Clinical Trial Report

Pilot study: physiological evidence that heat reduces pain and muscle damage in delayed-onset muscle soreness

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Practice points

- Heat applied immediately after strenuous exercise appears to both reduce pain and increase the speed of healing of damaged muscle tissue in this pilot study.
- At 24-h postexercise, the skin temperature over the exercised muscle was significantly elevated and the warmer the skin temperature, the less soreness the subjects reported.
- Heat applied for 8 h immediately after strenuous exercise reduced muscle soreness at 24-, 48- and 72-h postexercise.
- Groups that had heat applied after exercise maintained low concentrations of granulocyte white blood cells after the exercise. The control groups, in contrast, showed significantly higher granulocyte counts, an indicator of inflammation.

SUMMARY: **Aim:** To determine if heat applied after strenuous exercise reduces soreness and/or muscle damage. **Methods:** Self-reported soreness and blood biomarkers were measured in 20 subjects over 72 h after biceps exercise. Heat was applied both immediately and 24 h after exercise, or never (control). **Results:** Muscle soreness was lower in groups with more heat applied. Skin temperature and some biomarker analytes (granulocytes and mid-size white cells) support a hypothesis of less pain and faster healing in muscles when heat is applied after exercise. Ultrasound data showed less fascial swelling in groups with heat applied compared with controls. **Conclusion:** Data suggest that heat applied immediately and/or 24 h after exercise reduces muscle soreness and accelerates the healing process. A larger group of subjects is needed to draw better conclusions.

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Delayed-onset muscle soreness (DOMS) is common for anyone who makes the transition from little-to-intense exercise. DOMS is associated with an initial soreness starting within 3–24 h of heavy exercise, and can range from slight muscle tenderness and stiffness to debilitating pain. It may continue for 8–10 days postexercise [1]. The extent of DOMS depends on many things including fitness, age, genetics, training and the intensity of the activity [2]. Associated with muscle soreness is an elevation of related blood biomarkers including HSP27, HSP70, IL-6, IL-10, muscle myoglobin (MB), creatine phosphokinase (CPK) and lactate dehydrogenase (LDH), all of which generally indicate muscle damage [3,4].

Muscle strength is sometimes used as a marker of DOMS [5]. Unfortunately, the very act of measuring strength can damage a sore muscle further or, due to pain, reduce the subjects' willingness to exert a real maximum effort [6]. The effects of aging on muscle soreness have also been examined [7]. DOMS is worse the older the individual's age and can be caused by even a single bout of exercise.

Many different therapies are used clinically for DOMS [7], most commonly heat but also stretching and massage [8]. Historically, heat has been used to relieve the pain from muscle soreness. While the benefits of heat in reducing pain are well investigated [9], there has been little to quantify or even assess the benefits of heat on muscle healing after strenuous exercise. Heat is one of the modalities that activates voltage-gated calcium channels (TRPV channels) [10]. A common location for these channels is in skin sensory nerves and in vascular endothelial cells [11]. When tissues heat, there is an increase in calcium influx through these channels from the blood. Calcium inactivates P_2X_2 purine pain receptors that mediate the pain response to tissue damage. Preliminary work suggests low-level heat-wrap therapy is helpful in the prevention and early treatment of DOMS [9]. Heat increases muscle perfusion by the blood [12–14]. This has been suggested as a mechanism for the positive effect of heat after a workout on increasing healing [15]. However, heat also increases the basal metabolic rate in tissue, in itself promoting healing [16]. Other treatment methods, including whole-body vibration, have also been suggested as a preventative modality for DOMS [17].

However, while heat and other modalities such as capsaicin [18] or menthol [19] relieve pain, they may or may not promote healing. Pain blocking occurs through a different mechanism to healing in muscle and soft tissues. In fact, there is no clear evidence that any modality actually promotes healing.

When muscles are damaged, enzymes such as CPK, skeletal muscle LDH, MB, HSP27, HSP70, IL-6 and IL-10, which are normally found in muscle, are also found in dose–response-related levels in the blood [4]. Studies that have correlated DOMS to these biomarkers show a strong temporal correlation between the intensity of the discomfort and the elevation of these biomarkers in the blood [3,4]. While there are many treatment recommendations for DOMS, including cryotherapy, stretching, anti-inflammatory drugs, ultrasound and massage, among others, limited success has been reported in the literature [7]. The use of heat to modulate tissue recovery after exercise has not been correlated to these biomarkers. These chemical compounds might provide a quantitative measure of tissue healing in response to applied heat [20].

In the present study, heat was applied either immediately or 24-h postexercise, or both, compared with control subjects to whom no heat was applied. The outcome measures were blood CPK, LDH, MB, HSP27, HSP70, IL-6, IL-10 and ultrasound pictures of the muscle to quantify muscle edema and muscle damage.

Table 1. Group demographics.

	Age (years)	Height (cm)	Weight (kg)	Body fat (%)	BMI
Control group					
Mean	26.5	167.8	68.3	29.6	22.9
SD	13.3	11.3	23.3	9.2	9.7
Subjects with heat applied 24 h after exercise only					
Mean	25.4	168.3	71.3	29.6	25.0
SD	2.3	11.7	14.9	7.0	3.1
Subjects with heat applied both immediately after exercise and 24-h postexercise					
Mean	23.4	167.4	64.7	29.2	23.1
SD	0.6	4.2	6.4	6.4	2.3
Subjects with heat applied immediately after exercise only.					
Mean	25.8	165.4	74.5	34.3	27.3
SD	3.1	7.9	6.9	7.7	2.3

SD: Standard deviation.

Subjects

The subjects for this study were 20 healthy individuals between the ages of 20 and 40 years, randomly divided into four groups by using a random statistical table. The randomization was

generated in Visual Basic 6 (Microsoft Corp., WA, USA) using its built-in random number generator with a short program. Each group consisted of three women and two men. All subjects did not exercise their upper body extensively for at least 6 weeks prior to the study and their BMI was less than 40. Subjects had no cardiovascular or hepatic disease, no diabetes, upper limb neuropathy or recent upper limb injuries, and were not diagnosed with rhabdomyolysis, since this disease causes the release of MB and cytokines even without exercise. All had blood pressure measurements between 140/90 mmHg and 90/60 mmHg. Subjects were not on high doses of α- or β-agonist/antagonists, any type of NSAID, COX-2 inhibitors, calcium channel blockers, pregabalins (Lyrica®, Pfizer Corp., NY, USA) or pain reducers. Screening was carried out by a licensed staff member who conducted a history and physical examination. All methods and procedures were explained to each subject as approved by the Institutional Review Board of Azusa Pacific University and all subjects signed a statement of informed consent. The demographics of each group are listed in **Table 1**. There was no statistical difference between the groups ($p > 0.05$ for age, height and weight).

Methods

Body fat determination

Body fat percentage was measured by electrical impedance (Quantum X, RJL Systems, MN, USA) bioelectric impedance analyzer (RJL Systems). This and subcutaneous fat were measured to assure that heat transfer was uniform.

Subcutaneous fat thickness

A 2D–3D high-resolution ultrasound (Mindray M7, GA, USA) with an L34 head and a 1-cm standoff was used to determine subcutaneous fat thickness. By using the unit at a frequency of 10 MHz through the 1-cm standoff, the thickness of the skin and subcutaneous fat layer was measured. For consistency, a single investigator carried out all measurements on all subjects. Ultrasound imaging at a frequency of 10 million cycles per second (Mindray M5, Mindray International, Shenzhen, China) was also used to assess changes in muscle structure pre- and post-exercise.

Muscle strength measurement

Muscle strength was measured using a resistive strain gauge transducer. Four strain gauges were placed on opposite sides of a steel bar (two on each side). The dynamometer was mounted at a 45° angle so that the subject would primarily use their biceps. When the bar was bent, the strain gauges were deformed and an electrical output was recorded. The Wheatstone bridge was powered and amplified 5000-times by a BioPac DAC100 (BioPac Systems, CA, USA). An MP150 digitized the analog output (resolution 24 bits, frequency of 1000 samples per second). Data analysis and storage used Acknowledge 9.1 software from BioPac (BioPac Systems). Muscle strength was determined three-times. Each contraction was 3 s in duration with at least 1 min separating the contractions. Each subject chose which arm to use. The average of the three initial measurements was taken as the subject's maximum strength.

Exercise

To induce DOMS, the subjects exercised in four sets. Each set involved 25 bicep curls against resistance until fatigue. The resistance was 35% of their maximum strength.

Subjective pain measurement

A 10 cm visual analog scale was used. It was made with a horizontal line drawn across a piece of paper 10-cm long. One end was 'pain free' and the other

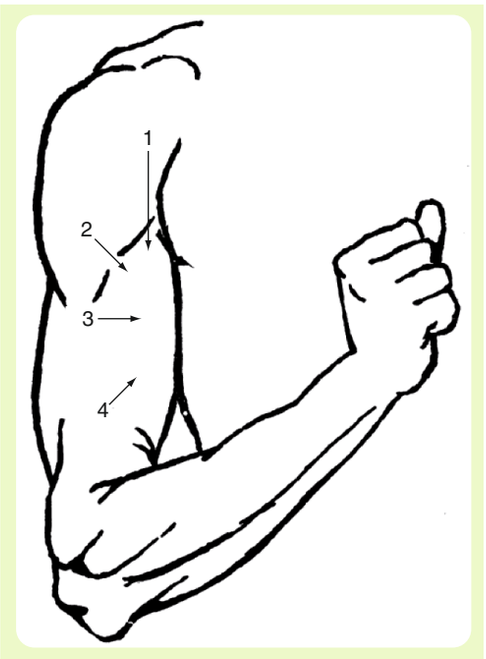

Figure 1. Placement of regions of interest on the arm to measure skin temperature.

'very, very sore'. The subject was asked to place a vertical slash across the line where appropriate. The location was converted into a number.

Blood sampling

Approximately 10 ml of venous peripheral blood was sampled from the antecubital vein. The vacutainer used was a serum separator. Blood was taken before, immediately after and at 3, 24, 48 and 72 h after each round of exercise. The blood was spun in a refrigerated centrifuge at 3000 rpm for 10 min to separate the serum or plasma from cells. The separated serum and plasma aliquots were stored at -80°C until analyses were conducted.

Plasma biomarker measurements

A complete blood count was performed using a Mindray BC3200 (Mindray International) to collect hematocrit and an automated three-part white blood cell count. Plasma CPK, LDH and CRP activity were measured spectrophotometrically on the Mindray BS120 discrete and random access Clinical Chemistry Analyzer (Mindray North America, NJ, USA) using the following test kits from Pointe Scientific, Inc. (MI, USA):

- Plasma CPK: #312C7522100;
- LDH: #12L7572100;
- High-sensitivity CRP: #C7568STD, #C7568CTL, #12C7656840 (standard, controls, reagents, respectively).

For the measurement of IL-6 and IL-10 cytokines, the inflammatory protein high-sensitivity CRP, plasma MB, HSP27 and HSP70, commercially available ELISA kits in the 96-well plate format were used according to the manufacturers' instructions:

- IL-6: HS600B (R&D Systems, MN, USA);
- IL-10: HS100B (R&D Systems);
- Plasma MB (MG017C; CalBiotech, CA, USA);
- HSP-27: ELH-HSP27-001 (Ray Biotech, GA, USA);
- HSP-70: ENZO ADI-EKS-715 (Ray Biotech);
- CRP: ELH-CRP-001 (Ray Biotech).

For all ELISA assays, the absorbance was measured spectrophotometrically with an ELISA microplate reader (Multiscan MCC 340; MTX Lab Systems, VA, USA) and the concentration of each plasma analyte/substance was dose interpolated from the derived calculated dose–response curve established within the same assay performance interval.

Skin temperature measurement

Skin temperature was measured by a Flir 650 thermal imager (Flir systems, MA, USA). Temperatures were measured at four locations above the biceps muscle. This was used here as a measure of inflammation. From these measurements, the individual regions were analyzed to give the average temperature for each region and by averaging the four regions, the temperature for the entire palmer surface of the upper arm was determined (Figure 1).

Procedures

The subjects were randomly divided into four groups. One group exercised but did not have heat applied at any time after exercise (control). A second group exercised and had a continuous, low-level heat wrap (ThermaCare® heat wraps, OH, USA) applied immediately after the exercise and left in place for 8 h. Heat was removed for 1 h before the skin temperature measurements

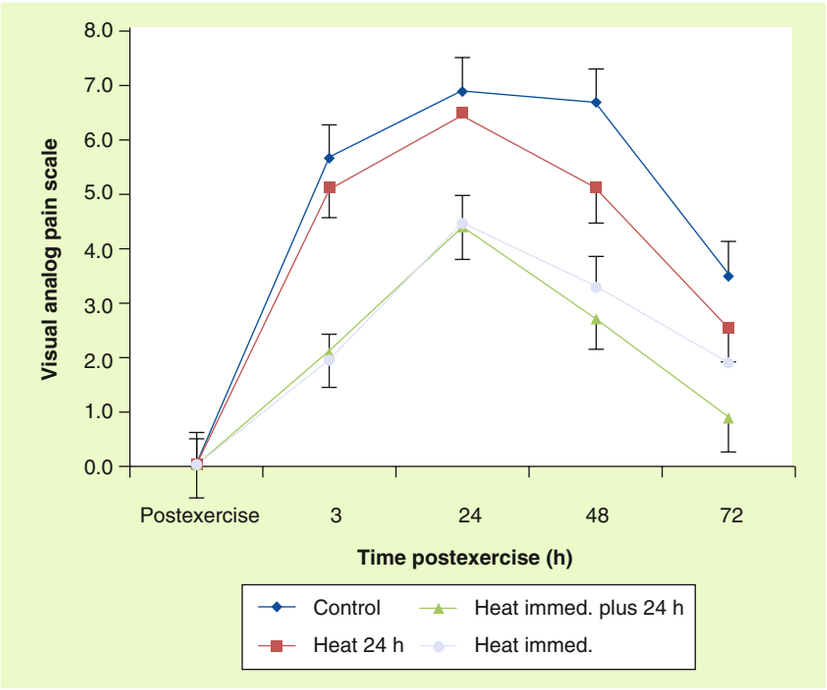

Figure 2. Average soreness in each group over 72-h postexercise. Each point is the mean of the group ± the standard deviation.
immed.: Immediately.

and then reapplied. A third group exercised and had a heat wrap applied 24 h after exercise, for 8-h duration. The fourth group had a heat wrap applied immediately after exercise and a second applied 24 h after exercising, remaining for 8 h each time. Heat was removed for 1 h before the skin temperature measurements and then reapplied. The subjects exercised as described in the methods section and blood was drawn before, immediately after and at 3, 24, 48 and 72 h after each exercise bout and analyzed as specified above. Subjects were asked to show, on the analog visual scale (described above), how sore they were. In the noncontrol groups, the assessment was made immediately before the heat wrap was applied.

Data analysis

Statistical analysis involved the calculation of means and standard deviations. Due to the small number of subjects, analysis of variance and T-tests are not valid. The Mann–Whitney test was used for group comparisons. The Wilcoxon signed-rank test was used to compare data over time for the same subjects. Blood analyte data were corrected for changes in plasma volume postexercise. The hematocrit from venous blood was converted to true whole-body hematocrit by multiplying the venous hematocrit value by 0.873. The change in plasma volume for subsequent days after the first were then calculated as below [21,22]. The formula used is:

$$C_a = \frac{Hct\,(100 - Hct1)\,C_b}{Hct1\,(100 - Hct2)}$$

Where:

- C_a = final analyte concentration;
- $Hct1$ = hematocrit on the control day;
- $Hct2$ = hematocrit on the test day;
- C_b = analyte test concentration.

Once corrected, any analyte in a group more than three standard deviations from the mean was removed.

Results

Strength

The average strength of the subjects was 11.8 ± 3.3 kg, with no significant difference between groups. Some subjects could finish all of the exercise and some could not. For the group averages together, the number of lifts was 77.2 ± 21.9 (coefficient of variation of 28.3%).

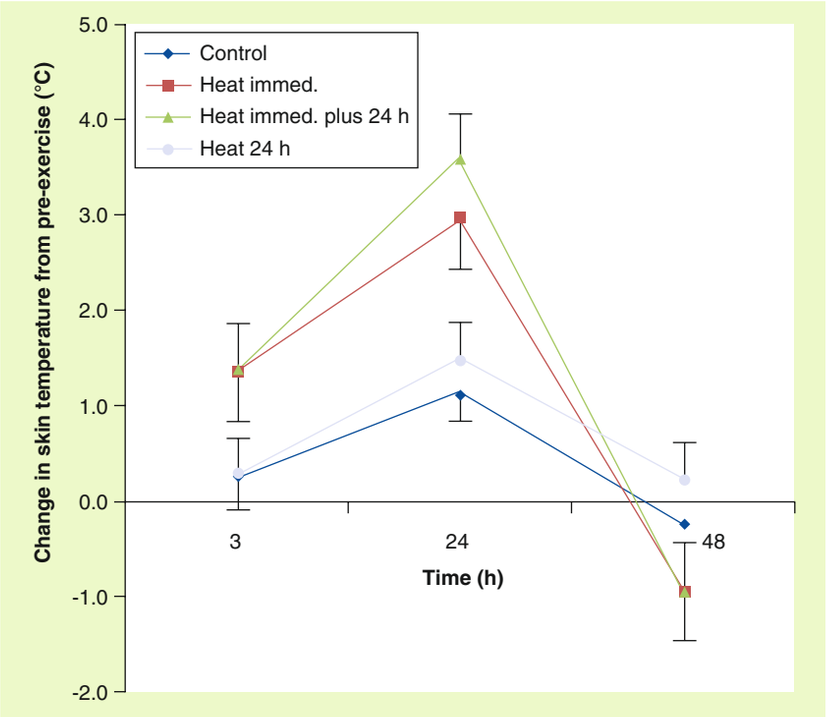

Figure 3. Average skin temperatures in region 3 for each group of five subjects. Each point is the mean of the group ± the standard deviation.
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The number of repetitions decreased as the exercise continued. For example, the first bout averaged 24.5 ± 1.1 out of 25 possible repetitions, but by the third exercise bout the average number of repetitions was 16.9 ± 7.1. There was no statistical difference between the groups in the number of lifts.

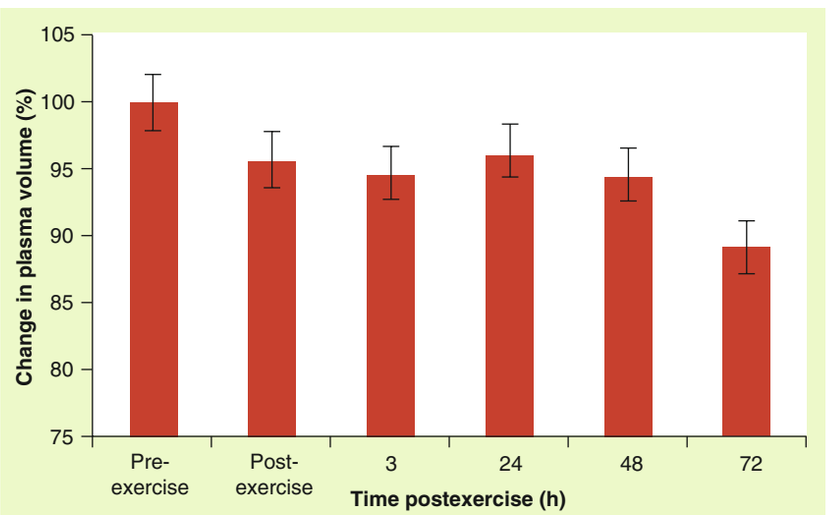

Figure 4. Corrected plasma volumes for the subjects pooled as one group. Each point is the mean of the group ± the standard deviation.

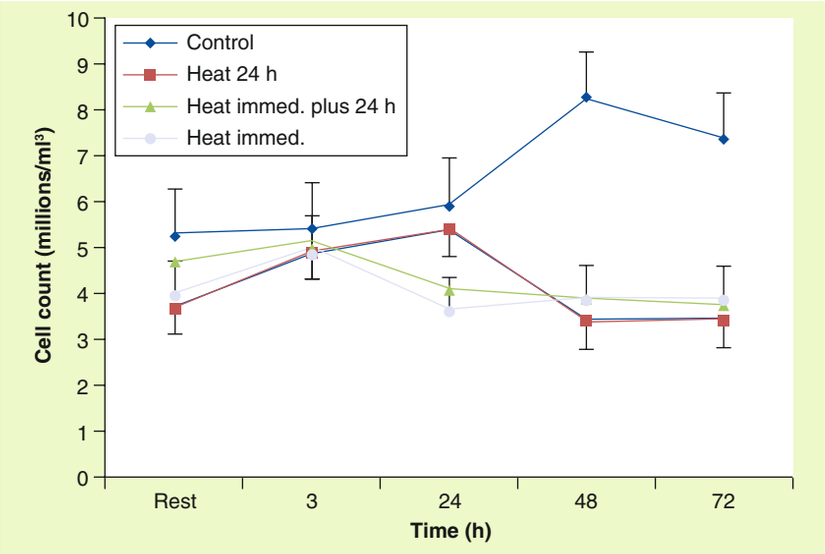

Figure 5. Granulocyte cell count in millions per ml³ pre- and post-exercise. Each point represents the mean of the group ± the standard deviation.
immed.: Immediately.

Soreness

Three hours after exercising, the average soreness for all groups was small, 2.11 ± 0.30 out of ten (ten was very, very sore), with no significant difference between groups. After 24 h, the average soreness was 5.6 ± 0.4 for all subjects. Soreness remained fairly constant at 48 h after exercise, averaging 5.23 ± 0.50 over all four groups and at 72 h it decreased to 2.4 ± 0.3 on a ten-point scale.

The data for the individual groups is plotted in Figure 2. As can be seen here, the soreness for the control and heat 24-h groups was significantly higher than the other two groups ($p = 0.4$). Soreness for all groups was significantly higher than postexercise ($p < 0.05$). At 24 h, there was no significant difference in soreness between the control and heat 24-h groups ($p > 0.05$) and between the immediate heat and immediate heat plus 24-h groups ($p > 0.05$). However, the control and heat after 24-h groups had significantly greater muscle soreness than the other two groups ($p < 0.05$); this same relationship was seen at 48 h, except the heat at 24-h group had less soreness than the control group ($p < 0.05$); this was also true at 72 h.

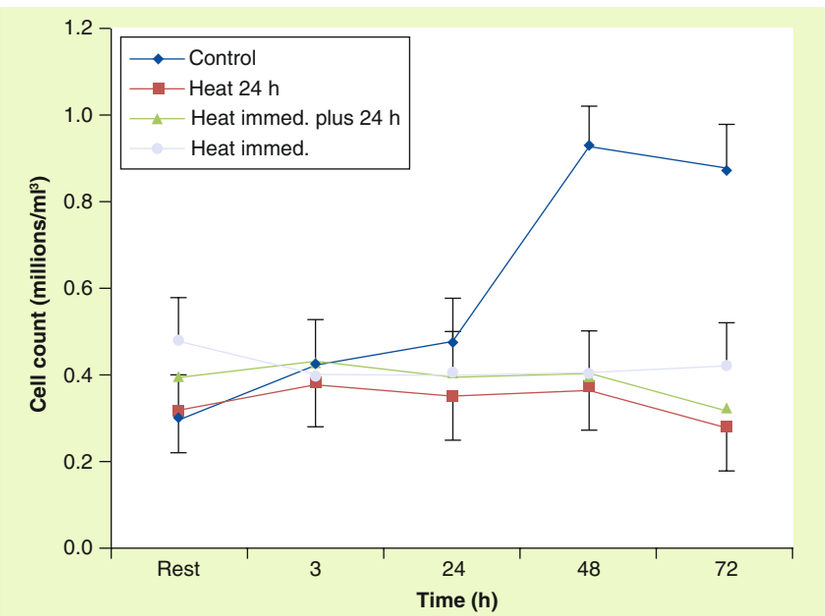

Figure 6. Mean mid-cell size white cell count (monophils, basophils and eosophils) in millions per ml³ pre- and post-exercise. Each point represents the mean of the group ± the standard deviation.
immed.: Immediately.

Skin temperature

Skin temperature was assessed at four locations as described in the methods section. For the whole limb, the differences between groups were small, with a slight trend toward increased temperatures at 24-h postexercise. The detailed data revealed that the majority of the change took place in the region just over the belly of the biceps (region 3 in Figure 1, data shown graphically in Figure 3). In this region, the muscle temperature increased at 3- and 24-h postexercise and decreased at 48 h.

The greatest increase in skin temperature occurred for the two groups that had heat applied immediately after exercise. At 3 and 24 h, the skin temperature here was significantly higher than that seen for the other two groups ($p < 0.05$), but the immediate heat and immediate heat plus 24-h groups were not different from each other ($p > 0.05$), as was the case at 24 h for the other two groups ($p > 0.05$). By 48 h, there was no difference between the groups ($p > 0.05$).

Hematocrit & plasma volume

The venous hematocrit was corrected to the whole-body true physiological hematocrit. From this measure, the plasma volume was calculated to correct the concentration of the analytes. All four groups showed a significant reduction in hematocrit over the 3 days after the

exercise ($p < 0.05$), but there was no difference between groups. From the change in hematocrit, the change in plasma volume after exercise was calculated and is shown in Figure 4.

White blood cells

The white blood cell count was measured both at rest and postexercise with a three-part differential analysis. The total white blood cell count in adults is typically 5–10 million/l, with increases indicating inflammation or infection [23]. White blood cells increase in concentration when there is tissue damage. The highest granulocyte cell count was in the control subjects, where the cells increased throughout the test period, indicating muscle signaling of tissue damage. The total numbers of granulocyte cells dropped or remained constant in all groups that had heat applied, as shown in Figure 5. There was no significant difference between the groups at rest and at 3-h postexercise ($p > 0.05$). However, the granulocytes were significantly higher in the control group than the other two groups at 48- and 72-h postexercise ($p < 0.05$). At 24-h postexercise, the control and heat 24-h groups were not different from each other ($p < 0.05$), but were greater than that seen in the other two groups ($p < 0.05$).

The mid-range white blood cells (monocytes, eosinophils and basophils) demonstrated little difference among the experimental groups, but increased dramatically in the control, nonheat group (Figure 6).

Lymphocyte levels showed little difference between groups (data not shown).

Blood biomarkers

MB

The normal range for MB is 0–90 ng/ml of blood and higher levels indicate damage to heart or skeletal muscle [24]. The average MB pre-exercise for all subjects was 28.8 ± 6.9 ng/ml, well within the reference range. There was no significant difference between the groups postexercise ($p > 0.05$) (Figure 7).

LDH

LDH is found in all tissues in the body, at levels up to 200 units/l in the blood. Slightly elevated levels are associated with damage to skeletal muscle and high levels can result from a number of different diseases [25]. Pre-exercise, the LDH blood level for all 20 subjects was 140.5 ± 26.7 mg/l, with no difference between groups. There was no significant increase post-exercise and no difference between the groups ($p > 0.05$) (Figure 8).

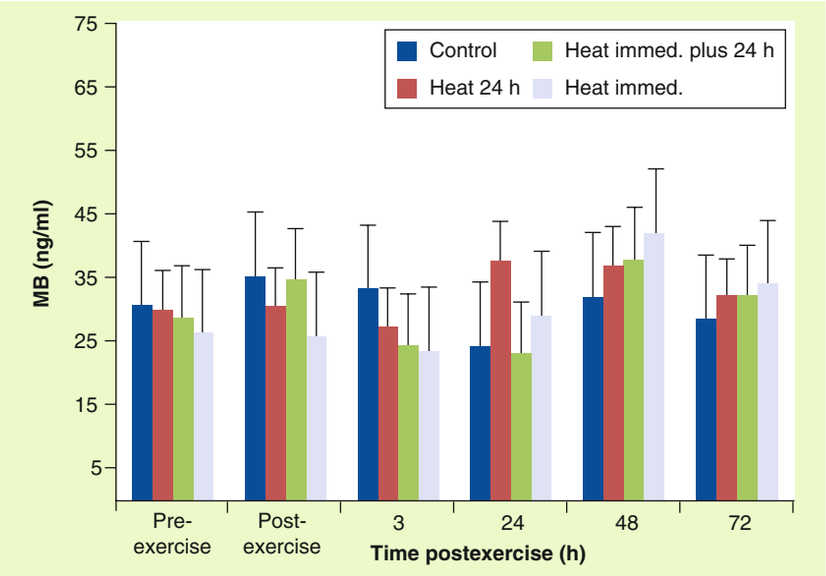

Figure 7. Average blood myoglobin for all subject groups ± the standard deviation.
immed.: Immediately; MB: Myoglobin.

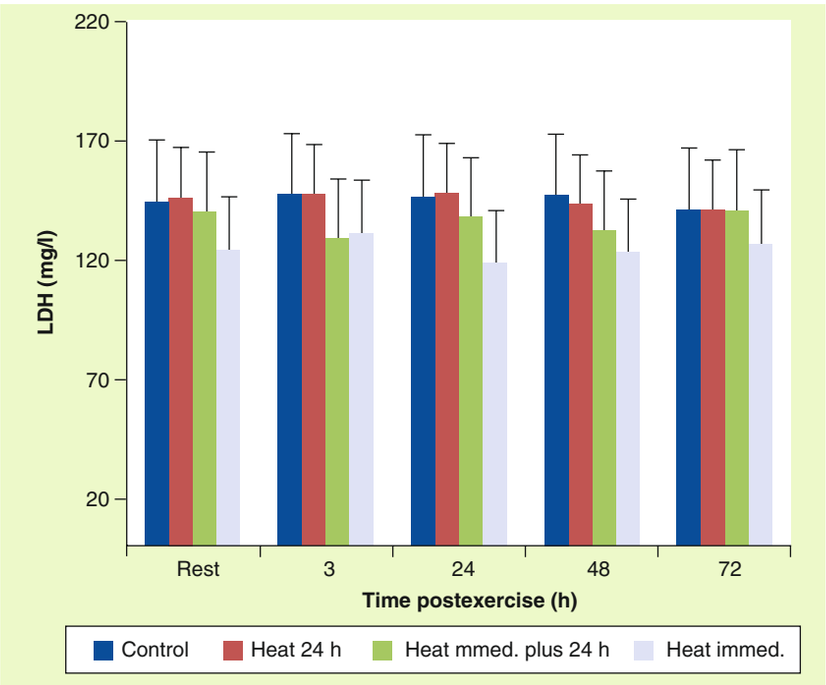

Figure 8. Average blood lactate dehydrogenase (normalized as a percentage change from the resting value) ± the standard deviation in the four groups of subjects.
immed.: Immediately; LDH: Lactate dehydrogenase.

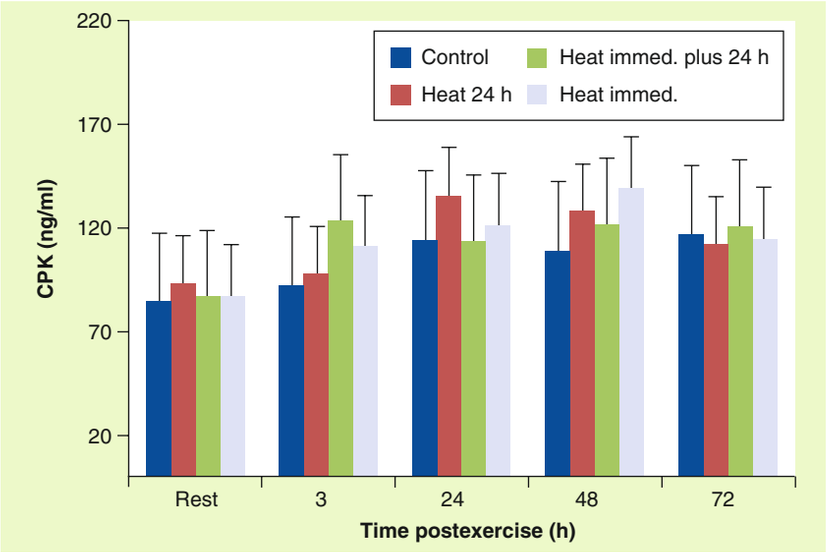

Figure 9. Average blood creatine phosphokinase levels pre- and post-exercise (normalized to resting values) as an average ± the standard deviation in the four groups of subjects.
CPK: Creatine phosphokinase; immed.: Immediately.

CPK

Normal blood levels of CPK are 30–135 units/l for females and 55–170 units/l for males and are known to increase after exercise. CPK is found primarily in heart and skeletal muscle, and the brain, and when these cells are injured, levels rise within 6 h, peak at 18 h after injury and return to normal in 2–3 days [25]. In this study, the 20 subjects averaged 120.2 ± 28.2 ng/ml pre-exercise, with no difference between groups ($p > 0.05$). There was no difference from rest after the exercise in any group ($p > 0.05$) **(Figure 9)**.

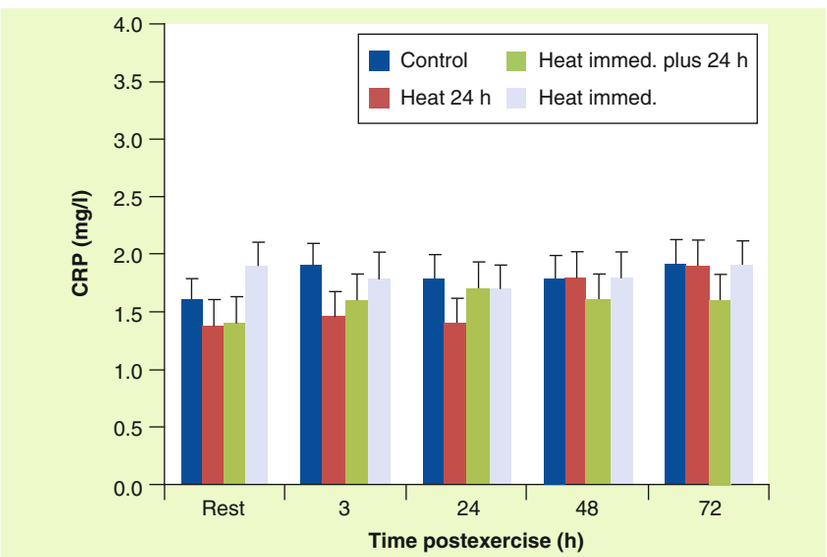

Figure 10. Average blood CRP levels over time. Values are in pg/ml and each point represents the mean of each group ± the standard deviation.
immed.: Immediately.

High-sensitivity CRP

The normal range for CRP is less than 10 mg/l, with higher levels caused by inflammation or infection, among other causes [25]. CRP levels rise during inflammatory processes [26]. There were no significant changes in CRP levels in the control or other groups over the test period ($p > 0.05$) **(Figure 10)**.

■ IL-6 & IL-10

There was no significant difference between the groups throughout the experiments for these two analytes ($p > 0.05$).

HSP27

Expression of HSPs increase when cells are exposed to stressors such as heat, cold, exercise, radiation and disease. HSPs have been shown to respond in a dose-dependent manner to the level of muscle damage [4]. HSP27 levels peaked at 24 h for all groups of subjects. At 3, 24 and 48 h, the immediate heat and immediate heat plus 24-h groups had significantly greater HSP27 than the other two groups ($p < 0.05$) and these two groups were not different from each other ($p < 0.05$). At 72 h, there was no difference between the groups ($p > 0.05$) **(Figure 11)**.

■ HSP70

Members of the HSP70 family are upregulated by tissue inflammation and toxicity, and aid in transmembrane transport of proteins [27]. HSP70 levels were tested with a high-sensitivity assay. HSP70 was not different between the groups or over time for any one group.

■ Ultrasound imaging

Ultrasound imaging showed a distinctive difference between the control and three experimental groups. All ultrasound images were read blindly. In the control group **(Figure 12)**, comparing images taken pre-exercise to those taken 48-h postexercise, a thickened band appeared in the fascia subcutaneously and just above the muscle (arrow). This is consistent with edema and damage to the fascial layers.

In the groups of subjects that had heat applied immediately or both immediately and 24-h postexercise, there was no difference in the ultrasound pictures. However, for subjects who had heat applied 24 h after exercise, there was some swelling **(Figure 13)**.

Discussion

The standard treatment after strenuous exercise used by many trainers is to apply cold for brief periods during the first 24 h, followed by heat 24-h postexercise to reduce soreness and promote healing. However, several recent studies in large clinical trials have found that while heat can significantly reduce pain, no such conclusions can be drawn about the use of cold [7,28]. Heat has also been shown to be effective for acute, nonspecific lower back pain [9,29,30]. Analgesics can also reduce pain, but do not promote healing [31].

The present authors were interested in two different mechanisms – reduction of soreness/pain and promotion of healing. In this pilot study, the authors investigated the use of heat only, without cold or analgesics, on DOMS. They looked at the effect of heat on both self-reported muscle soreness and on biological markers of muscle damage to measure the effects on pain relief and promotion of muscle healing, respectively. It was found that heat applied for 8 h immediately after strenuous exercise reduced muscle soreness at 24- and 48-h postexercise, and that heat applied both immediately and at 24-h postexercise reduced pain even further.

When heat was used immediately after exercise only, there was a carry-over effect such that at 24-h postexercise (a full 16 h after the heat packs were removed), the skin temperature was elevated by 3°C. Furthermore, the warmer the skin temperature was at 24-h postexercise, the less soreness the subjects reported.

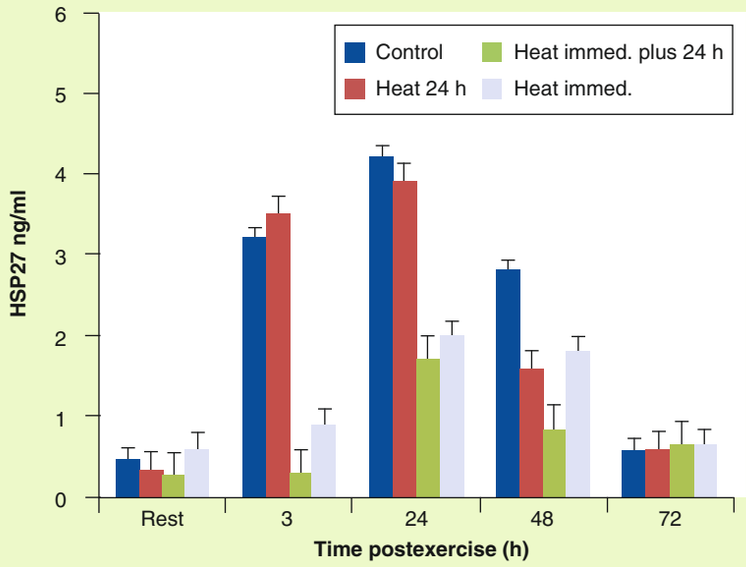

Figure 11. Average blood HSP27 levels pre- and post-exercise for each group of subjects ± the standard deviation.
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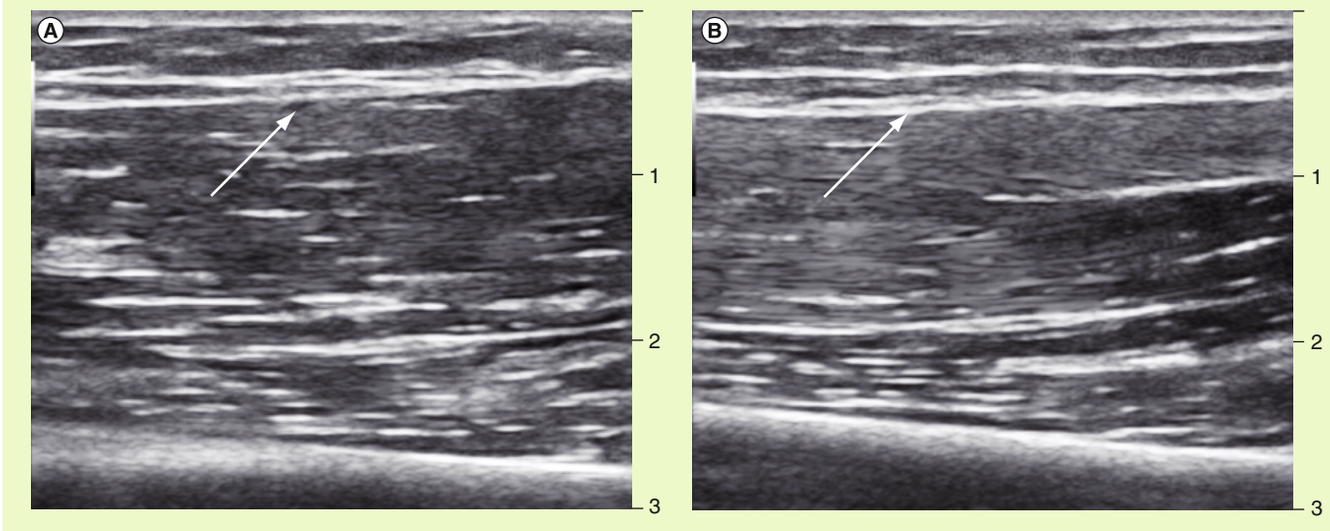

Figure 12. Ultrasound image of the biceps muscle. (A) Pre- and **(B)** 48 h post-exercise in a typical control subject. The y-axis represents the depth from the skin surface in cm.

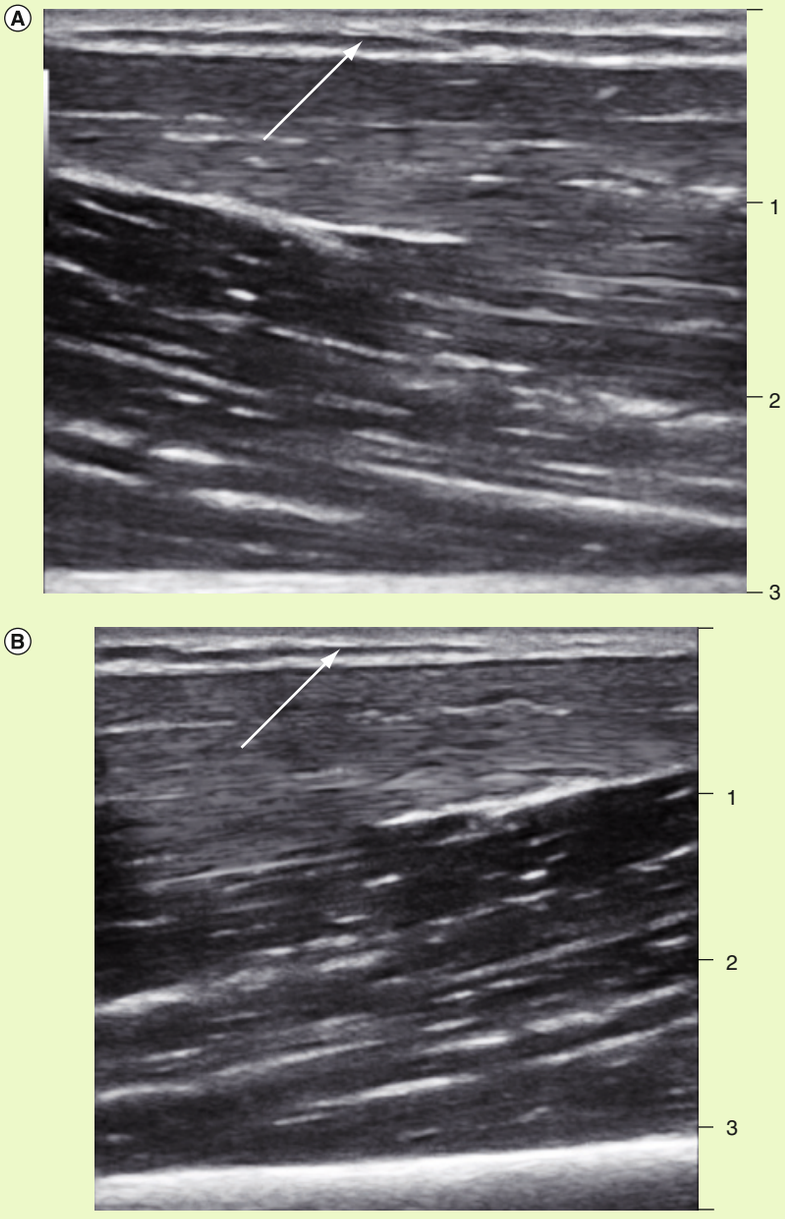

Figure 13. Ultrasound image of the biceps muscle. (A) Pre- and **(B)** 48 h post-exercise in a test subject who had heat applied both immediately and 24 h postexercise. The y-axis represents the depth from the skin surface in cm.

It is reasonable to assume, in the subjects where heat was applied just after exercise, that the skin itself will not, 24-h postexercise, show an elevated temperature due to exercise. Skin is a shell tissue and its temperature is usually about 6°C less than that of the core. This complies with the standard principle of heat flow: heat moves from high heat to low heat or, in other words, from the core, to the skin, to the environment. Heat can move from the body core to the skin and be removed by radiation, convection, conduction and evaporation. This allows the body to regulate core temperature. When an individual exercises, muscle generates heat. This heat then conducts to the skin, over the muscle, through the tissues. This is described by the Pennes equation [32]. Muscle, a core tissue, is usually warmer than skin by 3–4°C. If muscle blood flow remained elevated postexercise, the warmer core blood would keep the muscle warm and, hence, the overlying skin would stay warm. The increased skin temperature 24-h postexercise in all subjects is probably due to higher blood flow in muscle, due to inflammation and repair of tissue damage caused by the exercise. This would potentially be a benefit for healing in that higher blood flow and tissue temperatures increase tissue metabolism. One possible effect is that temperature increases the Q10 of biochemical reactions [33]. The Q10 of skeletal muscle is usually given as between two and three; this doubles metabolism if tissue is kept warm [34]. Furthermore, the circulation increase may deliver more oxygen and wash away metabolites, which also promotes healing.

When heat was applied immediately after exercise, the skin temperature was substantially higher than that observed without heat application (when assessed at 24-h postexercise). Logically, this would mean that the circulation is elevated 24-h postexercise. The fact that areas of the skin not above the muscle had no carry-over effect also supports the hypothesis that the heat is generated in the muscle. The net effect seems to be that increased heat and circulation allows the muscle to heal faster. Evidence of increased healing with heat is in several areas. First, heat reduced fascial edema measured with ultrasound. Ultrasound offers a way to quantify edema in tissue by measurement of water between fascial layers and in tissue [35]. It has even been used to view skin edema. As long as bone is not involved, it can image edema, and to some extent, damage, successfully. While the increase in fascial thickness was not measured here, it would be useful in future studies. It may allow edema to be quantified and tracked more closely.

Second, the white-cell count was reduced with heat applied immediately and at 24 h. Third, HSP27 was reduced by heat. The data from the other analytes was inconclusive, but the muscle group used here was small and dilution

of other analytes in the blood may have made their concentration unreadable as a result of the exercise.

Conclusion & future perspective

The preliminary data reported here suggest that there is both faster resolution of muscle damage and less subjective pain reported when heat is applied immediately after strenuous exercise. Heat applied for 8 h immediately after strenuous exercise reduced muscle soreness at 24-, 48- and 72-h postexercise. Lower granulocyte and mid-white-cell counts were observed after applying heat, indicating less damage to muscle as well. Faster healing was also directly observed by the reduced fascial edema in subjects who had heat applied after exercise. This was probably due to the increased circulation in the affected area, which promoted faster healing of damage to muscle tissue.

The limitations of this pilot study were:

- The study cohorts had only five subjects per group. The study needs to be repeated with larger groups to enhance statistical power and substantiate the conclusions;
- There is wide variability between subjects, particularly in the cytokine data. A single-gender study with larger group sizes would reduce this variability, and allow more detailed statistical analysis;
- The muscle group was very small. Using larger muscle groups would generate higher concentrations of analytes, help the analysis and reduce inconsistencies;
- In addition to using a larger muscle group, drawing blood directly from the exercised area would give more accurate results since the analytes would be more concentrated. There is a large variation in the level of many blood-borne biomarkers even in large muscle groups. As these biomarkers can be elevated by other body processes, their use has been problematic for DOMS [36]. Here, with a small muscle group, the problem of obtaining reasonable analyte concentrations in the blood was evident. Furthermore, muscle damage is just one theory causing DOMS. If it is due to neuromuscular or connective tissue damage, biomarkers will not correctly show the damage [37]. In fact, up to six mechanisms have been suggested to cause DOMS; it may be a combination of many of these [8].
- In many studies, DOMS lasts for 7 or more days. It might be useful to lengthen the data collection.

This study provides an indication that heat applied after strenuous exercise is effective in both reducing pain and potentially enhancing healing of muscles exercised to the point of DOMS. More comprehensive and detailed studies should be conducted to confirm and quantitate these effects.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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