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RESEARCH ARTICLE

Impact of heat therapy on recovery after eccentric exercise in humans

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Kim K, Kuang S, Song Q, Gavin TP, Roseguini BT. Impact of heat therapy on recovery after eccentric exercise in humans. *J Appl Physiol* 126: 965–976, 2019. First published January 3, 2019; doi: 10.1152/japplphysiol.00910.2018.—The purpose of this study was to investigate the effects of heat therapy (HT) on functional recovery, the skeletal muscle expression of angiogenic factors, macrophage content, and capillarization after eccentric exercise in humans. Eleven untrained individuals (23.8 ± 0.6 yr) performed 300 bilateral maximal eccentric contractions of the knee extensors. One randomly selected thigh was treated with five daily 90-min sessions of HT, whereas the opposite thigh received a thermoneutral intervention. Peak isokinetic torque of the knee extensors was assessed at baseline and daily for 4 days and fatigue resistance was assessed at baseline and 1 and 4 days after the eccentric exercise session. Muscle biopsies were obtained 2 wk before and 1 and 5 days after the eccentric exercise bout. There were no differences between thighs in the overall recovery profile of peak torque. However, the thigh exposed to HT had greater fatigue resistance than the thigh exposed to the thermoneutral intervention. The change from baseline in mRNA expression of vascular endothelial growth factor (VEGF) was higher at *day 1* in the thigh exposed to HT. Protein levels of VEGF and angiopoietin 1 were also significantly higher in the thigh treated with HT. The number of capillaries around type II fibers decreased similarly in both thighs at *day 5*. Exposure to HT had no impact on macrophage content. These results suggest that HT accelerates the recovery of fatigue resistance after eccentric exercise and promotes the expression of angiogenic factors in human skeletal muscle.

NEW & NOTEWORTHY We investigated whether exposure to local heat therapy (HT) accelerates recovery after a bout of eccentric exercise in humans. Compared with a thermoneutral control intervention, HT improved fatigue resistance of the knee extensors and enhanced the expression of the angiogenic mediators vascular endothelial growth factor and angiopoietin 1. These results suggest that HT hastens functional recovery and enhances the expression of regulatory factors involved in muscle repair after eccentric exercise in humans.

functional recovery; heat therapy

INTRODUCTION

Unaccustomed eccentric exercise evokes a myriad of manifestations that include reductions in muscle strength and power, soreness, swelling, and reduced range of motion (14). Most of these symptoms resolve within days after minor insults but can persist for several weeks after exposure to repeated maximal eccentric contractions (30). For example, a bout of high-force eccentric exercise with the knee extensors has been reported to cause a 40–50% strength loss (23, 38), from which full recovery can take >3 wk (23). In these severe cases, the sustained impairment in muscle function can negatively impact athletic performance and reduce adherence to training regimens (15, 16).

The genesis of the prolonged impairment in muscle function following intense eccentric exercise, most notably the marked reduction in the ability to generate power, appears to stem from multiple mechanisms, including failure of excitation-contraction coupling as well as impaired metabolism (6). Recent evidence derived from animal models of exercise-induced muscle damage indicates that abnormalities in microvascular structure and function might also play a determinant role in causing the observed performance decrements after eccentric contractions (17). In rat skeletal muscle, eccentric exercise impairs capillary hemodynamics and compromises the matching between O_2 delivery and utilization during contractions (17) and also promotes a reduction in capillarization (39). Combined, these detrimental changes can impair the ability to deliver O_2 and energetic substrates during the recovery period. As a possible compensatory response, robust changes in the expression of genes involved in capillary growth, including vascular endothelial growth factor (VEGF) and angiopoietin 1 (ANGPT1), are observed after muscle damage (51).

One important step in the reparative response following eccentric exercise-induced muscle damage is the accumulation of leukocytes in the tissue (34). Blood-borne monocytes are activated and begin to accumulate in the extracellular compartment within the muscle tissue after a bout of eccentric exercise (35). Recruitment of these immune cells, including macrophages, is coupled with increased muscle expression of cytokines and chemokines, including C-C motif chemokine ligand 2 (CCL2) and C-X3-C motif chemokine ligand 1 (CX3CL1) (35). Among other roles, it has been proposed that these inflammatory cells might contribute to the remodeling of muscle and its associated extracellular matrix to make the tissue less vulnerable to damage after subsequent insults (9).

Current therapeutic modalities employed to treat the symptoms manifested after a bout of intense eccentric exercise have proven to be largely ineffective. For example, cryotherapy, one widely popular postexercise recovery modality, appears to delay rather than improve recovery after a bout of eccentric exercise of the elbow flexors (50) as well as arm cycling exercise (8). In animal models of muscle injury, topical icing has been shown to delay the infiltration of inflammatory cells into the damaged muscle (45, 47) and attenuate the expression of proangiogenic factors (45).

In sharp contrast to the observations of an impairment in muscle recovery following exposure to cryotherapy, mounting

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evidence indicates that heat therapy (HT) accelerates postexercise recovery of contractile function after endurance exercise (8) and improves muscle regeneration after severe injury (19). Indeed, studies in animal models of muscle injury induced by crushing or injection of toxins revealed that repeated exposure to HT markedly improves skeletal muscle regeneration (19, 32, 44, 48). The beneficial effects of HT are thought to derive from several mechanisms, including accelerated infiltration of macrophages into the injury site (48). These findings indicate that HT might abrogate the manifestations induced by unaccustomed lengthening contractions in humans and promote faster functional recovery. Nonetheless, the majority of studies that support a favorable effect of HT have been performed in experimental models that cause extensive myofiber necrosis and require a major regenerative response. It remains unclear whether these observations hold true for the recovery following voluntary eccentric exercise performed with the lower limbs in humans.

We recently reported that a single session of HT in humans enhances the expression of genes that have been shown to be critical for skeletal muscle angiogenesis and the overall remodeling response to eccentric exercise, including VEGF, ANGPT1, and several members of the heat shock protein (HSP) family (20). In light of these findings and the aforementioned studies in models of muscle injury, it is conceivable that exposure to HT promotes a local milieu that accelerates recovery after maximal eccentric exercise in humans. In the present study, young subjects performed maximal, unaccustomed eccentric knee-extension exercise with both legs, and one thigh was randomly selected to receive local HT immediately after and over 4 consecutive days after the exercise bout. To gain insights into the potential mechanisms of action of HT, muscle samples were harvested to examine changes in macrophage content, skeletal muscle capillarization, as well the expression of several important factors involved in muscle remodeling. We hypothesized that, compared with the control thermoneutral intervention, exposure to HT would accelerate the recovery of muscle function and the accumulation of macrophages as well as enhance the expression of proangiogenic factors.

METHODS

Participants. The Institutional Review Board at Purdue University reviewed and approved all experimental procedures, and verbal and written informed consent were obtained from all participants. Eleven young male ($n = 9$) and female ($n = 2$) adults (means ± SE: age 23.8 ± 0.6 yr, body mass 72.4 ± 1.9 kg, height 175 ± 1.3 cm) volunteered to participate in this study. Participants were asked to fill out a medical history questionnaire. Individuals were excluded if they were obese (body mass index > 30 kg/m^2), used tobacco products, were diabetic, were taking any medication other than birth control, participated in leg resistance exercise in the previous 6 mo, and performed endurance exercise for >3 days/week. Female participants were tested during the early follicular phase of their menstrual cycle (days 1–7) or during the placebo or no-pill week if they were taking oral contraceptives. All participants were asked to refrain from taking oral or topical analgesics, vitamins, and antioxidants for the duration of the study.

Experimental design. Participants were asked to report to the laboratory on 10 different occasions over a 3- to 4-wk period. An overview of the experimental protocol is shown in Fig. 1. On the first experimental visit, a biopsy was obtained from the vastus lateralis muscle of one randomly selected thigh to serve as the resting baseline sample. The individuals were allowed to recover for at least 1 wk before the second experimental visit. The purpose of *visits 2* and *3* was to familiarize the participants with the functional test on the isokinetic dynamometer as well as with the assessment of muscle soreness. These visits were separated by at least 24 h. On *visit 4*, participants underwent baseline assessment of muscle strength, fatigability, and soreness as described in detail below. At least 48 h after *visit 4*, participants reported back to the laboratory to perform a bout of eccentric exercise on the isokinetic dynamometer. A 355-kcal defined formula diet (Ensure; Abbott Laboratories) was given to participants the day before the session, and they were instructed to consume it at least 2 h before the onset of the experiment. Upon completion of the eccentric exercise bout, each thigh was assigned to receive either HT or a thermoneutral control intervention with a counterbalanced design. The heat and control treatments began ~10 min after the end of the exercise bout and lasted 90 min. The treatment sessions were repeated daily for 4 consecutive days. Muscle biopsies were taken from both thighs at *day 1* (24 h) and *day 5* (120 h) after the eccentric exercise bout. Muscle function and perceived soreness were reassessed throughout the recovery period as outlined in Fig. 1. Participants were instructed to fast for 10–11 h before undergoing muscle biopsies and to eat a light meal before the other experimental visits. Participants were also asked not to perform massage or foam rolling in the thighs and to refrain from local application of ice or heat throughout the duration of the study.

Eccentric exercise. The exercise bout consisted of 300 maximal voluntary eccentric contractions at an angular velocity of 30°/s (20 sets, 15 repetitions/set) with the knee extensor muscles of each leg using an isokinetic dynamometer (Humac NORM; Computer Sports Medicine, Stoughton, MA). As demonstrated in detail by others (26, 38), this protocol has been shown to induce several manifestations of exercise-induced muscle damage, including impaired muscle function and a pronounced elevation in local pain. After warming up on an unloaded cycle ergometer for 5 min, subjects were positioned on the chair of the isokinetic dynamometer and initially performed 75 eccentric knee extensions (5 sets of 15 repetitions with 30 s rest between sets) with one randomly selected leg. After 2–3 min of rest, subjects followed the same protocol with the other leg and the procedure was repeated four times. Participants were asked to resist as the lever pulled their partially extended leg from 35° of knee flexion (0° = full extension) to 105° of knee flexion, resulting in a range of motion of 70°. Consistent verbal encouragement was provided throughout the exercise bout.

Heat treatment. Local heating and the thermoneutral intervention were applied with a water-circulating garment customized to cover the thighs and buttocks (Med-Eng, Ottawa, ON, Canada) as described

	Baseline	Muscle injury	Post-injury				
		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Muscle soreness	x	x	x	x	x	x	x
Muscle biopsy	x		x				x
Muscle strength	x		x	x	x	x	
Muscle fatigability	x		x			x	
Eccentric exercise		x					
HT/Control		x	x	x	x	x	

Fig. 1. Schematic overview of the study protocol. HT, heat therapy.

previously (20). The garment consists of a network of medical-grade polyvinyl chloride tubing sewn onto tight-fitting elastic fabric and connected to a water circulator. In the thigh assigned to receive HT, water at 54–55°C was circulated through the garment for 90 min to increase skin temperature to ~39.5–40°C (20). In the opposite thigh, thermoneutral water (32–33°C) was circulated to clamp skin temperature at ~33°C for the entire duration of the protocol. The length of the treatment sessions was based on our recent report that exposure to local HT for 90 min enhances the expression of angiogenic regulators and stress management genes in human skeletal muscle (20). Skin thermocouples were taped to each thigh and allowed skin temperature to be monitored continuously during the treatment sessions.

Muscle biopsies. Muscle biopsies (~200 mg) were taken from the vastus lateralis muscle after 30 min of rest in the supine position as described previously (20). Under local anesthesia (lidocaine hydrochloride; Hospira, Lake Forest, IL), a small incision was made in the skin and fascia, followed by the insertion of a 5-mm Bergström biopsy needle (Pelomi Medical, Albruslund, Denmark). The biopsy specimens were promptly weighed, cleared of visible fat and connective tissue, and divided into four sections. The portion designated for cryosectioning was placed in a disposable base mold, covered with Tissue-Tek optimal cutting temperature compound and frozen in liquid nitrogen-cooled isopentane (49). The other sections were immediately frozen in liquid nitrogen and stored at −80°C until RNA extraction or Western blot analysis. Biopsies were acquired from separate incisions, ~2–3 cm apart. Leg order for biopsy sampling was randomized for each time point. All muscle biopsies were performed between 7:30 AM and 9:00 AM.

Assessment of muscle strength and fatigability. Maximal knee extensor strength and fatigue resistance were assessed using an isokinetic dynamometer. Participants were familiarized with the testing procedures twice before the baseline assessment. Each session was preceded by a standardized protocol consisting of 5 min of pedaling on a cycle ergometer. After the warm-up, participants were positioned on the chair of the isokinetic dynamometer and straps were fastened at the waist and shoulders and across the thigh to maintain a stable body position. The dynamometer settings were recorded on the first familiarization session and replicated in subsequent tests. The familiarization protocol included a set of 5–10 concentric knee extensions at 60–70% of the estimated maximal effort at an angular velocity of 180°/s, a set of three maximal contractions at an angular velocity of 180°/s, a set of three maximal contractions at an angular velocity of 60°/s, and a final set of 28 consecutive maximal contractions at 180°/s.

Maximal isokinetic strength of the knee extensors was assessed at baseline (*visit 4*) and daily throughout the recovery period. Fatigue resistance was assessed at baseline and 1 and 4 days after the eccentric exercise bout. Testing was performed on both legs, with the order of the testing counterbalanced. In each experimental session, participants were allowed to warm up for 5 min on a cycle ergometer and were then positioned on the chair of the isokinetic dynamometer with the identical apparatus setting predetermined at the first familiarization visit. Maximal isokinetic strength was assessed at two angular velocities: 180°/s and 60°/s. Participants were asked to complete three maximal consecutive contractions at each angular velocity, with a resting period of 30 s between velocities and 3 min between limbs. Peak isokinetic torque was defined as the average of the two highest attained values. Once both limbs had been tested for maximal strength, participants were allowed to rest for ~3 min and were then asked to perform a bout consisting of 28 consecutive maximal contractions at 180°/s. A resting period of 10 min was allowed between limbs. The total work performed during the bout was computed and used as a measure of fatigue resistance of the knee extensors. The reliability of muscle performance assessment was determined by comparing the results of the second familiarization visit (*visit 3*) and the baseline testing visit (*visit 4*). Both measures of muscle strength and fatigue resistance were found to be highly reproducible [retest correlations: *1*) peak torque at 180°/s: 0.91, *2*) peak torque at 60°/s: 0.92, *3*) total work completed during the fatigue bout: 0.97].

Perceived muscle soreness. Participants were asked to evaluate knee extensor muscle soreness on a 10-cm visual analog scale (0 = no soreness, 10 = extreme soreness) after stepping on and off a 40-cm (women) or 45-cm (men) chair three times.

RNA extraction. Total RNA from the skeletal muscle biopsies was prepared with the TRIzol Reagent (Invitrogen, Life Technologies, Carlsbad, CA) and quantified spectrophotometrically (Nanodrop 3000; ThermoFisher), as described previously (20). The quality and integrity (RNA integrity number of 8.2 ± 0.1) of extracted RNA (276.9 ± 26.4 ng/μl) were evaluated with an RNA 6000 Nano LabChip kit on an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). cDNA was prepared with the RT^2 First Strand Kit following the manufacturer's instructions (Qiagen, Valencia, CA).

Skeletal muscle gene expression. The expression of nine select genes related to angiogenesis, myogenesis, inflammation, and stress management was determined with a custom RT^2 Profiler PCR array kit (Qiagen) and the Roche LightCycler 480 PCR System (Roche Diagnostics, Indianapolis, IN). Duplicate samples from each subject (baseline and *day 1* and *day 5* after eccentric exercise) were loaded in the same array plate. The list of genes analyzed is shown in Table 1 and included the housekeeping gene 18S ribosomal RNA (18S rRNA) as well as a reverse transcription control, a positive PCR control, and a genomic DNA control. Data were analyzed with the GeneGlobe Data Analysis Center (Qiagen). 18S rRNA was used as the internal reference gene and proved stable across all time points. The comparative C_t method (where C_t is threshold cycle) was used to calculate the changes in gene expression of each target mRNA relative to the baseline sample (22).

Protein extraction. Frozen muscle samples (~30 mg) were homogenized in ice-cold homogenization buffer containing 0.5 M Tris·HCl, pH 7.4, 1.5 M NaCl, 2.5% deoxycholic acid, 10% NP-40, and 10 mM EDTA (RIPA Lysis Buffer; EMD Milipore) with freshly added protease inhibitor cocktail (Sigma-Aldrich catalog no. P8340) at a 1:15 dilution of wet muscle weight with a bead mill homogenizer (Bead Ruptor 12; Omni International). The resulting homogenate was clarified by centrifugation (13,500 *g*) for 20 min at 4°C. The supernatant was collected, and the protein concentration of each sample (~5 μg/μl) was determined with a BCA protein assay kit (Thermo Scientific). All samples were subsequently diluted with 1× PBS and subsequently mixed with either reducing sample buffer (4× Laemmli sample buffer with 10% 2-mercaptoethanol) or nonreducing sample buffer (4× Laemmli sample buffer). Afterwards, samples were heated for 5 min at 95°C, divided into small aliquots, and stored at −80°C.

Western blot analysis. Twenty-five micrograms of proteins was separated by SDS-PAGE on precast Stain-Free 4–15% gels (Bio-Rad) and transferred to polyvinylidene fluoride membranes with the Trans-Blot Turbo Transfer System (Bio-Rad). Membranes were subsequently blocked with 5% nonfat milk in 1× Tris-buffered

Table 1. *Genes included in custom PCR array*

Gene Symbol	Official Full Name
HSPA1A	Heat shock 70-kDa protein 1A
HSPA1B	Heat shock 70-kDa protein 1B
HSP90AA1	Heat shock protein 90-kDa alpha class A member 1
HSP90AB1	Heat shock protein 90-kDa alpha class B member 1
VEGFA	Vascular endothelial growth factor A
ANGPT1	Angiopoietin 1
CCL2	Chemokine (C-C motif) ligand 2
CX3CL1	Chemokine (C-X3-C motif) ligand 1
MSTN	Myostatin
RRN18S	18S ribosomal RNA
RTC	Reverse transcription control
HGDC	Human genomic DNA contamination

saline-Tween 20 (TBST; 1% Tween 20) solution for 1 h at room temperature and incubated overnight at 4°C with primary antibodies diluted in blocking buffer. Details of the primary antibodies and recombinant proteins are provided in Table 2. The membranes were washed with 1× TBST at room temperature for 3 × 10 min, incubated with horseradish peroxidase-conjugated secondary antibodies diluted in 1× TBST for 1 h at room temperature, and then washed with 1× TBST at least 3 × 10 min before being exposed to an enhanced chemiluminescence solution (Clarity Western ECL; Bio-Rad) for 5 min. Membranes were visualized with a densitometer (ChemiDoc Touch Imaging System; Bio-Rad), and band densities were determined with image analysis software (Image Laboratory V5.0; Bio-Rad). PageRuler Prestained Protein Ladder (Thermo Fisher) was used as a molecular weight marker. Control for equal loading was performed with stain-free technology, and total protein normalization was used to calculate changes in the expression of each target protein relative to the baseline sample (12).

Immunohistochemistry. Transverse serial sections (10 μm) of muscle biopsy samples were cut with a Leica CM1850 cryostat (Leica, Wetzlar, Germany) at −25°C, mounted on frosted microscope slides (Thermo Scientific), air-dried for 0.5–1 h at room temperature, and stored at −80°C for subsequent analyses. Frozen sections were briefly exposed to room air and fixed with 4% paraformaldehyde for 5 min. After 2 × 3 min washes with 1× PBS, the slides were incubated with blocking buffer (5% goat serum, 2% bovine serum albumin, 0.1% Triton X-100, and 0.1% azide in PBS) for 1 h at room temperature (52). Thereafter, sections were incubated with primary antibodies diluted in blocking buffer for 3 h at room temperature. Macrophages were stained with an antibody for CD68 (mouse IgG2b, 1:100; R&D Systems catalog no. MAB20401), whereas laminin (rabbit IgG1, 1:500; Abcam catalog no. ab11575) was added for distinction of the myofiber sarcolemma. Identification of fiber type-specific capillaries was performed with antibodies against CD31 (mouse IgG1, 1:100; BD Biosciences catalog no. 550300), dystrophin (rabbit IgG1, 1:100; Abcam catalog no. ab15277), and myosin heavy chain type I (MHC I) (mouse IgG2b, supernatant 1:100; Developmental Studies Hybridoma Bank catalog no. BA-D5). After 2 × 5 min washes with 1× PBS, sections were stained with appropriate secondary antibodies [Alexa 350 goat anti-rabbit IgG (1:500, catalog no. A-11069), Alexa 488 goat anti-rabbit IgG (1:1,000, A-11008), Alexa 488 goat anti-mouse IgG2b (1:1,000, A-21141), and Alexa 568 goat anti-mouse IgG1 (1:1,000, A-21124); Thermo Fisher Scientific], diluted in 1× PBS for 1 h at room temperature. Nuclei were labeled with 4′,6-diamidino-2-phenylindole. After 4 × 5 min washes, slides were briefly dried and mounted with fluorescent mounting medium (Dako) and the edges were sealed with nail polish (Sally Hansen Hard as Nails). Negative controls for the primary antibodies against CD31 and CD68 were used to ensure specificity of staining. Slides were viewed at ×20 magnification with an Olympus BX53 fluorescence microscope equipped with an Olympus DP72 digital camera and cellSens Dimension software. The entire specimen cross section was initially selected with the stage navigator. The multichannel image was then acquired, and two images from each channel were merged with ImageJ software (National Institutes of Health). Histological analysis was not performed in 3 of 55 samples because of insufficient muscle yield.

Analysis of immunofluorescence images. Analyses of immunofluorescence images were carried out with Adobe Photoshop CC 2015. For the quantification of fiber type-specific capillarization, all internal fibers (not bordering on a fascicle) in a cross section were initially counted (an average of 173 ± 95 fibers for type I and 217 ± 121 for type II muscle fibers). A total of 50 type I and 50 type II muscle fibers were then randomly selected for analysis (37). All capillaries within a distance of 5 μm from each fiber were counted for the determination of the number of capillaries around each fiber (CAF) (55). To evaluate the distribution and perform the quantification of macrophages, an average of 736 ± 297 fibers were analyzed. Macrophages were identified when the fluorescent signal for CD68 clearly surrounded (11) or entirely covered a nucleus. All immunofluorescent images were masked for both group and time point before analysis.

Statistical analysis. All statistical analyses were conducted with SAS (version 9.4; SAS Institute), with results expressed as means ± SE. The Kolmogorov-Smirnov test was used to assess the distribution of the data. Data exhibiting skewed distribution were log-transformed before statistical analysis. A two-way repeated-measures ANOVA was employed to compare the changes in skeletal muscle performance and soreness, gene and protein expression responses, and changes in the content of $CD68^+$ immunoreactive macrophages and CAF between the leg exposed to HT and the control leg. Post hoc analysis (Tukey) was performed when appropriate. For all analyses, $P < 0.05$ was considered statistically significant.

RESULTS

Performance during eccentric exercise. Figure 2 depicts the amount of work completed during each of the 20 sets of eccentric contractions by the thigh that was later assigned to receive HT and by the thigh that was subsequently allocated to the thermoneutral control treatment. On average, the thigh randomized to receive HT performed slightly more work (4.6%) than the opposite thigh (control 1,649 ± 108 J vs. HT 1,724 ± 115 J). All participants were right-leg dominant (based on kicking preference). Six par-

Table 2. *Antibodies used for Western blotting*

Antigen	Primary Antibody	Secondary Antibody	Recombinant Protein
Heat shock protein 70s Reducing (~70 kDa)	Bio-Rad (VMA00042) 1:2,000	Bio-Rad (anti-mouse STAR207P) 1:10,000	Bio-Rad (VMA00042KT)
Heat shock protein 90A Reducing (~90 kDa)	Bio-Rad (VMA00081) 1:500	Bio-Rad (anti-mouse STAR207P) 1:10,000	Bio-Rad (VMA00081KT)
Heat shock protein 90B Reducing (~90 kDa)	Bio-Rad (VMA00082) 1:2,000	Bio-Rad (anti-mouse STAR207P) 1:10,000	Bio-Rad (VMA00082KT)
VEGF165 Nonreducing (~39 kDa)	R&D Systems (AF-293-NA) 1:200	R&D Systems (anti-goat HAF017) 1:10,000	R&D Systems (293-VE-010)
Angiopoietin 1 Reducing (~70 kDa)	R&D Systems (AF923) 1:1,000	R&D Systems (anti-goat HAF017) 1:10,000	R&D Systems (923-AN-025)
Myostatin Nonreducing (~26 kDa)	R&D Systems (AF788) 1:2,500	R&D Systems (anti-goat HAF017) 1:10,000	R&D Systems (788-G8-010)
CX3CL1 Reducing (~90 kDa)	R&D Systems (AF365) 1:2,500	R&D Systems (anti-goat HAF017) 1:10,000	R&D Systems (365-FR-025)
CCL2 Nonreducing (~10 kDa)	R&D Systems (MAB679-100) 1:1,000	R&D Systems (anti-goat HAF017) 1:10,000	R&D Systems (279-MC-010)

CCL2, C-C motif chemokine ligand 2; CX3CL1, C-X3-C motif chemokine ligand 1.

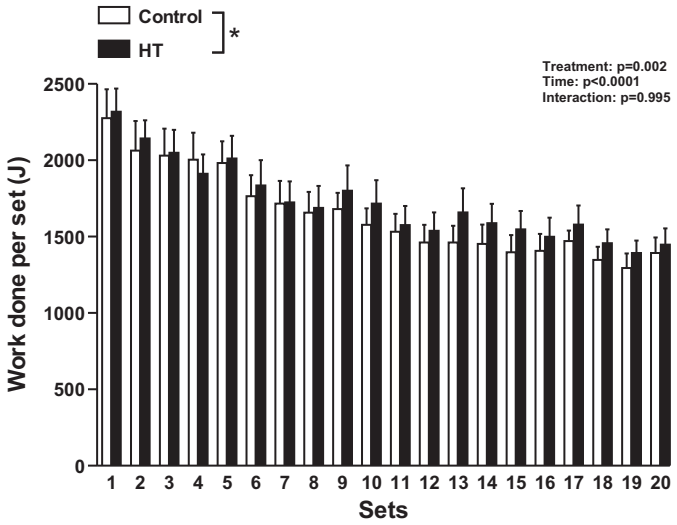

Fig. 2. Performance during eccentric exercise. Participants (n = 11) performed 300 bilateral eccentric contractions of knee extensors (20 sets of 15 reps). Data were analyzed with a 2-way repeated-measures ANOVA. Values are means ± SE. *Main effect for treatment (P = 0.002). HT, heat therapy.

ticipants were assigned to receive HT on their dominant leg, and five individuals had their dominant leg treated with the control regimen.

Changes in muscle function in response to muscle damage and recovery. The responses of isokinetic peak torque, total work completed during the fatigue bout, and perceived muscle soreness are displayed in Figs. 3–5. There were no significant differences between thighs for baseline measures of isokinetic peak torque at 180°/s (control 139 ± 2.3 Nm vs. HT 143 ± 1.9 Nm, P = 0.29) or 60°/s (control 181 ± 3.2 Nm vs. HT 181 ± 2.4 Nm, P = 0.37) and total work amount completed during the fatigue trial (control 3,531 ± 56 J vs. HT 3,575 ± 60 J, P = 0.60). Peak torque decreased, on average, by ~45% in the first 2 days after the eccentric exercise protocol and began to recover progressively starting at *day 3* (Fig. 3). There were no differences between thighs in the overall recovery profile of peak torque. Fatigue resistance, as determined by the total work amount completed during 28 consecutive maximal contractions at 180°/s, decreased by ~20% at *day 1* after the completion of the eccentric exercise bout and remained below baseline values at *day 4* (Fig. 4). The thigh exposed to HT had significantly greater fatigue resistance (i.e., lower reduction in total work relative to baseline) compared with the control thigh (P = 0.02, main effect). Perceived muscle soreness rose sharply after the eccentric exercise bout, reaching peak values at *day 2* (Fig. 5). Exposure to HT tended to decrease the magnitude of muscle soreness relative to the control treatment (P = 0.053).

Gene expression. Fold changes in gene expression from baseline for select angiogenic, myogenic, inflammatory, and stress management genes are shown in Fig. 6. At *day 1*, the mRNA expression of VEGF (control 0.79 ± 0.10 vs. HT 1.24 ± 0.12, $P < 0.05$) was significantly higher in the thigh exposed to HT compared with the control thigh. Conversely, at *day 5*, the mRNA expression of ANGPT1 (control 1.02 ± 0.13 vs. HT 0.78 ± 0.12, $P < 0.05$) was significantly lower in the thigh exposed to HT. The mRNA expression of CCL2 also was significantly lower in the thigh exposed to HT (P = 0.01, main effect).

Protein expression. Figure 7 displays the fold changes in the protein levels of select angiogenic, myogenic, inflammatory, and stress management factors relative to the baseline sample. A significant increase in the expression of chemokine CX3CL1 was observed in the thigh exposed to HT compared with the

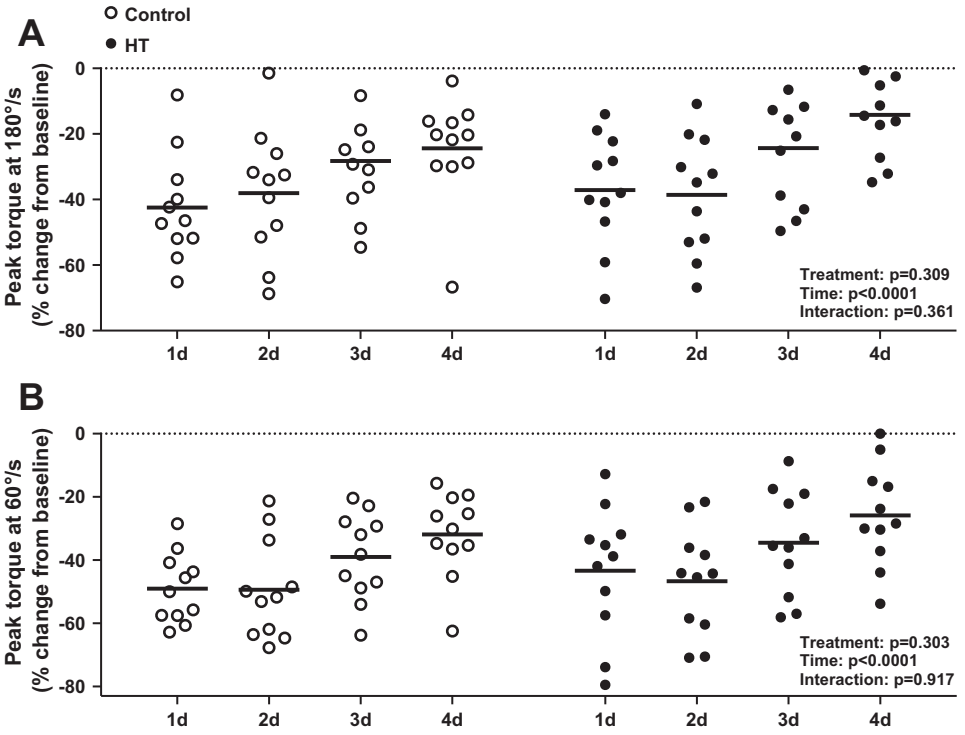

Fig. 3. Individual and group mean changes in peak torque during knee extensions at 180°/s (*A*) and 60°/s (*B*) after the eccentric exercise bout in the thigh exposed to the control thermoneutral intervention and the thigh treated with heat therapy (HT). Data were analyzed with a 2-way repeated-measures ANOVA. Values are means ± SE. d, Day.

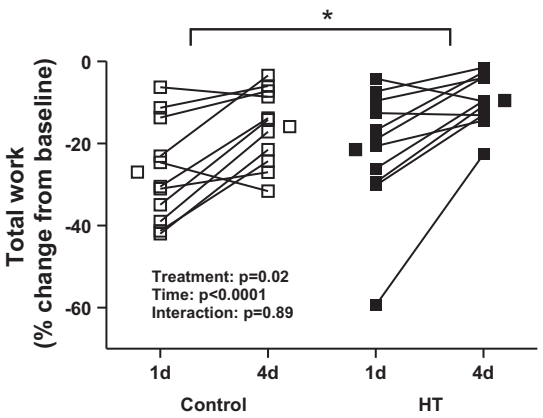

Fig. 4. Individual and group mean changes in fatigue resistance after the eccentric exercise bout in the thigh exposed to the control thermoneutral intervention and the thigh treated with heat therapy (HT). Data were analyzed with a 2-way repeated-measures ANOVA. Values are means ± SE. *Main effect for treatment ($P = 0.02$). d, Day.

control thigh on *day 1* (control 0.87 ± 0.07 vs. HT 1.21 ± 0.13, $P < 0.05$). The expression of VEGF ($P = 0.005$, main effect), ANGPT1 ($P = 0.045$, main effect), and CCL2 ($P = 0.02$, main effect) was higher in the thigh treated with HT compared with the control thigh.

Macrophage content. Skeletal muscle cross sections were analyzed for the presence of $CD68^+$ macrophages by immunohistochemistry (Fig. 8). The majority of $CD68^+$ immunoreactive macrophages were detected between fibers (Fig. 8*A*). Intracellular staining for CD68 was only detected in a few fibers. An increasing trend in the number of $CD68^+$ cells per 100 muscle fibers was noted after exposure to eccentric exercise ($P = 0.071$; Fig. 8*B*). On average, macrophage content increased by 44% on *day 1* and by 130% on *day 5*. There was no treatment effect or treatment × time interaction for this variable.

Skeletal muscle capillarization. One representative image of a muscle cross section stained for MHC I, CD31, and dystrophin is displayed in Fig. 9*A*. There was no treatment effect or treatment × time interaction for CAF. A significant decrease in CAF was observed at *day 5* after the eccentric exercise bout in type II fibers ($P < 0.05$).

DISCUSSION

The main findings of the present study are that compared with a thermoneutral intervention, exposure to HT after a bout of maximal eccentric exercise *1*) accelerated the recovery of fatigue resistance, *2*) increased the mRNA expression of VEGF at *day 1*, *3*) increased protein levels of CX3CL1 at *day 1*, and *4*) increased protein levels of proangiogenic factors VEGF and ANGPT1 and chemokine CCL2. Conversely, treatment with HT had no impact on the recovery of muscle strength, skeletal muscle macrophage content, and capillarization as well as HSP expression.

The persistent decline in muscle function following unaccustomed eccentric exercise, most notably in the ability of the muscles to generate power, has detrimental consequences for athletic performance and adherence to training regimens (6). A marked decline in power output and reduced work capacity during maximal dynamic exercise is commonly observed after eccentric exercise in humans (5, 41, 43). In agreement with these reports, we observed that the total work completed during 28 consecutive maximal contractions at 180°/s was reduced by ~20% on the day after a bout of eccentric exercise and was not fully restored after 4 days (Fig. 4). The genesis of this prolonged reduction in muscle work capacity is multifactorial, but it is recognized that changes in metabolic function play an important role (6). For instance, muscle glycogen resynthesis is impaired after eccentric exercise, possibly because of reduced glucose uptake (1, 18) as well as reductions in GLUT-4 content (3). Consequently, muscles exposed to eccentric exercise have to work at a higher relative workload during a subsequent bout of concentric exercise, resulting in increased glycogen utilization and decreased endurance (2). One important finding of the present study is that work capacity was greater in the thigh that received HT compared with the control thigh (Fig. 4). Although we did not investigate the specific mechanisms behind this effect, it is conceivable that HT accelerated the resynthesis of glycogen, resulting in greater dynamic power output during the maximal work capacity test. Two pieces of evidence lend strong support to this hypothesis. Slivka and coworkers first demonstrated that local muscle heating during recovery from a bout of exhaustive cycling exercise increased the rate of glycogen resynthesis in active male subjects (46). More recently, Cheng and coworkers showed that glycogen resynthesis after fatiguing stimulation in mouse intact single muscle fibers was accelerated by increasing muscle temperature, which in turn led to improved fatigue resistance (8). It is important to emphasize, nonetheless, that these studies focused on the recovery after exhaustive endurance exercise and it remains to be determined whether HT accelerates glycogen resynthesis after maximal eccentric exercise.

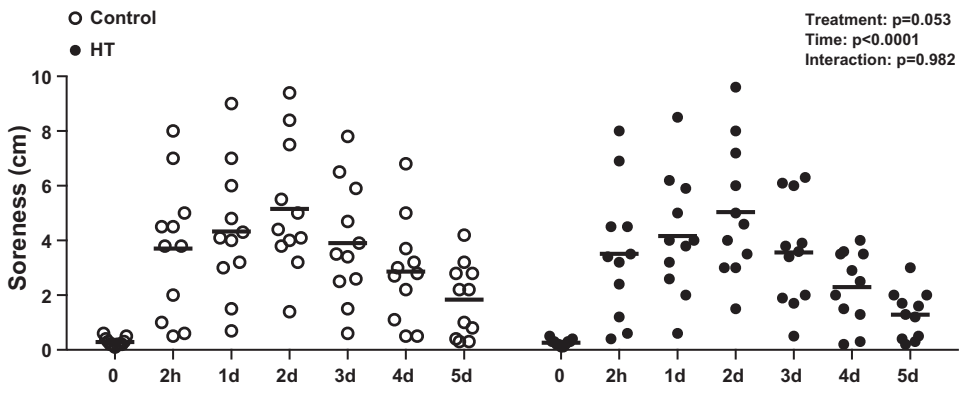

Fig. 5. Individual and group mean changes in perceived muscle soreness after the eccentric exercise bout in the thigh exposed to the control thermoneutral intervention and the thigh treated with heat therapy (HT). Data were analyzed with a 2-way repeated-measures ANOVA. Values are means ± SE. d, Day; h, Hour.

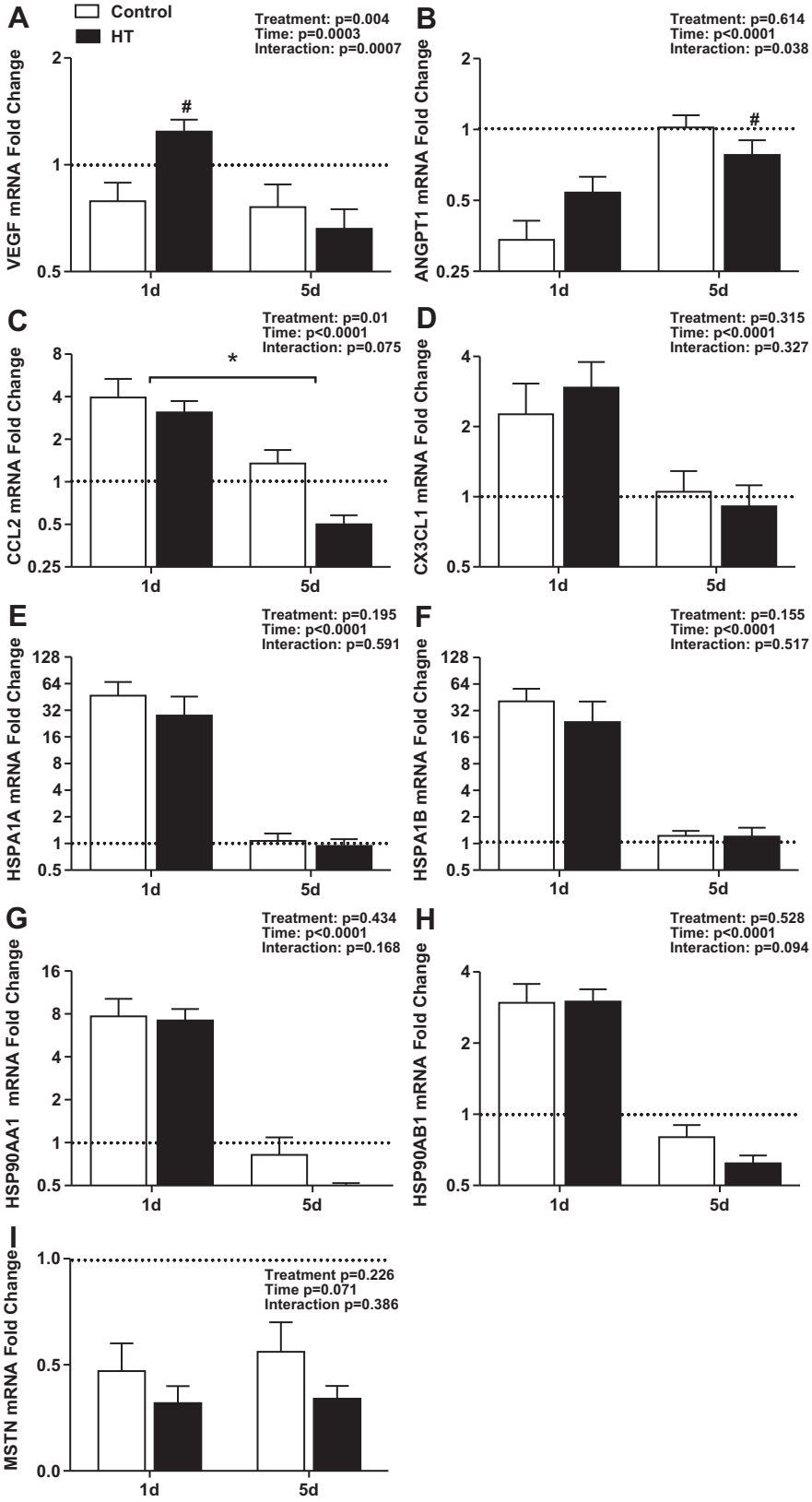

Fig. 6. Fold changes in skeletal muscle mRNA expression relative to the baseline sample of select angiogenic, myogenic, inflammatory, and stress management factors. *A*: vascular endothelial growth factor (VEGF). *B*: angiopoietin 1 (ANGPT1). *C*: C-C motif chemokine ligand 2 (CCL2). *D*: C-X3-C motif chemokine ligand 1 (CX3CL1). *E*: heat shock 70-kDa protein 1A (HSPA1A). *F*: heat shock 70-kDa protein 1B (HSPA1B). *G*: heat shock protein 90-kDa alpha class A member 1 (HSP90AA1). *H*: heat shock protein 90-kDa alpha class B member 1 (HSP90AB1). *I*: myostatin (MSTN). Biopsy samples were obtained 2 wk before and 1 and 5 days (d) after the eccentric exercise bout. The baseline sample was assigned a value of 1 and is represented by the dashed line. Data were analyzed with a 2-way repeated-measures ANOVA. Values are means ± SE. *Main effect for treatment ($P = 0.01$). $^{\#}P < 0.05$ vs. control. HT, heat therapy.

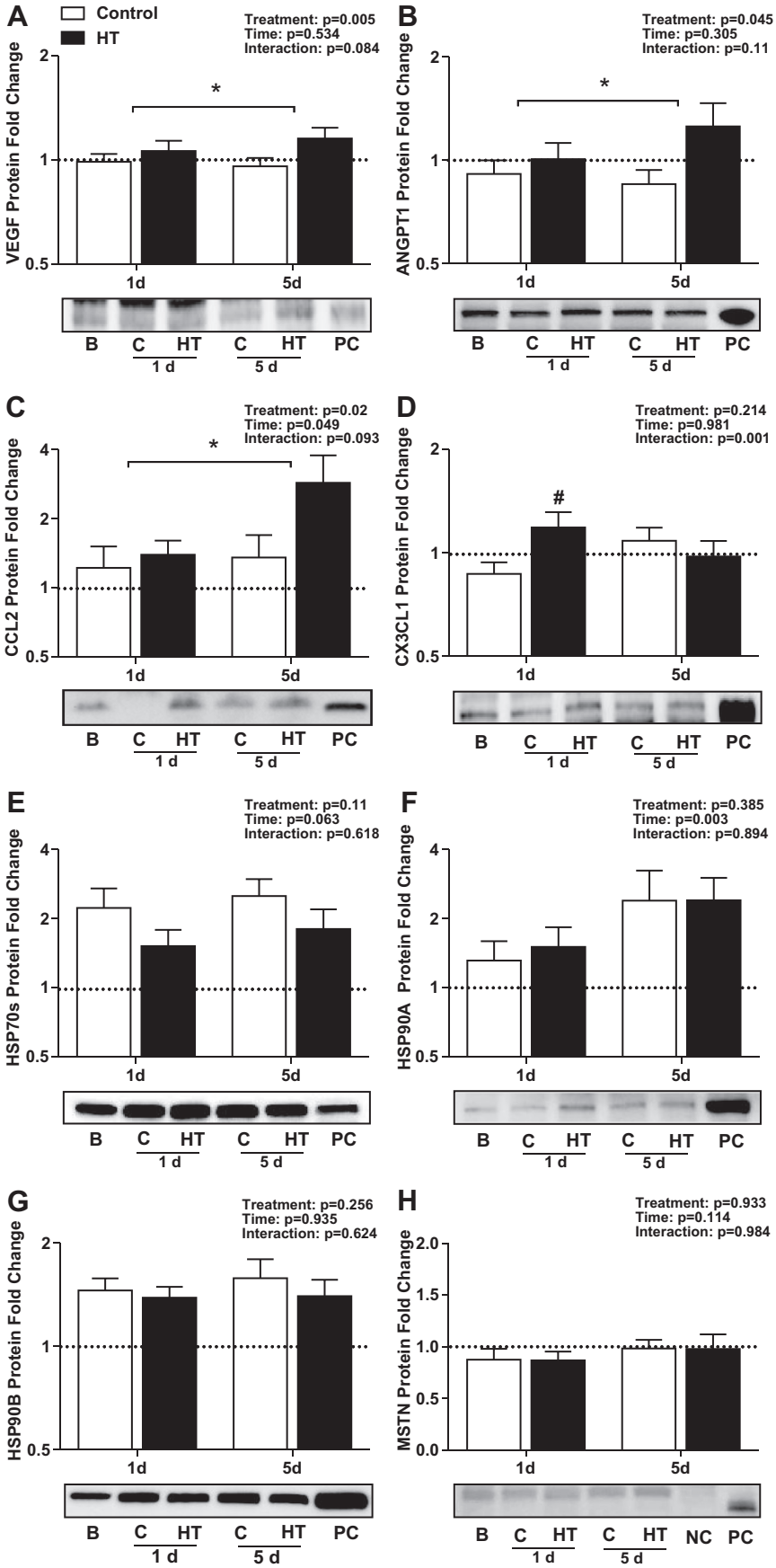

Fig. 7. Fold changes in skeletal muscle protein expression relative to the baseline sample of select angiogenic, myogenic, inflammatory, and stress management proteins. *A*: vascular endothelial growth factor (VEGF). *B*: angiopoietin 1 (ANGPT1). *C*: C-C motif chemokine ligand 2 (CCL2). *D*: C-X3-C motif chemokine ligand 1 (CX3CL1). *E*: heat shock 70-kDa protein (HSP70s). *F*: heat shock protein 90-kDa alpha class A member 1 (HSP90A). *H*: heat shock protein 90-kDa alpha class B member 1 (HSP90B). *H*: myostatin (MSTN). Biopsy samples were obtained 2 wk before and 1 and 5 days (d) after the eccentric exercise bout. The baseline sample was assigned a value of 1 and is represented by the dashed line. Representative Western blots are shown below each panel. Data were analyzed with a 2-way repeated-measures ANOVA. Values are means ± SE. *Main effect for treatment ($P < 0.05$). $^{\#}P < 0.05$ vs. control. B, baseline; C, control; HT, heat therapy; NC, negative control; PC, positive control.

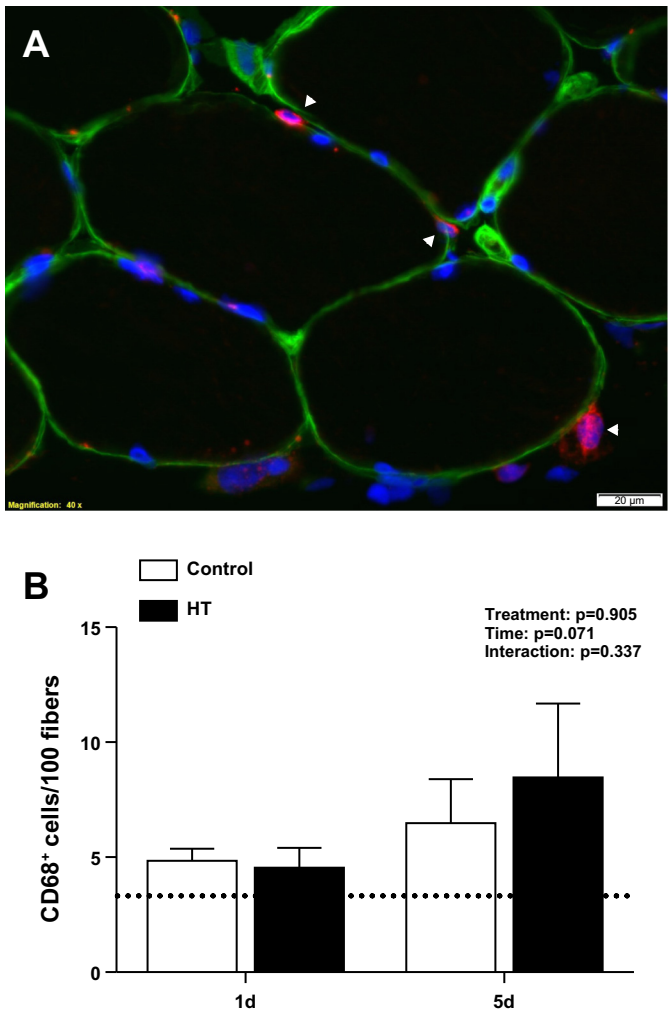

Fig. 8. *A*: representative skeletal muscle cross section displaying immunoreactivity for 4′,6-diamidino-2-phenylindole (DAPI; blue), CD68 (red), and laminin (green). A predominant portion of $CD68^+$ immunoreactive macrophages were located between fibers (arrowheads). *B*: no. of $CD68^+$ cells per 100 fibers at 1 and 5 days (d) after the eccentric exercise bout in the thigh treated with the control intervention and the thigh treated with heat therapy (HT). The baseline content of $CD68^+$ cells is represented by the dashed line (3.25 ± 0.35 cells per 100 fibers). A 2-way repeated-measures ANOVA was employed to compare the changes in the number of $CD68^+$ cells across the time course and between thighs. Values are means ± SE.

The finding that perceived soreness tended to decrease in the thigh exposed to HT compared with the thigh that received the control treatment is in line with a growing number of studies that indicate that local heating early after vigorous eccentric exercise reduces local pain. For example, Mayer and coworkers reported that treatment with a heat wrap starting 4 h after a bout of eccentric lumbar extension exercise was more effective at reducing local pain than cryotherapy (24). Petrofsky and coworkers recently showed that a heat wrap placed over the quadriceps immediately after a bout of eccentric exercise significantly reduced perceived pain compared with a control intervention (36). This hypoalgesic effect of HT is thought to be caused in part by heat-induced increases in blood flow (13) and the consequent accelerated removal of factors that sensitize muscle nociceptors. One view is that mechanical hyperalgesia following eccentric exercise is caused by the production by muscle fibers and/or muscle satellite cells of nerve growth factor and glial cell line-derived neurotrophic factor (27). Of note, treatment with hot packs has been shown to reduce muscle nerve growth factor content and substantially reduced physical inactivity-induced mechanical hyperalgesia in rats (29).

The beneficial effects of HT on skeletal muscle recovery after damage have also been proposed to stem from the accelerated recruitment of inflammatory cells to the injury site (48). A recent study in a model of muscle crush injury in rats revealed that HT applied immediately after injury speeded the infiltration of M1 macrophages that express the cell surface molecule CD68 (48). These findings are relevant because M1 macrophages have been shown to enhance muscle regeneration in rodents by interacting with proliferating satellite cells (40) and reducing fibrosis (31). An increase in the number of $CD68^+$ immunoreactive macrophages has been consistently observed in the endomysium and perimysium after a bout of lengthening contractions in humans (4, 10, 34). One prevailing view is that these cells are likely recruited to repair tears within the extracellular matrix (10). Consistent with previous reports, we observed that $CD68^+$ macrophages were predominantly located in the extracellular compartment after exposure to exercise (Fig. 8*A*). Our hypothesis, based on the aforementioned observation in a rodent model of muscle injury (48), was that HT would speed the arrival and consequently increase the content of $CD68^+$ macrophages. Chemoattractants produced by muscle cells, including CX3CL1 and CCL2, appear to be critical for the recruitment of inflammatory cells to injured skeletal muscle (7). Of note, treatment with HT evoked increased protein expression of CCL2 and CX3CL1 during recovery (Fig. 7, *C* and *D*). However, despite the changes in CCL2 and CX3CL1 levels, exposure to HT did not impact the dynamics and magnitude of macrophage recruitment.

We previously reported that a single 90-min session of HT enhances the skeletal muscle mRNA expression of proangiogenic factors VEGF and ANGPT1 in humans (20). This observation prompted us to test the hypothesis that HT creates a milieu that favors capillary growth and mitigates the detrimental effects of eccentric exercise on the muscle microcirculation. Eccentric exercise has been shown to induce pronounced alterations in the skeletal muscle microcirculation in rats and in humans. Kano and coworkers first reported that downhill running, which forces eccentric contractions within the rat spinotrapezius, caused a significant increase in the proportion of capillaries that do not support continuous red blood cell flow (17). The decrease of microvascular oxygen pressure during electrically stimulated contractions was also accelerated in muscles of animals subjected to eccentric exercise, which is compatible with a slowed exercise hyperemic response to muscle contractions (17). Along the same lines, Larsen and coworkers recently demonstrated that a single bout of eccentric contractions of the dorsiflexor muscles slows microvascular reactivity during brief contractions in humans (21). In addition to causing abnormalities in vascular function, there is evidence that eccentric exercise negatively impacts microvascular structure. Rizo-Roca and coworkers reported that a double session of strenuous eccentric exercise in trained rats caused a marked decline in capillary density and capillary-to-fiber ratio in the soleus muscle (39). Of note, the reduction in capillarization was evident within 1–3 days after the exercise bouts (39). In humans, Yu and coworkers also reported that a single bout of

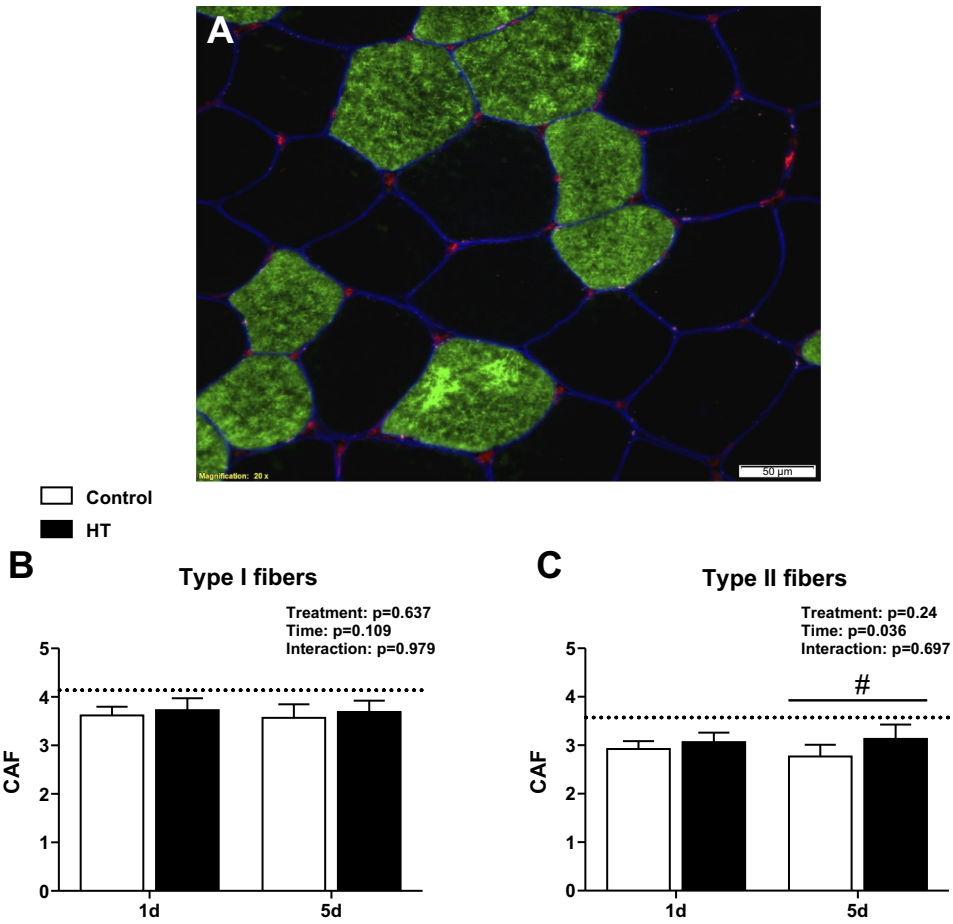

Fig. 9. *A*: representative skeletal muscle cross section displaying immunoreactivity for dystrophin (blue), CD31 (red), and myosin heavy chain type I (MHC I; green). *B* and *C*: no. of capillaries around each fiber (CAF) for type I (*B*) and type II (*C*) fibers. The baseline CAF is represented by the dashed line (type I: 4.19 ± 0.18, type II: 3.57 ± 0.24 capillaries around a fiber). A 2-way repeated-measures ANOVA was employed to compare the changes in CAF across the time course and between thighs. Values are means and SD. #$P < 0.05$ vs. baseline. d, Day.

downstairs running induced capillary regression in some, but not all, individuals during the recovery period (56). In the present study, we observed a decline in the number of capillaries around type II muscle fibers 5 days after the eccentric bout (Fig. 9*C*), but contrary to our predictions, exposure to HT did not prevent this effect. However, HT did induce increases in the expression of VEGF and ANGPT1, and it is possible that these factors might facilitate capillary growth later in the recovery period.

A hallmark response to strenuous exercise is the upregulation in skeletal muscle of stress management proteins, most notably the members of the HSP family. These molecular chaperones are thought to be fundamental in facilitating the cellular remodeling processes evoked by muscle injury as well as mediating the adaptations to exercise training (28). For example, overexpression of HSP70 enhances recovery from damage induced by lengthening contractions in mice (25), whereas ablation of this protein severely impairs muscle regeneration after injury caused by cardiotoxin injection or muscle reloading following a period of muscle disuse injury (42). In light of our recent observation that HT elicits increased expression of several members of the HSP family (20), we had anticipated that HT would augment the response of HSP90 and HSP70 to eccentric exercise. Although we did observe the expected increase in the expression of these factors after eccentric exercise, these changes were comparable between the muscles exposed to HT and the control treatment. It is tempting to speculate that the potent stress response triggered by the maximal bout of eccentric exercise prevented the detection of an additive effect induced by HT. Further studies are necessary to examine whether this same pattern remains later in the recovery process (i.e., beyond 5 days), when the stress response is resolved and HSP levels begin to return to baseline levels (28).

Limitations. One particular challenge in the design of studies aimed at assessing the impact of HT on recovery after exercise is that it is not possible to blind subjects to the exposure to heat. Although participants in the present study were not informed of the study hypothesis and a control intervention was used for comparison, we did not examine the potential influence of the placebo effect on the observed responses. Accumulating evidence indicates that therapeutic effects of popular recovery modalities, such as cryotherapy, stem at least partially from a placebo effect (53, 54). These important observations emphasize the need for future studies on the effects of HT to implement an effective placebo-controlled design.

Recovery from intense eccentric exercise entails a complex, well-orchestrated response that may persist for several weeks. Insights into the mechanisms underlying the beneficial effects of HT were limited in the present study because muscle biopsies were harvested only in the immediate (*day 1*) and subacute (*day 5*) phases of recovery. Indeed, some of the manifestations of exercise-induced muscle damage, including the decline in force and power output, increased soreness, and reduced capillarization, were not fully resolved by *day 5*.

Future studies should examine the impact of repeated local HT on the later phases of recovery.

Perspectives and significance. Our results show that exposure to HT immediately after and for 4 consecutive days after a maximal bout of eccentric exercise in humans hastens recovery of fatigue resistance and tends to reduce perceived soreness. These findings are in line with a growing body of literature supporting a beneficial impact of HT on recovery after noninjurious exercise (8) as well as severe muscle injuries (19, 32, 33, 44, 48). Additional studies are warranted to determine whether repeated exposure to local HT amplifies the skeletal muscle adaptations to exercise training in humans.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

K.K. and B.T.R. conceived and designed research; K.K., T.P.G., and B.T.R. performed experiments; K.K., Q.S., and B.T.R. analyzed data; K.K., S.K., T.P.G., and B.T.R. interpreted results of experiments; K.K. and B.T.R. prepared figures; K.K. and B.T.R. drafted manuscript; K.K., S.K., Q.S., T.P.G., and B.T.R. edited and revised manuscript; K.K., S.K., Q.S., T.P.G., and B.T.R. approved final version of manuscript.

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