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original research

Short-Wave Diathermy Pretreatment and Inflammatory Myokine Response After High-Intensity Eccentric Exercise

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Context: Various modalities have been used to pretreat skeletal muscle to attenuate inflammation.

Objective: To determine the effects of short-wave diathermy (SWD) preheating treatment on inflammation and stress markers after eccentric exercise.

Design: Controlled laboratory study.

Setting: University laboratory setting.

Patients or Other Participants: Fifteen male (age = 22 ± 4.9 years, height = 179.75 ± 9.56 cm, mass = 82.22 ± 12.67 kg) college-aged students.

Intervention(s): Seven participants were selected randomly to receive 40 minutes of SWD heat treatment (HT), and 8 participants served as the control (CON) group and rested without SWD. Both groups completed 7 sets of 10 repetitions of a high-intensity eccentric exercise protocol (EEP) at 120% of the 1-repetition maximum (1-RM) leg extension.

Main Outcome Measure(s): We biopsied muscles on days 1, 3 (24 hours post-EEP), and 4 (48 hours post-EEP) and collected blood samples on days 1, 2 (4 hours post-EEP), 3, and 4. We determined 1-RM on day 2 (24 hours post-SWD) and measured 1-RM on days 3 and 4. We analyzed the muscle samples for interleukin 6 (IL-6), tumor necrosis factor α, and heat shock protein 70 and the blood for serum creatine kinase.

Results: We found a group × time interaction for intramuscular IL-6 levels after SWD ($F_{2,26} = 7.13$, $P = .003$). The IL-6 decreased in HT ($F_{1,6} = 17.8$, $P = .006$), whereas CON showed no change ($P > .05$). We found a group × time interaction for tumor necrosis factor α levels ($F_{2,26} = 3.71$, $P = .04$), which increased in CON ($F_{2,14} = 7.16$, $P = .007$), but saw no changes for HT ($P > .05$). No group × time interactions were noted for 1-RM, heat shock protein 70, or creatine kinase ($P > .05$).

Conclusions: The SWD preheating treatment provided a treatment effect for intramuscular inflammatory myokines induced through high-intensity eccentric exercise but did not affect other factors associated with intense exercise and inflammation.

Key Words: inflammation, cytokines, interleukin-6, tumor necrosis factor α

Key Points

- The 1-repetition maximum, heat shock protein 70, and creatine kinase values did not differ between the heat-treatment and control groups after a high-intensity eccentric exercise protocol (EEP).
- The short-wave diathermy preheating treatment resulted in a treatment effect for intramuscular inflammatory myokines induced through a high-intensity EEP.
- Forty minutes of short-wave diathermy 24 hours before an EEP resulted in decreased intramuscular interleukin 6 levels from baseline to 72 hours in the heat-treatment group and increased intramuscular tumor necrosis factor α levels from 48 to 72 hours in the control group.

Eccentric exercises are performed by eliciting a muscle contraction while the muscle lengthens; these exercises are commonly used for strengthening and rehabilitating skeletal muscle. This type of exercise is known for greater production of force and can damage the muscle if performed in excess, as illustrated by changes to the Z lines, myofilament structures, and sarcolemma.[1] Other changes may include an increase in creatine kinase (CK), the onset of delayed-onset muscle soreness, and a reduction in muscular strength.[2,3] High volumes of eccentric exercise have been shown to elicit increases in intramuscular myokines (muscle-derived cytokines) with both proinflammatory and anti-inflammatory properties[4–6] and in neutrophils.[7] Myokines, such as interleukin 6 (IL-6), interleukin 8, and interleukin 15, have been associated with inflammation and healing[8] and, in the case of IL-6, have been shown to regulate other myokines.[9–11] Researchers[12,13] have reported that these myokines are released by the skeletal muscle cells during periods of stress.

Investigators[14–16] have suggested that the use of preheat treatment may influence the physiologic response to exercise. Various modalities, including active warm-ups,[17] warm-water immersion,[18] microwave diathermy,[19]

Table. Experiment Timeline

Time, h	Procedure
0 (baseline)	Blood draw 1
	Muscle biopsy 1
	Short-wave diathermy
24	1-repetition maximum (baseline) 1
	Eccentric exercise protocol
	Blood draw 2 (4 h after eccentric exercise protocol)
48	Blood draw 3
	Muscle biopsy 2
	1-repetition maximum 2
72	Blood draw 4
	Muscle biopsy 3
	1-repetition maximum 3

and ultrasound,[20] have been evaluated for their ability to influence the inflammatory response in skeletal muscle. Skurvydas et al[18] reported decreases in serum CK and soreness in participants who stood in a warm-water bath before a series of box jumps. Similarly, Evans et al[14] observed that short-wave diathermy (SWD) treatment immediately before high-intensity eccentric exercise of the elbow flexors resulted in lower serum CK values and reduced soreness compared with active warm-up techniques. These data suggest that SWD may provide a protective effect against muscle damage when used before eccentric exercise on muscles in the upper body. Results reported by Touchberry et al[21] agree and show an increase in heat shock protein 70 (HSP70) in women after a 20-minute SWD treatment. Heat shock protein 70 is associated with a protective effect on human skeletal muscle[22,23] and is responsible for controlling protein quality and attenuating damage to cell structure.[24–26] It can be induced through intramuscular heating. These data also indicated that SWD may elicit a physiologic protective response against muscle damage from eccentric exercise. However, to date, no researchers have explored the effect of pretreatment with SWD on the inflammatory response after a high-intensity lower body eccentric exercise bout.

Short-wave diathermy uses a frequency of 27.12 MHz and does not overheat the skin with direct contact.[27] It is commonly selected as a modality to treat larger musculoskeletal areas. Short-wave diathermy can increase intramuscular temperatures to a depth of 3 to 5 cm[15] and has been associated with increased flexibility and decreased muscle soreness.[14] The nuances of intramuscular heating by various modalities and the mechanism that may attenuate symptoms after heavy training loads need to be understood. Therefore, the purpose of our study was to examine the effect of SWD preheat treatment on the inflammatory response after a high-intensity eccentric exercise protocol (EEP). Specifically, we assessed the response of HSP70, IL-6, and tumor necrosis factor α (TNF-α) myokines and circulating cytokines that are associated with inflammation. Based on our previous research, we hypothesized that HSP70 would increase after SWD treatment compared with the untreated skeletal muscle. Furthermore, we hypothesized that SWD treatment would attenuate the activation of inflammatory myokines and cytokines in muscle treated by SWD compared with the untreated muscle.

Figure 1. Patient and short-wave diathermy placement during heat treatment.

METHODS*

Participants

Fifteen moderately active men (age = 22 ± 4.9 years, height = 179.75 ± 9.56 cm, mass = 82.22 ± 12.67 kg, body mass index = 25.4 ± 2.7) volunteered for this investigation. Participants reported exercising fewer than 3 times per week and not being involved in a regular resistance or aerobic training schedule. Using the random selection function of Excel (Office 2007; Microsoft Corporation, Redmond, WA), we randomly assigned participants to either the SWD heat treatment (HT; n = 7) or control (CON; n = 8) group.

Participants were instructed to refrain from using anti-inflammatory medications and to avoid physical activity for 24 hours before the first testing session and for the duration of the 4-day protocol. An outline of the study protocol is provided in the Table. All participants provided written informed consent, and the study was approved by the University of Kansas Human Subjects Committee–Lawrence Campus.

The SWD Treatment

We performed all SWD treatments with the ME300 Auto*Therm SWD (Mettler Electronics Corp, Anaheim, CA) using a 22-cm induction coil drum. This unit operates at a frequency of 27.12 MHz with a wavelength of approximately 11 m. The device was calibrated according to the manufacturer's guidelines. The adjustable power analog meter ranges from 0 to 100 units, and the device was set at 35 for each participant. We placed participants in the

* Portions of the Methods section were adapted with permission from Vardiman JP, Jefferies L, Touchberry C, Gallagher P. Intramuscular heating through fluidotherapy and heat shock protein response. *J Athl Train.* 2013;48(3):353–361.

side-lying position with the treatment limb resting at 0° of knee flexion. We placed a single layer of terry cloth towel over the thigh and positioned the drum of the diathermy unit flat on the lateral aspect of the thigh 3 to 5 cm proximal to the first biopsy site (Figure 1). The SWD was paused at 35 minutes so we could clean the area with antiseptic and inject 3 mL of local anesthetic (1% lidocaine) in the vastus lateralis. The drum was replaced for the remaining 5 minutes. At the completion of the 40-minute SWD treatment, intramuscular temperature was determined using a needle thermocouple (Physitemp Instruments, Inc, Clifton, NJ).[28] Touchberry et al[21] reported an inconsistent heat-stress protein response after 20 minutes of SWD treatment using the same device. Therefore, we increased the SWD treatment time to 40 minutes to allow sufficient intramuscular heating for a consistent heat-stress protein response in the tissue. The temperature was analyzed using a microprobe thermometer (model BAT-12; Physitemp Instruments, Inc). This device is calibrated and certified by the manufacturer to be accurate to 0.1°C ± 1% within the readable range of −100°C to 200°C. After determining the intramuscular temperature, we cleaned the insertion site with antiseptic and covered it with a bandage.

The 1-Repetition Maximum and Eccentric Exercise

At 24 hours after the SWD treatment for the EEP, participants reported to the laboratory. They performed a 10-minute warm-up on a cycle ergometer (Lifetime Fitness, Schiller Park, IL). Warm-up sets of the isotonic leg-extension exercise were then performed with 8 to 10 repetitions of 75% of predicted 1-repetition maximum (1-RM) and 3 to 5 repetitions of 85% of predicted 1-RM. The weight was increased by 10 pounds (0.45 kg) after each successful attempt at a 1-RM until a concentric 1-RM was achieved. Participants rested for 2 minutes between 1-RM attempts. Given that the overall aim of the exercise bout was to induce inflammation, the EEP immediately followed the 1-RM testing. The EEP consisted of 7 sets of 10 repetitions of the eccentric leg extensions at 120% of the previously obtained concentric 1-RM. A 2-minute rest was allowed between sets. We raised the resistance arm to full knee extension for each repetition and instructed participants to lower the weight to the stopping point at a controlled rate of 2 to 4 seconds; a metronome (Franz Manufacturing Company, Inc, East Haven, CT) was used to ensure the exercise cadence was correct. All participants repeated the warm-up and 1-RM testing protocol on days 3 and 4 after muscle biopsies and blood collection.

Blood Collection

We collected blood samples using standard venipuncture methods from the cubital fossa of the elbow at 0 (baseline), 24 (4 hours post-EEP), 48, and 72 hours[29] and placed aliquots in serum and plasma microcentrifuge tubes that contained ethylenediaminetetraacetic acid. The samples rested at room temperature for 15 minutes and then were centrifuged at 4°C at 2500*g* for 20 minutes. We removed the serum and stored it at −80°C.

Creatine kinase levels were measured with a CK reagent kit (Pointe Scientific, Inc, Canton, MI). The samples were read at 1, 2, and 3 minutes using a Synergy microplate reader (BioTek Instruments, Inc, Winooski, VT) at an absorbance setting of 340 nm. The average within-sample coefficient of variation (%) for all time points was 2.01% (range = 0%–6.88%).

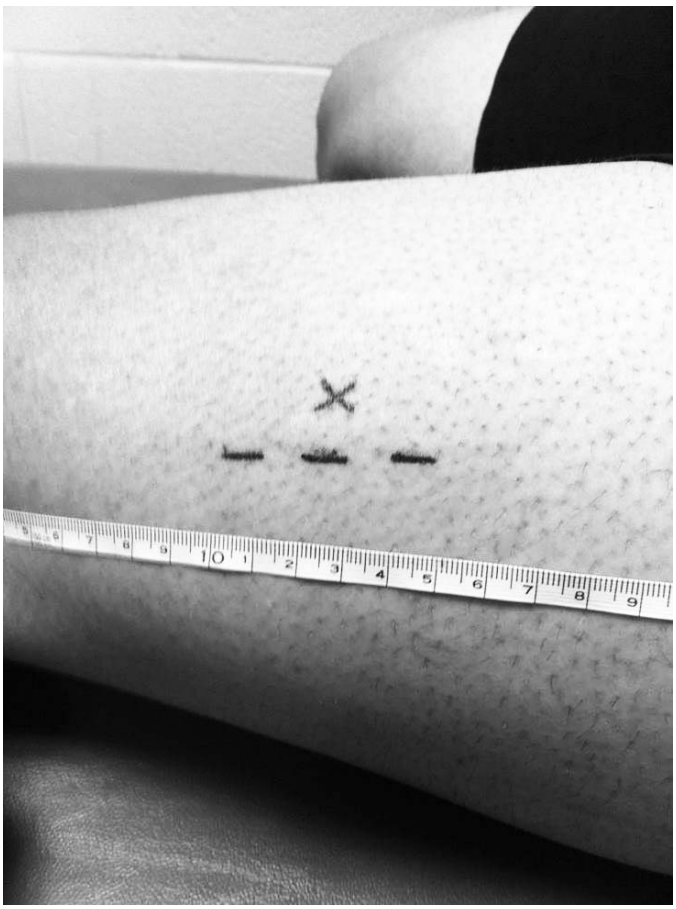

Figure 2. Muscle biopsy incision sites are indicated with horizontal hash marks. The incisions were made distal to proximal, starting at the far left and proceeding to the far right. The thermocouple insertion site was medial to the muscle biopsy sites and is indicated with the X.

Skeletal Muscle Biopsy

We performed 3 biopsies of the vastus lateralis muscle of the randomly selected limb at 0 (baseline), 48, and 72 hours. At the site of each biopsy, participants received a standard antiseptic application followed by an injection of 3 mL of local anesthetic (1% lidocaine). They rested for 5 minutes to guarantee that the area was anesthetized adequately. Using a scalpel with a number 11 blade, we made each incision 0.5 cm wide and 1 cm deep on the lateral aspect of the lower one-third of the thigh (Figure 2). The initial incision and biopsy site was approximately 7 to 10 cm from the lateral joint line of the knee, and each corresponding biopsy was 2 to 3 cm proximal to the previous biopsy and remained within the region of the SWD treatment. All samples were collected with 5-mm Bergström biopsy needles (Pelomi Medical, Albrutslund, Denmark) that provided approximately 100 mg of muscle.[30] All samples were placed in liquid nitrogen and stored at −80°C until analysis.

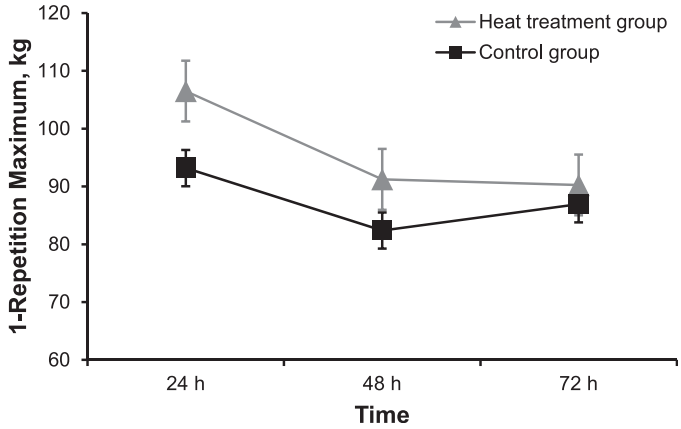

Figure 3. Change from baseline 1–repetition-maximum values at 24 and at 48 hours. Actual means ± standard errors of the means are shown.

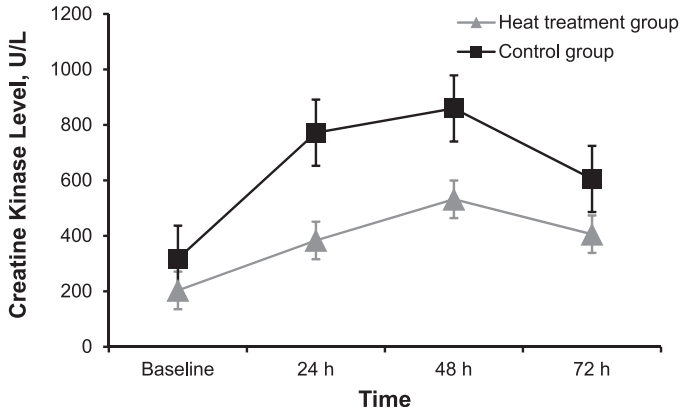

Figure 4. Plasma creatine kinase levels measured at baseline (0 hours) and at 24 (4 hours after the eccentric exercise protocol), 48, and 72 hours. Actual means ± standard errors of the means are shown.

The IL-6, TNF-α, and HSP70 Analysis

The muscle samples were homogenized in cell extraction buffer (Biosource, Carlsbad, CA) using a glass-on-glass tissue grinder. The homogenized samples were centrifuged at 4°C at 2400*g* for 5 minutes, and the supernatant was separated from the pellet. We diluted the samples (1:1000) and determined the total protein concentration using a bicinchoninic acid protein assay (Pierce, Rockford, IL). The samples were diluted with 5× buffer (IL-6) and with 1× buffer (TNF-α and HSP70)[31] and were heated for 3 minutes in a 100°C water bath. Samples were loaded at an equal protein concentration of 80 μL and were run on a 5% stacking and 10% separating gel at 0.05 mA for 1 hour. Proteins were transferred to polyvinylidene difluoride membranes at 0.20 A for 2 hours. Membranes were blocked for 1 hour using 5% nonfat dry milk in Tris-buffered saline on a rocker at room temperature. We incubated membranes overnight on a rocker in 1:1000 IL-6 (Santa Cruz Biotechnology, Inc, Santa Cruz, CA), TNF-α antibody (Cell Signaling Technology, Inc, Beverly, MA), and anti-HSP70 (SPA-810; StressGen Biotechnologies, Ann Arbor, MI) that was normalized to tubulin (Cell Signaling Technology, Inc) using 1% nonfat dry milk in Tris-buffered saline with Tween 20 (200 mmol/L Tris base, 1.37 mEq/L NaCl, pH = 7.6; Thermo Fisher Scientific, Lenexa, KS). After overnight incubation, the membranes were rinsed 4 times for 5 minutes in Tris-buffered saline with Tween 20 before 1-hour incubation in horseradish peroxidase–conjugated secondary antibody and then rinsed again 4 times. Membranes were incubated in chemiluminescence (Amersham ECL Western Blotting detection reagent and analysis system; GE Healthcare UK Limited, Buckinghamshire, United Kingdom). We visualized the IL-6, TNF-α, HSP70, and tubulin protein bands (ProteinSimple, Santa Clara, CA) and quantified the protein bands using densitometry (AlphaView FluorChemHD2 v.3.4.0.0; ProteinSimple).[32,33]

Statistical Analysis

Data are presented as means ± SD. The IL-6 and TNF-α levels are described in logarithmic normalized arbitrary units. We compared the changes in IL-6, TNF-α, and HSP70 levels and 1-RM between the HT and CON participants using a 2 × 3 repeated-measures analysis of variance (ANOVA). We compared the change in CK levels between the HT and CON participants using a 2 × 4 factorial ANOVA. We conducted post hoc 1-way ANOVAs for follow-up analysis of interactions. For all analyses, the α level was set at .05. All statistical analyses were completed using SPSS (version 20.0; IBM Corporation, Armonk, NY).

RESULTS

The 1-RM and CK

We did not observe a group × time interaction for 1-RM between HT and CON ($F_{2,26} = 0.923$, $P = .41$; Figure 3). Similarly, no group × time interaction was found for logarithmic normalized CK levels for HT or CON ($F_{3,39} = 0.55$, $P = .65$; Figure 4). Whereas CK levels were not different between groups, the levels observed after eccentric exercise at each time point were above the recognized normal values (24–195 U/L).[34] The increase in CK levels for both groups suggested that the EEP was a sufficient insult to cause muscle damage.

The IL-6, TNF-α, and HSP70

A group × time interaction was seen for IL-6 levels ($F_{2,26} = 7.13$, $P = .003$). Post hoc analysis showed that IL-6 levels decreased from baseline (1.40 ± 3.01) to 72 hours (0.97 ± 3.06) in the HT group ($F_{2,12} = 4.85$, $P = .03$) but did not change in the CON group ($F_{2,14} = 2.43$, $P = .13$; Figure 5). A group × time interaction was seen for TNF-α ($F_{2,26} = 3.71$, $P = .04$). Post hoc analysis showed TNF-α levels increased from baseline (0.53 ± 2.70) to 48 hours (0.65 ± 2.79) and 72 hours (0.81 ± 2.61) in the CON group ($F_{2,14} = 7.16$, $P = .007$; Figure 6). We found no changes in TNF-α levels for the HT group ($F_{2,12} = 1.03$, $P = .38$). We observed no group × time interaction for intramuscular levels of HSP70 ($F_{2,22} = 1.08$, $P = .36$; Figure 7).

DISCUSSION

The aim of this investigation was to examine the influence of SWD preheating treatment on skeletal muscle before a single bout of high-intensity eccentric exercise to

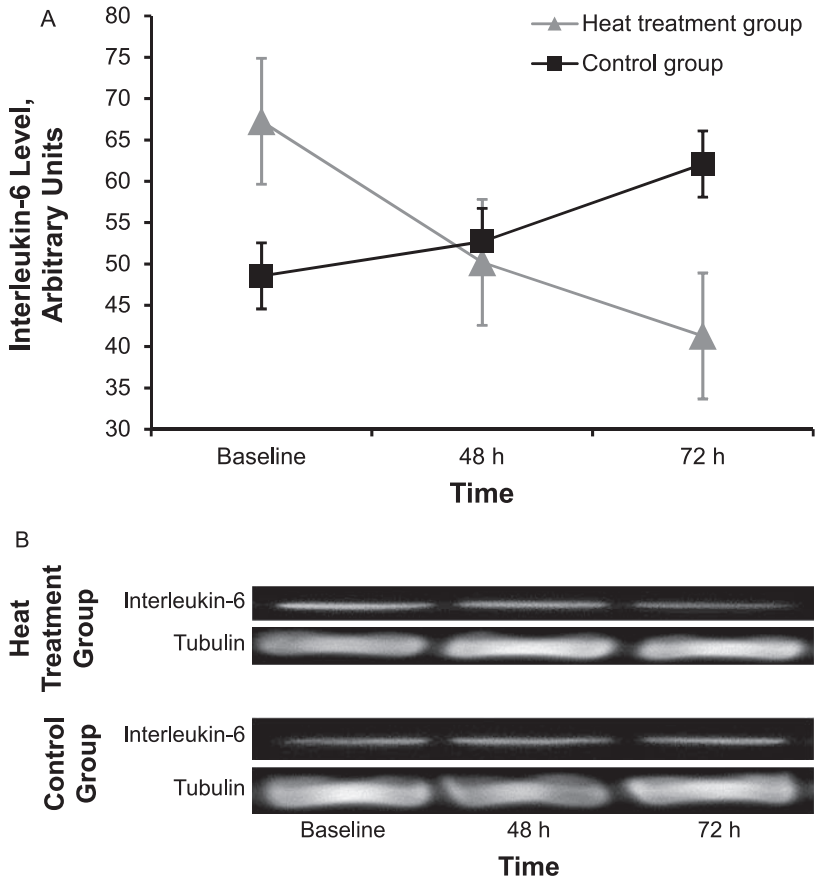

Figure 5. A, Interleukin 6 levels measured at baseline (0 hours) and at 48 and 72 hours. Values represent interleukin 6 normalized to tubulin in arbitrary units (mean ± standard error of the mean). B, The interleukin 6 and tubulin protein bands for the heat treatment and control groups.

specifically identify its effect on 1-RM strength, intramuscular inflammatory myokines and HSP70 levels, and circulating CK levels. Our study revealed that 40 minutes of SWD 24 hours before an eccentric exercise bout resulted in decreased intramuscular IL-6 levels in HT participants after SWD at 72 hours compared with baseline, whereas we found no change in the levels for CON participants. We also observed that intramuscular TNF-α levels increased at 48 and 72 hours in the CON group but no changes occurred in the HT participants. No changes were seen in 1-RM value, intramuscular HSP70 level, or circulating CK level after SWD pretreatment in either group.

Sprenger et al[35] showed that circulating IL-6 values were elevated immediately postexercise and gradually declined over time. This finding is contrary to the intramuscular IL-6 response that we observed. Our data showed that the intramuscular IL-6 content decreased mildly at 24 hours post-EEP (48-hour time point) and at 48 hours post-EEP (72-hour time point) in the HT group. However, we noted no changes in the IL-6 levels for the CON group. Bruunsgaard et al[36] reported that muscle damage resulted in a pronounced increase in plasma IL-6 2 hours after eccentric exercise on a bicycle ergometer; however, in a recent examination of the systemic IL-6 response to eccentric-resistance exercise, Philippou et al[37] observed that serum IL-6 and myoglobin (another marker of muscle damage) peaked at 48 hours postexercise. These investigators presented conflicting results concerning the IL-6 response after intense-exercise protocols. The use of a cycle ergometer and an isokinetic knee-extensor protocol could be factors in the different responses between these studies. The TNF-α levels increased at 48 and 72 hours for CON participants; no changes were observed in the HT participants. Several authors[38–47] have examined the effects of eccentric exercise on TNF-α expression in skeletal muscle and have reported mixed results. Compared with our data, Liao et al[41] and Nieman et al[42–44] showed much larger changes in TNF-α levels after eccentric exercise. Thus, the dissimilarities in TNF-α response may be due to the amount of damage that occurred from the eccentric exercise. In contrast to our data, Buford et al[38] showed an increase in CK levels after 45 minutes of downhill treadmill running but no changes in TNF-α expression. We observed an increase in TNF-α at 48 and 72 hours in the CON group but not in the HT group. To our knowledge, no researchers have examined the effects of pre-exercise HT on intramuscular levels of TNF-α. Our results suggest that SWD before eccentric exercise may have a treatment effect that attenuates the expression of TNF-α after an intense bout of eccentric exercise. Contrary to our results, Welc et al[48] reported an increase in TNF-α after HT of skeletal muscle in mice. However, their study had no exercise component, making direct comparison difficult.

No increase occurred in intramuscular HSP70 levels in the HT and CON groups. Thus, SWD appears to be

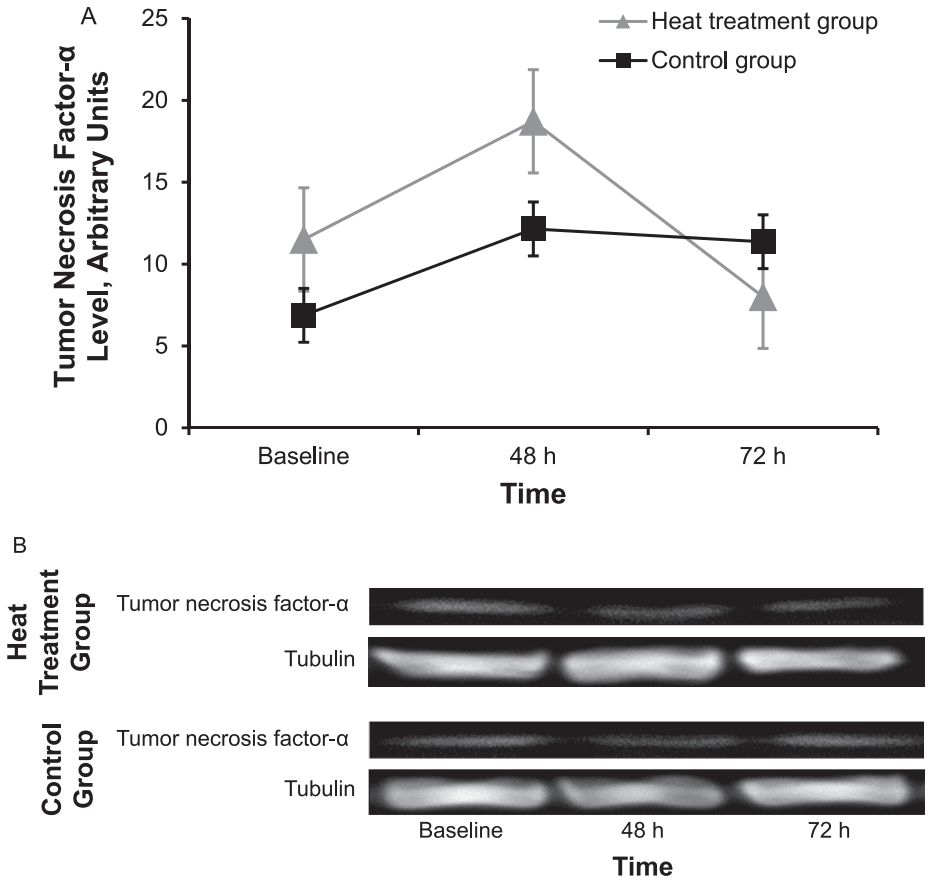

Figure 6. A, Tumor necrosis factor α levels measured at baseline (0 hours) and at 48 and 72 hours. Values represent tumor necrosis factor α normalized to tubulin in arbitrary units (mean ± standard error of the mean). B, The tumor necrosis factor-alpha symbol and tubulin protein bands for the heat treatment and control groups.

ineffective in inducing HSP70. These data are in contrast to the results of other investigators who have shown increases in HSP70 using various clinical modalities, including SWD, in both humans and rodents.[20,21,32,33] The lack of HSP70 response after SWD treatment is not that remarkable, as this has been reported in males.[21] Researchers also have reported no changes in HSP70 after fluidotherapy[32] or continuous or pulsed ultrasound.[49] The duration of SWD treatment that we used would be considered extensive in the clinical setting. Despite this duration, the intramuscular temperature measured after 40 minutes of treatment was slightly greater than 38°C. We did not monitor intramuscular temperatures throughout the study, which prevented us from verifying that higher temperatures were not achieved. However, previous findings have indicated that a 1°C temperature decay occurs 7.65 ± 4.96 minutes after the end of a 20-minute SWD treatment.[50] The results of this study and previous studies suggest that it is extremely difficult using standard treatment modalities to increase the temperature of human skeletal muscle sufficiently to induce HSP70.[21,32] Nussbaum and Locke[20] suggested that heat shock proteins are more likely to be induced in response to mechanical disturbances to the cells than to increased temperature in skeletal muscle. This does not explain why increases were not seen in HSP70 post-EEP or post-SWD. Whereas we did not measure muscle temperature after the EEP, the temperature was unlikely to have exceeded 40°C, the minimal temperature shown to induce HSP70. Authors of most studies have observed increases in HSP70 after eccentric exercise in human skeletal muscle[51,52] and in mouse extensor digitorum longus muscle[53]; however, Touchberry et al[33] reported no changes in HSP70 after eccentric exercise in rats. Another likely mechanism for the increased HSP70 may be a cytoprotective response to the eccentric-exercise–induced myofibrillar damage.[54,55]

We measured the serum CK levels and found no group × time interaction. In contrast to these data, Nosaka et al[19] reported that 15 minutes of microwave diathermy 1 day before eccentric exercise of the elbow flexors resulted in less CK activity and strength losses in the HT group at 1, 2, 3, and 4 days postexercise. An increase in intramuscular temperatures to levels that would be considered therapeutic occurred in both studies,[19,56] but different muscle groups were tested and different devices were used. The elbow flexors tested by Nosaka et al[57] represent smaller musculature than the thigh musculature we tested. Given that the elbow flexors are a smaller muscle group, the level of muscle damage could have been more pronounced than what we observed in the larger thigh musculature. Furthermore, the smaller cross-sectional area of the elbow-flexor group possibly allowed more thorough heating of the tissues, contributing to results that we did not observe.

Evans et al[14] reported that 10 minutes of SWD may provide a protective effect when used immediately before

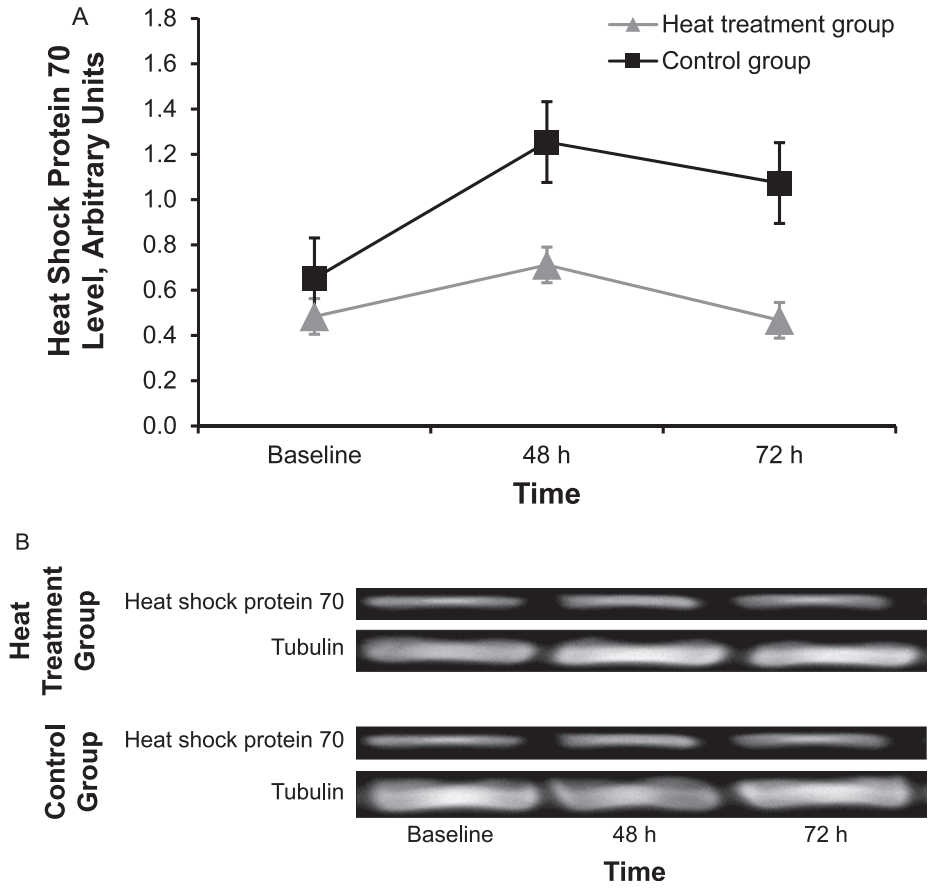

Figure 7. A, Heat shock protein 70 levels measured at baseline (0 hours) and at 48 and 72 hours. Values represent heat shock protein 70 normalized to tubulin in arbitrary units (mean ± standard error of the mean). B, The heat shock protein 70 and tubulin protein bands for the heat treatment and control groups.

eccentric exercise of the elbow flexors. They also noted that individuals who completed an active warm-up (concentric contractions) exhibited a greater CK response after the EEP. These participants also presented with greater inflammation, as determined by circumference measurements, than those who completed diathermy treatment. Overall, the HT group exhibited changes in muscular strength, soreness, or damage when compared with the active–warm-up and no–warm-up groups. Evans et al[14] cautioned readers to consider these findings preliminary because of the small number of HT participants. Whereas researchers have exhibited different results than our investigation, differences also existed in the methods in each study. For example, Evans et al[14] performed SWD treatment immediately before the eccentric exercise as opposed to 24 hours before exercise. The volume of the exercise-testing protocols differed among studies; participants performed 24 and 50 eccentric contractions in the studies of Nosaka et al[19] and Evans et al,[14] respectively, and 70 eccentric contractions in our study.

When examining strength using 1-RM values for leg extensions, we did not find a group × time interaction after the EEP and SWD. Our participants were considered moderately active and reported that they exercised fewer than 3 times per week and were not involved in a regular resistance or aerobic training schedule. Researchers[21,32] examining the influence of HT on the response to muscle damage have studied untrained individuals. Whereas we found no differences in intramuscular HSP70, CK, or 1-RM between the HT and CON groups, we found a difference in IL-6 and TNF-α levels. These data, combined with sufficient anecdotal data referencing muscle soreness from the present EEP, indicated a sufficient exercise stimulus to cause a change. To limit any question of the participants' physical conditioning, future researchers should study untrained individuals in future investigations.

CONCLUSIONS

Our study did not reveal differences in 1-RM, CK, or HSP70 values between the HT and CON groups after an EEP. Future research on this topic, specifically focusing on the role of treatment timing on inflammation, could provide useful insight into proper warm-up protocols for optimal performance and injury prevention.

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