

**Authors’ contributions**

Concept - N.A; Design - N.A., N.K.; Supervision- N.A., N.K.; Resource - N.A., N.K.; Materials - N.A., N.K.; Data Collection and/or Processing - N.A., N.K.; Analysis and/or Interpretation- N.A., N.K.; Literature Search - N.A., N.K.; Writing- N.A., N.K.; Critical Reviews - N.A., N.K. All authors have read and approved the manuscript

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Tables

Table 1: Demographics parameters

| Parameters       | Group 1          | Group 2          | Group 3          | Group 4          | P     |
|------------------|------------------|------------------|------------------|------------------|-------|
|                  | Mean ± Standard Deviation Or N (Frequency %) | Mean ± Standard Deviation Or N (Frequency %) | Mean ± Standard Deviation Or N (Frequency %) | Mean ± Standard Deviation Or N (Frequency %) |
| Age Gender       | 21.60±1.09       | 21.50±1.06       | 21.50±0.82       | 22.60±0.51       | 0.829 |
| Male             |                  |                  |                  |                  |       |
| Female           |                  |                  |                  |                  |       |
| Body Mass Index  | 23.00±2.18       | 23.50±3.41       | 23.40±1.42       | 24.40±1.22       | 0.197 |
| Thickness        | 12.60±2.90       | 12.40±2.80       | 13.50±1.73       | 13.20±1.32       | 0.586 |
| Dominant Side    |                  |                  |                  |                  |       |
| Right            | 14 (93.3 %)      | 14 (93.3 %)      | 13 (86.7 %)      | 13 (86.7 %)      | 0.936 |
| Left             | 1 (6.7 %)        | 1 (6.7 %)        | 2 (23.3 %)       | 2 (23.3 %)       |       |

Table 2: Skin temperature before and after cold pack application
|       | Group 1 |       | Group 2 |       | Group 3 |       | Group 4 |
|-------|---------|-------|---------|-------|---------|-------|---------|
|       | n       | p     | Ma      | p     | Min     | p     | Max     | p     |
| x     | 30.     | 28.   | 30.     | 28.   | 29.     | 29.   | 30.     | 29.   |
| ±     | 40±     | 90±   | 30±     | 80±   | 50±     | 30±   | 70±     | 29±   |
| i     | 1.6     | 1.2   | 1.1     | 1.8   | 1.8     | 1.5   | 1.4     | 1.5   |
|       | 3       | 7     | 0       | 1     | 0       | 4     | 8       | 4     |
| <0.   | 23.     | <0.001| 22.     | <0.   | 24.     | <0.001| 23.     | <0.   | 24.     | <0.001| 22.     | <0.   | 23.     | <0.001|
| ±     | 001     | 10±   | 50±     | 001   | 10±     | 80±   | 001     | 70±   | 001     | 10±   | 001     | 70±   | 001     | 10±   |
| i     | 2.2     | 2.3   | 2.1     | 2.5   | 2.5     | 3.2   | 3.2     | 3.2   | 3.2     | 3.2   | 3.2     | 3.2   | 3.2     | 3.2   |
|       | 5       | 1     | 4       | 2     | 2       | 0     | 3       | 5     |
| <0.   | 25.     | <0.001| 25.     | <0.   | 26.     | <0.001| 25.     | <0.   | 26.     | <0.001| 24.     | 0.0   | 26.     | 0.0   |
| ±     | 001     | 20±   | 001     | 10±   | 20±     | 001   | 50±     | 001   | 80±     | 02    | 10±     | 01    | 001     | 80±   |
| i     | 1.2     | 1.2   | 1.5     | 1.7   | 1.7     | 2.1   | 2.0     | 2.0   | 2.0     | 2.0   | 2.0     | 2.0   | 2.0     | 2.0   |
|       | 5       | 2     | 9       | 1     | 1       | 2     | 5       | 5     |
| <0.   | 26.     | <0.001| 26.     | <0.   | 27.     | <0.001| 26.     | <0.   | 27.     | <0.001| 25.     | <0.   | 26.     | 0.0   |
| ±     | 001     | 10±   | 001     | 30±   | 001     | 20±   | 001     | 60±   | 001     | 60±   | 001     | 60±   | 001     | 60±   |
| i     | 1.5     | 1.4   | 1.3     | 1.2   | 1.2     | 1.6   | 1.4     | 1.4   | 1.4     | 1.4   | 1.4     | 1.4   | 1.4     | 1.4   |
|       | 2       | 5     | 4       | 7     | 3       | 0     | 6       | 6     |
| <0.   | 27.     | <0.001| 26.     | <0.   | 27.     | <0.001| 26.     | 0.0   | 27.     | <0.001| 25.     | <0.   | 27.     | 0.0   |
| ±     | 001     | 30±   | 001     | 90±   | 001     | 90±   | 70±     | 001   | 90±     | 06±   | 01      | 06±   | 01      | 06±   |
| i     | 1.3     | 1.4   | 1.5     | 1.3   | 1.2     | 1.2   | 1.2     | 1.2   | 1.2     | 1.2   | 1.2     | 1.2   | 1.2     | 1.2   |
|       | 4       | 0     | 3       | 5     | 5       | 0     | 3       | 3     |
| <0.   | 28.     | <0.001| 26.     | <0.   | 28.     | <0.001| 26.     | 0.0   | 27.     | <0.001| 26.     | <0.   | 27.     | 0.0   |
| ±     | 001     | 001   | 20±     | 80±   | 03      | 90±   | 20±     | 001   | 30±     | 02    | 001     | 30±   | 02      | 001   |
| i     | 1.3     | 1.4   | 1.2     | 1.2   | 1.2     | 1.2   | 1.2     | 1.2   | 1.2     | 1.2   | 1.2     | 1.2   | 1.2     | 1.2   |
|       | 0       | 5     | 5       | 8     | 0       | 6     | 9       | 9     |
| <0.   | 28.     | <0.001| 27.     | <0.   | 28.     | <0.001| 27.     | 0.0   | 28.     | 0.006 | 26.     | 0.0   | 28.     | 0.0   |

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|   | 001 | 001 | 001 | 001 | 001 | 001 | 001 |
|---|-----|-----|-----|-----|-----|-----|-----|
| ± | 70± | 40± | 50± | 20± | 17  | 30± | 90± |
| ! | 1.6 | 1.2 | 1.2 | 1.3 | 1.2 | 0.8 | 0.9 |
|   | 5   | 1   | 8   | 3   | 9   | 9   | 4   |

Min, Minimum; Max, Maximum; Mean ± Standart Deviation; p, Significance compared to pre-application

**Table 3: Muscle tone (frequency) and stiffness before and after cold pack application**
|       | Group 1 |       | Group 2 |       | Group 3 |       | Group 4 |       |
|-------|---------|-------|---------|-------|---------|-------|---------|-------|
| p     | Stiffness | p     | Frequency | Stiffness | p     | Frequency | Stiffness | p     | Frequency | Stiffness | p     |
| 206   | 12.7    | ±36.  | 212     | 13.4   | ±36.  | 217     | 13.5    | ±20.  | 0±0.     | 233±     |
| ±36.  | 20      | 58    | ±36.  | 0±0.   | 81    | 60      | 90      | 0     | 0         |
| 0.02  | 221     | <0.0  | 13.4   | 229    | <0.0  | 13.7   | <0.0    | 235   | <0.0     | 13.8    | 0.37   |
| ±35.  | 01      | 0±1.  | 01     | ±39.  | 0±0.  | 01     | ±22.    | 01    | 0±1.     | 9       | 27.2   |
| 00    | 96      | 80    | 84     | 10     | 90    | 06      | 0       |       |
| 0.46  | 221     | 0.12  | 12.9   | 221    | <0.0  | 13.4   | 0.92    | 226   | 0.04     | 13.5    | 0.19   |
| ±43.  | 7       | 0±1.  | 01     | ±37.  | 0±0.  | 0       | ±22.    | 01    | 0±0.     | 7       | 24.5   |
| 70    | 61      | 70    | 90     | 90     | 77    | 0       |         |
| 0.01  | 222     | 0.00  | 12.9   | 219    | <0.0  | 13.7   | 0.22    | 229   | 0.00     | 13.6    | 0.47   |
| ±40.  | 4       | 0±1.  | 9      | ±36.  | 0±1.  | 2       | ±25.    | 3     | 0±0.     | 6       | 20.1   |
| 90    | 48      | 50    | 50     | 5      | 60    | 0       |         |
| 0.69  | 213     | 0.09  | 13.0   | 216    | 0.09  | 13.5   | 0.59    | 229   | 0.00     | 13.7    | 0.11   |
| ±35.  | 5       | 0±1.  | 7      | ±40.  | 0±0.  | 3       | ±19.    | 1     | 0±0.     | 5       | 23.6   |
| 90    | 76      | 09    | 84     | 20     | 71    | 0       |         |
| 0.77  | 211     | 0.13  | 13.1   | 219    | 0.02  | 13.6   | 0.43    | 228   | 0.04     | 13.7    | 0.30   |
| ±41.  | 6       | 0±1.  | 6      | ±41.  | 0±0.  | 1       | ±23.    | 4     | 0±0.     | 8       | 18.6   |
| 71    | 71      | 80    | 91     | 40     | 63    | 0       |         |
| 0.61  | 218     | 0.54  | 12.7   | 214    | 0.09  | 13.5   | 0.74    | 227   | 0.01     | 13.2    | 0.39   |
| ±1.6  | 2       | 0±1.  | 0      | ±38.  | 0±0.  | 1       | ±38.    | 2     | 0±0.     | 2       | 21.2   |
| 0     | 30      | 10    | 85     | 20     | 66    | 0       |         |

Mean ± Standart Deviation; p, Significance compared to pre-application
Table 4: Muscle elasticity* (decrement) before and after cold pack application

| Group 1 | Group 2 | Group 3 | Group 4 |
|---------|---------|---------|---------|
| Decrement | p | Decrement | p | Decrement | p | Decrement | p |
| 1.17±0.21 | 1.31±0.28 | 1.12 ±0.19 | 1.27±0.12 |
| 1.25±0.20 | 0.040 | 1.36±0.31 | 0.003 | 1.21 ±0.17 | 0.004 | 1.49±0.17 | 0.012 |
| 1.21±0.20 | 0.181 | 1.32±0.31 | 0.577 | 1.20±0.17 | 0.03 | 1.38±0.15 | 0.05 |
| 1.25±0.20 | 0.040 | 1.34±0.25 | 0.03 | 1.17±0.19 | 0.190 | 1.42±0.16 | 0.04 |
| 1.14±0.14 | 0.266 | 1.31±0.11 | 0.904 | 1.19±0.16 | 0.04 | 1.40±0.16 | 0.03 |
| 1.15±0.16 | 0.411 | 1.30±0.33 | 0.774 | 1.17±0.19 | 0.190 | 1.43±0.17 | 0.04 |
| 1.13±0.20 | 0.265 | 1.24±0.26 | 0.287 | 1.16±0.15 | 0.173 | 1.36±0.17 | 0.29 |

Mean ± Standard Deviation; p, Significance compared to pre-application;* The higher the decrement parameter, the less the elasticity