Intramuscular Heating Through Fluidotherapy and Heat Shock Protein Response

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Context: Therapeutic modalities that can increase intramuscular temperature commonly are used to treat injuries in the clinical setting. Researchers recently have suggested that the physiologic changes occurring during an increase in temperature also could provide a cytoprotective effect for exercise-induced muscle damage.

Objective(s): To determine if the Fluidotherapy treatment increases the inducible expression of heat shock protein (HSP), to identify the rate of heating that occurs in the lower extremity with Fluidotherapy treatment, and to evaluate the relationship between the inducible expression of HSP and temperature.

Design: Controlled laboratory study.

Setting: Laboratory.

Patients or Other Participants: Six male (age = 21.67 ± 1.63 years, height = 180.09 ± 4.83 cm, mass = 87.60 ± 10.51 kg) and 6 female (age = 24.60 ± 4.59 years, height = 151.05 ± 35.76 cm, mass = 55.59 ± 14.58 kg) college-aged students.

Intervention(s): One lower extremity was randomly selected to receive the heat treatment, and the other extremity received no treatment.

Main Outcome Measure(s): We measured intramuscular temperature every 10 minutes, determining peak intramuscular temperature by 2 identical sequential measurements, and we analyzed the time to peak temperature. We analyzed the amount of HSP70 expression and HSP27P:T (ratio of HSP27

to the total HSP27 expression) in the gastrocnemius and soleus muscles and measured baseline skinfold thickness and estradiol levels.

Results: Fluidotherapy increased intramuscular temperature by $5.66 \pm 0.78^{\circ}\text{C}$ ($t_{11} = 25.67$, $P < .001$) compared with baseline temperature, with a peak temperature of $39.08^{\circ}\text{C} \pm 0.39^{\circ}\text{C}$ occurring at 84.17 ± 6.69 minutes. We did not find a heat treatment effect for HSP70 or HSP27P:T in the gastrocnemius or soleus muscles ($P > .05$). Peak temperature and the percentage change of HSP70 were positively correlated for the gastrocnemius and soleus muscles ($P < .05$). We found no other correlations for skinfold thickness, sex, or estradiol levels ($P > .05$). No effect of sex for skinfold thickness or estradiol levels at baseline was discovered ($P > .05$).

Conclusions: This Fluidotherapy protocol increased the intramuscular temperature to a therapeutic level; however, it did not stimulate inducible HSP70 or HSP27P:T in the soleus and gastrocnemius muscles regardless of sex or skinfold thickness. These data confirmed that Fluidotherapy is an effective heating modality but suggested it is not an effective method for stimulating an HSP response in the lower limb.

Key Words: heat shock protein 27, heat shock protein 70, exercise-induced muscle damage

Key Points

- Heat shock protein concentrations did not increase with Fluidotherapy treatment in the lower extremity.
- Therapeutic levels of temperature within commonly accepted treatment times occurred with Fluidotherapy.
- Fluidotherapy effectively increased the temperature of extremity tissue.

Therapeutic heat treatments using various heat modalities have been applied in clinical, athletic, and research settings to target soft tissue healing,^{1,2} increase range of motion,¹ and reduce pain.^{1–4} The efficacy of a thermal modality is determined by its ability to adequately increase temperatures in a targeted tissue to achieve the outcome goal. An increase of 1°C to 5°C provides analgesic and cellular responses that aid in positive treatment outcomes.^{5,6} The physiologic changes that occur with heat treatment (HT) include vasodilatation, increased cellular metabolism, and induction of phagocytosis.⁷ One related but underexplored mechanism of tissue healing from HT modalities is the induction of heat shock proteins (HSP).

The family of stress proteins known as HSP are cytoprotective agents that promote cell survival during stressors.^{8,9} The HSPs commonly are referred to as the chaperone proteins because of their abilities to control protein quality and decrease harm to the cellular structures.^{10–14} In particular, HSP70 assists in protein folding and is involved in stabilization and repair of protein damaged due to thermal or oxidative stress.¹⁵ The HSP family also has been linked to reductions in oxidative stress that may influence muscle hypertrophy,^{16–18} attenuation of atrophy from immobilization,^{17,19,20} and prevention of apoptosis.^{21–24} For example, during a reloading protocol in which the limbs were immobilized, Selsby et al¹⁷ found that HSP expression was approximately 25% greater and regrowth of atrophied tissue was approximately 30%

greater in the heating than nonheating group. Furthermore, the phosphorylation of HSP27 is one of the first steps to help stabilize filamental proteins after oxidative stress.²⁵ Therefore, by inducing HSP and the corresponding protection at the cellular level, researchers have theorized that the extent of secondary injury could be limited. Different approaches, including exercise,^{14,26} ischemia,^{9,27} hyperthermia^{8,13,14,28,29} and mechanical stress,³⁰ have been used to induce HSP expression. However, few researchers have investigated the use of therapeutic modalities capable of producing sufficient heating or mechanical stress for induction of HSP in skeletal muscle. The modalities that have been investigated include ultrasound,³⁰ microwave diathermy,³¹ and shortwave diathermy.³² The HSP induction from ultrasound is likely due to mechanical disruption rather than an increase in temperature.³⁰ Recently, data have shown that shortwave diathermy, a commonly used therapeutic modality, potentially can induce HSP expression in human tissue.³² However, further studies need to be conducted to elucidate the HSP-inducing properties of microwave and shortwave diathermy. Researchers have assumed that another commonly used therapeutic modality, Fluidotherapy (Chattanooga Group, Inc, Hixson, TN), can treat large surface areas, but no one has evaluated its ability to increase intramuscular temperature or to induce HSP expression.

Fluidotherapy circulates a dry cellulose medium (Cellex Dry Heat Medium; Chattanooga Group, Inc) with temperature-regulated warmed air that provides uniform heat transfer across the surface of the treatment area. This dry-source convection heat transfer provides tactile stimulation that helps prevent potentially painful high temperatures²⁻⁴ while adequately heating the treatment area. The width and depth of the large, single-extremity unit enable treatment of upper and lower extremities, including the elbow and knee. No one has determined if Fluidotherapy can comfortably reach temperatures and depth of tissues to increase an inducible HSP response. Therefore, the primary purpose of our study was to determine if the Fluidotherapy treatment increases the inducible expression of HSP. The secondary purposes were to identify the rate of heating that occurs in the lower extremity with Fluidotherapy treatment and to evaluate the relationship between the inducible expression of HSP and temperature.

METHODS

Participants

Six moderately active men (age = 21.67 ± 1.63 years, height = 180.09 ± 4.83 cm, mass = 87.60 ± 10.51 kg) and 6 women (age = 24.60 ± 4.59 years, height = 151.05 ± 35.76 cm, mass = 55.59 ± 14.58 kg) volunteered for this investigation. We defined *moderately active* as participating in no more than 1 hour of physical activity 4 days each week. Each participant was involved in 3 to 4 bouts of exercise per week that lasted 30 to 60 minutes each. Exclusion criteria for all participants were obesity (body mass index ≥ 28 kg/m²), known metabolic disease, cardiovascular disease, hypertension, circulatory disease, previous episode of heat-related illness, and diagnosis of any condition that required suppression of a fever. The participants completed a medical history questionnaire and

a prebiopsy screening and were advised of the risks and benefits associated with this study. They provided written informed consent, and the study was approved by the University of Kansas Human Subjects Committee—Lawrence Campus.

Before testing, participants were instructed to refrain from using any anti-inflammatory medications and to avoid physical activity for 48 hours before and throughout the study. When they arrived at the laboratory, participants had their blood drawn to evaluate estradiol levels, and skinfold thickness measurements were taken from the middle one-third of the leg. The women were tested within the first 7 days of the menses cycle (early follicular phase) to mitigate the amount of estradiol that would be present during the investigation.³³ Each participant's lower extremities were identified as HT or control (CON) legs using the Random Selection function on Excel (Office 2007; Microsoft Corporation, Redmond, WA). Biopsies of the muscles were performed from the lateral aspect of the gastrocnemius and soleus muscles using percutaneous needles in the CON leg at baseline and from the HT leg 24 hours after HT. This time was selected based on research indicating HSP expression was induced 24 hours after HT.³²

The HT procedure was performed using a Fluidotherapy 210 full-extremity unit. We evaluated intramuscular temperature with an indwelling thermocouple that we inserted 3 cm into the muscle under local anesthesia. Intramuscular temperature was recorded every 10 minutes, and peak temperature was determined by 2 identical and sequential temperature measurements. After we determined peak temperature, the HT ended, the participant was removed from the Fluidotherapy unit, and the thermocouple was removed.

Heat Treatment

The Fluidotherapy unit was set at 48.9°C using the digital temperature control on the device. Throughout the HT, we observed temperature fluctuations from 47.2°C to 51.1°C on the digital display. To measure the intramuscular temperature, we injected each participant with 3 mL of local anesthetic (1% lidocaine) in the lateral aspect of the lower one-third of the calf 5 minutes before insertion of the needle thermocouple (Physitemp Instruments, Inc, Clifton, NJ; Figure 1). The thermocouple was inserted via a 20-mm needle into the gastrocnemius-soleus muscle complex to a depth of 3 cm.^{34,35} We covered the insertion site of the thermocouple with an occlusive dressing to prevent exposure to the circulating medium in the device. The participant was seated with his or her back slightly extended and the treatment extremity in the Fluidotherapy device to maximize tissue surface area exposure to the media (Figure 2). We analyzed the temperature using a microprobe thermometer (model BAT-12; Physitemp Instruments, Inc). This device was calibrated and certified by the manufacturer for accuracy to $0.1 \pm 1\%$ within the readable range of -100°C to 200°C . We recorded the temperature every 10 minutes and determined peak temperature by identical and consecutive readings. When this temperature was reached, the heat treatment stopped, and the participant was removed from the HT. After the participant was removed, we removed the thermocouple,

Figure 1. Incision and temperature probe locations. The hash marks on the far right and left indicate the incision locations approximately 5 to 6 cm apart. The X in the center of the hash marks indicates the temperature probe location in the calf.

cleaned the insertion site with antiseptic, and covered it with a self-adhesive bandage.

Skeletal Muscle Biopsy

We performed biopsies of the muscles at baseline from the CON extremity and 24 hours after Fluidotherapy from the HT extremity. At the site of each biopsy, each participant received standard antiseptic application and then an injection of 3 mL of local anesthetic (1% lidocaine). The participant then rested for 5 minutes to ensure the area was anesthetized sufficiently. Using a scalpel with a number 11 blade, we made 2 incisions approximately 5 cm apart, 0.5 cm wide, and 1 cm deep at the lateral aspect of the lower one-third of the leg (Figure 1). For the HT extremity, the incisions were made 2 to 3 cm proximal to the thermocouple insertion site for the biopsies of the gastrocnemius muscle and 2 to 3 cm distal to the thermocouple insertion site for the biopsy of the soleus muscle (Figure 1). All samples were collected using

percutaneous needles (ProMag Ultra; Angiotech, Vancouver, BC) that provided 8 to 19 mg of muscle sample from both muscles in the CON and HT extremities for each participant for analysis. Next, we placed muscle sections in liquid nitrogen and stored them at -80°C until analysis.

HSP70 and HSP27P:T Analysis Using SDS-PAGE and Western Blotting

The muscle samples were homogenized in cell extraction buffer (Invitrogen, Carlsbad, CA) containing protease and phosphatase inhibitors (Thermo-Scientific, Waltham, MA) using a glass-on-glass tissue grinder. We centrifuged the homogenate at 4°C at 2400g for 5 minutes, then we separated the supernatant from the pellet, diluted the sample (1:525), and determined total protein concentration using a bicinchoninic acid protein assay (Pierce, Rockford, IL). The remaining supernatant was removed and placed into individual microcentrifuge tubes and stored at -80°C until further analysis. The muscle homogenates were diluted with sample buffer³⁶ and heated for 5 minutes in a 100°C water bath. We loaded samples at an equal protein concentration of 80 μL in duplicate and ran them on a 10% separating gel in a Mini-PROTEAN 3 electrophoresis system (Bio-Rad, Hercules, CA) at 20 mA for 20 minutes, then at 50 mA for 60 minutes. Next, proteins were transferred to a polyvinylidene fluoride membrane at 200 mA for 90 minutes. Membranes were blocked using 5% nonfat dairy milk in tris-buffered saline with 0.1% Tween 20 (Thermo Fisher Scientific, Lenexa, KS; TBST; 200 mmol/L tris base, 1.37 mEq/L NaCl, pH = 7.6) at room temperature for 60 minutes. We incubated membranes overnight with the primary antibodies (1:1000): anti-HSP70 (SPA-810; Stressgen, Ann Arbor, MI), which was normalized for tubulin; anti-HSP27 (SPA-800; Stressgen); and anti-HSP27 phosphorylated (HSP27P; SPA-523; Stressgen) in TBST/1% nonfat dry milk. After overnight incubation, the membranes were rinsed 3 times for 10 minutes in TBST before the final 60-minute incubation in secondary antibody (SAB-100; Stressgen). The membranes were rinsed 3 times for 10 minutes in TBST before incubation with chemiluminescence (GE Healthcare, Piscataway, NJ). We visualized and quantified the protein bands using densitometry (Alpha Innotech, San Leandro, CA).³² Images were normalized for background, and repeated densitometry measurements were averaged for each band of interest. After measuring HSP27P, we stripped and reprobed the membranes to measure the total HSP27 from the samples. The HSP27P:T represents the ratio of HSP27P to the total HSP27 expression.

Blood Collection

We collected blood samples from the forearm antecubital area of participants and placed aliquots in serum and plasma centrifuge tubes containing ethylenediaminetetraacetic acid. The samples rested at room temperature for 15 minutes and then were centrifuged at 4°C and 2500g for 20 minutes. The serum was removed and immediately stored at -80°C .

Estradiol levels were measured with an estradiol enzyme immunoassay kit (model 582251; Cayman Chemical Company, Ann Arbor, MI). It had a Synergy HT Multi-

Figure 2. Participant positioning in the Fluidotherapy device. Each participant was placed in a reclined position in the Fluidotherapy device until his or her peak temperature was reached.

Mode Microplate Reader (BioTek Instruments, Inc, Winooski, VT) with an absorbance setting of 410 nm.

Skinfold Measurement

We measured the skinfold thickness of the calf 3 times to the nearest millimeter with a Lange skinfold caliper (Cambridge Scientific Industries, Inc, Cambridge, MD). The measurements were taken in the middle one-third of the calf muscle of the treatment limb. The mean of 3 measurements was reported.

Statistical Analysis

Data are presented as means $\pm$ standard deviations (SDs). We measured changes in temperature and differences in HSP expression and concentrations using a paired-samples *t* test with an α level set at .05. Sex differences in levels of estradiol and skinfold thickness were measured using 1-way analysis of variance (ANOVA). Sex differences in HSP70 expression and HSP27P:T concentration in the CON and HT extremities were measured with 2-way repeated-measures ANOVA with an α level set at .05. We used Pearson product moment correlation analysis to assess relationships between the following in both muscle types: sex and percentage difference in HSP expression (HSP-DIFF), peak temperature and HSP-DIFF, skinfold thickness

and HSP-DIFF, estradiol levels and HSP-DIFF, peak temperature and sex, and peak temperature and skinfold thickness. We used SPSS (version 16.0; IBM Corporation, Armonk, NY) for all statistical analyses.

RESULTS

Intramuscular Temperature

Fluidotherapy heat treatment increased intramuscular temperature by $5.66^{\circ}\text{C} \pm 0.78^{\circ}\text{C}$ to $39.08^{\circ}\text{C} \pm 0.39^{\circ}\text{C}$ ($t_{11} = 25.67$, $P < .001$). The average heat treatment lasted 84.17 ± 6.69 minutes (Table 1). Intramuscular temperature increased by $1.83^{\circ}\text{C} \pm 0.51^{\circ}\text{C}$ at 10 minutes and by $3.39^{\circ}\text{C} \pm 0.55^{\circ}\text{C}$ at 20 minutes (Figure 3). Peak temperature was reached as early as 70 minutes, but all participants had reached peak temperature by 90 minutes of treatment.

HSP70 and HSP27P:T

We found no overall interactions between sex and HSP70 expression in the gastrocnemius ($F_{1,10} = 0.01$, $P = .93$) or the soleus ($F_{1,10} = 0.38$, $P = .55$) or HSP27P:T concentrations in the gastrocnemius ($F_{1,10} = 1.78$, $P = .21$) or the soleus ($F_{1,10} = 1.29$, $P = .28$; Table 2). We observed no differences in HSP70 expression between the HT extremity and CON extremity in the gastrocnemius (t_{11}

Table 1. Temperature Change, Peak Temperature, Time to Peak Temperature, Skinfold Thickness, and Estradiol by Sex (Mean $\pm$ SD)

Sex	Temperature Change, $^{\circ}\text{C}$	Peak Temperature, $^{\circ}\text{C}$	Time to Peak Temperature, min	Skinfold Thickness, mm	Estradiol, pg/mL
Male (n = 6)	5.75 $\pm$ 0.83	38.97 $\pm$ 0.29	83.33 $\pm$ 5.16	11.10 $\pm$ 1.87	46.44 $\pm$ 8.92
Female (n = 6)	5.58 $\pm$ 0.80	39.21 $\pm$ 0.47	85.00 $\pm$ 8.37	14.47 $\pm$ 3.88	39.07 $\pm$ 11.15

= 0.65, $P = .55$) or soleus ($t_{11} = 1.06$, $P = .31$; Figure 4) or HSP27P:T concentrations in the gastrocnemius ($t_{11} = 0.66$, $P = .53$) or soleus ($t_{11} = 1.60$, $P = .14$; Figure 5).

Estradiol and Skinfold Measurement

We noted no differences in estradiol levels ($F_{1,10} = 1.60$, $P = .24$) and skinfold measurements ($F_{1,10} = 3.67$, $P = .08$) between the male and female participants (Tables 1 and 2).

Correlational Data

We found a correlation ($P < .05$) between peak temperature and the percentage difference in HSP70 expression in both the gastrocnemius and soleus muscles. However, we did not find correlations between sex or skinfold thickness and the difference between CON and HT expressions of inducible HSP70 and HSP27P:T expressions in either the gastrocnemius or soleus muscles (Table 2).

DISCUSSION

Intramuscular Temperature

To achieve the therapeutic benefits of a heat treatment, intramuscular temperature needs to increase by 1°C to 5°C .³⁷ Similar thermal modality treatments, such as a warm whirlpool, commonly are used in the clinical setting and have a recommended treatment time from 15 to 20 minutes.³⁷ Garrett et al³⁴ compared the tissue temperature change after a 20-minute diathermy and ultrasound protocol and found shortwave diathermy treatment heated muscle tissue more than a 20-minute ultrasound treatment to the

same diameter of tissue. Their data indicated that Fluidotherapy adequately increased intramuscular temperature at a depth of 3 cm to the accepted therapeutic range within the average 20-minute treatment time. Furthermore, they showed that Fluidotherapy can increase intramuscular temperatures to the upper levels recommended. As shown in Figure 3, tissue temperature increased by almost 2°C after 10 minutes of treatment and incrementally increased to $3.40^{\circ}\text{C} \pm 0.55^{\circ}\text{C}$ by 20 minutes. Although standard modality treatments do not continue for 30 to 40 minutes, these data showed that vigorous heating can be achieved with Fluidotherapy if treatment is continued to 40 minutes.

Using these data, we developed a Fluidotherapy heating curve (Figure 6). This curve should be used as a recommendation for treatment times for the lower extremity. More data are needed for recommended modality set-up and treatment times for the elbow, forearm, wrist, and hand.

HSP70 and HSP27P:T

Although our data showed Fluidotherapy treatment can increase intramuscular temperatures to the recommended therapeutic level, inducible HSP expression did not increase. Researchers generally have considered and reported that the up-regulation of HSP proteins in cells is induced when the body temperature is elevated by 3°C to 5°C ³⁸ or above 40°C in animal models.³⁹ However, Nussbaum and Locke⁴⁰ showed that the increases in HSP from repeated ultrasound treatments were not due to increased temperature but likely due to the mechanical properties introduced to the cell. As discussed, shortwave diathermy has been shown to increase HSP expression in human skeletal muscle.³² However, Touchberry et al³² did

Figure 3. The mean change in temperature at all time points. Temperatures of participants were not monitored after peak temperature was reached. Therefore, the number of participants for the 80-minute time point is 11 and for the 90-minute time point is 6.

Table 2. Pearson Product Moment Correlation Matrix for Percentage Difference in Heat Shock Protein 70 and Heat Shock Protein 27 by Sex, Peak Temperature, and Skinfold Thickness

Variable	r^2 Values			
	Heat Shock Protein 70 Soleus	Heat Shock Protein 70 Gastrocnemius	Heat Shock Protein 27 (P:T) Soleus	Heat Shock Protein 27 (P:T) Gastrocnemius
Sex	0.04	0.00	0.15	0.15
Peak temperature, °C	0.28 ^a	0.24 ^b	0.013	0.07
Skinfold thickness, cm	0.00	0.01	0.02	0.02

^a Indicates $P = .04$.

^b Indicates $P < .05$.

not collect intramuscular temperatures during the short-wave diathermy treatments, so we cannot conclude that the induction of HSP was related directly to increased muscle temperature.

Goto et al⁴¹ reported similar temperature increases to approximately 38°C in the vastus lateralis during repeated bouts of heat treatment with a steam-generating sheet. As did Goto et al,⁴¹ we found Fluidotherapy treatment resulted in an increase in peak temperature but did not produce an HSP response in the gastrocnemius or soleus muscle 24 hours posttreatment. Therefore, these data suggest that modalities capable of producing higher temperatures in shorter amounts of time or that render mechanical changes to the tissues should be used to induce HSP expression.

Our findings did not indicate an effect of sex in HSP response from the Fluidotherapy treatment. Researchers have suggested that no sex differences exist between basal levels of HSP70 and HSP27p in rodents,⁴² regardless of time in the estrous cycle, or in HSP responses to low-intensity or high-intensity eccentric exercise in human

skeletal muscle.^{43,44} In contrast, researchers have shown that estrogen may decrease the HSP70 expression in skeletal muscle⁴⁵ after heat shock³² and exercise for male but not female participants.⁴⁶ To control for intraparticipant variation of estradiol levels in female participants, we collected all data in the first 7 days of the menstrual cycle. Interestingly, we found no difference in estradiol levels between men and women.

Investigators³² have suggested that muscle mass may play a role in improving the contact area of a modality, increasing the efficacy of the treatment. Our findings indicate that this is not applicable with a convection modality, such as Fluidotherapy. The infrared thermal energy that Fluidotherapy produced possibly was not adequate to penetrate to the skeletal muscle tissue in participants who had thicker adipose tissue layers. Although we found an increase (5.66°C) in muscle temperature and correlations ($P < .05$) between peak temperature and HSP70, we saw no difference between men and women.

Figure 4. The pretest and posttest HSP70 levels in the gastrocnemius and soleus muscles for the heat treatment and control extremities by sex. Abbreviations: F, females; Gastroc, gastrocnemius; M, males.

Figure 5. The pretest and posttest HSP27 phosphorylated antibodies to HSP27 total expression levels in the gastrocnemius and soleus muscles for the heat treatment and control extremities by sex. Abbreviations: F, females; Gastroc, gastrocnemius; M, males.

Therapeutic modalities have been shown to penetrate approximately 2.5 cm when treatment times were extended. We saw no difference between skinfold measurements in men and women and no correlation between skinfold thickness and peak temperature. Our data suggested that sex and skinfold thickness did not affect the thermal

treatment efficacy of Fluidotherapy and that Fluidotherapy effectively increased intramuscular temperatures. Although we found correlations between the peak temperature and percentage change in HSP70 in the gastrocnemius and soleus muscles, our data indicated that Fluidotherapy did not induce a skeletal muscle HSP response.

Figure 6. Fluidotherapy heating curve. A pictorial description of the intramuscular heating for the lower extremity in a Fluidotherapy device.

CONCLUSIONS

Fluidotherapy treatment did not result in increased HSP concentrations in the lower extremity. Although we noted a correlation between peak temperature and percentage change in HSP70 and an increase in intramuscular temperature of approximately 5.6°C, we did not find an increase in HSP proteins. However, our data showed that therapeutic levels of temperature within commonly accepted treatment times did occur with Fluidotherapy treatment. Specifically, temperature was increased to lower therapeutic levels by 10 minutes, moderate levels by 20 minutes, and peak temperatures between 70 and 90 minutes. Clinically, this showed that Fluidotherapy was an effective tool for increasing extremity tissue temperature. Further research is needed to determine if HSP response depends on the type of heat transfer that a modality provides or if conduction-based or convection-based modalities require a specific intramuscular temperature to provide both therapeutic effects and a corresponding increase in HSP. Finally, we believe that Fluidotherapy is an effective treatment choice for increasing intramuscular temperatures in the lower extremity but is not an ideal modality to produce HSP response.

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