SWACSM Abstract

A Comparison of Heat Treatment-Induced Skeletal Muscle Adaptations Relative to Exercise Training

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ABSTRACT

In vitro and animal studies indicate that the response to heat stress is associated with beneficial adaptations that promote cell health and survival. Few studies to date have examined this finding in human subjects, and it is unclear how the adaptation compares in magnitude to exercise training. **PURPOSE**: To investigate the skeletal muscle adaptations (namely mitochondrial biogenesis and capillarization) of 6 weeks of deep-muscle heat treatment relative to exercise training. We hypothesized that heat treatment (HT), applied through pulsed shortwave diathermy (2 hr, 3 days/week) over a 6-week intervention period would lead to increased mitochondrial content and capillarity within skeletal muscle, though to a lesser extent than single-leg knee extension exercise training (EX; 40 min, 3 days/week). **METHODS**: We randomized 28 sedentary but otherwise healthy, young adults (ages 18–36; *n* = 13 female, *n* = 15 male) to receive either HT, EX, or sham heating sessions (CON; 2 hr, 3 days/week) over 6 weeks. Diathermy increased muscle temperature by 3.2 ± 0.33 °C ($P < 0.0001$) within 20 minutes. Muscle biopsies were taken from the vastus lateralis at baseline, after 3 weeks of intervention and again after 6 weeks of intervention. **RESULTS**: Following 3 and 6 weeks of heat treatment, we did not observe significant changes in mitochondrial biogenesis or capillarization. However, exercise training was sufficient to elicit an increase in individual capillary-to-fiber ratio ($P = 0.0003$), capillary density ($P = 0.0428$), and the Capillary to Fiber Perimeter Exchange Index ($P = 0.0089$). Significant increases in the expression of mitochondrial protein Complexes I ($P = 0.0073$) and IV ($P = 0.0015$), were observed in the exercise group, but not the heat or control groups. **CONCLUSIONS**: 6 weeks of localized HT, when applied to young healthy individuals, is insufficient to induce mitochondrial biogenesis or capillarization in skeletal muscle. Additionally, our findings provide support for the extensive body of literature that connects exercise training to beneficial skeletal muscle adaptations.

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BACKGROUND: Skeletal muscle is a dynamic and adaptable tissue that contributes to mobility, exercise capability, and the performance of activities of daily living[1].It is recognized that endurance training elicits robust adaptations in skeletal muscle[2], two of the primary underlying and most well-characterized of these adaptations being increased microvascular density[3] and mitochondrial biogenesis[4,5,6]. It is understood that these adaptations arise from the variety of stresses imposed on the muscle by exercise, including oxidative, metabolic, mechanical, and heat stresses[7,8]. A limited number of studies suggest that repeated exposure to mild heat treatment (HT) stimulates mitochondrial biogenesis[9] and pro-angiogenic[10] microvascular remodeling in human skeletal muscle. However, it is currently unknown how the magnitude of these adaptations compare to those observed via exercise training. The **PURPOSE** of this study was to examine the effect of 6 weeks of deep muscle heating on skeletal muscle mitochondrial biogenesis and microvascular remodeling, relative to the adaptations observed through exercise training. I hypothesized that 6 weeks of heat treatment would increase mitochondrial content and capillarity within skeletal muscle relative to a sham heat treatment, but to a lesser extent than what would be observed through the exercise training intervention.

METHODS

Subjects: Twenty-eight untrained adults (15 male, 13 female; 18-36 years) completed this study. Inclusion criteria included participation in no structured physical activity (PA) for the preceding 3 months and passing a health screening questionnaire. Exclusion criteria included known cardiovascular/metabolic diseases, pregnancy, or taking any prescription medication excluding birth control. The study was approved by the Brigham Young University Institutional Review Board.

Study Design: Subjects were randomly assigned to a control (CON), passive heat treatment (HT), or exercise (EX) group. One week after the initial muscle biopsy, subjects began reporting to the laboratory 3 days a week for a 120-min HT or CON session, or 40-min EX session on the right leg. HT was administered using pulsed shortwave diathermy. Two diathermy drums were placed directly on the vastus lateralis (VL) and turned on at 27.12 MHz and 800 bursts per second. In order to heat muscles of the quadricep group evenly, the drums were alternated from a more proximal to a more distal position every 20 min over each 2-hr HT bout. CON subjects had nearly identical visits to those in the HT group. The same settings that were used for HT were programmed for the CON group, however, the diathermy was not turned on. The EX subjects performed 40 min of knee extension (KE) exercise 3 days per week. The intensity of exercise during each visit alternated from light intensity to 4-min bouts of high-intensity exercise. Six minutes of light-intensity exercise was performed at the start and end of each session to allow the subject to adequately warm up and cool down. Subjects were also randomly selected from each group to have thermocouple temperature probes inserted into the VL during their first intervention visit to assess intramuscular temperature. Midpoint and post-training biopsies were obtained from the right VL at 3 and 6 weeks, respectively. Tissue samples were stored at −80 °C. All biopsies were taken from the right VL

and moved proximally from the first incision ~1 cm for each subsequent biopsy. For analyses, all samples from each subject were measured on the same plate to account for any variability between plates.

Immunohistochemistry: Eight-micrometer cross-sections of muscle tissue samples were cut using a cryostat at −25°C, mounted on Superfrost slides, and air-dried for 10 min. After fixation and blocking steps, the samples were incubated in the appropriate primary antibodies (CD31 and Dystrophin) in a humidified chamber overnight at 4 °C. Following several washes, sections were incubated in the appropriate secondary antibodies (Alexa Fluor 488 and Cy3) for 30 min at 37 °C.

Protein Analyses: Frozen samples were weighed and homogenized in T-PER buffer at a ratio of 9 µL per mg of tissue with an added protease and phosphatase inhibitor cocktail. Homogenates were centrifuged and then stored at –80 °C. To quantify biomarkers of microvascular remodeling, homogenates were measured with a Luminex Magpix multiplexing system using a 17-plex Human Angiogenesis/Growth Factor Magnetic Bead Panel. Mitochondrial protein Complexes I-V and PGC-1α protein expression were analyzed using an automated capillary electrophoresis system Wes (ProteinSimple). The following primary antibodies were used: Total OXPHOS Human WB Antibody Cocktail and Anti-PGC-1.

Statistics: A mixed model ANOVA was used to examine the main effects of time (pre vs. mid vs. post) and intervention (control vs. heat vs. exercise) in conjunction with the interaction of group × time. A Tukey-Kramer HSD test was performed post hoc when appropriate. Alpha was set at $P < 0.05$. Data are expressed as means ± SD.

RESULTS

Intramuscular Temperature: VL temperature in the HT group increased 3.2 ± 0.33 °C ($P < 0.0001$) within 20 min of application of shortwave diathermy, where it remained elevated for the remaining 1.5-hr session. In the EX group, temperatures increased by 1.8 ± 0.42 °C ($P < 0.0001$) by 10 min and remained elevated for the following 30 min. In the CON group, temperatures did not change significantly over the 2 hour session.

Muscle Capillarization: No significant main effect of group ($P = 0.2014$) was detected for the individual capillary-to-fiber ratio (C/F_i) following 6 weeks of intervention. A significant main effect of time ($P < .0001$) was observed, and post hoc analysis revealed this difference to be in the EX group from pre to post ($P = 0.0003$). Post hoc analysis on our group × time interaction ($P = .0408$) revealed a significant difference between EX and CON groups following 6 weeks of intervention. There were no significant changes in C/F_i at 3 or 6 weeks in either the HT or CON groups. When data were analyzed per volume (capillary density (CD)), no significant main effect of group ($P = 0.2445$) was detected following 6 weeks. A significant main effect of time ($P = 0.0028$) was observed, and post hoc analysis revealed this difference to be in the EX group from pre to post ($P = 0.0428$). There were no significant changes in CD at 3 or 6 weeks in either HT or CON groups. Similarly, analysis of the Capillary to Fiber Perimeter Exchange Index (CFPE Index) revealed no significant main effect of group ($P = 0.4182$) following 6 weeks of intervention. A significant main effect of time ($P = 0.0006$) was observed,

and post hoc analysis revealed this difference to be in in the EX group from pre to post (P = 0.0089). There were no significant changes in the CFPE Index at 3 or 6 weeks in either the HT or CON groups.

Molecular Indicators of Angiogenesis: In total, 14 of the 17 analytes were detected within skeletal muscle. Overall, HT had little effect on expression of these markers, but EX elicited significant changes in several of the markers. Angiopoietin-2 (ANG-2), Fibroblast Growth Factor (FGF-1), Hepatocyte Growth Factor (HGF) and Vascular Endothelial Growth Factor A (VEGF-A) increased significantly from baseline following 6 weeks of EX training.

Mitochondrial Biogenesis: We measured the concentrations of 3 mitochondrial respiratory chain subunits (Complex I, II, and IV), as well as protein expression of PGC-1α, an upstream regulator of mitochondrial biogenesis. Following 6 weeks of intervention, no significant main effect of group (P = 0.1305) was observed in the expression of Complex I. A significant main effect of time (P = 0.0307) was observed, and post hoc analyses revealed these differences to be in the EX group following 3 weeks (P = 0.0129) and 6 weeks (P = 0.0073) of intervention. No significant main effect of group (P = 0.4773) or main effect of time (P = 0.4608) was detected in Complex II protein expression. No significant main effect of group (P = 0.0982) was observed in the expression of Complex IV following 6 weeks of intervention. A significant main effect of time (P = 0.0005) was observed, and post hoc analyses revealed these differences to be in the EX group following 3 weeks (P = 0.0133) and 6 weeks (P = 0.0015) of exercise training. There were no changes in the protein expression of mitochondrial Complexes I, II, and IV in HT or CON groups. Finally, no significant main effect of group (P = 0.7614) or main effect of time (P = 0.6678) was observed for PGC-1α protein expression.

DISCUSSION

The purpose of this study was to examine the skeletal muscle adaptations induced by HT, namely microvascular remodeling and mitochondrial biogenesis, relative to EX training. Contrary to our hypotheses, we found that 6 weeks of HT administered to the knee extensor muscle group using shortwave diathermy, did not elicit significant increases in either skeletal muscle capillarization or mitochondrial content. We also report that 6 weeks of high-intensity interval single-leg KE exercise was sufficient to induce both mitochondrial biogenesis and microvascular remodeling in the VL.

Kuhlenhoelter et al. (2016) first reported that after an acute bout of lower body heating or unilateral thigh heating, the mRNA expression of angiogenic regulators in young adults increased significantly. Subsequently, Kim et al. (2020) reported that the expression of pro-angiogenic factor VEGF was enhanced after a single session, as well as after 5 days of repeated HT. These studies suggested that a long-term application of HT may induce angiogenesis in skeletal muscle. Hesketh et al. (2019) reported improvements in skeletal muscle capillarization similar to those induced by moderate intensity continuous training (MICT) following 6 weeks of whole body HT in healthy, untrained adults. In contrast to their findings, we did not observe increases in any index of capillarity or the expression of pro-angiogenic factors after our 6-week intervention of localized deep-muscle HT. This can potentially be explained by the differing time courses, subject

characteristics, and HT modalities used by the prior studies. For example, the higher level of PA that was accepted by Hesketh et al. (2019) among their participants may have contributed to the angiogenic benefits that they observed following HT intervention. This is evidenced through the increased maximal rate of oxygen consumption observed in the HT and MICT groups alike. Whereas their exclusion criteria included exercising more than 150 min/week, we did not allow any structured PA. Additionally, most of the recent heat studies (Hesketh et al., 2019; Kim et al., 2020) have used heating modalities that heated a greater portion of muscle mass and/or likely elicited some systemic effects in addition to those observed within the skeletal muscle specifically. For example, Hesketh et al. (2019), who reported increased capillary density following a similar 6 weeks of intervention, utilized a protocol in which their participants sat in a heat chamber, a systemic form of HT. On the other hand, Kim et al. (2020), whose form of HT was more localized, elicited no significant improvements in VEGF, ANG1, or capillary contacts. It is therefore reasonable to question whether or not our localized HT did not promote greater systemic effects that may contribute to skeletal muscle adaptations. For instance, existing literature associates sauna bathing, a form of systemic passive HT, with better cardiovascular and circulatory function in humans[10,13]. Perhaps skeletal muscle adaptations are more likely to be elicited through systemic, rather than localized HT, and this raises an interesting question for future research.

Consistent with existing literature, 6 weeks of EX training led to improvements in skeletal muscle capillarization. Expression of CD31, as well as the expression of pro-angiogenic factors such as VEGF-A, HGF, and ANG-2 all increased significantly with EX training. This finding provides further support to previous studies that have reported beneficial microvascular skeletal muscle adaptations following at least 6 weeks of EX training[10, 14, 15].

Contrary to our hypothesis, no changes were observed that would be consistent with mitochondrial biogenesis after 6 weeks of HT intervention. Our hypothesis was primarily based on the results of Hafen et al. (2018), who used the same HT modality and reported increases in both PGC-1α and mitochondrial respiratory protein Complexes I and V. However, increases in mitochondrial function in response to HT has not necessarily been a universal finding in the literature. Both Kim et al. (2020) and Hesketh et al. (2019) reported that local HT had no effect on respiratory chain protein content. Like us, both of these studies implemented extended heating protocols (6–8 weeks). Together, the findings of our study and those recently conducted by Kim et al. (2020) and Hesketh et al. (2019), indicate that 6 weeks of repeated bouts of HT may be insufficient to increase long-term mitochondrial content in healthy, untrained adults. It is worth noting that Hafen et al. (2018) did not see an increase in maximal enzymatic activity of citrate synthase, a common surrogate marker of mitochondrial content. However, Hafen et al. (2018) reported significant augmentation of maximal coupled and uncoupled respiratory capacity using high-resolution respirometry. This finding may indicate that HT, rather than signaling for increased mitochondrial content, could possibly lead to improved efficiency of existing mitochondria.

In contrast to the findings of HT, 6 weeks of our EX intervention was sufficient to induce increased mitochondrial content. Significant increases were observed in Complexes I and IV, at both 3 and 6 weeks of EX. These results add to the extensive body of literature that supports exercise training as a means of improving skeletal muscle mitochondrial biogenesis[16, 17, 18]. A recent study by MacInnis et al. (2017) reported that following 6 sessions of single-leg cycling, Complex IV protein content increased by 24% post-training. This is in line with our finding that 6 weeks of single leg KE led to a significant increase in Complex IV protein expression post-training ($P = 0.0015$).

We chose to apply local HT for 120 minutes, as this dose has previously been shown, when applied over a shorter time course (6–14 consecutive days), to increase mitochondrial biogenesis and PGC-1α expression, attenuate the reduction in angiogenic signaling associated with limb disuse (unpublished data), and increase the expression of heat shock proteins[9, 20, 21]. One unintended consequence of this protocol was that some participants reported the inability to maintain their habitual levels of PA throughout the study. The additional 2 hr of sitting, 3 days/week, could have decreased the amount of time that participants may have spent on PA such as walking to campus, grocery shopping, etc. The results of existing step reduction studies demonstrate the potency of decreased ambulation as a means of skeletal muscle deconditioning[22, 23, 24]. While our protocol likely did not lead to such a drastic step reduction, it was perhaps ample enough to overshadow any potential benefits of the HT. Perhaps the additional sedentary time was not enough to elicit maladaptation below baseline (hence the lack of significant decreases in HT and CON groups), but it may have been sufficient to prevent any potential mitochondrial or pro-angiogenic remodeling following 6 weeks of HT.

In conclusion, 6 weeks of localized HT, applied to the knee extensor muscles through pulsed shortwave diathermy, appears to be insufficient to induce skeletal microvascular remodeling and increased mitochondrial biogenesis in healthy, untrained adults. Further research is necessary to determine if HT may illicit beneficial skeletal muscle adaptations in other populations (i.e., elderly, injured, or bedridden adults), serving as more of a protective mechanism against muscle maladaptation. Additionally, our findings among the EX group align with existing literature, in that 6 weeks of EX training is sufficient to induce skeletal muscle mitochondrial biogenesis, as well as increased capillarization.

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