Determining the effect of acute and chronic passive heating on endothelial and muscular function and the response of serum heat shock proteins

by

Kaylin Dix Didier

B.S., University of Oklahoma, 2012
M.S., University of Oklahoma, 2015

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Kinesiology
College of Human and Health Sciences

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2020

Abstract

Passive heating has been utilized as a therapeutic intervention for skeletal muscle disorders and improvements in cardiovascular function. Despite the growing body of research in the field of passive heating interventions, the underlying mechanisms for the physiological changes and the performance outcomes are not fully understood. The overarching aim of this dissertation was to determine the effect of acute and chronic whole-body heat treatments on endothelial and muscular function, exercise tolerance, and to determine the time course response of heat shock proteins (HSPs). In our first investigation (Chapter 2), we found that one bout of whole-body heating improved endothelial function measured by flow-mediated dilation. However, our data showed that the expression of HSP90α was not affect ~24 hours post the heat treatment. In the second investigation (Chapter 3) we found that one bout of whole-body passive heating was not a sufficient stimulus to produce changes in exercise tolerance (duration) during isometric knee extension at 40% maximal voluntary contraction. Furthermore, we demonstrated that neuromuscular recovery from exercise was not impacted by the heat treatment. However, we did find that diffusive oxygen delivery into the muscle during exercise was significantly decreased after heating. The third investigation (Chapter 4) was aimed at determining the effects of eleven consecutive days of whole-body passive heating on endothelial function, exercise tolerance, and the time course of HSPs over the chronic heating treatments. We demonstrated that the repeated bouts of heating did not improve endothelial function or exercise tolerance. However, during the vascular occlusion test we found that the perfusive and diffusive components of oxygen delivery in to the skeletal muscle were accelerated. The findings of these studies together indicate that physiological changes are occurring post passive heating, however these changes are not enough sufficient to increase exercise tolerance or performance. This

dissertation contributes to the growing body of research on passive heat treatments and underlying mechanisms as an intervention for improving exercise tolerance.

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Passive heating has been utilized as a therapeutic intervention for skeletal muscle disorders and improvements in cardiovascular function. Despite the growing body of research in the field of passive heating interventions, the underlying mechanisms for the physiological changes and the performance outcomes are not fully understood. The overarching aim of this dissertation was to determine the effect of acute and chronic whole-body heat treatments on endothelial and muscular function, exercise tolerance, and to determine the time course response of heat shock proteins (HSPs). In our first investigation (Chapter 2), we found that one bout of whole-body heating improved endothelial function measured by flow-mediated dilation. However, our data showed that the expression of HSP90α was not affect ~24 hours post the heat treatment. In the second investigation (Chapter 3) we found that one bout of whole-body passive heating was not a sufficient stimulus to produce changes in exercise tolerance (duration) during isometric knee extension at 40% maximal voluntary contraction. Furthermore, we demonstrated that neuromuscular recovery from exercise was not impacted by the heat treatment. However, we did find that diffusive oxygen delivery into the muscle during exercise was significantly decreased after heating. The third investigation (Chapter 4) was aimed at determining the effects of eleven consecutive days of whole-body passive heating on endothelial function, exercise tolerance, and the time course of HSPs over the chronic heating treatments. We demonstrated that the repeated bouts of heating did not improve endothelial function or exercise tolerance. However, during the vascular occlusion test we found that the perfusive and diffusive components of oxygen delivery in to the skeletal muscle were accelerated. The findings of these studies together indicate that physiological changes are occurring post passive heating, however these changes are not enough sufficient to increase exercise tolerance or performance. This

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Dedication

To my family, Grant, Dixie, Gatlin, and Christine Didier:

The support you have given me during this process is undeserved, but I cannot thank you enough for believing in me. You have always encouraged me to pursue what I am passionate about and the completion of this dissertation is just the beginning of me doing that. I love you all more than words can express.

Chapter 1 - Introduction

Passive heating has been utilized as a therapeutic intervention for skeletal muscle disorders and improvements in cardiovascular function in the form of hot baths and saunas for centuries (Ablin, Hauser, & Buskila, 2013; Barfield & Hodder, 1987; Beever, 2010). Increasing core temperature elicits increases in blood flow, shear stress, and expression of heat shock proteins (HSPs), which have been linked to improved endothelial and skeletal muscle function. However, multiple factors such as heat modality, duration of heating, occurrence, and magnitude of the stress all play a role in the induction of physiological changes from passive heating treatments (Kim, Monroe, Gavin, & Roseguini, 2020).

Acute and chronic exposure to passive heating have demonstrated changes in endothelial function. Acute bouts of heating in previous research have demonstrated that 30 min after a 45 min lower limb heating session, there was an increased macro- and microvascular dilator function in an aged population (Romero et al., 2017). One bout of lower limb heating has shown to increase antegrade shear rate similar to responses seen during exercise in elderly subjects with and without peripheral artery disease (Thomas, van Rij, Lucas, & Cotter, 2017). Similar to acute studies, Ohori et al. showed that repeated exposure to sauna treatment increased exercise tolerance and endothelial function in patients with chronic heart failure (Ohori et al., 2012). Brunt and colleagues also demonstrated that repeated bouts (5-6x per week) of whole body passive heating over an 8 week period improved micro- and macro-vasculature function in young, sedentary adults (Brunt, Eymann, Francisco, Howard, & Minson, 2016; Brunt, Howard, Francisco, Ely, & Minson, 2016). Significant increase in endothelial function first occurred at the two-week time mark, thus indicating that 8-10 bouts of passive heating during a two-week time span was sufficient stimulus to induce physiological adaptations.

Single and repeated exposure to passive heating has led to beneficial skeletal muscle adaptations (Kim, Kuang, Song, Gavin, & Roseguini, 2019; Kim, Reid, et al., 2020; Racinais, Wilson, & Periard, 2017). Goto et al. utilized a ten-week heating protocol to increase maximal isometric torque of the quadriceps (Goto et al., 2011). Similarly, muscle strength, capillarization, and eNOS are all found to be increased post 8 weeks of local heating of the investigated muscle (Kim, Reid, et al., 2020). The ability of locally applied passive heat to quicken functional recovery and improved fatigue resistance after muscle-damaging exercise has been demonstrated in previous research (Kim et al., 2019). Increases in endothelial function, as described above, and muscle adaptations from acute and chronic passive heating could lead to improvements in exercise tolerance and daily activities.

The overall aim of this dissertation was to determine the effect of acute (one bout) and chronic (11 consecutive days) whole-body passive heat treatments on endothelial and muscular function, exercise tolerance, and to determine the time course of response of heat shock proteins (HSPs). Specifically, we aimed to investigate the after-effects of acute and chronic whole-body passive heating on endothelial function measured by flow-mediated dilation and exercise tolerance (duration). During these assessments we sought to determine the changes in perfusive and diffusive delivery of O_2 into the skeletal muscle after each condition of passive heating. Further, we aimed to determine the effect of acute and chronic heating on neuromuscular recovery after isometric knee extension at 40% maximal voluntary contraction. Finally, this dissertation aimed to determine the effect of serum HSP90α and HSP72 as underlying mechanisms for improvements in physiological and performance outcomes.

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Chapter 2 - The acute effects of passive heating on endothelial function, skeletal muscle oxygen delivery, and extracellular HSP90α

Summary

Passive heating has been a therapeutic tool used to elevate core temperature and induce increases in cardiac output, blood flow, and shear stress. We aimed to determine the effects of a single bout of passive heating on endothelial function and serum HSP90 levels in young, healthy subjects. 8 healthy subjects were recruited to participate in one bout of whole-body passive heating via immersion in a 40°C hot tub to maintain a 1°C increase in rectal temperature for 60 min. One bout of passive heating increased shear-rate corrected endothelium-dependent dilation pre- (0.004 ± 0.002 $\%SR_{AUC}$) to post-heating (0.006 ± 0.003 $\%SR_{AUC}$) (p=0.034). Serum HSP90α was not different from pre- (36.7 ± 10.3 ng/mL) to post-heating (40.6 ± 15.9 ng/mL) (p=0.39). Muscle oxygen uptake during the occlusion test from pre- (0.17 ± 0.11 ml O2 min^{-1} $(100g)^{-1}$) to post-heating (0.14 ± 0.9 ml O2 min^{-1} $(100g)^{-1}$) (p=0.28) and mean arterial pressure pre- (74 ± 11 mmHg) to post-heating (73 ± 11 mmHg) were not influence by the heating intervention. Finally, time to peak response post cuff release significantly delayed for % O_2 sat (p=0.007) and deoxy-[heme] (p=0.018), with no effect on oxy-[heme] (p=0.19) and total-[heme] (p=0.41). One bout of passive heating improved endothelium-dependent dilation in young, healthy subjects. This data suggests that passive heat treatments may provide a simple intervention for improving vascular health in several populations.

Introduction

Exposure to heat therapy has been utilized for centuries in the form of hot baths and saunas for medicinal purposes (Ablin, Hauser, & Buskila, 2013; Barfield & Hodder, 1987; Beever, 2010). Clinical populations, such as chronic heart failure patients, have shown improvements in cardiovascular function with the utilization of infrared saunas (Ohori et al., 2012; Sobajima et al., 2015; Sobajima et al., 2013). Repeated bouts of passive heating have demonstrated a significant relationship between increased endothelial function and increased exercise tolerance (Ohori et al., 2012). Previous studies have also demonstrated that heat therapy can promote beneficial hemodynamic changes, increased skeletal muscle capilliarization, and upregulation of eNOS in healthy, young individuals (Chiesa, Trangmar, & Gonzalez-Alonso, 2016; Hesketh et al., 2019). For example, Brunt et al. have shown that repeated bouts of whole body passive heating led to improved micro- and macro-vasculature function in young, sedentary adults (Brunt, Eymann, Francisco, Howard, & Minson, 2016; Brunt, Howard, Francisco, Ely, & Minson, 2016).

Mechanisms behind the improved vascular function can likely be attributed to multiple factors. The first response to increases in core temperature is increased blood flow and shear stress on the vasculature leading to increased endothelial NO synthase (eNOS) and increased NO bioavailability in the vascular smooth muscle (Crandall & Wilson, 2015). A single bout of lower limb heating has been utilized to produce increases in antegrade shear rate in elderly subjects with and without peripheral artery disease, suggesting a therapeutic intervention to produce responses similar to those seen during exercise (Thomas et al 2016). Second, heat shock proteins (HSPs) are elevated due to increased core body temperature. Brunt et al. demonstrated that one hour post whole body heating HSP90 levels were increased and could lead to the protective

cellular adaptations seen after chronic heating (Brunt et al., 2019). The HSP90 family is specifically thought to be responsible for endothelial and vascular improvement, and has been shown to act as a molecular chaperone for eNOS, while assisting in stabilization and refolding of other proteins during stress. Recent ex vivo and in vitro studies have shown an association between eNOS and HSP90 (Averna et al., 2008; Pritchard et al., 2001). Cardena et al. showed that HSP90 is a physiological binding partner and regulator of eNOS such that when HSP90 and eNOS are bound there is a significant increase in the bioavailability of NO and consequently vasodilation (Garcia-Cardena et al., 1998).

The majority of heating studies in humans have looked at the effect of repeated bouts of heating on vascular and skeletal muscle adaptations. To our knowledge, the combination of the response of endothelial function and underlying mechanisms to an acute bout of heating have yet to be examined. Therefore, the purpose of this study was to investigate the effects of a single bout of passive heating on endothelial function and serum HSP90 levels. We hypothesized that an acute bout of whole body passive heating would 1) increase endothelial function measured by flow-mediated dilation of the popliteal artery and 2) increase circulating levels of serum HSP90.

Methods

Ethical Approval

The current study was approved by The Institutional Review Board for Research Involving Human Subjects at Kansas State University (#9697), in accordance with the standards set by the Declaration of Helsinki, except that data from the study was not registered in a database. Written informed consent was obtained from all participants prior to any data collection.

Subjects

8 healthy subjects (5 men) were recruited to participate in the study. Subject characteristics were (mean ± SD): age: 25 ± 7 years, height: 173 ± 6 cm, and weight: 73.3 ± 12.9 kg. All subjects completed a detailed medical health history questionnaire to ensure that they were free of known cardiovascular, pulmonary, and metabolic diseases. The experimental protocols were explained to the subjects prior to entering the study. Subjects were instructed to abstain from vigorous activity 24 h prior, alcohol 12 h prior, and caffeine or food consumption 2 h prior to the scheduled testing time.

Experimental Design

Subjects visited the laboratory a minimum of 3 times and were first familiarized with all testing procedures and equipment prior to testing. On day 1, a vascular occlusion test (VOT) was performed on the popliteal artery to measure flow mediated dilation (FMD) and post-occlusive reactive hyperemia (PORH). Day 2, subjects were passively heated in a hot tub for 1 hour. Day 3 (within 24 hours of completion of heating), subjects repeated the VOT.

Intervention

For the heating intervention, subjects were immersed up to the shoulders in a 40°C hot tub for 60 min, until rectal temperature (T_{rec}) increased 1°C or reached 38.5°C. Once the target T_{rec} was reached the subject was instructed to come out of the water to waist-level to maintain T_{rec} for the remainder of the time. Following the end of hot water immersion, subjects were monitored for another 10 mins of recovery. Core temperature was monitored using a sterile rectal thermometer probe (YSI Series 400, Yellow Spring Instruments, Yellow Springs OH, USA) inserted ~10 cm past the anal sphincter. While in the tub, subjects were allowed to drink water *ad libitum*.

Flow-mediated dilation and post-occlusive hyperemia

Subjects lay prone with a small pillow under the ankle. Measurement of the popliteal artery (dilation and blood flow) was made immediately proximal to the bifurcation (at or right above the popliteal fossa). The pneumatic cuff was placed around the thigh, 2-4 inches proximal to the popliteal fossa and the cuff was inflated to 250 mmHg for 5 min (Hokanson, Bellevue, WA, USA) (Parker, Ridout, & Proctor, 2006). A wide belt was placed over the cuff to ensure inward compression of the leg vasculature. Occlusion was confirmed via Doppler ultrasound of the popliteal artery distal to the inflated cuff. Popliteal artery diameter and blood velocity were measured via Doppler ultrasonography for 1 min of baseline prior to cuff inflation and 3 min following cuff release.

Experimental Measures

Blood pressure

Systolic, mean, and diastolic blood pressures (SBP, MAP, and DBP, respectively) were measured at baseline via an automated sphygmomanometer (GE Datex-Ohmeda Light), with the subject seated in an upright position and the right arm resting on a table at heart level.

Doppler Ultrasound

Measurements of popliteal artery diameter and blood velocity were simultaneously obtained using non-invasive 2D Doppler ultrasound equipped with a linear array transducer operating in duplex mode at a frequency of 10M Hz and 4.0 MHz, respectively (Logiq S8, GE Medical Systems, Milwaukee, WI). Doppler velocity measurements were corrected for an angle of insonation less than 60°. Baseline and post-occlusion popliteal artery diameters were calculated at 15 frames per second and averaged into 3 sec bins. Popliteal artery diameter and PABV were time-aligned (same 3 sec bins) and used to calculate popliteal artery blood flow (PABF), defined as, PABF (mL/min) = PABV x π x radius^2 x 60. Vascular compliance (Kragelj, Jarm, Erjavec, Presern-Strukelj, & Miklavcic) was calculated as the ratio of PABF to MAP, VC (mL/min x 100mmHg) = (PABF/MAP) x 100. VC area under the curve (VC_{AUC}) was calculated as the integral of VC values above baseline VC (average of 30 sec prior to cuff inflation). VC values are reported as % changes from pre heating.

FMD was calculated as the percent change in diameter from baseline to peak dilation following cuff release. To estimate the stimulus for dilation after occlusion, shear rate (SR) was calculated as PABV/diameter and used to determine the area under the curve above baseline from time of cuff release to peak dilation (SR_{AUC}). FMD was normalized for shear rate by dividing FMD by SR_{AUC} as previously described (Padilla et al., 2009; Pyke & Tschakovsky, 2005).

Near-Infrared Spectroscopy

The oxygenation characteristics of the *medial head of the gastrocnemius* were determined using a frequency-domain multi-distance NIRS (Near-infrared spectroscopy) system (Oxiplex TS, ISS, Champaign, IL, USA). This system provides a calculation of absolute concentrations of deoxygenated [Hb+Mb] (deoxy-[Hb+Mb]), oxygenated [Hb+Mb] (oxy-[Hb+Mb]), total-[Hb+Mb], and % saturation. The principles and algorithms of the NIRS technology were reviewed by Gratton et al. (1997) and have previously been described by (Ferreira, Harper, & Barstow, 2006; Hammer et al., 2019). The applications of NIRS to skeletal muscle research have been reviewed in depth (Barstow, 2019). Briefly, this device consists of eight light-emitting diodes (LED) operating at wave-lengths of 690 and 830 nm (four LEDs per wavelength) with one detector fiber bundle and LED-detector separation distances of 2.0, 2.5, 3.0, and 3.5 cm. The NIRS data were collected at 50 Hz and stored for post-hoc analysis. The original deoxy-[Hb+Mb], oxy-[Hb+Mb], and total-[Hb+Mb] concentrations were multiplied by a factor of four to return the values from units of [Hb+Mb] concentration into the original units of heme concentrations as measured by the instrument, and are hereby denoted as deoxy-[heme], oxy-[heme], and total-[heme], respectively (Hammer et al., 2018). After locating the *medial head of the gastrocnemius* of the left leg using EMG and palpation, the NIRS probe was secured longitudinally along the belly of the muscle. The NIRS probe was calibrated prior to each test according to the manufacturer's recommendations using a calibration block with known absorption and scattering coefficients. Calibration was confirmed on a separate block with different absorption and scattering coefficients. Muscle oxygen uptake ($m\dot{V}O_2$) was calculated as

$$m\dot{V}O_2 \text{ (ml } O_2 \text{ min}^{-1} \text{ (100g)}^{-1}) = \text{abs}[((Hb_{diff}) \times 60)/(10 \times 1.04) \times 4] \times 22.4/1000$$

During occlusion the difference in slopes between oxy[heme] vs. deoxy[heme] for sec 30-60 were used to calculate Hb_{diff} using the following equation: Hb_{diff} = (oxy[heme]-deoxy[heme])/2 (van Beekvelt, van Engelen, Wevers, & Colier, 2002).

Blood Sampling

Venous blood samples were collected pre-heating and within 24 hours of the heating intervention. 4 ml of blood were collected in a tube containing a clot-inducing plug. This tube was inverted, serum was allowed to clot for 30 min, then centrifuged at 1000 x g for 4°C. The separated serum was collected and stored at -20°C prior to analysis for HSP90α protein.

Blood Analysis

An enzyme-linked immunosorbent assay (ELISA) method (HSP90α high sensitivity ELISA kit' (Enzo, CAT. ADI-EKS-895) was used to determine the relative expression of the HSP90α protein in the serum. We added 100 µL of prepared standards and samples (diluted 1:50) in duplicate to wells of the AntiHsp90α Immunoassay Plate. The plate was incubated for 1 hour before washing the wells. 100 µL of HRP Conjugate was added to each well, incubated for 1 hour, and then washed again. 100 µL of TMB Substrate was added to each well, incubated for 20 mins, and them 100 µL Stop Solution was added. The resulting yellow color was read at 450 nm and the amount of signal is directly proportional to the level of HSP90α in the sample.

Statistics

Statistical analyses were performed using a commercially available software package (Sigma Plot 12.5/SigmaStat 3.5, Systat Software, Point Richmond, CA). One-tailed paired t-test was used for the comparison of HSP90α, FMD, shear-corrected FMD, AUC PABF peak, PABF time to peak, and SR_{AUC} pre- and post-heating. Two-tailed paired t-tests were used to determine the differences in AUC VC, VC peak, and VC time to peak. Likewise, two-tailed t-tests were used to compare time to peak and peak Δ after cuff release in % O_2 saturation, oxy-[heme], total-

[heme], and deoxy-[heme]. All data were expressed as mean ± SD, unless otherwise stated. Statistical significance was declared when $p<0.05$.

Results

Passive Heating and Blood pressure

Passive heating resulted in an increase in T_{rec} as intended of 1.2 ± 0.45°C from 37.2 ± 0.45°C baseline to 38.4 ± 0.24°C at the end of passive heating. We observed no significant difference in systolic, diastolic, or MAP between pre and post heating (p=0.86, p=0.79, and p=0.79, respectively) (Table 2.1.).

HSP90α

Mean and individual HSP90α concentrations for pre and post heating are displayed in Figure 2.1A and B. and. There was no significant difference between pre- (36.7 ± 10.3 ng/mL) and post-heating (40.6 ± 15.9 ng/mL) serum HSP90α (p=0.39).

Endothelial function

There were no significant differences in FMD between pre and post heating (p=0.11) (Figure 2.2A). FMD corrected for the shear rate stimulus was not significantly increased post heating (p=0.034), displayed in Figure 2.2B. SR_{AUC} showed no significant difference between pre and post heating (p=0.29). There were no significant differences in peak PABF and PABF time to peak between pre- and post-heating (p= 0.07 and p=0.32, respectively) (Table 2.1). There were no significant differences in VC AUC (p=0.49), peak VC (p=0.087), time to reach peak (p=0.72) between pre and post heating.

NIRS

m$\dot{V}$O2 for pre and post heating was 0.17 ± 0.11 and 0.14 ± 0.9 ml O2 min-1 (100g)-1, respectively (p=0.28). The peak changes in % O_2 saturation, oxy-[heme], total-[heme], and deoxy-[heme] were not significantly different pre- to post-heating (p>0.1 for all). The time to peak from cuff release for % O_2 saturation and deoxy-[heme] post-heating were significantly

delayed compared to pre-heating ($p=0.007$ and $p=0.018$, respectively). Also, the time to peak from cuff release for total-[heme] and oxy-[heme] were not significantly different pre- to post-heating ($p=0.19$ and $p=0.41$, respectively).

Discussion

In the current study, we investigated the effect of one bout of whole body passive heating on endothelial function and serum HSP90α levels. Our major findings were that one bout of passive heating increased endothelium-dependent dilation, however the presence of HSP90α in circulation was not changed. Muscle oxygen uptake during the occlusion test and blood pressure were not influence by the heating intervention. Finally, time to peak response post cuff release significantly delayed for % O_2 sat and deoxy-[heme], with no effect on oxy-[heme] and total-[heme].

HSP90 is a ubiquitous protein that acts as a molecular chaperone and facilitates the signaling of mechanotransduction pathways that lead to the activation of eNOS. Pritchard et al. showed that HSP90 plays a pivotal role in the eNOS-dependent production of NO and when HSP90 was inhibited the eNOS production of NO was decreased (Pritchard et al., 2001; Pritchard et al., 2002). Our data showed that serum HSP90α levels were not changed ~24 hours post one bout of passive heating. This finding is in agreement with previous research that showed intracellular HSP90 was not significantly increased one hour post whole body passive heating (Brunt et al., 2019). However, Brunt et al. did show an abundance of HSP90 in peripheral blood mononuclear cells (PMBC) collected one hour post heating (Brunt et al., 2019). Others have also found that heating acclimation increased baseline HSP90 in PMBC after 10 days, furthermore ex vivo exposure to heat produced an 89% increase in HSP90 above baseline on day 1 (McClung et al., 2008). These findings suggest that when HSP90 is released into the circulation, there is a protective layer surrounding the protein. This hypothesis is agreement with Clayton et al., who found that human cells exposed to heat stress (42°C for 3 hours) produced increased HSP90 encapsulated within the exosome lumen (Clayton, Turkes, Navabi, Mason, & Tabi, 2005). This

may account for the lack of changes in free circulating protein concentrations pre- to post- whole body heating in the present study.

FMD is a measurement commonly utilized in clinical and research settings to evaluate endothelial function. Measurement of the popliteal artery may provide a better indicator of peripheral artery disease (Parker et al., 2006), which is a clinical population that has experienced improved endothelial function after passive heating (Thomas, van Rij, Lucas, & Cotter, 2017). Our data showed significant improvement in endothelial-dependent dilation after one bout of whole body passive heating when corrected for shear-rate (Figure 2.2B). Shear stress is a possible mechanism for producing FMD. Endothelial cells possess mechanorecptors that detect changes in shear stress amplitude, magnitude, direction, and frequency (Baeyens, Bandyopadhyay, Coon, Yun, & Schwartz, 2016). Increased flow leads to the secretion of NO and the relaxation the vascular smooth muscle leading to dilation of the vessel. Tinken and colleagues showed that when the rise in shear stress was controlled, improvements in the brachial artery dilation after acute heating was attenuated (Tinken et al., 2009). Previous research looking at endothelial function demonstrated that 30 min post a 45 min lower limb heating protocol there was an increase in both macro- and microvascular dilator function in the aged population, however young healthy subjects only showed increased microvascular function and no change in macrovascular function (Romero et al., 2017). The absence of an increase in the macrovascular dilator function in young subjects may be due to inadequate severity of the heat stress. The duration, intensity (temperature), and frequency of exposure to passive heating are likely to play a large role in the stimulation of mechanistic and physiological adjustments and adaptations in subjects.

During the VOT, NIRS was utilized to evaluate tissue deeper than the cutaneous tissue and quantify the absolute values of deoxy-[heme] and total-[heme]. Changes in total-[heme] have been interpreted to reflect changes in microvascular [Hb] (i.e., hematocrit), reflective of diffusive O_2 delivery, while absolute values for deoxy-[heme] have been used to estimate fractional O_2 extraction and perfusive O_2 delivery (Davis & Barstow, 2013). Previous data from our lab showed that NIRS examines the microvasculature of the skeletal muscle compared to the macrovascular response of the conduit artery (Didier et al., 2020). In the current study, we found that one bout of heating did not provide enough stimulus to provoke a change in the peak diffusive or perfusive O_2 delivery during a VOT. Previous research displayed increased microvascular function post 8 weeks of passive heating by evaluating the cutaneous vasculature (Brunt, Eymann, et al., 2016). This measurement may provide information for systemic changes from heat exposure but not changes in the skeletal muscle (Barstow & Wong, 2008; Didier et al., 2020; Rizzoni, 2008). However, our data showed that the time to peak for total-[heme] and % O_2 saturation were significantly delayed post whole body passive heating. These findings suggest that ~24 hours post heating the time course for microvascular hematocrit changes in the skeletal muscle was significantly delayed. It is currently unclear what underlying mechanistic changes would lead to this delay post heating.

We designed our experimental approach to examine the effect of one bout of passive heating on endothelial function and O_2 delivery in the skeletal muscle in young healthy adults. A number of considerations should be noted in the current study. We did not control for menstrual cycle in the 3 women subjects. We recognize this approach does not control for the hormonal differences between sexes, however we performed intra-subject comparisons within 48 hours. Recent studies have shown that FMD and microvascular vasodilation were not changed across

the menstrual cycle (D'Urzo, King, Williams, Silvester, & Pyke, 2018; Ketel et al., 2009; Shenouda, Priest, Rizzuto, & MacDonald, 2018). While we understand that the current study is underpowered on some measurements and adding subjects would be beneficial, due to the current COVID-19 pandemic we were unable to collect additional data. Another limitation of the study is the timing of the measurement of serum HSP90, which was evaluated approximately 24 hours post the whole-body heating session to time align with post measurements. However, this timing was not able to determine if the heat stress was severe enough to elicit upregulation of HSP90 during or immediately post the heat treatment.

The present study demonstrated that one bout of whole body passive heating induced increased endothelial function measured via FMD in young, healthy subjects. However, HSP90α was not upregulated approximately 24 hours post the bout of passive heating. Future studies are needed to determine the time course of upregulation of HSP90α in circulation and the minimum amount of heat stress (duration, intensity, and occurrence) needed to provide enough stimulus to invoke both increased mechanistic and functional changes. Observing changes in young, healthy subjects in the current study suggests that clinical populations, such as aged or individuals with peripheral artery disease may see improvements in endothelial function after acute bouts of whole body passive heating.

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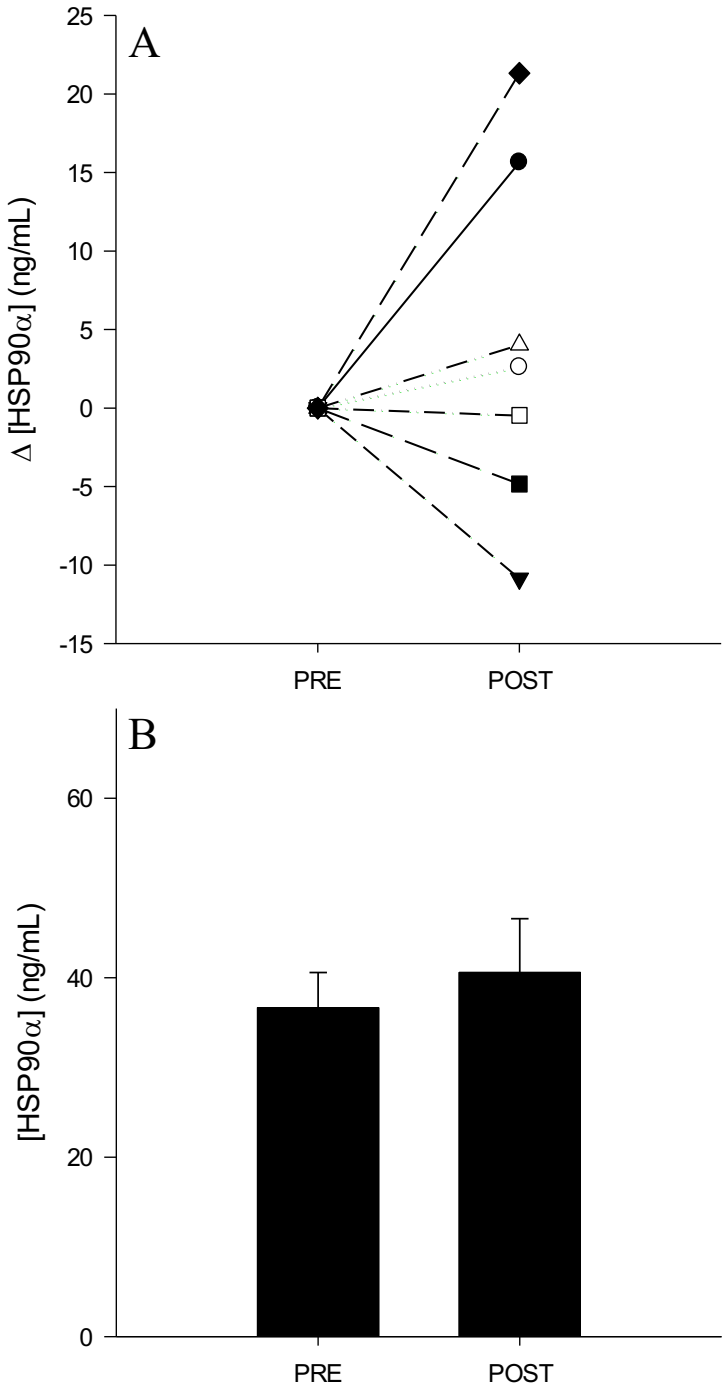

Figure 2.1 Heat shock protein 90 response to acute heating

A) Individual changes of serum HSP90α from pre to post passive heating. **B)** Mean ± SE values of serum HSP90α. No significant differences were detected between pre and post heating.

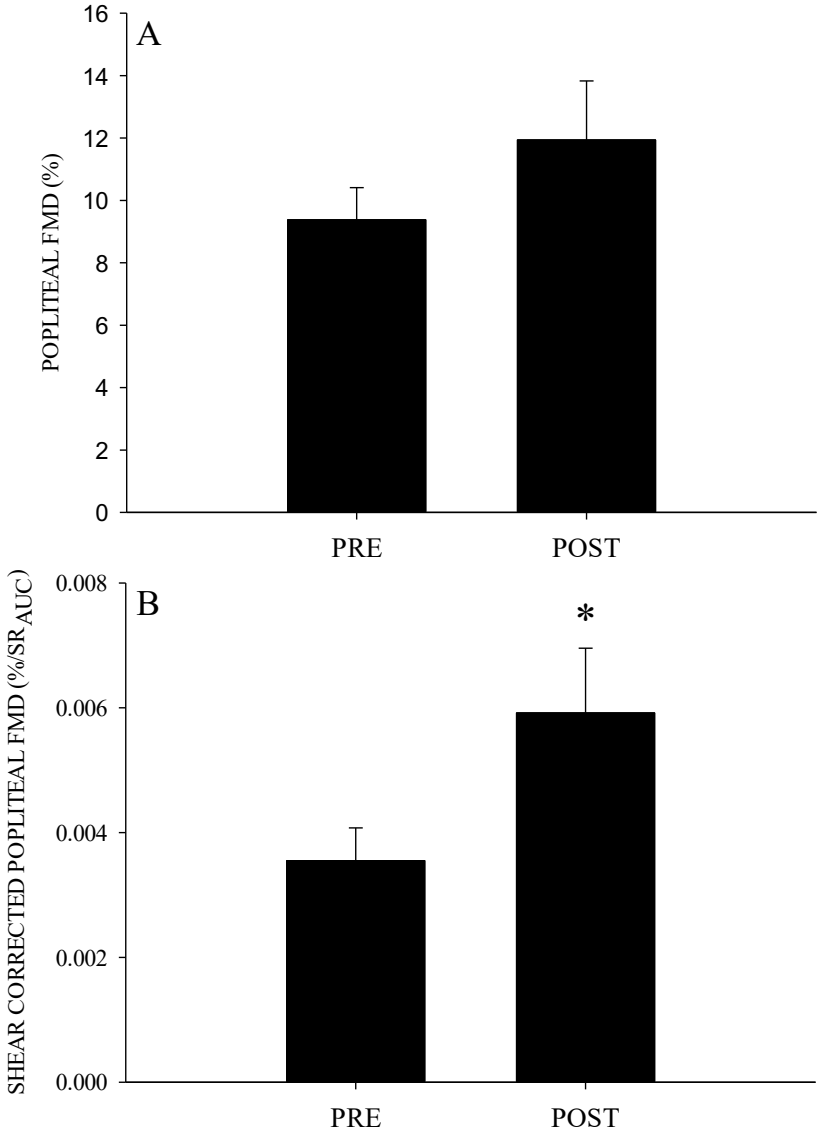

Figure 2.2 Changes in popliteal artery flow-mediated dilatation (FMD)

FMD presented as a percentage change from baseline diameter (A), and shear-corrected FMD (B), pre and post passive heating. Data are mean ± SE. SR_{AUC}, area under the curve of the shear rate stimulus for vasodilation. There was no significant difference in FMD, however shear-corrected FMD was significantly increased after one bout of passive heating compared to pre-heating. * Significantly different from pre heating (p=0.03).

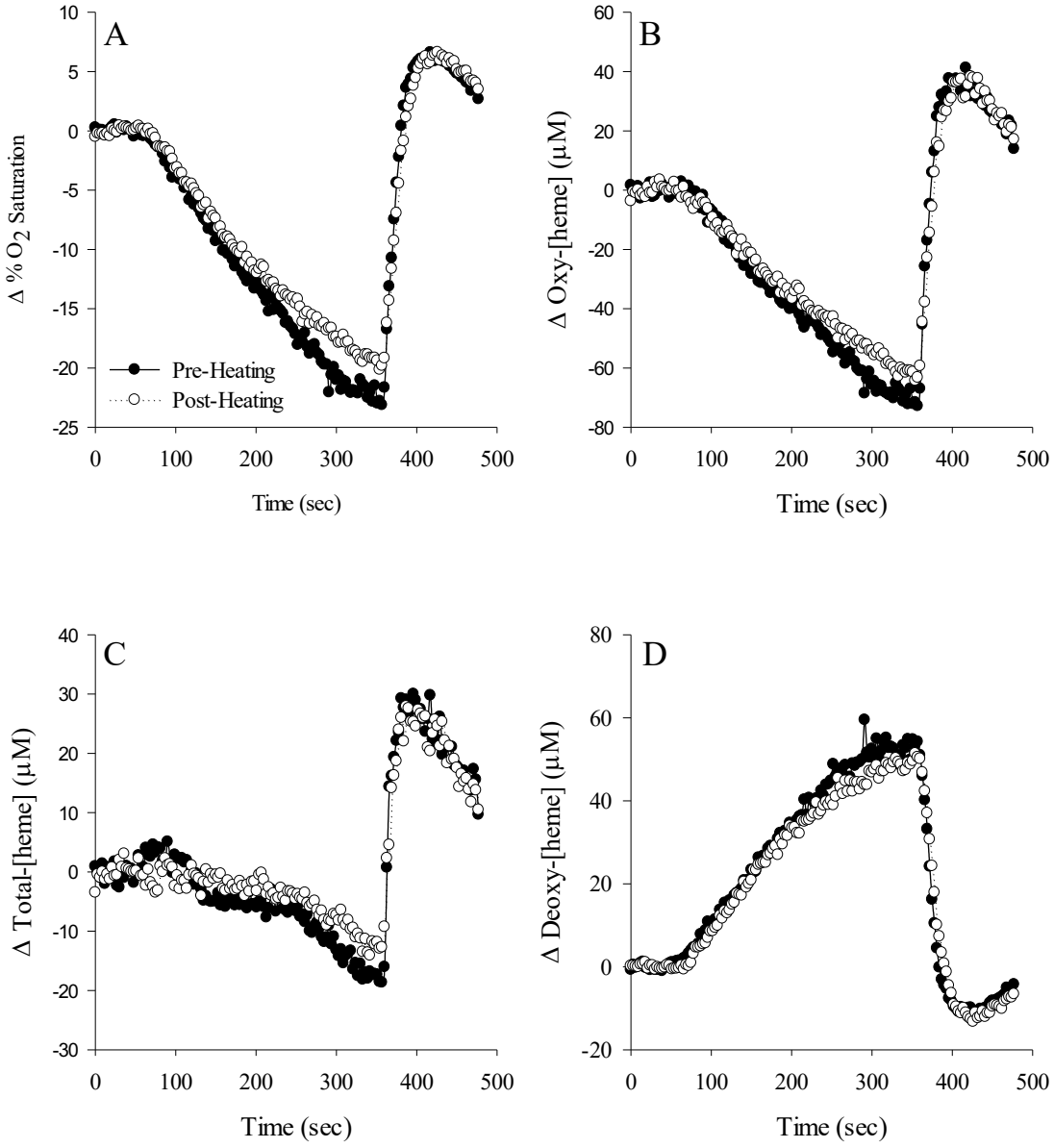

Figure 2.3 Mean changes in oxygenation characteristics

A-D: the mean responses during the VOT protocol for (A) % O_2 Saturation, (B) Oxy-[heme], (C) Total-[heme], and (D) Deoxy-[heme]. Time to peak was significantly delayed post-heating for % O_2 saturation and deoxy-[heme] ($p<0.01$ and $p<0.04$, respectively), while there was no significant effect for total-[heme] or oxy-[heme] ($p>0.05$).

Table 2.1 Cardiovascular responses

	PRE	POST
SR_{AUC} (10^3 s^{-1})	2.89 ± 1.05	2.51 ± 1.76
VC (% change)		
Peak	100	89.8 ± 12.5
AUC	100	87.6 ± 46.7
PABF (mL/min)		
Peak	771 ± 415	731 ± 400
Time to peak	20 ± 20	16 ± 11
Δ O_2 % Saturation		
Peak	6.5 ± 2	6.7 ± 1.7
TTP	39 ± 8.9	43.5 ± 8.2*
Δ Oxy-[heme] (µM)		
Peak	44.5 ± 26.1	41.1 ± 17.4
TTP	38.6 ± 11.2	40.5 ± 9.6
Δ Total-[heme] (µM)		
Peak	34.6 ± 20.5	31.7 ± 12.6
TTP	35.3 ± 13.2	31.5 ± 13.9
Δ Deoxy-[heme] (µM)		
Peak	-11 ± 8	-14.2 ± 9.5
TTP	41.3 ± 18.1	51.4 ± 16.3*
Blood pressure (mmHg)		
Systolic	125 ± 10	125 ± 14
Diastolic	73 ± 11	72 ± 11
Mean	74 ± 11	73 ± 11

Data are mean ± SD. SR_{AUC}, area under the curve above baseline of the shear rate stimulus from release of the arterial occlusion to peak dilation; VC, vascular compliance; PABF, popliteal artery blood flow. * Significantly different compared to pre-heating.

Chapter 3 - Influence of acute passive heating effects on neuromuscular function, exercise tolerance, and expression of serum HSP70

Summary

Previous investigations of passive heating have shown improved muscular strength and recovery from exercise. The purpose of this study was to determine the effect of one bout of passive heating on exercise tolerance, muscle recovery, and the underlying mechanisms. We hypothesized that one bout of passive heating would lead to an increases in exercise duration, faster muscle recovery post exercise, and greater expression of serum heat shock protein 70 (HSP70) levels. Eleven healthy subjects were recruited to performed intermittent isometric knee extension tests to exhaustion at 40% maximal voluntary contraction (MVC) pre and post one bout of whole-body passive heating. The heating modality utilized was water immersion in a 40°C hot tub to maintain a 1°C increase in rectal temperature for 60 min. Peripheral and central fatigue recovery were measured at the end of each exercise test. Exercise duration during isometric knee extension at 40% MVC from pre- (429 ± 72 sec) to post-heating (580 ± 109 sec) after a singular session of whole-body passive heating was not changed (p=0.115). HSP70 at rest pre- (8.29 ± 10.9) to post-heating (10.1 ± 15 ng/mL) was not different (p=0.47). The time course of peripheral fatigue recovery from the exercise protocol was not impacted by the heat treatment (p=0.63). The time course of central fatigue recovery from the exercise protocol was not impacted by the heat treatment (p=0.42). RMS was significantly decreased post-heating despite no change in the time to exhaustion (p=0.018). Total-[heme] (diffusive O_2 delivery) at the end of exercise was significantly decreased after heating (p=0.046). This data suggests that one bout of

passive heating was not sufficient stimulus to increase exercise tolerance or alter neuromuscular fatigue profiles, however diffusive O_2 delivery in the skeletal muscle was decreased.

Introduction

Heat therapy has been utilized for generations in the treatment of muscle disorders, comfort of participants, and other medicinal healing (Ablin, Hauser, & Buskila, 2013; Barfield & Hodder, 1987; Beever, 2010; Stadnyk, Rehrer, Handcock, Meredith-Jones, & Cotter, 2018). Ohori et al. showed that repeated exposure to sauna treatment increased exercise tolerance and endothelial function in patients with chronic heart failure (Ohori et al., 2012). Likewise, beneficial skeletal muscle adaptations have also been seen after single and repeated exposure to passive heating (Kim, Kuang, Song, Gavin, & Roseguini, 2019; Kim, Reid, et al., 2020; Racinais, Wilson, & Periard, 2017). Ten weeks of single-leg heating results in an increase in maximal isometric torque and cross-sectional area of the quadriceps muscle (Goto et al., 2011). Skeletal muscle treated with local heat therapy exhibited accelerated functional recovery and improved fatigue resistance after muscle-damaging exercise (Kim et al., 2019). The combination of increased endothelial and skeletal muscle function after passive heating could lead to functional improvements (i.e. increased exercise duration or increased strength), which may provide a therapeutic intervention for clinical populations. Previous research has investigated neuromuscular function immediately post whole body passive heating and found that central and peripheral factors both contribute to a decline in force production, with central fatigue accounting for <42% of the total decrease (Periard, Caillaud, & Thompson, 2011). However, despite the induction of central fatigue via the passive heat treatment, voluntary activation (VA) was not affected during a prolonged maximal voluntary contraction (MVC) (Periard et al., 2011).

Mechanistic stimuli produced by passive heating, such as increased cardiac output, metabolic alterations, and activation of signaling pathways, initiate secondary signaling that may lead to muscular adaptations (Chiesa, Trangmar, & Gonzalez-Alonso, 2016; Hesketh et al.,

2019). Examples include shear-stress on vascular walls, calcium influx, and ATP turnover. Heat shock proteins (HSPs) are elevated in circulation and skeletal muscle during physiological stress, such as heat (Gupte, Bomhoff, Touchberry, & Geiger, 2011) and exercise (Febbraio & Koukoulas, 2000; Walsh et al., 2001). Specifically, HSP72 (the inducible form of HSP70) has been shown to lead to increases in protein content, protein synthesis, and the number of mitochondria (Henstridge et al., 2014). The expression of HSP72 is controlled by intracellular heat shock transcription factor-1 (HSF-1) and is dependent of the intensity and duration of the stress (Blake, Gershon, Fargnoli, & Holbrook, 1990; Ruell, Hoffman, Chow, & Thompson, 2004). Rats that have an overexpression of HSP72 have an increased exercise performance (Henstridge et al., 2014). Racinais et al. (Racinais, Wilson, & Periard, 2017) found that increasing core temperature through passive heating provided sufficient stress to improve muscle function in humans. They demonstrated that repeated daily bouts over 11 days of passive heating led to an increase in knee extension torque produced for the same muscle recruitment by iEMG (Racinais, Wilson, & Periard, 2017). However, the authors did not measure HSP72 in the blood (extracellular) or skeletal muscle (intracellular), to which they attributed as a possible mechanism for the increased function. A different approach that has been utilized is the assessment of mechanistic changes without functional outcomes. Hafen et al. showed that six days of repeated local exposure increased the expression of HSP70 in the skeletal muscle (Hafen, Preece, Sorensen, Hancock, & Hyldahl, 2018). Given that previous studies have investigated either the effects of repeated bouts of heating on functional outcomes or the mechanistic changes, the effect of acute exposure on the combination of changes in function and mechanistic stimulus needs to be assessed.

Therefore, the purpose of this study was to investigate the effect of passive heating on exercise tolerance, muscle recovery, and the underlying mechanisms. We hypothesized that one bout of passive heating would lead to 1) increased exercise tolerance (T_{lim}; increased time to exhaustion), 2) faster muscle recovery post exercise, and 3) greater extracellular HSP72 levels in the blood.

Methods

Ethical Approval

The current study was approved by The Institutional Review Board for Research Involving Human Subjects at Kansas State University (#9697), in accordance with the standards set forth by the Declaration of Helsinki, except that data from the current study was not registered in a database. Written informed consent was obtained from all participants prior to enrollment in the study and any data collection.

Subjects

Eleven healthy subjects (6 men) were recruited to participate in the study. Subject characteristics were (mean ± SD): age: 25 ± 6 years, height: 172 ± 6 cm, and weight: 76.9 ± 18.2 kg. All subjects completed a detailed medical health history questionnaire to ensure that they were free of known cardiovascular, pulmonary, and metabolic diseases. The experimental protocols were explained to the subjects prior to entering the study. Subjects were instructed to abstain from vigorous activity 24 h prior, alcohol 12 h prior, and caffeine or food consumption 2 h prior to the scheduled testing time.

Experimental Design

Subjects visited the laboratory a minimum of 3 times and were first familiarized with all testing procedures and equipment prior to testing. Day 1, subjects performed constant-torque isometric knee extension exercise at 40% MVC until task failure. Day 2, subjects were passively heated in a hot tub for 1 hour and had increased core temperature of a minimum 1°C. Day 3 (within 24 hours of Day 2), subjects repeated the constant-torque test until task failure.

Constant-torque test

Subjects performed isometric knee extension MVCs with 1 min between each attempt until they were able to produce 2 MVCs within 5%. Pre- and post-heating, subjects performed constant-torque tests to task failure) at 40% MVC, using a 60% duty cycle (3 sec contraction; 2 sec relaxation) per a pre-recorded audio cue. Task failure was defined as the inability to maintain contraction pace or produce the required force for three consecutive contractions. If exercise continued for >20 min, the exercise intensity was considered to be below critical torque (CT) and the test was terminated and post exercise measurements were made.

Intervention

For the heating intervention, subjects were immersed up to the shoulders in a 40°C hot tub for 60 min, where rectal temperature (T_{rec}) increased 1°C or reached 38.5°C. Once the target T_{rec} was reached the subject was instructed to come out of the water to waist-level to maintain T_{rec} for the remainder of the time. Following the end of hot water immersion, subjects were monitored for another 10 mins of recovery. Core temperature was monitored using a sterile rectal thermometer probe (YSI Series 400, Yellow Spring Instruments, Yellow Springs OH, USA) inserted ~10 cm past the anal sphincter. While in the tub, subjects were allowed to drink water *ad libitum*.

Experimental Measures

Neuromuscular function

Neuromuscular function testing was conducted similar to previous protocols used in our laboratory (Broxterman et al. 2015b, Alexander et al. 2019). Briefly, testing was performed on the right leg prior to and following the constant torque exercise test. The right ankle was secured to a force transducer (LBG1, BLH Electronics, Waltham, MA) and ankle height was adjusted for

each subject such that a 90° angle of pull was maintained. Adhesive electrodes (4 x 6 cm) were used to electrically stimulate the right quadriceps muscle via the femoral nerve. The anode was attached to the gluteal fold and the cathode was positioned over the approximate location of the femoral nerve (Babault et al. 2001). Before beginning each exercise protocol, the placement of the cathode that produced the greatest force development with electrical stimulation was determined and used for pre- and post-exercise testing. Force was sampled at 1000 Hz and displayed on a computer screen (LabVIEW, National Instruments, Austin, TX). The quadriceps muscle was stimulated using a high-voltage constant-current electrical stimulator (DS7AH, Digitimer, Welwyn Garden City, UK). Paired stimuli (doublets) were delivered at 400 V with 100 μsec square-wave pulse durations and a 10 msec pulse interval. Maximal stimulation was assessed prior to each exercise bout. Stimulation intensity was initiated at 25 mA and was increased in 25 mA increments until the measured force and compound muscle action potential (M-wave) ceased to increase. The stimulator current was then increased an additional 30% to ensure the stimuli were supramaximal. Prior to each exercise test, subjects performed a series of six, 3 sec MVCs, beginning every 30 sec. Doublet muscle stimulations were delivered 5 sec prior to each MVC, 1.5 sec into the MVC, and 5 sec after each MVC to obtain measurements of unpotentiated, superimposed, and potentiated doublet (Q_{tw}) forces, respectively. Changes in MVC, Q_{tw}, and %VA have been used extensively to quantify global, peripheral, and central fatigue, respectively (Alexander et al., 2019; Bigland-Ritchie, Furbush, & Woods, 1986; Broxterman et al., 2015). This neuromuscular assessment was completed a second time starting immediately following task failure.

Electromyography

Surface EMG measurements were obtained during each session using a commercially available system (Trigno EMG, Delsys Inc., Boston, MA). The EMG sensor contained four electrodes (5 x 1 mm) arranged in a 2 x 2 orientation to make single differential measurements. The belly of the right vastus lateralis was identified and the sensor was secured using an adhesive film. The EMG data were collected at a sampling rate of 1000 Hz and band-pass filtered (13–400 Hz) using a fifth-order Butterworth filter. The EMG signal corresponding to each muscle contraction was detected using previously developed (in house) software (MATLAB R2011a, The Mathworks, Natick, MA). The amplitude characteristics were described using the root mean squared (RMS) to provide an index of motor unit recruitment. The frequency characteristics were described via median power frequency (MedPF) to provide an index of the muscle action potential conduction velocity. The EMG data were analyzed using binned averages of 2 contractions.

Near-Infrared Spectroscopy

The oxygenation characteristics of the *vastus lateralis* were determined using a frequency-domain multi-distance NIRS (Near-infrared spectroscopy) system (Oxiplex TS, ISS, Champaign, IL, USA). This system provides a calculation of absolute concentrations of deoxygenated [Hb+Mb] (deoxy-[Hb+Mb]), oxygenated [Hb+Mb] (oxy-[Hb+Mb]), total-[Hb+Mb], and % saturation. The principles and algorithms of the NIRS technology were reviewed by Gratton et al. (Gratton, Fantini, Franceschini, Gratton, & Fabiani, 1997) and have previously been described by Ferreira et al. (Ferreira, Harper, & Barstow, 2006). Barstow has comprehensively described the application of NIRS to skeletal muscle (Barstow, 2019). Briefly, this device consists of eight light-emitting diodes (LED) operating at wave-lengths of 690 and 830 nm (four LEDs per

wavelength) with one detector fiber bundle and LED-detector separation distances of 2.0, 2.5, 3.0, and 3.5 cm. The NIRS data were collected at 50 Hz and stored for post-hoc analysis. The original deoxy-[Hb+Mb], oxy-[Hb+Mb], and total-[Hb+Mb] concentrations were multiplied by a factor of four to return the values from units of [Hb+Mb] concentration into the original units of heme concentrations and are hereby denoted as deoxy-[heme], oxy-[heme], and total-[heme], respectively (Hammer et al., 2018). Changes in total-[heme] have been interpreted to reflect changes in microvascular [Hb] (i.e., hematocrit), reflective of diffusive O_2 delivery, while absolute values for deoxy-[heme] have been used to estimate fractional O_2 extraction and perfusive O_2 delivery (Davis & Barstow, 2013). After locating the *vastus lateralis* of the right leg using EMG and palpation, the NIRS probe was secured longitudinally along the belly of the muscle. The NIRS probe was calibrated prior to each test according to the manufacturer's recommendations using a calibration block with known absorption and scattering coefficients. Calibration was confirmed on a separate block with different absorption and scattering coefficients.

Blood Sampling

Venous blood samples were collected pre heating and within 24 hours of the heating intervention. Four ml of blood were collected in a tube containing a clot-inducing plug. The tube was inverted multiple times, sat for 30 min to allow blood to clot, and then centrifuged at 1000 x g for 4°C for 15 min. The separated serum was collected and stored at -20°C prior to analysis for HSP72 protein.

Blood Analysis

An enzyme-linked immunosorbent assay (ELISA) method (HSP70 high sensitivity ELISA kit, (Enzo, CAT. ADI-EKS-715)) was used to determine the relative expression of the HSP72 protein in the serum. Serum samples and standard were added to the immunoassay plate provided and then incubated for 2 hours. The wells were precoated with a monoclonal antibody for HSP70. The plate was washed, leaving only bound HSP70 on the plate. A yellow solution of polyclonal antibody, specific to HSP70, was used to bind the HSP to the plate and then incubated for 1 hour. The plate was then washed to remove excess antibody and a blue solution of HRP conjugate was added to each well. The plate was the incubated for 1 hour before being washed. Tetramethylbenzidine (TMB) substrate solution was added and a HRP-catalyzed reaction generated blue color to appear in the solution. The plate was incubated for 30 minutes, then stop solution (hydrochloric acid in water) was added to stop the reaction. The resulting yellow color was read at 450 nm with the amount of signal directly proportional to the level of HSP70 in the sample.

Statistics

Statistical analyses were performed using a commercially available software package (Sigma Plot 12.5/SigmaStat 3.5, Systat Software, Point Richmond, CA). Two-tailed paired t-test was used for the comparison of T_{lim} pre and post heating. Changes in Q_{tw}, MVC, and %VA were compared using two-way repeated measures ANOVA with repeated factor of test/twitch condition and between subject's condition (pre or post heating). A two-way repeated measures ANOVA was used to test for differences in EMG between the first contraction compared to the average of the last three contractions (condition, first vs. last). One-way repeated measures ANOVA (condition, first vs. last) was used to determine the effect of heating on % change in

RMS, MedPF, total-[heme], deoxy-[heme], oxy-[heme], % O_2 saturation, and blood pressure. If significant main effects were determined, a Student Newman's post hoc analysis was performed to determine where significant differences existed. All data were expressed as mean ± SD, unless otherwise stated. Statistical significance was declared when $p<0.05$.

Results

T_{lim} for pre- and post-heating

Mean ± SD pre MVC and 40% MVC were 67.1 ± 23.1 and 27.4 ± 9.2 kg. T_{lim} for pre- and post-acute heating were 429 ± 72 sec and 580 ± 109 sec ($p = 0.115$). Individual and mean changes in T_{lim} are shown in Figure 3.1. 1 subject's post heating test was terminated after 20 min (* indicated in Figure 3.1).

Heat-shock proteins

Mean and individual HSP70 concentrations for pre and post heating are displayed in Figure 2. HSP70 values at rest pre and post heating were 8.29 ± 10.9 and 10.1 ± 15 ng/mL. There was no significant difference between pre and post heating for HSP70 ($p = 0.47$).

NIRS

Muscle oxygenation responses during the exercise protocol are shown in Figure 3.5. For both pre- and post-heating, end exercise values (μM) for Total-[heme] and Deoxy-[heme] were significantly increased compared to baseline ($p < 0.01$, $p < 0.005$). Pre- and post-heating end exercise values for O_2 Sat % was significantly decreased compared to baseline ($p < 0.02$).

There was no difference in end exercise values for O_2 Sat %, Oxy-[heme], and Deoxy-[heme] between pre and post heating. However, the end exercise value for Total-[heme] was significantly less post heating compared to pre ($p = 0.046$), suggesting that the diffusion of O_2 in the exercising muscle was reduced. There was no difference in % change from baseline to end exercise for O_2 Sat %, Total-[heme], Oxy-[heme], and Deoxy-[heme] between pre and post heating.

Neuromuscular Function

Root mean square (RMS) and median power frequency (MedPF) responses during 40% MVC isometric knee extension pre- and post-acute heating are shown in Figure 3.4. RMS significantly increased during both pre- and post-heating exercise tests ($p < 0.001$ and $p = 0.012$). The % change in RMS at end exercise was significantly less post-heating compared to pre-heating ($p = 0.018$). MedPF did not significantly change during exercise pre- or post-heating. The % change from start to end of exercise in MedPF was no significantly different between pre- and post-heating ($p = 0.092$).

The post-exercise % changes in potentiated twitch (Q_{tw}) for pre- and post-acute heating are shown in Figure 3.3a. Compared to resting baseline, the Q_{tw} was significantly reduced for twitches 1-6 following the exercise test for both pre- and post-heating (all $p < 0.05$), but there was no significant difference between pre- and post-heating. Within the pre-test, compared to twitch 1 the % change in Q_{tw} was significantly less for twitches 4, 5, and 6 ($p = 0.038$, $p = 0.005$, and $p = 0.005$). Similarly, within the post-test, compared to twitch 1 the % change in Q_{tw} was significantly less for twitches 4, 5, and 6 ($p = 0.036$, $p = 0.041$, and $p = 0.007$), indicating significant partial recovery of Q_{tw} starting ~90 secs (Twitch 4) post-exercise.

Pre- and post-heating changes in MVC post-exercise are shown in Figure 3.3b. MVC was significantly reduced following exercise for pre- and post-heating for twitches 1-6 compared to baseline (all $p < 0.05$), but unlike Q_{tw}, there was no significant recovery of MVC over the monitored 150 sec (out to Twitch 6).

Post-heating %VA at end of exercise for twitches 3 and 5 was significantly less compared to post-heating baseline ($p < 0.001$ and $p = 0.007$). The %VA post-exercise post heating for twitches 3 and 5 was also significantly less compared to post-exercise pre-heating ($p = 0.032$ and

p = 0.010). The %VA post-exercise pre-heating for twitch 6 was also significantly less compared to baseline (p = 0.048). However, when looking at %VA as a % change there was no significant differences post-exercise compared to baseline for pre or post heating (Figure 3.3c.); nor were there significant differences between pre- and post-heating.

Discussion

The aims of this study were to determine the effects of one bout of passive heating on 1) exercise duration, 2) muscle recovery, and 3) the underlying mechanisms. We found that exercise duration during isometric knee extension at 40% MVC was not significantly increased after a singular session of whole-body passive heating. The time course of immediate neuromuscular recovery from this exercise was not impacted by the heat treatment. However, we did find that the change in total-[heme] (diffusive O_2 delivery) during exercise was significantly decreased after heating (Figure 3.5B). RMS, which provides an index of motor neuron recruitment, was significantly decreased post-heating despite no change in the time to exhaustion (Figure 3.4A). The expression of serum HSP70 in the circulation was not changed ~24 hours post the heat treatment. This suggests that changes seen in the skeletal muscle after one bout of passive heating do not provide enough stimulus to increase exercise tolerance.

Exercise Tolerance

The data from our study showed that exercise duration was not significantly increased after one bout of passive heating. However, time to exhaustion increased from 429 sec at pre-heating to 580 sec at post-heating, which is an average increase of 2.5 min of exercise time after the heating intervention. Previous research has shown that chronic heart failure patients demonstrated significant increase in exercise tolerance after 3 weeks of repeated heat treatments (Ohori et al., 2012). This increase in endothelial function was significantly correlated with the increases in exercise tolerance that was observed. We previously demonstrated (Chapter 2) that one bout of passive heating led to an increase in endothelial function. Duration, intensity, occurrence, and modality of the heat treatments all play a role response seen in subjects (Kim, Monroe, Gavin, & Roseguini, 2020). The lack of change seen in the current study may be due to

multiple factors, such as being underpowered or the occurrence of the passive heat treatment being inadequate to stimulate improvements in functional outcomes.

Fatigue

Fatigue during exercise has been investigated as peripheral (Q_{tw}; at or distal to the neuromuscular junction) and central (% VA; proximal to the neuromuscular junction) fatigue (Bigland-Ritchie et al. 1978, 1986; Kent-Braun 1999; Burnley 2009) using electrical stimulation. Decreases in Q_{tw} are used as evidence of peripheral fatigue following exercise (Bigland-Ritchie et al. 1986). Our data showed a reduction in Q_{tw} after pre- and post-heating exercise. This reduction Q_{tw} is consistent with previous data collected across a multitude of work rates (Burnley 2009 2012). However, our data demonstrated a recovery in peripheral fatigue ~90 sec post exercise in both pre- and post-heating conditions. Impaired handling or sensitivity of intracellular Ca^{2+} is one mechanism that contributes to peripheral fatigue. Previous work measured immediately post heating has indicated that heat stress may stimulate myoplasmic Ca^{2+} accumulation and Ca^{2+} from the sarcoplasmic reticulum (Kokura et al., 2007; van der Poel & Stephenson, 2007), but our data shows peripheral fatigue recovery occurring quickly after exercise. This suggests that [Ca^{2+}] accumulation from a passive heat treatment was not sustained 24 hours post one bout of passive heating.

Decreases in % VA are indicative of central fatigue and in this study % VA following task failure was not decreased pre- or post-heating. However, we do show large variability in %VA and cannot discount central fatigue as a contributor to exercise failure. The decreased RMS at task failure post-passive heating indicated decreased excitatory input to the motorneuron pool. This is likely due to decreased motor cortical input (Taylor, Amann, Duchateau, Meeusen, & Rice, 2016). It has been shown that as fatigue develops during submaximal exercise additional

motor units are progressively recruited. Previous research has suggested that mechanisms of central fatigue impact performance, as shown by participants reporting that as submaximal exercise continues more effort is required to reach the same force output (Garland, Enoka, Serrano, & Robinson, 1994), which is we expect from exercise at 40% MVC. Our results for central fatigue are consistent with previous work that examined fatigue post-exercise that was ≥30% MVC (Bigland-Ritchie et al., 1986; Bigland-Ritchie, Jones, Hosking, & Edwards, 1978). However, others have found that during isometric knee extension exercise (38, 42, and 46% MVC) to task failure %VA was decreased (Burnley, Vanhatalo, & Jones, 2012). Racinais and colleagues suggested that subjects acclimated to heat treatments for 11 days showed supraspinal adaptations and the ability to maintain VA during a sustained contraction was improved (Racinais, Wilson, Gaoua, & Periard, 2017). Previous studies have examined central fatigue immediately post passive heating and showed no change in %VA (Periard et al., 2011), which is in agreement with our findings of no change in %VA pre- to post-exercise or in pre- to post-heating . During isometric exercise less blood flow occlusion occurs compared to sustained MVC exercise, thus K^+ build-up is decreased. This decrease in metabolite accumulation could reduce the firing of group III and IV afferents.

Improved efficiency

Decreased changes in total-[heme] suggest that the diffusion of O_2 into the exercising muscle was reduced after one bout of passive heating. This finding, along with no change in the perfusive O_2 delivery, as represented by the changes in deoxy-[heme], suggests that the O_2 uptake of the exercising muscle was decreased. It is currently unclear what mechanisms are responsible to the improved efficiency of the skeletal muscle. Previous research has found that passive heating increased endothelium-specific eNOS content and prevented a decline in

capillarity index or increased capillary density (Hesketh et al., 2019; Kim, Reid, et al., 2020). These findings are consistent with Brunt et al., who demonstrated an increase in eNOS and endothelial tubule formation in cultured endothelial cells exposed to serum from participants that had undergone repeated passive heating (Brunt et al., 2019). It has been proposed that these findings lead credence to NO as a mediator of skeletal muscle angiogenesis induced by passive heating (Kim, Monroe, et al., 2020). Hafen and colleagues found that repeated exposure to heat increased the mitochondrial respiratory capacity and content of the skeletal muscle in humans (Hafen et al., 2018). This may be due to upregulation of AMP-activated protein kinase and peroxisome proliferator-activated receptor γ–coactivator 1α, which have been stimulated by heat stress and are mediators of mitochondrial biogenesis and expression (Liu & Brooks, 2012).

HSP expression

Exposure to heat-stress, such as passive heating, upregulates HSF-1 leading to trimerization and translocation to the nucleus, then binding to sites in HSP gene promoters, which activates gene transcription (McClung et al., 2008). Our data showed that serum HSP70 was not increased after one bout of whole body passive heating (Figure 3.2). This may be due to multiple factors, such as timing and analysis of the sample. Recent work from Racinais and colleagues found that one bout of whole body heating induced a significant increase in HSP72 in the skeletal muscle, however single-leg heating did not elicit an increase (Racinais 2020). Brunt et al. reported that HSP72 was not elevated in serum one hour post heating, but did show that HSP72 was upregulated intracellularly in peripheral blood mononuclear cells (PBMCs) after one hour (Brunt, Wiedenfeld-Needham, Comrada, & Minson, 2018). However, another research group found that after 2 days of exercise-heat exposure there were no changes in PBMC HSP72 expression at rest (Marshall, Campbell, Roberts, & Nimmo, 2007). The majority of stress-

induced HSP72 has been found to be released in to the blood via an exosome release pathway. The release of HSP72 enveloped in exosomes provides a protective lipid bi-layer that aids communication between cells over a long distance. Bausero et al. showed that while extracellular HSP72 can be found freely in circulation there was a significant increase in synthesis and expression of HSP72 within exosomes (Bausero, Gastpar, Multhoff, & Asea, 2005).

Limitations

A critical limitation to the current study was that adaptations were obtained with a large external heat load, leading to an increase of 1°C in core temperature. Adaptations in skeletal muscle have been seen to occur when the muscle temperature increases 3-4°C (Hafen et al., 2018). However, previous studies have seen adaptations in both skeletal and vascular function after whole body heating ((Brunt, Howard, Francisco, Ely, & Minson, 2016; Kim, Reid, et al., 2020; Racinais, Wilson, & Periard, 2017)). Another limitation to this study was that subjects could not be blinded to the intervention and outcomes measured may have been impacted by the placebo effect. However, since we did not observe any significant changes in time to exhaustion, it was unlikely that knowledge of the intervention impacted the results. Measurement of HSP70 in this study was conducted utilizing serum samples obtained pre-exercise on both the pre- and post-heating days. We wanted to determine the effects of a passive heating bout on HSP70 concentration and the impact this could have on skeletal muscle function. However, to detect changes in the concentration of circulating HSP70 measurements may need to examine exosomes that contain HSP70 or biopsy the skeletal muscle.

Conclusions

Our results show that exposure to a single bout of whole-body passive heating was not an adequate stimulus to increase exercise duration, enhance neuromuscular recovery, or

upregulate serum HSP70. However, we did demonstrate that there was a decrease in the diffusive O_2 delivery, suggesting that O_2 uptake was decreased in the exercising skeletal muscle. These findings add to the growing body of research on the effects and mechanisms of passive heating in humans. Additional studies are warranted to examine repeated bouts of passive heating on exercise tolerance and skeletal muscle adaptations.

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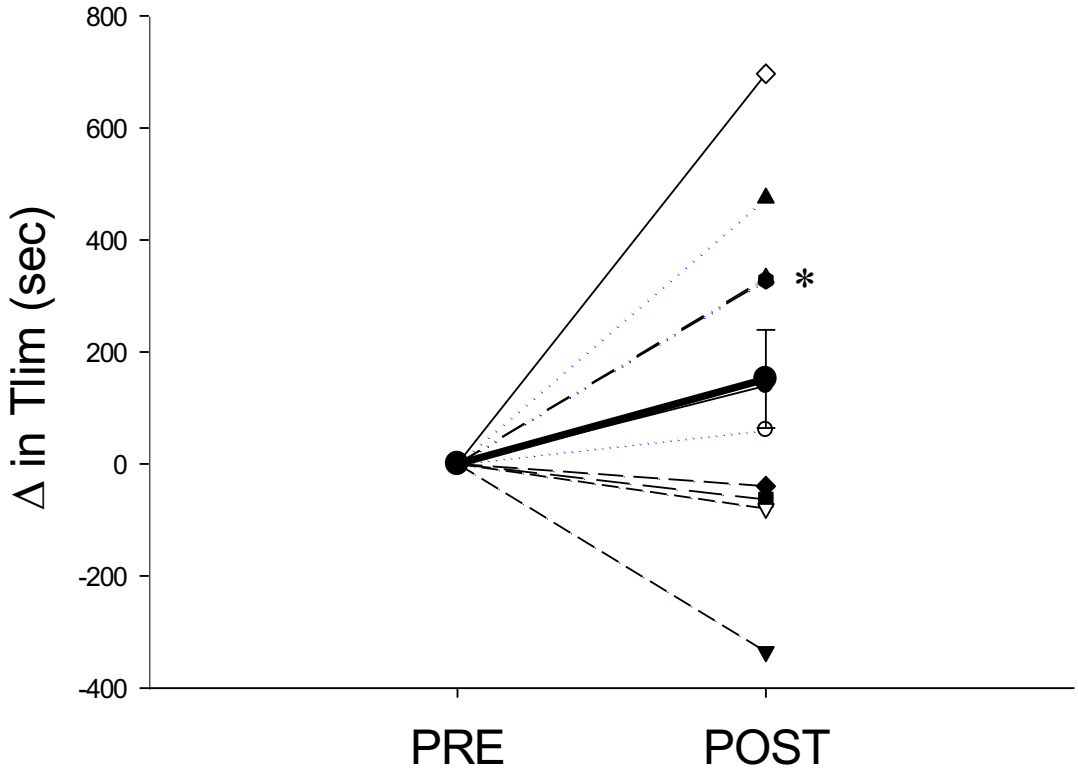

Figure 3.1 Changes in T_{lim} from pre- to post-heating

Individual and Mean ± SE changes in T_{lim} from pre to post heating. No differences were detected. * T_{lim} > 20 minutes and thus exercise was terminated.

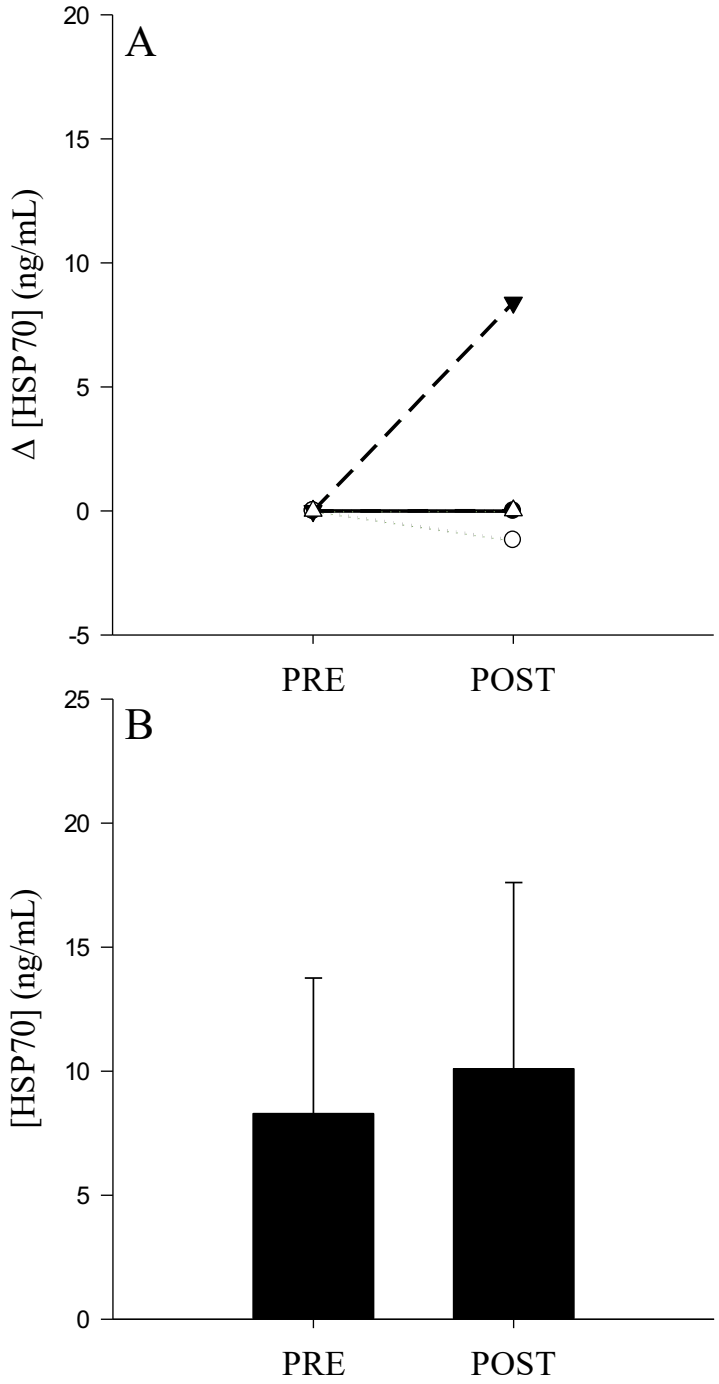

Figure 3.2 Heat Shock Protein (HSP) 70.

A) Individual responses of serum HSP70 pre and post passive heating. **B)** Mean ± SE values of serum HSP70. No differences were detected between pre and post heating.

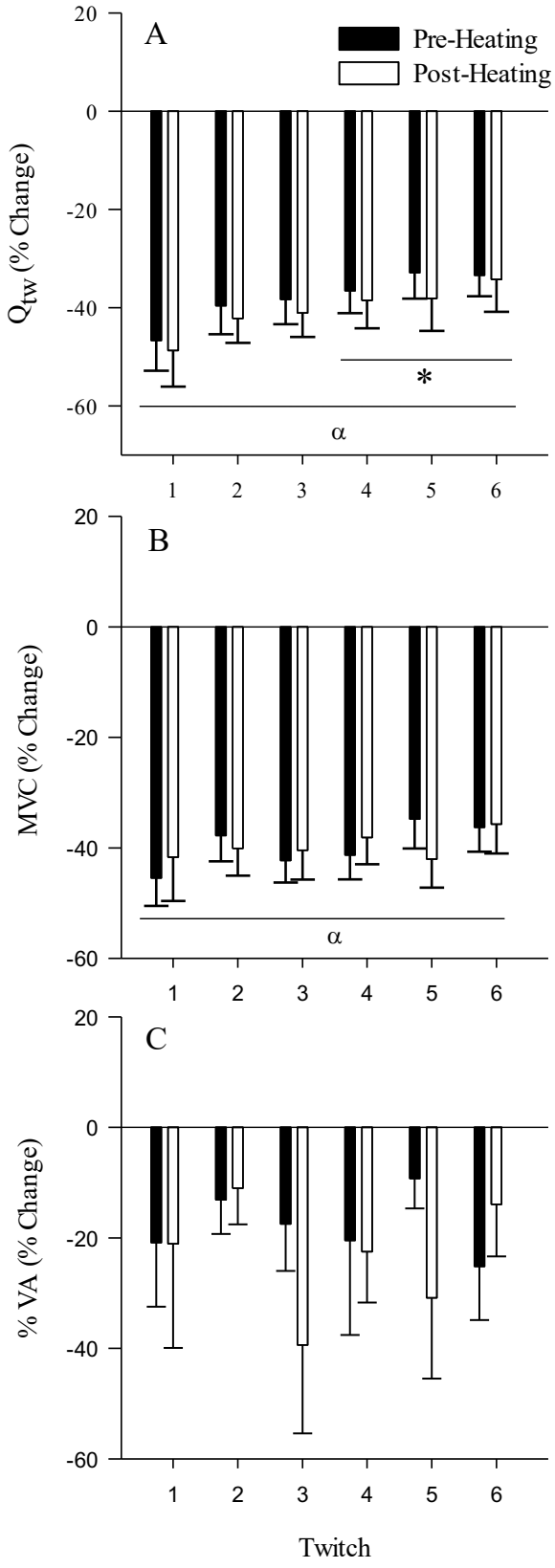

Figure 3.3 Changes in neuromuscular function pre- to post-exercise.

Percent change in **A)** potentiated twitch (Q_{tw}), **B)** maximal voluntary contraction (MVC), and **C)** % voluntary activation (%VA) post-exercise for all twitches (Mean ± SE). Q_{tw} and MVC following exercise pre- and post-heating were significantly decreased ($p<0.05$). No differences in %VA were detected in the percent change pre- to post-exercise or between pre and post heating. $^{\alpha}$ different from pre exercise ($p<0.05$); * different from Twitch 1 ($p<0.05$).

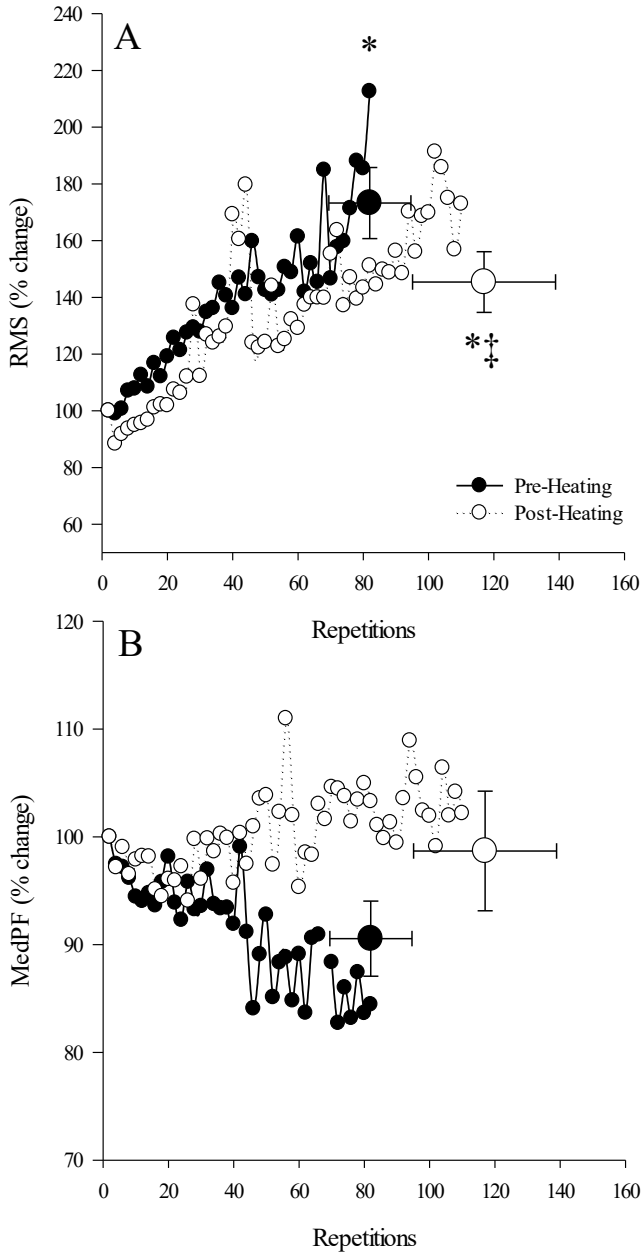

Figure 3.4 Electromyography (EMG) data pre- and post-heating.

Root mean square (RMS; **Panel A**) and median power frequency (MedPF; **Panel B**) throughout exercise for pre- and post-heating. Changes shown as a percent of the first contraction and averaged into 2 contraction bins. RMS increased throughout pre- and post-heating so that end exercise was significantly greater than at the beginning of exercise ($P < 0.05$) and % change in

post-heating was significantly less than pre-heating ($p = 0.018$). MedPF was not significantly changed at end exercise compared to the beginning for both pre and post heating ($p = 0.22$, nor were there differences in % change of MedPF pre- and post-heating at end exercise ($p = 0.092$).

*Significantly greater than beginning of exercise; ‡Significantly different from pre-heating.

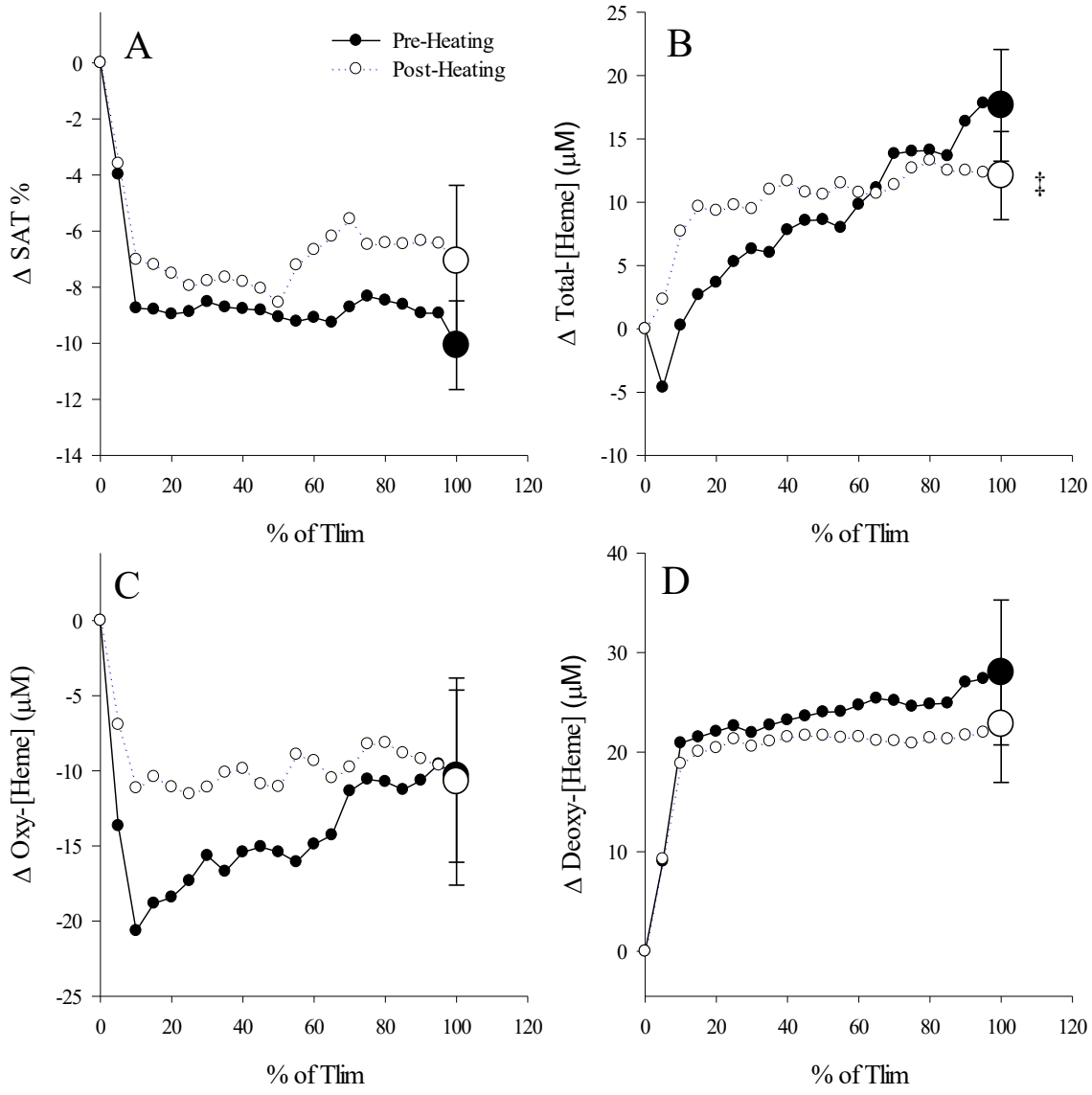

Figure 3.5 Muscle oxygenation responses during the exercise protocol.

A–D: the mean responses for percentage saturation (*top left*), total-[heme] (*top right*), oxy-[heme] (*bottom left*), and deoxy-[heme] (*bottom right*) from rest to end exercise. Data points at end exercise are Mean ± SE. ‡Significantly different from pre-heating ($P<0.05$).

Chapter 4 - The response of endothelial function, exercise tolerance, and serum heat shock proteins to repeated bouts of passive heating

Summary

Improvements in endothelial function and skeletal muscle physiology has been stimulated from utilizing passive heat treatments. The aim of this study was to determine the influence of 11 consecutive days of passive heating on endothelial and muscular function, along with the time course of serum heat shock protein (HSP) expression. We hypothesized that repeated bouts of whole-body passive heating over 11 days would increase popliteal endothelial function measured by flow-mediated dilation (FMD), increase exercise duration, accelerate fatigue recovery post exercise, and 4) upregulate the expression of serum HSP90α and HSP72. Seven healthy subjects were recruited to performed intermittent isometric knee extension tests to exhaustion at 40% maximal voluntary contraction (MVC) pre and post 10-11 consecutive days of whole-body passive heating and after the first bout of passive heating. The heating modality utilized was water immersion in a 40°C hot tub to maintain a 1°C increase in rectal temperature for 60 min. Peripheral and central fatigue recovery were measured at the end of each exercise test. Our findings show that exercise tolerance for pre- (503 ± 276 sec), acute- (635 ± 362 sec), and chronic-heating (799 ± 459 sec) ($p=0.111$). Partial recovery of peripheral fatigue (Q_{tw}) within 60 secs (Twitch 2) post exercise pre-heating ($p<0.01$ for all), but no significant change in Q_{tw} from Twitch 1-6 after acute- and chronic-heating ($p>0.05$ for all). There were no significant differences in % changes in %VA post-exercise compared to baseline for all conditions ($p>0.05$ for all). RMS significantly increased during pre- ($p=0.002$) and chronic-heating ($p=0.001$) exercise tests, but did not significantly change during the acute-heating exercise test ($p=0.077$). A subset of 5 subjects were recruited to assess FMD and expression of HSP90α pre- and post the

11 days of passive heating and after the first bout of passive heating. We found that repeated exposure to whole-body passive heating did not provide sufficient stimulus to induce improvements in FMD ($p \geq 0.15$ for all conditions) or serum HSP90α pre- (72.7 ± 18.1), acute- (78.3 ± 37.9), and chronic- heating (76.6 ± 36.7 ng/mL) ($p=0.77$). The results of this study indicate that 11 days of whole-body passive heating may not provide sufficient stimulus to elicit functional changes in performance. However, our data did suggests that repeated bout of heating may lead to faster recovery of central and peripheral fatigue.

Introduction

Historically, heat therapy has been used to induce improvements in both muscle and cardiovascular function (Ablin, Hauser, & Buskila, 2013; Barfield & Hodder, 1987). Passive heating used as an intervention is an innovative alternative to exercise for populations that are unable to exercise or stress the body. Previous research demonstrated that 8 weeks of passive heating induced improvements in vascular function, as seen by increased endothelial function, reduced blood pressure, and reduced arterial stiffness (Brunt, Eymann, Francisco, Howard, & Minson, 2016; Brunt, Howard, Francisco, Ely, & Minson, 2016). Increasing core body temperature stimulates increases in cardiac output, blood flow, and shear stress placed on the vasculature (Crandall & Wilson, 2015). The upregulation of endothelial NO synthase, prompted by shear stress, leads to increases in NO bioavailability. Elevations in core temperature from exercise and passive heating have shown to upregulate the production of heat shock proteins (HSPs). HSP90 acts as a molecular chaperone for eNOS and a physiological binding partner and regulator of eNOS. When HSP90 and eNOS are bound there is a significant increase in the bioavailability of NO, an important vasodilator, leading to improvements in endothelial function and increases in vasodilation (Garcia-Cardena et al., 1998). We previously demonstrated in Chapter 2 that a single bout of passive heating via hot water immersion provided sufficient stimulus to increase popliteal artery flow-mediated dilation (FMD; an indication of endothelial function).

Repeated exposure to heating has also been shown to improve muscle function. Racinas et al. provided evidence that 11 days of consecutive whole body heating leads to improved skeletal muscle contraction function during electrically produced and voluntary contractions (Racinais, Wilson, & Periard, 2017). Another change in skeletal muscle seen after repeated exposure to

heating is improved fatigue resistance, accelerated recovery, and muscle repair after muscle damage (K. Kim, Kuang, Song, Gavin, & Roseguini, 2019). Goto et al. found that repeated heating increased force development and cross-sectional area (Goto et al., 2011). Muscle strength, along with capillarization, and eNOS has been found to be increased post 8 weeks of local heating of the investigated muscle (K. Kim, Reid, et al., 2020). Increased presence of NO has been shown to decrease VO_2 in the skeletal muscle during constant work rate exercise (Larsen, Weitzberg, Lundberg, & Ekblom, 2007), due to an improved mitochondrial efficiency and a decreased ATP cost of force production (Larsen et al., 2011). Animal studies have shown heating as a stimulus to promote muscle hypertrophy and protein synthesis after 36 hours (Ohno et al., 2011; Uehara et al., 2004). HSP72, the inducible form of HSP70, facilitates in the folding of proteins and refolding of damaged proteins. In contrast to adaptations to repeated heating, however, we found no improvements in exercise tolerance or muscle recovery 24 hours post 1 bout of whole-body heating.

Given the improvement in endothelial function (Chapter 2) and lack of in muscle function (Chapter 3) 24 hours following one exposure to whole-body heating, we investigated the effects of chronic exposure (repeated bouts of heating over several days) on muscle function during and immediately following exercise. Therefore, the aim of this study was to determine the influence of 11 consecutive days of passive heating on endothelial and muscular function, along with the time course of serum HSP expression. We hypothesized that repeated bouts of whole body passive heating over 11 days would 1) increase FMD response of the popliteal artery, 2) increase exercise tolerance at a given absolute work rate, 3) accelerate muscle recovery post exercise, and 4) upregulate the expression of serum HSP90α and HSP70.

Methods

Ethical Approval

The current study was approved by The Institutional Review Board for Research Involving Human Subjects at Kansas State University (#9697), in accordance with the standards set by the Declaration of Helsinki. Data from the study was not registered in a database. Written informed consent was obtained from all participants prior to any data collection. All subjects completed a detailed medical health history questionnaire to ensure that they were free of known cardiovascular, pulmonary, and metabolic disease.

Subjects

Seven healthy subjects (5 men) were recruited to participate in the study; a subset of 5 subjects were analyzed for endothelial function and serum HSP90α. Originally 11 subjects were recruited for this chronic study. Preliminary data for all 11 are reported in Chapters 2 and 3. However, current epidemiological precautions regarding COVID-19 precluded 4 of the subjects from finishing the chronic portion of the study including post-testing. Subject characteristics were (mean ± SD): age: 25 ± 5 years, height: 176 ± 3 cm, and weight: 86.0 ± 16.4 kg. The experimental protocols were explained to the subjects prior to entering the study. Subjects were instructed to abstain from vigorous activity 24 h prior, alcohol 12 h prior, and caffeine or food consumption 2 h prior to the scheduled testing time.

Experimental Design

Following familiarization with all testing procedures and equipment prior to testing, subjects completed the vascular occlusion test (VOT) and constant-torque isometric knee extension exercise at 40% MVC until task failure. Subjects then visited the laboratory 10-11 days consecutively, 60 minutes per day for whole-body passive heating sessions. Subjects then

repeated the initial testing protocols ~24 hours post the first day of heating and then at a minimum of 24 hours after the last heating session to ensure the chronic (11 day), rather than acute (1 day), effects of passive heating were being investigated. Pre-, acute-, and chronic-heating tests and heating sessions were completed within a two week period.

Heating Intervention

For the heating intervention, subjects were immersed up to the shoulders in a 40°C hot tub for 60 min, until rectal temperature (T_{rec}) increased 1°C or reached 38.5°C. Once the target T_{rec} was reached the subject was instructed to come out of the water to waist-level to maintain T_{rec} for the remainder of the time. Following 1 hour of hot water immersion, subjects were monitored for another 10 mins of recovery. Core temperature was monitored using a sterile rectal thermometer probe (YSI Series 400, Yellow Spring Instruments, Yellow Springs OH, USA) inserted ~10 cm past the anal sphincter. While in the tub, subjects were allowed to drink water *ad libitum*.

Experimental Measures

Flow-mediated dilation (FMD) and post-occlusive hyperemia (PORH)

Subjects lay prone with a small pillow under the ankle, while responses of the popliteal artery (dilation and blood flow) was measured immediately proximal to the bifurcation (at or right above the popliteal fossa). The pneumatic cuff was placed around the thigh, 5-10 cm proximal to the popliteal fossa and the cuff was inflated to 250 mmHg for 5 min (Hokanson, Bellevue, WA, USA) (Parker, Ridout, & Proctor, 2006). An external strap was placed over the cuff to ensure inward compression of the leg vasculature. Occlusion was confirmed via Doppler ultrasound of the popliteal artery distal to the inflated cuff. Popliteal artery diameter and blood

velocity were measured via Doppler ultrasonography for 1 min of baseline prior to cuff inflation and 3 min following cuff release.

Blood pressure

Systolic, mean, and diastolic blood pressures (SBP, MAP, and DBP, respectively) were measured at baseline via an automated sphygmomanometer (GE Datex-Ohmeda Light) with the subjects seated in an upright position and the right arm resting on a table at heart level.

Doppler Ultrasound

Measurements of popliteal artery diameter and blood velocity were simultaneously obtained using non-invasive 2D Doppler ultrasound equipped with a linear array transducer operating in duplex mode at a frequency of 10M Hz and 4.0 MHz, respectively (Logiq S8, GE Medical Systems, Milwaukee, WI). Doppler velocity measurements were corrected for an angle of insonation less than 60°. Baseline and post-occlusion popliteal artery diameters were calculated at 15 frames per second and averaged into 3 sec bins. Popliteal artery diameter and PABV were time-aligned (same 3 sec bins) and used to calculate popliteal artery blood flow (PABF), defined as, PABF (mL/min) = PABV x π x radius^2 x 60. Vascular compliance was calculated as the ratio of PABF to MAP where VC (mL/min x 100mmHg) = (PABF/MAP) x 100. VC area under the curve (VC_{AUC}) was calculated as the integral of VC values above baseline VC (average of 30 sec prior to cuff inflation). VC values are reported as % changes from pre-heating.

FMD was calculated as the percent change in diameter from baseline to peak dilation following cuff release. To estimate the stimulus for dilation after occlusion, shear rate (SR) was calculated as PABV/diameter and used to determine the area under the curve above baseline from time of cuff release to peak dilation (SR_{AUC}). FMD was normalized for shear rate by

dividing FMD by SR_{AUC} as previously described (Padilla et al., 2009; Pyke & Tschakovsky, 2005).

Near-Infrared Spectroscopy

The oxygenation characteristics of the *medial head of the gastrocnemius* (FMD test) and *vastus lateralis* (constant-load test) were determined using a frequency-domain multi-distance NIRS (Near-infrared spectroscopy) system (Oxiplex TS, ISS, Champaign, IL, USA). This system provides a calculation of absolute concentrations of deoxygenated [Hb+Mb] (deoxy-[Hb+Mb]), oxygenated [Hb+Mb] (oxy-[Hb+Mb]), total-[Hb+Mb], and % saturation. The principles and algorithms of the NIRS technology were reviewed by (Gratton, Fantini, Franceschini, Gratton, & Fabiani, 1997) and have previously been described by Ferreira et al. (Ferreira, Harper, & Barstow, 2006). The applications of NIRS to skeletal muscle research have been reviewed in depth (Barstow, 2019). Briefly, this device consists of eight light-emitting diodes (LED) operating at wave-lengths of 690 and 830 nm (four LEDs per wavelength) with one detector fiber bundle and LED-detector separation distances of 2.0, 2.5, 3.0, and 3.5 cm. The NIRS data were collected at 50 Hz and stored for post-hoc analysis. The original deoxy-[Hb+Mb], oxy-[Hb+Mb], and total-[Hb+Mb] concentrations were multiplied by a factor of four to return the values from units of [Hb+Mb] concentration into the original units of heme concentrations and are hereby denoted as deoxy-[heme], oxy-[heme], and total-[heme], respectively (Hammer et al., 2018). After locating the *medial head of the gastrocnemius* of the left leg or *vastus lateralis* of the right leg using EMG and palpation, the NIRS probe was secured longitudinally along the belly of the muscle. The NIRS probe was calibrated prior to each test according to the manufacturer's recommendations using a calibration block with known absorption and scattering

coefficients. Calibration was confirmed on a separate block with different absorption and scattering coefficients. Muscle oxygen uptake ($m\dot{V}O_2$) was calculated as

$$m\dot{V}O_2 \text{ (ml } O_2 \text{ min}^{-1} \text{ (100g)}^{-1}) = \text{abs}[((Hb_{diff}) \times 60)/(10 \times 1.04) \times 4] \times 22.4/1000$$

During occlusion the difference in slopes between oxy[heme] vs. deoxy[heme] for sec 30-60 were used to calculate Hb_{diff} using the following equation: Hb_{diff} = (oxy[heme]-deoxy[heme])/2 (van Beekvelt, van Engelen, Wevers, & Colier, 2002).

Constant-load test

Subjects performed isometric knee extension MVCs with 1 min between each attempt until they were able to produce 2 MVCs within 5%. Pre and post the heating intervention, subjects performed constant torque tests till exhaustion) at 40% MVC, using a 60% duty cycle (3 sec contraction; 2 sec relaxation) per a pre-recorded audio cue. Task failure (T_{lim}) was determined as the time from beginning of exercise till the subject failed to maintain contraction pace or produce the required force for three consecutive contractions. If exercise continued for >20 min, the exercise intensity was considered to be sub-severe intensity and the test was terminated and post exercise measurements were made.

Neuromuscular function

Neuromuscular function testing was conducted similar to previous protocols used in our laboratory (Broxterman et al. 2015b, Alexander et al. 2019). Briefly, testing was performed on the right leg prior to and following the constant torque exercise test. The right ankle was secured to a force transducer (LBG1, BLH Electronics, Waltham, MA) and ankle height was adjusted for each subject such that a 90° angle of pull was maintained. Adhesive electrodes (4 x 6 cm) were used to electrically stimulate the right quadriceps muscle via the femoral nerve. The anode was attached to the gluteal fold and the cathode was positioned over the approximate location of the

femoral nerve (Babault et al. 2001). Before beginning each exercise protocol, the placement of the cathode that produced the greatest force development with electrical stimulation was determined and used for pre- and post-exercise testing. Force was sampled at 1000 Hz and displayed on a computer screen (LabVIEW, National Instruments, Austin, TX). The quadriceps muscle was stimulated using a high-voltage constant-current electrical stimulator (DS7AH, Digitimer, Welwyn Garden City, UK). Paired stimuli (doublets) were delivered at 400 V with 100 μsec square-wave pulse durations and a 10 msec pulse interval. Maximal stimulation was assessed prior to each exercise bout. Stimulation intensity was initiated at 25 mA and was increased in 25 mA increments until the measured force and compound muscle action potential (M-wave) ceased to increase. The stimulator current was then increased an additional 30% to ensure the stimuli were supramaximal. Prior to each exercise test, subjects performed a series of six, 3 sec MVCs, beginning every 30 sec. Doublet muscle stimulations were delivered 5 sec prior to each MVC, 1.5 sec into the MVC, and 5 sec after each MVC to obtain measurements of unpotentiated, superimposed, and potentiated (Q_{tw}) doublet forces, respectively. Changes in MVC, Q_{tw}, and % voluntary activation (%VA) have been used extensively to quantify global, peripheral, and central fatigue, respectively (Alexander et al., 2019; Bigland-Ritchie, Furbush, & Woods, 1986; Broxterman et al., 2015). MVC was determined as the greatest force attained prior to the superimposed muscle doublet stimulation. This neuromuscular assessment was completed a second time starting immediately following task failure.

Electromyography

Surface EMG measurements were obtained during each session using a commercially available system (Trigno EMG, Delsys Inc., Boston, MA). The EMG sensor contained four electrodes (5 x 1 mm) arranged in a 2 x 2 orientation to make single differential measurements.

The belly of the right vastus lateralis was identified and the sensor was secured using an adhesive film. The EMG data were collected at a sampling rate of 1000 Hz and band-pass filtered (13–400 Hz) using a fifth-order Butterworth filter. The EMG signal corresponding to each muscle contraction was detected using previously developed (in house) software (MATLAB R2011a, The Mathworks, Natick, MA). The amplitude characteristics were described using the root mean squared (RMS) to provide an index of muscle activation and motor neuron firing rate. The frequency characteristics were described via median power frequency (MedPF) to provide an index of the muscle action potential conduction velocity. The EMG data were analyzed using binned averages of 2 contractions.

Blood Sampling

Venous blood samples were collected pre-heating, pre-acute testing, every other day during the 10-11 heating protocol, and pre-chronic testing. All samples were taken ~24 hours post the last heating intervention. Four mL of blood were collected in a tube containing a clot-inducing plug. This tube was inverted, serum was allowed to clot for 30 min, then centrifuged at 1000 x g for 4°C. The separated serum was collected and stored at -20°C prior to analysis for HSP70 and HSP90 protein.

Blood Analysis

HSP70

An exzyme-linked immunosorbent assay (ELISA) method (HSP70 high sensitivity ELISA kit' (Enzo, CAT. ADI-EKS-715)) was used to determine the relative expression of the HSP70 protein in the serum. This assay was suitable for measuring HSP70 (HSP72) in serum. Serum samples and standard were added to the immunoassay plate provided and then incubated for 2 hours. The wells were precoated with a monoclonal antibody for HSP70. The plate was

washed, leaving only bound HSP70 on the plate. A yellow solution of polyclonal antibody, specific to HSP70, was used to bind the HSP to the plate and then incubated for 1 hour. The plate was then washed to remove excess antibody and a blue solution of HRP conjugate was added to each well. The plate was the incubated for 1 hour before being washed. Tetramethylbenzidine (TMB) substrate solution was added and a HRP-catalyzed reaction generated blue color to appear in the solution. The plate was incubated for 30 minutes, then stop solution (hydrochloric acid in water) was added to stop the reaction. The resulting yellow color was read at 450 nm with the amount of signal directly proportional to the level of HSP70 in the sample.

HSP90α

An exzyme-linked immunosorbent assay (ELISA) method (HSP90α high sensitivity ELISA kit' (Enzo, CAT. ADI-EKS-895) was used to determine the relative expression of the HSP90 protein in the serum. We added 100 µL of prepared standards and samples (diluted 1:50) in duplicate to wells of the AntiHsp90α Immunoassay Plate. The plate was incubated for 1 hour before washing the wells. 100 µL of HRP Conjugate was added to each well, incubated for 1 hour, and then washed again. 100 µL of TMB Substrate was added to each well, incubated for 20 mins, and them 100 µL Stop Solution was added. The resulting yellow color was read at 450 nm and the amount of signal is directly proportional to the level of HSP90α in the sample.

Statistics

Statistical analyses were performed using a commercially available software package (Sigma Plot 12.5/SigmaStat 3.5, Systat Software, Point Richmond, CA). Changes in T_{lim}, blood pressure, and HSP90α were compared using one-way repeated measures. Two-way repeated measures ANOVA was used to determine changes in Q_{tw}, MVC, and %VA with repeated factor of twitch and between condition (pre, acute, and chronic heating). A two-way repeated measures

ANOVA was used to test for differences in EMG between the first contraction compared to the average of the last three contractions (condition, first vs. last). One-way repeated measures ANOVA was used to determine the effect of heating on % change in RMS, MedPF, total-[heme], deoxy-[heme], oxy-[heme], and % O_2 saturation. A subset of 5 subjects was used to investigate the effect of 11 days of passive heating on endothelial function. A one-tailed t-tests were utilized to compare pre- vs acute-heating, pre- vs chronic-heating, and acute- vs chronic-heating. Measurements assessed were FMD, shear-corrected FMD, AUC VC, VC peak, VC time to peak (TTP), SR_{AUC}, peak PABF, and PABF TTP. If significant main effects were determined, a Student Newman's post hoc analysis was performed to determine where significant differences existed. All data were expressed as mean ± SD, unless otherwise stated. Statistical significance was declared when $p<0.05$.

Results

Passive heating and blood pressure

Passive heating resulted in the 1°C in T_{rec}. Baseline T_{rec} was 36.9 ± 0.35°C and at the end of passive heating T_{rec} was 38.3 ± 0.41°C, for a change in T_{rec} of 1.3 ± 0.56°C. Thus we were successful in raising T_{rec}. We observed no significant difference in systolic, diastolic, and mean arterial pressure between pre and post heating (p=0.31, p=0.82, and p=0.81, respectively) (Table 4.1).

Heat-shock proteins

Serum HSP90α values at rest for pre-, acute-, and chronic- heating were 72.7 ± 18.1, 78.3 ± 37.9, and 76.6 ± 36.7 ng/mL (Fig. 4.1; p=0.77). Serum HSP90α was measured every other visit throughout the protocol (Fig. 4.2). These were no significant difference in serum HSP90α among days (p=0.97).

During the 11 days of heating serum HSP70 was measured every other visit; however, many of the values of HSP70 were below the threshold for detection, therefore no statistical comparison was possible.

Endothelial function

Fig 4.3. shows the response of FMD to the heating intervention. No differences in FMD and FMD corrected for the shear rate stimulus were detected between pre- vs acute-heating (p=0.47, p=0.15), pre- vs chronic-heating (p=0.16, p=0.22), and acute- vs chronic-heating (p=0.23, p=0.27). SR_{AUC} showed no significant difference between pre- vs acute-heating (p=0.49), pre- vs chronic-heating (p=0.46), and acute- vs chronic-heating (p=0.41) (Table 4.1). There were no significant differences in peak PABF and PABF TTP between for pre- vs acute-

heating (p=0.13, p=0.27), pre- vs chronic-heating (p=0.047, p=0.42), and acute- vs chronic-heating (p=0.064, p=0.078) (Table 4.1.). There were no significant differences in pre- vs acute-heating (p=0.47) and for VC AUC (p=0.19), however VC AUC for chronic-heating was significantly decreased compared to pre-heating (p=0.046). Peak VC was not different for pre- vs acute-heating (p=0.19) or acute- vs chronic-heating (p=0.069), conversely chronic-heating was significantly decreased compared to pre-heating vs (p=0.035). Time to reach peak VC was not significantly different between all conditions (p=0.27, p=0.42, p=0.077).

T_{lim}

Pre MVC and the target force of 40% MVC were 73.3 ± 27 and 29.3 ± 10.8 kg. T_{lim} for pre-, acute-, and chronic-heating were 503 ± 276, 635 ± 362 and 799 ± 459 sec (p=0.111). Individual and mean change in T_{lim} are shown in Figure 4.4. Chronic-heating test for 3 subjects were terminated after 20 min.

Neuromuscular Function

Root mean square (RMS) and median power frequency (MedPF) responses during 40% MVC isometric knee extension for pre-, acute-, and chronic-heating are shown in Fig 4.8. RMS significantly increased during pre- and chronic-heating exercise tests (p=0.002 and p=0.001, respectively). RMS did not significantly change during the acute-heating exercise test (p=0.077). MedPF did not significantly change during exercise pre-, acute-, and chronic-heating exercise (p=0.86).

The % changes from pre-exercise to post-exercise in potentiated twitch (Q_{tw}) for pre-, acute-, and chronic-heating are shown in Figure 4.5. Compared to resting baseline, the Q_{tw} was significantly reduced for twitches 1-6 following the exercise test for all conditions (all $p<0.05$), but there was no significant difference in the % change in Q_{tw} for twitches 1-6 between all

conditions. Within the pre-test, compared to twitch 1 the % change in Q_{tw} was significantly less for twitches 2, 3, 4, 5, and 6 ($p=0.008$, $p=0.010$, $p=0.006$, $p<0.001$, and $p<0.001$, respectively). In contrast, within the acute and chronic tests there was no significant difference in twitches 1-6. These findings together indicate significant partial recovery of Q_{tw} (peripheral fatigue) within 60 secs (Twitch 2) post exercise pre-heating, but no significant change in Q_{tw} from Twitch 1-6 after acute- and chronic-heating.

Pre- and post-heating % changes in MVC from pre- to post-exercise are shown in Figure 4.6. MVC was significantly reduced following exercise for all conditions for twitches 1-6 compared to baseline (all $p<0.05$). In addition, there was significant difference in % change in MVC between pre- and chronic-heating during twitch 1 ($p=0.020$).

There were no significant differences in % changes in %VA post-exercise compared to baseline for all conditions (Fig 4.7). Post-exercise the %VA for twitches 3 and 5 were significantly decreased compared to baseline for acute-heating ($p<0.001$ and $p=0.008$, respectively). There were no significant differences in % change in %VA between all conditions.

NIRS during exercise

Muscle oxygenation responses during the exercise protocol are shown in Figure 4.10. End exercise values for total-[heme] were not significantly different between all conditions ($p=0.067$). There was no difference in end exercise values for O_2 Sat %, oxy-[heme], and deoxy-[heme] between pre-, acute-, and chronic-heating. Likewise, there was no difference in % change from baseline to end exercise for O_2 Sat %, total-[heme], oxy-[heme], and deoxy-[heme] between all conditions. The pre-heating end exercise value for deoxy-[heme] was significantly increased compared to baseline ($p=0.042$), however total-[heme] was not significantly changed ($p=0.051$). Pre-heating end exercise values for O_2 Sat % was significantly decreased compared to

baseline ($p<0.004$). Acute-heating end exercise values for total-[heme] and deoxy-[heme] were significantly increased compared to baseline ($p=0.044$ and $p=0.020$, respectively). Chronic-heating end exercise values for O_2 Sat % and oxy-[heme] were not significantly different compared to baseline. Chronic-heating end exercise values for total-[heme] and deoxy-[heme] were significantly increased compared to baseline ($p=0.021$ and 0.046, respectively). Chronic-heating end exercise values for O_2 Sat % was significantly decreased compared to baseline ($p=0.074$).

NIRS during VOT

m$\dot{V}O_2$ for pre- (0.14 ± 0.1 ml O_2 min^{-1} $(100g)^{-1}$), acute- (0.15 .1 ml O_2 min^{-1} $(100g)^{-1}$), and chronic-heating (0.16 ± 0.2 ml O_2 min^{-1} $(100g)^{-1}$)and there was no significant difference between conditions ($p \geq 0.3$). The peak Δ in % O_2 saturation, oxy-[heme], total-[heme], and deoxy-[heme] were not significantly different between all conditions ($p>0.05$) (Table 4.1). The TTP from cuff release for % O_2 saturation acute-heating was significantly slower compared to pre- and post-heating ($p=0.049$ and $p=0.006$, respectively) (Table 4.1). The TTP from cuff release for chronic-heating total-[heme] was significantly faster than pre- and acute-heating ($p=0.011$ and $p=0.019$ respectively). The TTP from cuff release for oxy-[heme] after chronic-heating was significantly faster than pre-heating ($p=0.024$) and acute-heating ($p=0.041$), however there was no difference in TTP between pre- vs. acute-heating ($p=0.2$). Chronic-heating TTP from cuff release for deoxy-[heme] was significantly faster than acute-heating ($p=0.048$), however TTP pre-heating was not different than acute- ($p=0.09$) and chronic-heating ($p=0.41$).

Discussion

The aim of the current study was to determine the effect of 11 consecutive days of passive heating on endothelial and muscular function, along the time course of serum HSP expression. The first major finding of this study was that after repeated bouts of passive heating endothelium-dependent dilation was not increased during a VOT, however TTP was faster for the perfusive and diffusive components of skeletal muscle oxygenation. Secondly, we found that exercise duration during isometric knee extension at 40% MVC was not affected by passive heating. The third finding in the current study is that neuromuscular recovery post exercise displayed partial recovery of Q_{tw} within 60 secs for the pre-heating condition, but no recovery of Q_{tw} after acute- and chronic-heating. After chronic-heating the reduction in MVC post-exercise was significantly less compared to pre- and acute-heating. We also found that the time course of serum HSP90α during the 11 days of passive heating was not altered at rest and serum HSP70 was measured, but not detected, so no conclusions could be made.

Endothelial function

Previous studies have utilized VOTs to evaluate FMD and reactive hyperemia, providing an index of vascular endothelial function (Didier et al., 2020; Ederer et al., 2016). Previous work in our lab (Chapter 2) showed a significant improvement in FMD corrected for shear-rate after one bout of passive heating, however the current study showed that 11 consecutive days of passive heating did not stimulate a change in FMD compared to pre- or acute-heating. This absence of change may be due to only having a subset of 5 subjects and being underpowered. Brunt et al. showed that within 2 weeks (4-5x per week) of an 8 week heating protocol that FMD of the brachial artery was significantly increased (Brunt, Howard, et al., 2016). In contrast, lower limb heating 3x per week showed increased FMD after 4 weeks but was returned to baseline

values after 8 weeks of heating (Carter et al., 2014). The regression of improvement in that study may be due to the stimulus of heat not being severe enough to induce longstanding changes in endothelial function, as the core body temperature during this study only reached ~38°C 3x per week. Improvements seen in endothelial function post repeated exposure to heating are likely a result of multiple responses. One of those responses is the increased shear stress, due to increased blood flow, placed on the vascular walls during heating. Repeated exposure to increased shear stress has shown to increase FMD (Tinken 2010). Likewise, when the shear stress on the vascular walls is mediated during heating the increase in FMD is not prevalent (Tinken 2009). Brooks et al. found that shear stress stimulated the upregulation HSP transcription without increasing temperature (Brooks, Lelkes, & Rubanyi, 2002). The collective responses of shear stress and HSPs may be part of the underlying mechanisms to increase endothelial function via passive heating interventions.

Changes in total-[heme] have been interpreted to reflect changes in microvascular [Hb] (i.e., hematocrit), reflective of diffusive O_2 delivery, while absolute values for deoxy-[heme] have been used to estimate fractional O_2 extraction and perfusive O_2 delivery (Davis & Barstow, 2013). However, it is important to note that total-[heme] signal reflects changes in RBC concentration (Ferreira, Lutjemeier, Townsend, & Barstow, 2006; Kindig, Richardson, & Poole, 2002). We found that during the VOT the time to peak for diffusive O_2 delivery into the skeletal muscle was significantly faster after chronic-heating compared to both pre- and acute-heating. The perfusive O_2 time to peak was significantly faster after chronic-heating compared to acute-heating. These findings together indicate that chronic-heating leads to quicker increase in RBC concentration and diffusion of O_2 into the muscle post-occlusion.

Heat Shock Proteins

Heat exposure prompts the expression of HSPs, which play a variety of roles in the cardiovascular system and skeletal muscle. HSPs help to regulate other proteins in the body that are responsible for increased NO signaling (Pritchard et al., 2001), reduced oxidative stress (Baek et al., 2000), and reduced vascular inflammation (I. K. Kim, Shin, & Baek, 2005). We specifically looked at HSP90α and HSP70 in serum samples as underlying mechanisms for physiological adaptations to whole-body passive heating. The upregulation of HSP90 has been shown to play an important role in the production of NO through the eNOS pathway. Previous studies have shown that inhibition of HSP90 led to decreases in eNOS-dependent production of NO (Pritchard et al., 2001; Pritchard et al., 2002). In the current study we show no change in serum HSP90α at rest during the 11 day protocol (Figure 4.1 and 4.2). Previous studies have shown that HSP90 encapsulated in peripheral blood mononuclear cells (PBMCs) are increased both 1 hour and 10 days post heating (Brunt et al., 2019; McClung et al., 2008).

The upregulation of HSP72 has led to increases in protein content, protein synthesis, and the number of mitochondria in skeletal muscle (Henstridge et al., 2014). The expression of HSP72 is dependent on the intensity and duration of the stress and is regulated by intracellular heat shock transcription factor-1 (Blake, Gershon, Fargnoli, & Holbrook, 1990; Ruell, Hoffman, Chow, & Thompson, 2004). The expression of HSP70 in the serum during the current study was below the threshold for detection. This is finding is not irregular, as previous research has shown serum HSP70 levels to be below the threshold for detection (Brunt, Wiedenfeld-Needham, Comrada, & Minson, 2018). However, earlier studies have demonstrated that after repeated bouts of local passive heating the expression of HSP70 in the skeletal muscle is the increased (Hafen, Preece, Sorensen, Hancock, & Hyldahl, 2018). Rats that have an overexpression of HSP72 have an increased exercise performance (Henstridge et al., 2014). Similar to HSP90, HSP70 has been

shown to be upregulated intracellularly in peripheral blood mononuclear cells (Brunt et al., 2018). Goto and colleagues found improvements in skeletal muscle function without increase in HSP72 (Goto et al., 2011). Overexpression of HSP72 has been linked to increased exercise performance and habitual activity in the mouse model (Henstridge et al., 2014).

Exercise Tolerance

Our data showed that 11 days of repeated passive heating did not increase exercise duration at 40% MVC. However, we did find that 3 of the 7 subjects increased their time to exhaustion over 20 minutes (an average increase of 12 min pre- to chronic-heating), thus possibly increasing their critical torque (CT). CT is the threshold between heavy and severe exercise intensities, where exercise at or below CT reaches a general steady state (Jones, Wilkerson, DiMenna, Fulford, & Poole, 2008) and exercise above CT exhaustion is achieved (Burnley, 2009). Infrared heat treatment for 3 weeks demonstrated significant increase in exercise tolerance in subjects with chronic heart failure (Ohori et al., 2012). The increase in exercise tolerance was significantly correlated with increased FMD, suggesting improvements in exercise tolerance was linked to improvements in endothelial function. Racinais et al. found that 11 days of passive heating increased the torque produced through knee extension for same muscle recruitment by iEMG during baseline (Racinais, Wilson, & Periard, 2017).

Neuromuscular Recovery

Fatigue recovery after isometric knee extension at 40% MVC was examined as components of peripheral (Q_{tw}, which is at or distal to the neuromuscular junction) and central (% VA, which is proximal to the neuromuscular junction) fatigue (Bigland-Ritchie et al., 1986; Burnley, Vanhatalo, & Jones, 2012). Our findings indicate partial recovery of Q_{tw} within 60 secs post-exercise pre-heating, but no significant change in Q_{tw} from Twitch 1-6 after acute- and

chronic-heating. There was no difference in the amount of peripheral fatigue between conditions during recovery. These findings may indicate that partial recovery of peripheral fatigue after acute- and chronic-heating occurred immediately post exercise. Our findings are consistent with previous work that showed a reduction in Q_{tw} across a multitude of isometric knee extension work rates (38, 42, and 46% MVC) (Burnley et al., 2012). Pre- and chronic-heating conditions exhibited maximal RMS at task failure, which is an indication of increased motor cortical input (Taylor, Amann, Duchateau, Meeusen, & Rice, 2016). The findings of no changes in RMS from first contraction to last contraction of exercise for the acute-heating condition is consistent with our previous findings (Chapter 3).

Eleven days of whole-body passive heating has demonstrated supraspinal adaptations and the ability to maintain VA during a sustained contraction was improved (Racinais, Wilson, Gaoua, & Periard, 2017). Our data showed that %VA was not changed pre- to post-exercise in any of the conditions. When a measurement of central fatigue was previously assessed immediately post passive heating there was no change in %VA (Periard, Caillaud, & Thompson, 2011). We also found that the reduction in MVC immediately post-exercise was significantly less after the chronic-heating condition compared to the pre-heating condition. This finding suggests that partial global fatigue recovery was also initiated immediately post exercise termination. To our knowledge, we are the first study to examine the relationship between neuromuscular recovery after exercise post repeated bouts of whole-body passive heating.

Limitations

The current study contains a number of limitations that need to be considered. The subset of subjects in which endothelial functional measurements were assessed was underpowered. We agree that adding subjects would be advantageous, however due to the current restriction

guidelines for the COVID-19 pandemic we were unable to collect additional data. Additionally, measurement of HSPs in this study was conducted utilizing serum samples obtained at rest pre-exercise on testing days and pre-heating on heating days. The timing of these serum samples was ~24 hours post the last bout of heating, thus to measure HSPs in circulation future studies need to examine the half-life of the HSP response to passive heating and the presence of HSPs incased in protective coverings (i.e., exosomes). Heating duration, intensity, occurrence, and the modality (whole-body vs. local) all play a role in the stimulation of HSPs (K. Kim, Monroe, Gavin, & Roseguini, 2020). We chose to utilize the whole-body water immersion model in this study because of the ease of use and the ability to increase core temperature the desired amount. HSPs are a ubiquitous proteins that are involved in many functional roles (Kregel, 2002), thus local heating may not provide adequate stress to the body to upregulate these chaperone proteins. During the two weeks of testing and heating we did not control the menstrual cycle of the two women subjects. Previous studies have shown that the menstrual cycle did not have an effect on microvascular dilation and FMD (D'Urzo, King, Williams, Silvester, & Pyke, 2018). Another limitation to this study was that subjects could not be blinded to the intervention and outcomes measured may have been impacted by the placebo effect. However, since we did not observe any significant changes in time to exhaustion, it was unlikely that knowledge of the intervention impacted the results.

Conclusions

In conclusion our findings show that exercise tolerance was not impacted by the heating intervention and serum HSP70 levels were undetectable. Nevertheless, our data showed that after the chronic-heating condition there were improvements in the speed of central and peripheral recovery post-exercise. However, we did find that repeated exposure to whole-body passive

heating did not provide sufficient stimulus to induce improvements in endothelial function or serum HSP90α. The results of this study contribute novel findings to the body of knowledge surrounding passive heating and the impact on skeletal muscle function and recovery.

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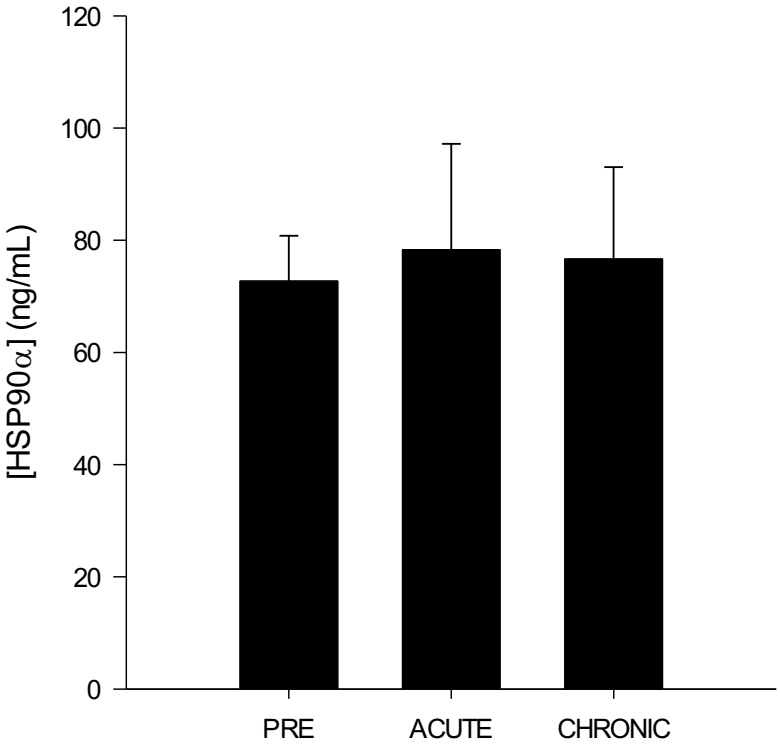

Figure 4.1 Mean ± SE values of serum HSP90α

No differences were detected between pre-, acute-, and chronic-heating.

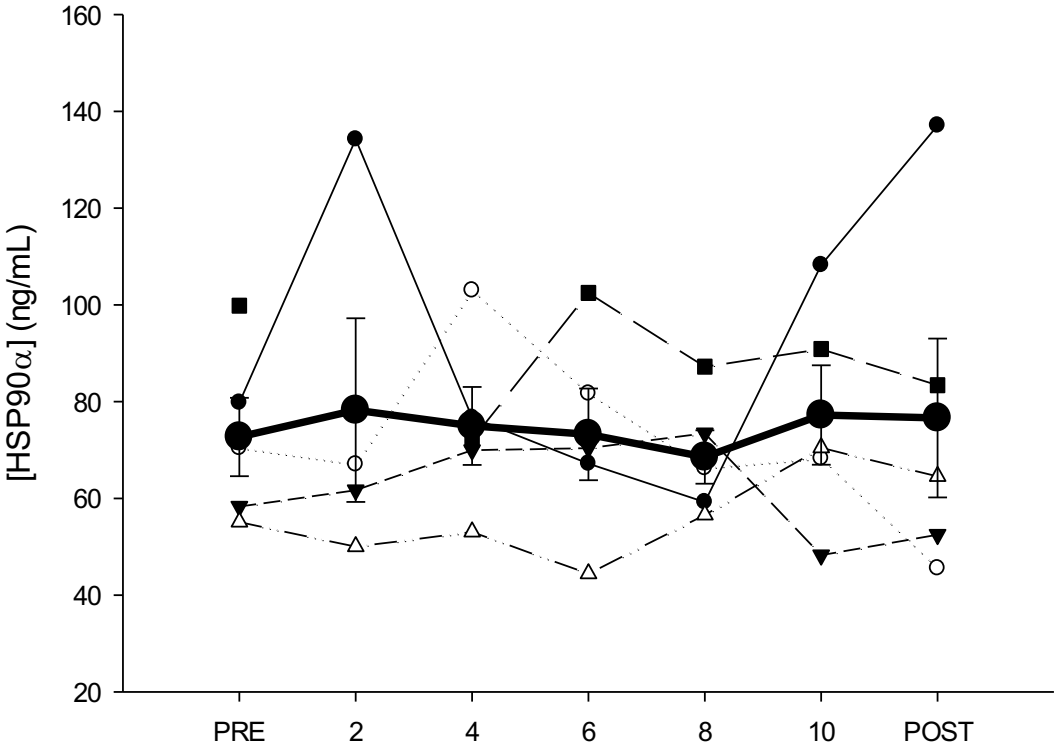

Figure 4.2 Individual and Mean ± SE change in serum HSP90α

No differences were detected in HSP90α over the 11 days of passive heating.

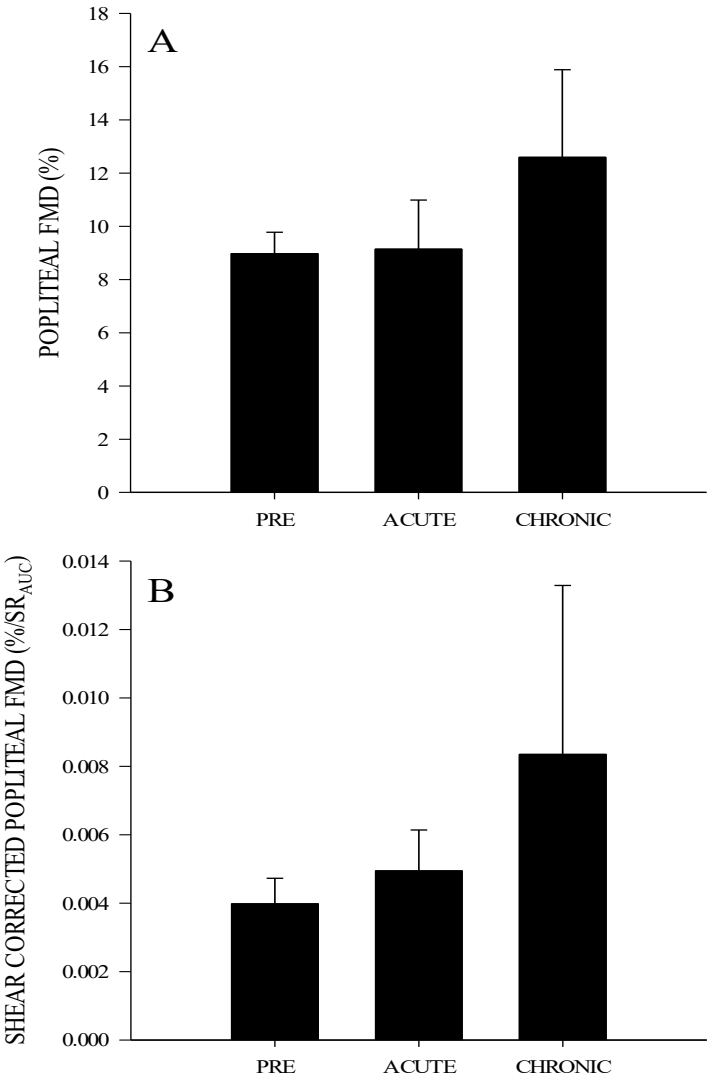

Figure 4.3 Changes in popliteal artery flow-mediated dilatation (FMD)

FMD presented as a percentage change from baseline diameter (A), and shear-corrected FMD (B), pre, acute, and chronic passive heating. Data are mean ± SE. SR_{AUC}, area under the curve of the shear rate stimulus for vasodilatation. There was no significant difference in FMD or shear-corrected FMD between pre, acute, and chronic heating.

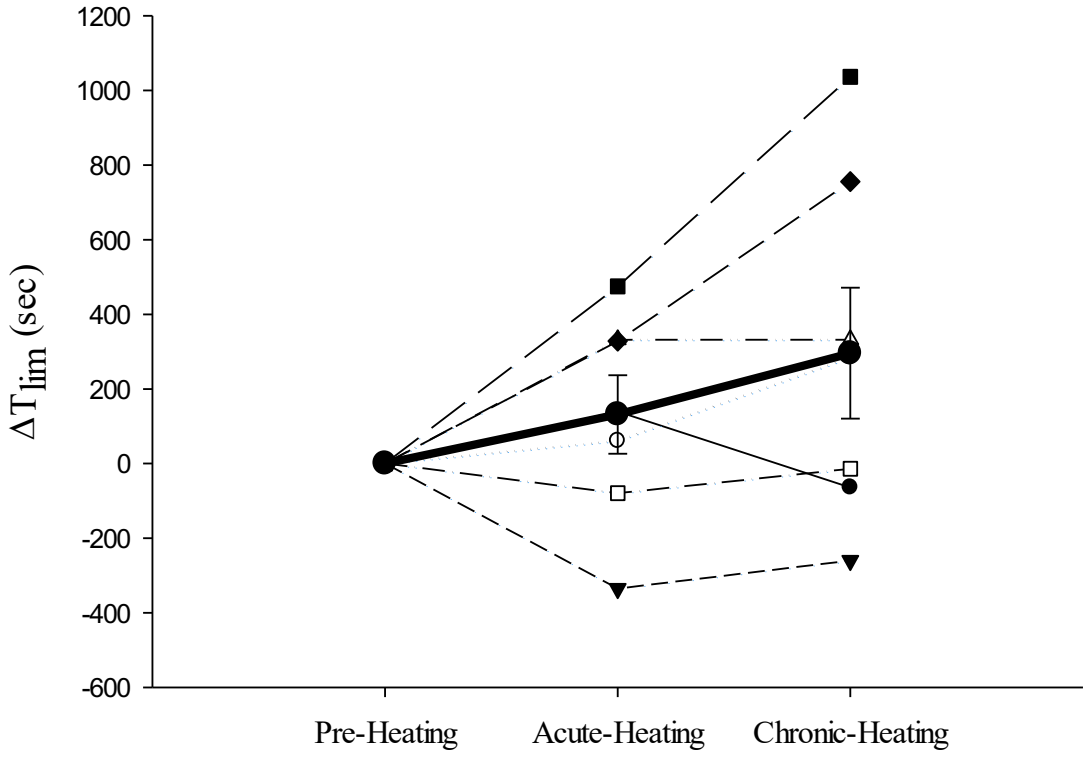

Figure 4.4 . Individual and Mean ± SE change in T_{lim}

No differences were detected in T_{lim} pre-, acute-, and chronic-heating.

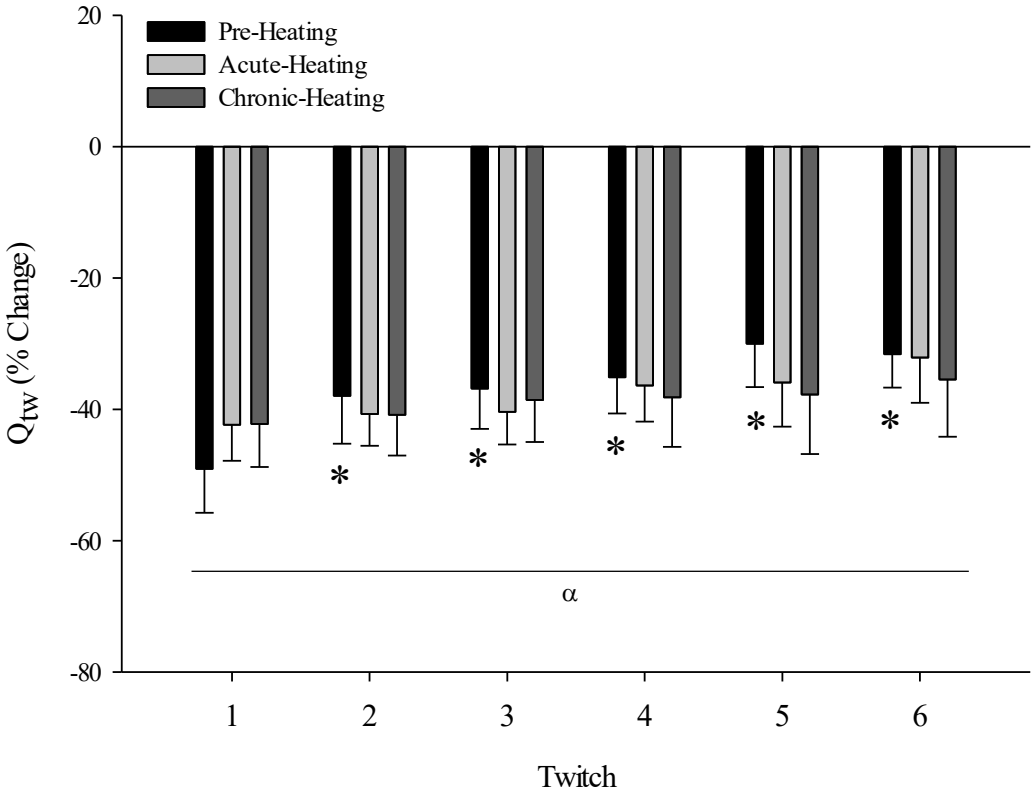

Figure 4.5 Twitch force pre- to post-exercise

Percent change in potentiated twitch (Q_{tw}) post-exercise for all twitches (Mean ± SE). Q_{tw} following exercise for pre, acute, and chronic heating was significantly decreased ($p<0.05$). α different from pre exercise ($p<0.05$); * different from Twitch 1 ($p<0.05$).

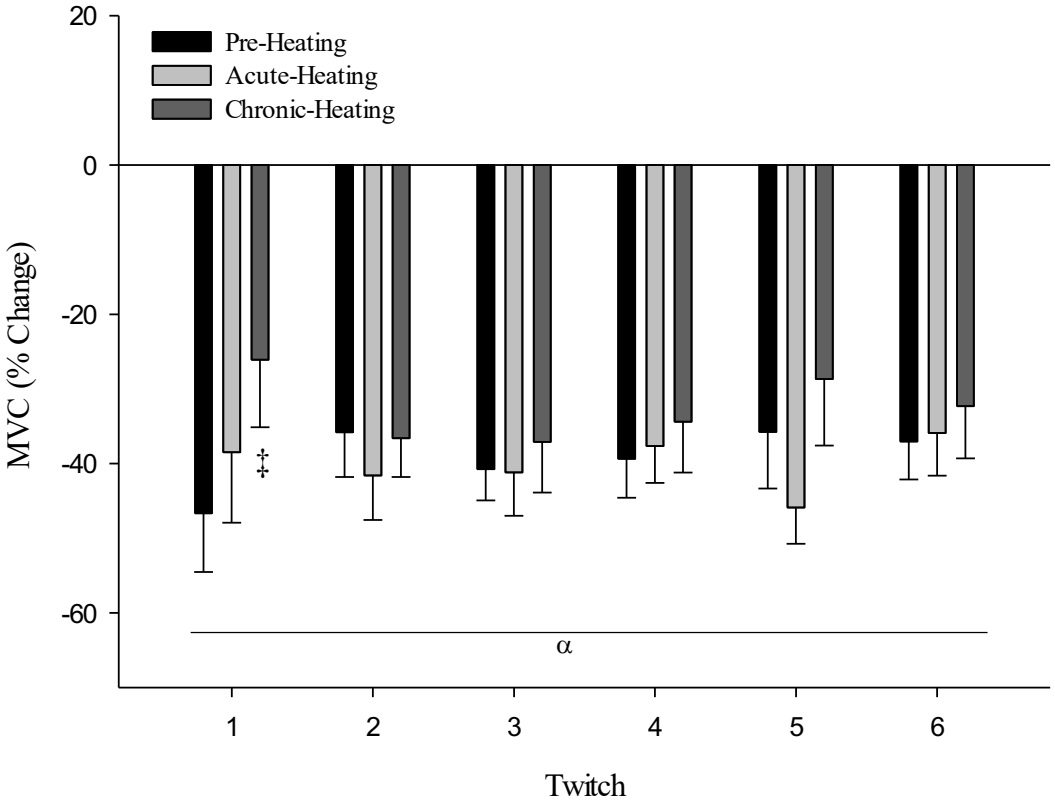

Figure 4.6 Maximal voluntary contraction (MVC) pre- to post-exercise

Percent change in MVC post-exercise for all twitches (Mean ± SE). MVC following exercise for pre, acute, and chronic heating was significantly decreased ($p<0.05$). During twitch 1 following exercise chronic heating MVC was significantly less decreased compared to pre heating. $^{\alpha}$ different from pre exercise ($p<0.05$); ‡ different from pre heat.

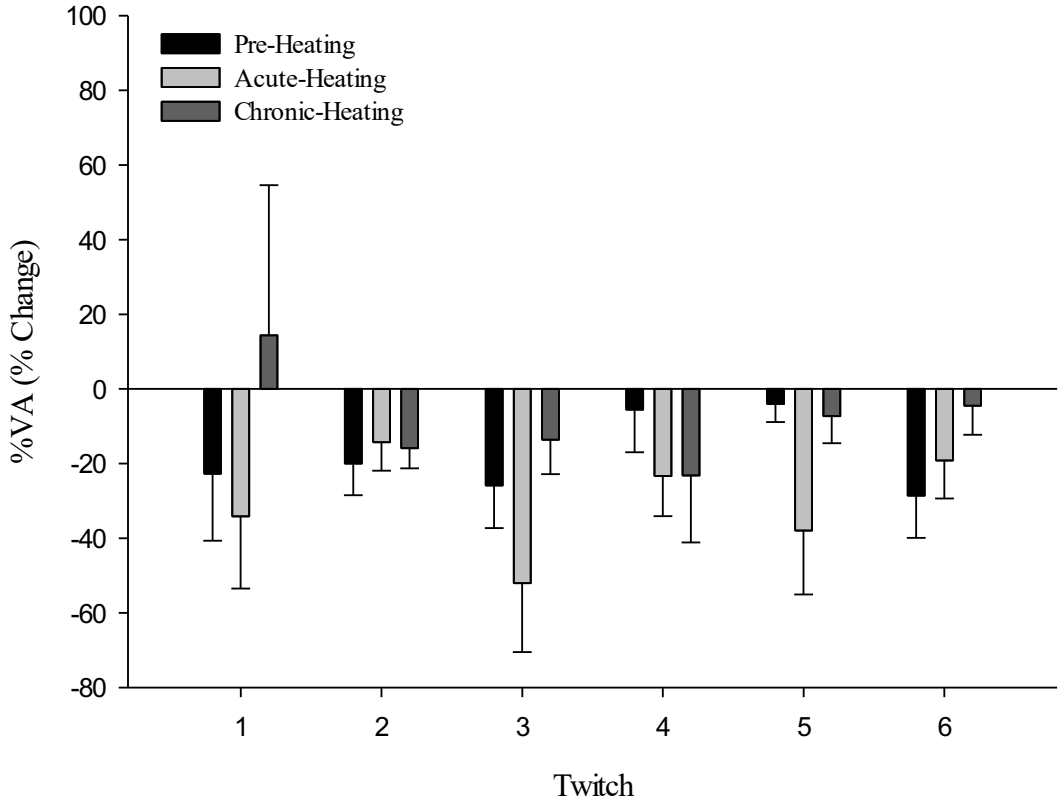

Figure 4.7 Voluntary activation (VA) pre- to post-exercise

Percent change in VA post-exercise for all twitches (Mean ± SE). No differences were detected in the percent change pre- to post-exercise between pre, acute, and chronic heating.

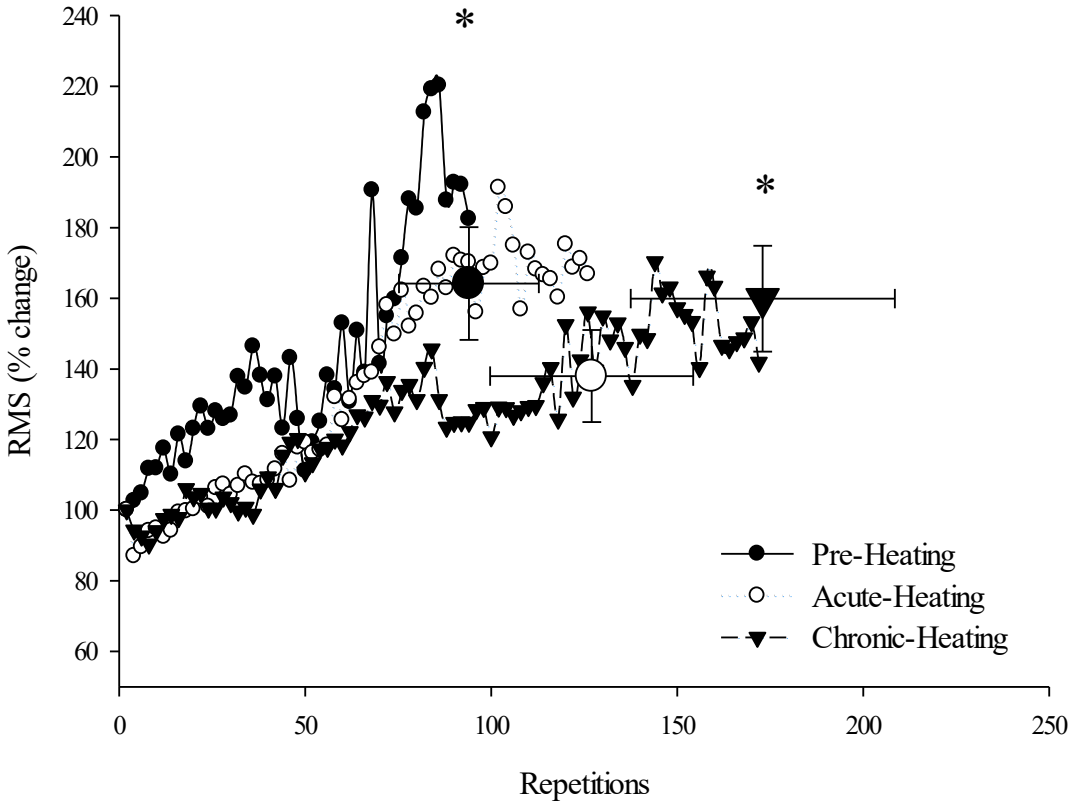

Figure 4.8 Root mean square (RMS) throughout exercise

Changes RMS as a percent of the first contraction for pre, acute, and chronic heating and at end exercise. Data were averaged into 2 contraction bins. RMS increased throughout pre and chronic heating so that end exercise was significantly greater than at the beginning of exercise ($P < 0.05$). *Significantly greater than beginning of exercise ($p<0.05$).

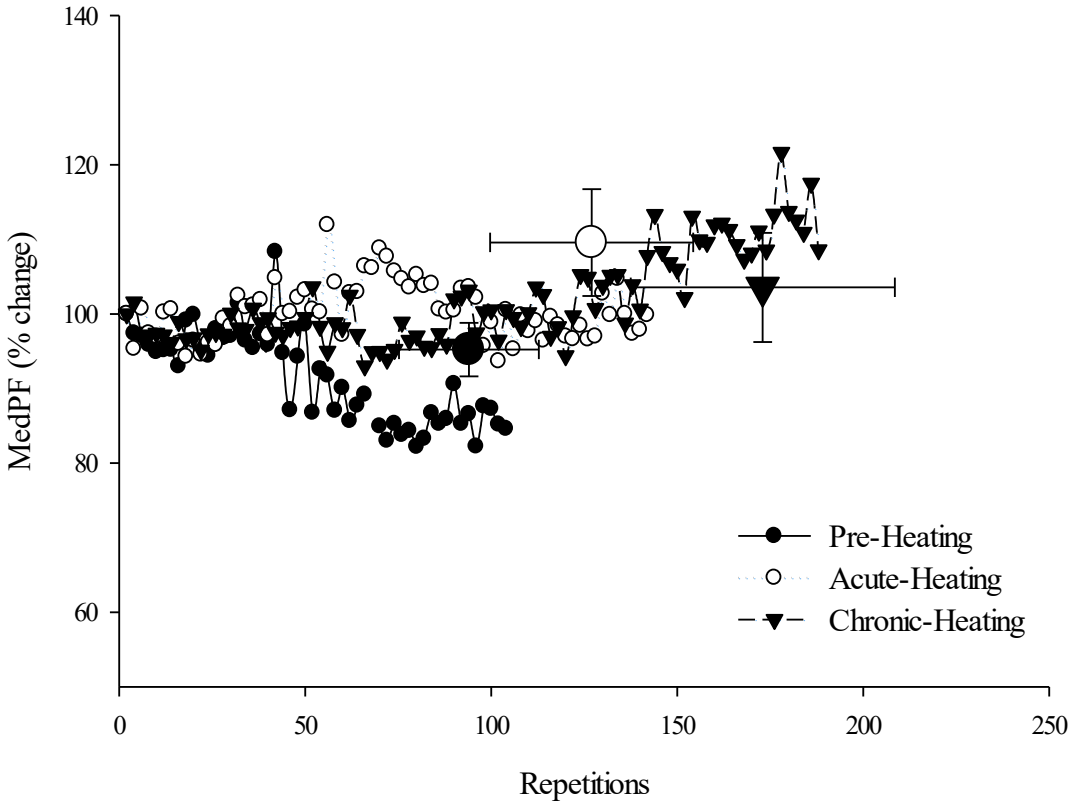

Figure 4.9 Median power frequency (MedPF) throughout exercise

Changes in MedPF as a percent of the first contraction pre-, acute-, and chronic-heating and at end exercise. Data were averaged into 2 contraction bins. MedPF was not significantly decreased at end exercise compared to the beginning for pre-, acute-, and chronic-heating. There were no differences among tests at end exercise.

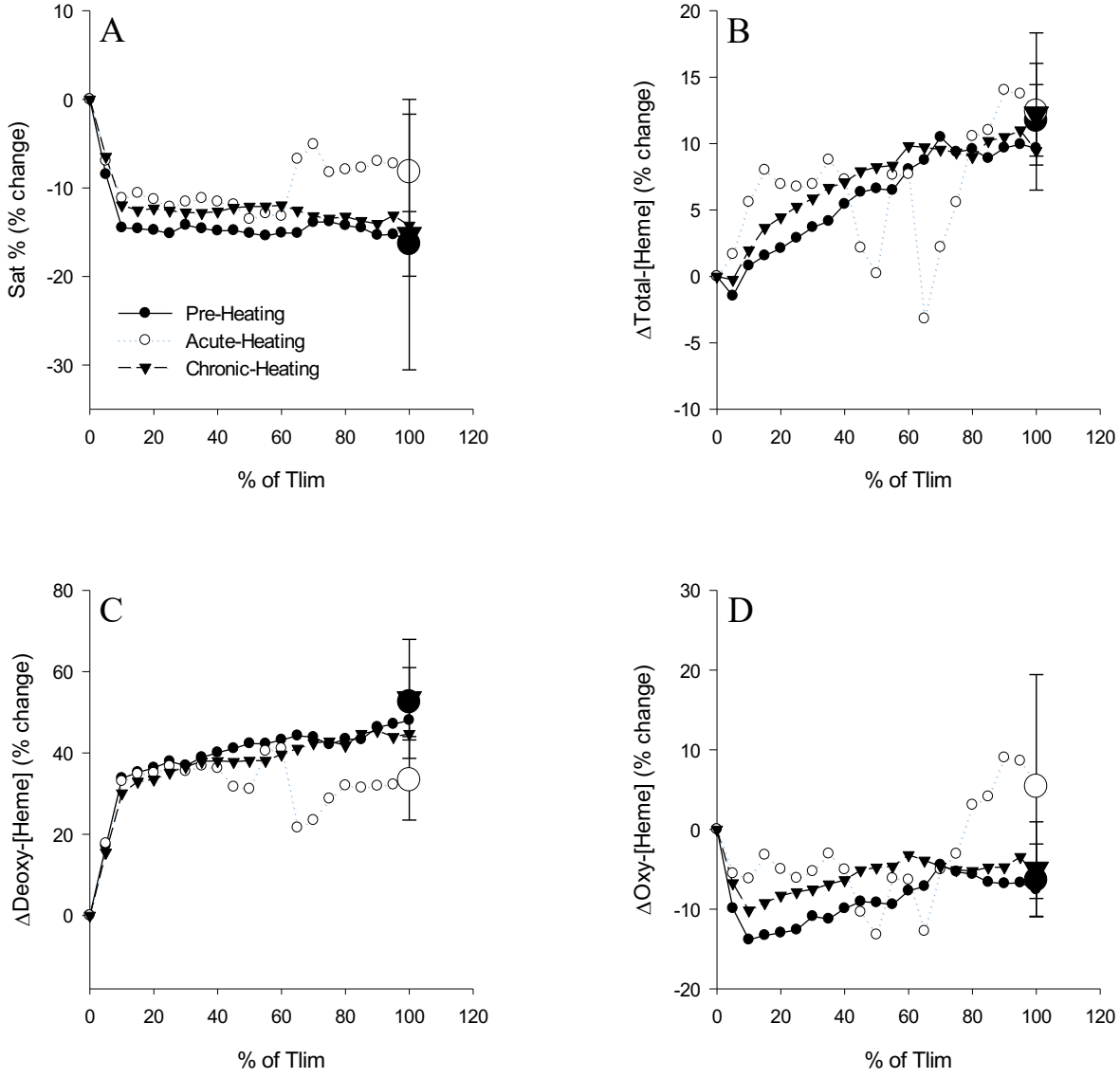

Figure 4.10 Muscle oxygenation responses during the exercise protocol

A–D: the mean responses for A) % O_2 saturation, B) total-[heme], C) deoxy-[heme], and D) oxy-[heme] from rest to end exercise. Data points at end exercise are Mean ± SE. No significant difference between pre and post heating in % change in muscle oxygenation values.

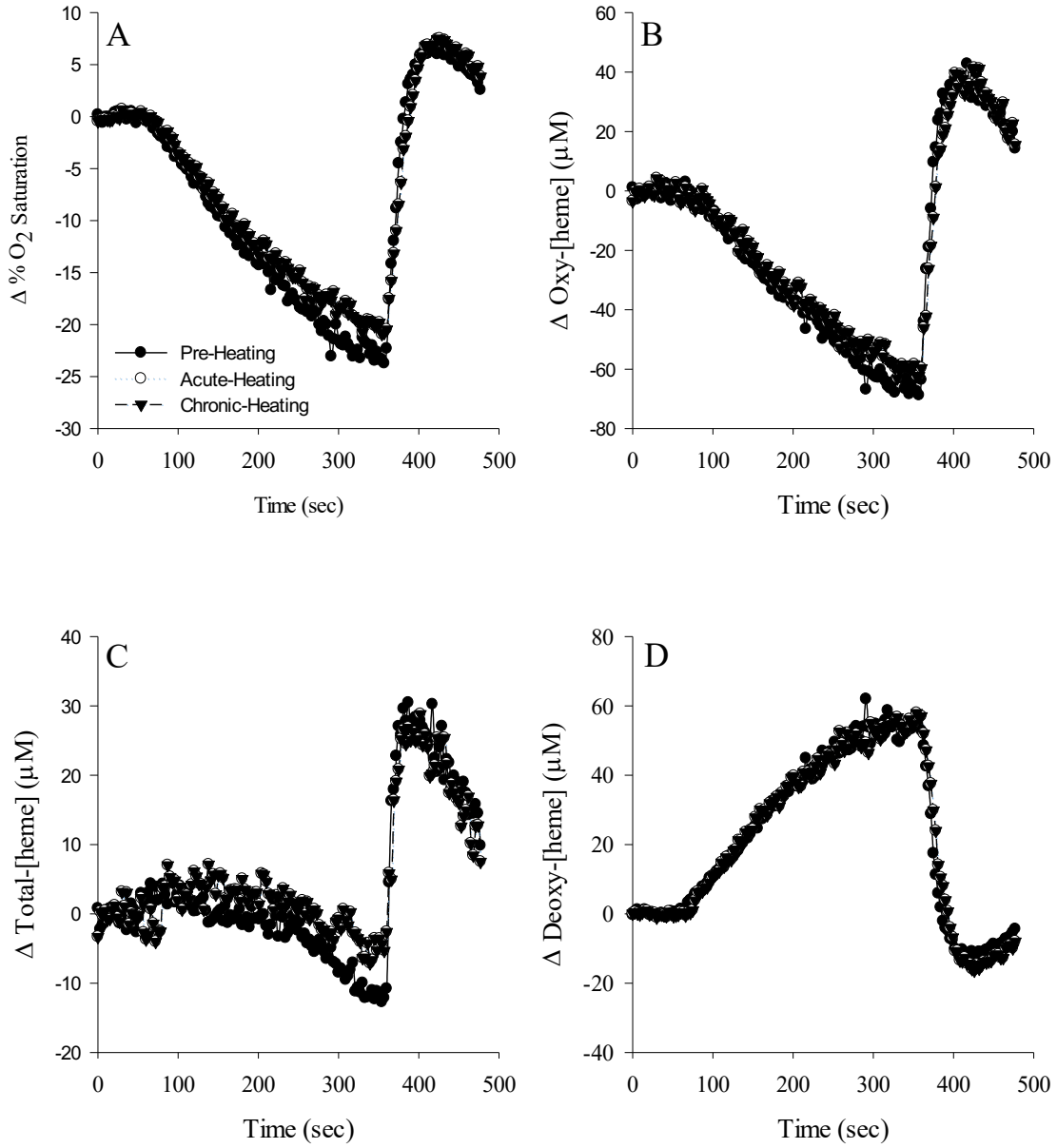

Figure 4.11 Changes in oxygenation characteristics during a VOT

. A-D: the mean responses during the VOT protocol for (A) % O_2 Saturation, (B) Oxy-[heme], (C) Total-[heme], and (D) Deoxy-[heme]. Time to peak was significantly faster after chronic-heating compared to acute-heating in % O_2 saturation, oxy-[heme], total-[heme], and deoxy-[heme] ($p<0.05$).

Table 4.1 Cardiovascular responses

	PRE	ACUTE	CHRONIC
SR_{AUC} (10^3 s^{-1})	2.46 ± 0.87	2.43 ± 2.01	2.56 ± 1.78
VC (% change)			
Peak	100	94.6 ± 10.8	63.8 ± 18.2*
AUC	100	91.7 ± 45.3	70.4 ± 31.44*
PABF (mL/min)			
Peak	771 ± 415	731 ± 400	450 ± 155
Time to peak	20 ± 20	16 ± 11	22 ± 9*
Δ O_2 % Saturation			
Peak	6.7 ± 2.6	7.4 ± 1.5	6.6 ± 1.4
TTP	41.4 ± 8.6	46.2 ± 5.8*	34.8 ± 10.7‡
Δ Oxy-[heme] (μM)			
Peak	44.6 ± 31.4	41.7 ± 18.7	43.6 ± 23.8
TTP	40.2 ± 13.8	42.6 ± 10.9	32.4 ± 12.8*‡
Δ Total-[heme] (μM)			
Peak	33.9 ± 23.3	32.2 ± 14.1	28.1 ± 37.3
TTP	37.9 ± 15.1	33 ± 17.5	22.8 ± 18.7*‡
Δ Deoxy-[heme] (μM)			
Peak	-12 ± 10.4	16.1 ± 11.4	-16.1 ± 12.9
TTP	45.6 ± 21.8	53.4 ± 16.1	43.2 ± 13‡
Blood pressure (mmHg)			
Systolic	124 ± 8	122 ± 14	119 ± 12
Diastolic	76 ± 11	75 ± 9	74 ± 8
Mean	76 ± 11	75 ± 9	74 ± 8

Data are mean ± SD. SR_{AUC}, area under the curve above baseline of the shear rate stimulus from release of the arterial occlusion to peak dilatation; VC, vascular compliance; PABF, popliteal artery blood flow; TTP, time to peak from cuff release. Subset of 5 subjects for SR_{AUC}, VC, and PABF data. *Significantly different from pre-heating; ‡Significantly different from acute-heating.

Chapter 5 - Conclusions

The overall aim of this dissertation was to determine the effect of acute and chronic whole-body heat treatments on endothelial and muscular function, exercise tolerance, and to determine the time course response of heat shock proteins (HSPs). We demonstrated that one bout of whole-body passive heating improved endothelial function measured by flow-mediated dilation (FMD) (Chapter 2), but eleven consecutive days did not improve FMD (Chapter 4). Furthermore, during the vascular occlusion test after chronic heating we found that the perfusive and diffusive components of oxygen delivery in to the skeletal muscle were accelerated. When trying to establish HSP90α as an underlying mechanism for endothelial function we found that the expression of serum HSP90α was not affected ~24 hours post the heat treatment and the expression of the protein did not change at rest over the eleven day period.

When examining the muscular function response to heating we found that neither one bout (Chapter 3) nor 11 days (Chapter 4) of whole-body passive heating were a sufficient stimulus to produce changes in exercise tolerance (duration) during isometric knee extension at 40% maximal voluntary contraction (MVC). When investigating the neuromuscular recovery from exercise we found that one bout of heating did not affect peripheral or central fatigue recovery. However, our data indicated that the chronic heating condition (11 days) led to earlier partial peripheral fatigue recovery immediately post exercise termination. HSP70, which is the stress induced protein associated with skeletal muscle adaptation, showed no change ~24 hours post passive heating and was not detectable during the eleven day heating intervention. Finally, we found that diffusive oxygen delivery into the muscle during exercise was significantly decreased after heating.

The findings of these studies together indicate that physiological changes are occurring post passive heating, however these changes are not enough sufficient to increase exercise tolerance or performance. While this dissertation contributes novel evidence to the growing body of research on passive heat treatments and underlying mechanisms as an intervention for improving exercise tolerance, further studies are needed to determine the amount of heat stress (duration, intensity, and occurrence) to provide sufficient stimulus to invoke both increased mechanistic and functional changes. Implications of our data suggest that increased stress via passive heating may be relevant for clinical populations, such as aged or individuals with peripheral artery disease, who may see improvements after whole body passive heating.

Appendix A - Curriculum Vitae

Kaylin D. Didier

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Manhattan, KS 66502

Education

2015-Present **Ph.D. in Kinesiology**
Dissertation Title: *Impact of Heat Shock Proteins on Daily Activity Tolerance in Younger and Older Humans*
Kansas State University
Manhattan, KS
Expected Graduation Date: 2020

2013-2015 **M.S. in Exercise Physiology**
Thesis Title: *Effects of Chemotherapy and Radiation Exposure on Brachial Artery Blood Flow during Dynamic Handgrip Exercise*
The University of Oklahoma
Norman, OK

2008-2012 **B.S. in Health and Exercise Science, Native American Studies minor**
The University of Oklahoma
Norman, OK

Academic Awards

2020	Martin Frank Diversity Travel Award
2019	Graduate Student Council Research Travel Award
2019	College of Human Ecology Research Travel Award
2018	Graduate Student Council Research Travel Award
2018	College of Human Ecology Research Travel Award
2018	Graduate Student Council Research Travel Award
2018	College of Human Ecology Research Travel Award
2017	Graduate Student Council Research Travel Award
2017	College of Human Ecology Research Travel Award
2016	Graduate Student Council Research Travel Award
2016	Timothy R. Donoghue Graduate Scholarship
2016	FASEB MARC Poster Presenter Travel Award
2016	Graduate Student Council Travel Award
2015	Timothy R. Donoghue Graduate Scholarship
2015	American Physiological Society Minority Travel Fellowship Award
2015	Robberson Research & Creative Endeavors Grant
2008-2020	Choctaw Nation of Oklahoma Higher Education Scholarship

Grant Applications

2019	College of Human Ecology Dissertation Research Award (**Funded $998**)
2019	Arts, Humanities & Social Sciences Small Research Grant (Not Funded)
2018	American Herat Association Predoctoral Grant (Not Funded)

Publications

1. Shane M. Hammer, Andrew M. Alexander, **Kaylin D. Didier**, Lillie M. Huckaby, Thomas J. Barstow. “Limb blood flow and muscle oxygenation responses during handgrip exercise above vs. below critical force” Microvascular Research, 2020
2. **Kaylin D. Didier**, Shane M. Hammer, Andrew M. Alexander, Jacob T. Caldwell, Shelbi L. Sutterfield, Joshua R. Smith, Carl J. Ade, Thomas J. Barstow. “Microvascular blood flow during post-occlusive reactive hyperemia assessed by diffuse correlation spectroscopy” Experimental Physiology, 2019
3. Shane M. Hammer, Lillie M. Huckaby, Andrew M. Alexander, **Kaylin D. Didier**, Dennis Huebner, Dana K. Townsend, Thomas J. Barstow. “Effects of assuming constant tissue scattering on muscle oxygenation measurements during ischemia and vascular reperfusion” Journal of Applied Physiology, 2019
4. Andrew M. Alexander, Shelbi L. Sutterfield, Karly N. Kriss, **Kaylin D. Didier**, Shane M. Hammer, Jacob T. Caldwell, Alex C. Dzewaltowski, Carl J. Ade, Thomas J. Barstow. “Prediction of emergency capsule egress performance” Journal of Aeronautics & Space Exploration, 2019
5. Shelbi L. Sutterfield, Andrew M. Alexander, Shane M. Hammer, **Kaylin D. Didier**, Jacob T. Caldwell, Thomas J. Barstow, Carl J. Ade. “Prediction of planetary critical mission task performance: Implications for long-duration spaceflight aerobic fitness levels” In review: Medicine & Science in Sports & Exercise, 2019
6. Andrew M. Alexander, **Kaylin D. Didier**, Shane M. Hammer, Alex C. Dzewaltowski, Karly N. Kriss, Garrett M. Lovoy, Joseph L. Hammer, Joshua R. Smith, Carl J. Ade, Ryan M. Broxterman, Thomas J. Barstow. “Exercise tolerance through severe and extreme intensity domains” Journal of Applied Physiology, 2018
7. Shane M. Hammer, Andrew M. Alexander, **Kaylin D. Didier**, Joshua R. Smith, Jacob T. Caldwell, Shelbi L. Sutterfield, Carl J. Ade, Thomas J. Barstow. “The noninvasive simultaneous measurement of tissue oxygenation and microvascular hemodynamics during incremental handgrip exercise” Journal of Applied Physiology, 2018.
8. Joshua R. Smith, Shelbi L. Sutterfield, Dryden R. Baumfalk, **Kaylin D. Didier**, Shane M. Hammer, Jacob T. Caldwell, and Carl J. Ade “Left Ventricular Strain Rate is Reduced during Voluntary Apnea in Healthy Humans” Journal of Applied Physiology, 2017.
9. Joshua R. Smith, **Kaylin D. Didier**, Shane M. Hammer, Andrew M. Alexander, Stephanie P. Kurti, Steven W. Copp, Thomas J. Barstow, Craig A. Harms. “Effect of cyclooxygenase inhibition on the inspiratory muscle metaboreflex-induced cardiovascular consequences” Journal of Applied Physiology, 2017.
10. Joshua R. Smith, Ryan M. Broxterman, Shane M. Hammer, Andrew M. Alexander, **Kaylin D. Didier**, Thomas J. Barstow, Stephanie P. Kurti, and Craig A. Harms. "Cardiovascular consequences of the inspiratory muscle metaboreflex: effects of age and sex" American Journal of Physiology Heart & Circulatory Physiology, 2017.
11. Joshua R. Smith, Ryan M. Broxterman, Shane M. Hammer, Andrew M. Alexander, **Kaylin D. Didier**, Stephanie P. Kurti, Thomas J. Barstow, and Craig A. Harms. “Sex differences in the cardiovascular consequences of the inspiratory muscle metaboreflex” American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 2016.
12. Carl J. Ade, Michael G. Brown, Austin K. Ederer, Rachel N. Hardy, Landon K. Reiter, and **Kaylin D. Didier**. Influence of prior anterograde shear rate exposure on exercise-induced brachial artery dilation. Physiol Rep 3: 2015.
13. Austin K. Ederer, **Kaylin D. Didier**, Landon K. Reiter, Michael G. Brown, Rachel N. Hardy, Jacob T. Caldwell, Chris D. Black, Larson RD, and Carl J. Ade. Influence of Adjuvant Therapy in Cancer Survivors on Endothelial Function and Skeletal Muscle Deoxygenation. PLoS One 11: e0147691, 2016.

14. **Kaylin D. Didier**, Austin K. Ederer, Landon K. Reiter, Michael G. Brown, Rachel N. Hardy, Chris D. Black, Michael G. Bemben, and Carl J. Ade. Peripheral vascular responses to small muscle mass exercise in cancer survivors treated with adjuvant therapy. Journal of the American Heart Association.

Manuscripts in Review

1. Shane M. Hammer, Andrew M. Alexander, Kaylin D. Didier, Thomas J Barstow. "Influence of blood flow occlusion on muscular recruitment and fatigue during maximal-effort small muscle-mass exercise" Journal of Physiology.
2. **Kaylin D. Didier,** Andrew M. Alexander, Shane M. Hammer, Korynne S. Rollins, Thomas J. Barstow. "Influence of acute passive heating on [HSP72] and neuromuscular function during and post exercise" In preparation
3. **Kaylin D. Didier,** Andrew M. Alexander, Shane M. Hammer, Korynne S. Rollins, Thomas J. Barstow. "Effect of acute passive heating on endothelial function and skeletal muscle oxygen delievery" In preparation
4. Shane M. Hammer, **Kaylin D. Didier**, Andrew M Alexander, Thomas J. Barstow. "Total heme concentration measured via near-infrared spectroscopy and biochemical assay in swine" In preparation
5. Andrew M. Alexander, Shane M. Hammer, Lillie M. Huckaby, **Kaylin D. Didier**, Thomas J. Barstow. "Neuromuscular recovery from extreme and severe intensity exercise" In preparation
6. Andrew M. Alexander, Joshua R. Smith, Stephanie P. Kurti, Sam R. Emerson, Shane M. Hammer, **Kaylin D. Didier**, Thomas J. Barstow, Craig A. Harms. "Sex differences in the gas exchange threshold pre- and post-puberty" In preparation

Presented Research Abstracts

1. **Kaylin D. Didier**, Lillie M. Huckaby, Andrew M. Alexander, Shane M. Hammer, Camryn N. Webster, Thomas J. Barstow. "Effects of passive heating on perfusive and diffuse microvascular oxygen delivery" Presented: American College of Sports Medicine. Orlando, FL. May 2019
2. Shane M. Hammer, Andrew M. Alexander, **Kaylin D. Didier**, Lillie M. Huckaby, Camryn N. Webster, Thomas J. Barstow. "Effect of acute hyperglycemia on microvascular hemodynamics and tissue oxygenation during handgrip exercise" Oral Presentation: American College of Sports Medicine. Orlando, FL. May 2019
3. Andrew M. Alexander, Shane M. Hammer, **Kaylin D. Didier**, Lillie M. Huckaby, Camryn N. Webster, Thomas J. Barstow. "Sex differences in recovery from extreme and severe intensity exercise" Presented: American College of Sports Medicine. Orlando, FL. May 2019
4. Camryn N. Webster, Shane M. Hammer, Andrew M. Alexander, **Kaylin D. Didier**, Lillie M. Huckaby, Thomas J. Barstow. "Oxygen utilization during the contraction-relaxation of isometric knee extension exercise" Presented: American College of Sports Medicine. Orlando, FL. May 2019
5. Lillie M. Huckaby, Andrew M. Alexander, **Kaylin D. Didier**, Shane M. Hammer, Camryn N. Webster, Thomas J. Barstow. "The effect of passive stretch on vascular control during exercise" Presented: American College of Sports Medicine. Orlando, FL. May 2019
6. Shane M. Hammer, **Kaylin D. Didier**, Andrew M. Alexander, Lillie M. Huckaby, Thomas J. Barstow. "Tissue oxygenation and microvascular hemodynamics in recovery from incremental handgrip exercise" Presented: Integrative Physiology of Exercise. San Diego, CA. September 2018
7. Andrew M. Alexander, Shane M. Hammer, **Kaylin D. Didier**, Lillie M. Huckaby, Thomas J. Barstow. "Recovery from extreme and severe intensity exercise" Presented: Integrative Physiology of Exercise. San Diego, CA. September 2018

8. **Kaylin D. Didier**, Andrew M. Alexander, Shane M. Hammer, Lillie M. Huckaby, Thomas J. Barstow. "Impact of passive heating on critical torque" Presented: Integrative Physiology of Exercise. San Diego, CA. September 2018
9. Lillie M. Huckaby**,** Shane M. Hammer, Andrew M. Alexander, **Kaylin D. Didier**, Dana K. Townsend, Dennis M. Huebner, Thomas J. Barstow. "Effects of constant scattering on muscle oxygenation measurements during ischemia and vascular reperfusion" Presented: Integrative Physiology of Exercise. San Diego, CA. September 2018
10. **Kaylin D. Didier**, Andrew M. Alexander, Shane M. Hammer, Lillie M. Huckaby, Thomas J. Barstow. "Impact of passive heating on critical torque" Presented: Integrative Physiology of Exercise. San Diego, CA. September 2018
11. Andrew M. Alexander, Shane M. Hammer, **Kaylin D. Didier**, Lillie M. Huckaby, Thomas J. Barstow. "Recovery from extreme and severe intensity exercise" Presented: Integrative Physiology of Exercise. San Diego, CA. September 2018
12. Shane M. Hammer, **Kaylin D. Didier**, Andrew M. Alexander, Lillie M. Huckaby, Thomas J. Barstow. "Tissue oxygenation and microvascular hemodynamics in recovery from incremental handgrip exercise" Presented: Integrative Physiology of Exercise. San Diego, CA. September 2018
13. **Kaylin D. Didier**, Shane M. Hammer, Kelsey J. Phelps, John M. Gonzalez, Thomas J. Barstow. "Near-infrared spectroscopy derived total heme vs. assay derived total heme" Presented: American College of Sports Medicine. Minneapolis, MN. June 2018
14. Shane M. Hammer, Jacob T. Caldwell, **Kaylin D. Didier**, Andrew M. Alexander, Carl J. Ade, Thomas J. Barstow. "Perfusive and diffusive microvascular oxygen delivery during simulated hypovolemia and dynamic forearm exercise" Presented: American College of Sports Medicine. Minneapolis, MN. June 2018
15. Andrew M. Alexander, **Kaylin D. Didier**, Shane M. Hammer, Thomas J. Barstow. "Oxygenation characteristics during knee extension exercise in severe and extreme domain" Presented: American College of Sports Medicine. Minneapolis, MN. June 2018
16. Jacob T. Caldwell, Shane M. Hammer, Hunter K. Post, Andrew M. Alexander, **Kaylin D. Didier**, Garrett M. Lovoy, Carl J. Ade. "Duty cycle impairs functional sympatholysis during moderate intensity hand-grip exercise". Presented: Experimental Biology. San Diego, CA. April 2018
17. Shelbi L. Sutterfield, Joshua R. Smith, Dryden R. Baumfalk, **Kaylin D. Didier**, Carl J. Ade. "Acute Changes in Left Ventricular Mechanics During Voluntary Apnea" Presented: Experimental Biology. Chicago, IL. April 2017.
18. Andrew M. Alexander, Shane M. Hammer, **Kaylin D. Didier**, Dryden R. Baumfalk, Thomas J. Barstow. "Muscle Recruitment Patterns Above Critical Power" Presented: Experimental Biology. Chicago, IL. April 2017.
19. Joshua R. Smith, **Kaylin D. Didier**, Shane M. Hammer, Andrew M. Alexander, Stephanie P. Kurti, Thomas J. Barstow, Craig A. Harms. "Contribution of Prostaglandins to the Inspiratory Muscle Metaboreflex-induced Cardiovascular Consequences" Presented: Experimental Biology. Chicago, IL. April 2017.
20. Shane M. Hammer, Andrew M. Alexander, **Kaylin D. Didier**, Dryden R. Baumfalk, Thomas J. Barstow. "Oxygenation Characteristics of the Vastus Lateralis During Cycling Exercise Performed Above Critical Power" Presented: Experimental Biology. Chicago, IL. April 2017.
21. **Kaylin D. Didier**, Carl J. Ade, Shane M. Hammer, Thomas J. Barstow. "Peak Total - [Hemoglobin + Myoglobin] During Incremental Dynamic Handgrip Exercise and Post Occlusive Hyperemia" Presented: Experimental Biology. Chicago, IL. April 2017.
22. Andrew M. Alexander, Shane M. Hammer, **Kaylin D. Didier**, Dryden R. Baumfalk, Joshua R. Smith, Thomas J. Barstow. "Upper Limits of Exercise Tolerance" Presented: American College of Sports Medicine. Denver, CO. June 2017.

23. **Kaylin D. Didier**, Shane M. Hammer, Andrew M. Alexander, Jacob T. Caldwell, Shelbi L. Sutterfield, Carl J. Ade, Thomas J. Barstow FACSM. "Micro-vascular Blood Flow During Post-Occlusive Reactive Hyperemia Assessed By Diffuse Correlation Spectroscopy" Presented: American College of Sports Medicine. Denver, CO. June 2017.
24. Joshua R. Smith, Ryan M. Broxterman, Shane M. Hammer, Andrew M. Alexander, **Kaylin D. Didier,** Thomas J. Barstow, Stephanie P. Kurti, and Craig A. Harms. "Effect of Aging on the Inspiratory Muscle Metaboreflex" Presented: Experimental Biology. San Diego, CA. 2016.
25. Joshua R. Smith, Ryan M. Broxterman, Shane M. Hammer, Andrew M. Alexander, **Kaylin D. Didier**, Thomas J. Barstow, Stephanie P. Kurti, and Craig A. Harms. "Sex Differences in the Inspiratory Muscle Metaboreflex" Presented: American College of Sports Medicine. Boston, MA. June 2016
26. **Kaylin D. Didier**, Samuel L. Wilcox, Ryan M. Broxterman, Shane M. Hammer, Andrew M. Alexander, Thomas J. Barstow FACSM. "The Relationship Between Muscle Activation and VO_2 During Incremental Ramp Exercise" Presented: American College of Sports Medicine. Boston, MA. June 2016
27. Carl J. Ade, Jacob T. Caldwell, **Kaylin D. Didier**, Rachel N. Hardy, Landon K. Reiter, Austin K. Ederer. "Alterations in endothelial function and fractional O_2 extraction in long-term cancer survivors treated with chemotherapy and radiation" Presented: Experimental Biology. Boston, MA. March 2015
28. **Kaylin D. Didier**, Landon K. Reiter, Austin K. Ederer, Jacob T. Caldwell, Carl J. Ade. "Effects of Chemotherapy and Radiation on Brachial Artery Blood Flow during Dynamic Handgrip Exercise" Presented: Experimental Biology. Boston, MA. March 2015
29. Landon K. Reiter, Jacob T. Caldwell, **Kaylin D. Didier**, Austin K. Ederer, Rachel N. Hardy, Carl J. Ade. "Impact of Chemotherapy and Radiation Treatment on Endothelial-Dependent Vasodilation and Circulating Endothelial Microparticles" Presented: Stephenson Cancer Research Symposium. Oklahoma City, OK. January 2015
30. Austin K. Ederer, Jacob T. Caldwell, **Kaylin D. Didier**, Rachel N. Hardy, Landon K. Reiter, Carl J. Ade. "Alterations in endothelial function and fractional O_2 extraction in long-term cancer survivors treated with chemotherapy and radiation" Presented: Stephenson Cancer Research Symposium. Oklahoma City, OK. January 2015
31. **Kaylin D. Didier**, Landon K. Reiter, Austin K. Ederer, Jacob T. Caldwell, Carl J. Ade. "Effects of Chemotherapy and Radiation on Brachial Artery Blood Flow during Dynamic Handgrip Exercise" Presented: Stephenson Cancer Research Symposium. Oklahoma City, OK. January 2015

Teaching Experience

2013-2015	**Graduate Teaching Assistant**	Department of Health and Exercise Science The University of Oklahoma Norman, OK
2015-Present	**Graduate Teaching Assistant**	Department of Kinesiology Kansas State University Manhattan, KS
	Courses Instructed	Biobehavioral Bases of Physical Activity Lab Physiology of Exercise Lab Exercise Testing and Prescription Lab Anatomy and Physiology
2017	**Guest Lecturer**	Molecular Exercise Physiology Department of Kinesiology Kansas State University Manhattan, KS
2019	**Guest Lecturer**	Anatomy and Physiology Department of Kinesiology Kansas State University Manhattan, KS

Professional Affiliations

Professional Memberships

2013-Present	American Physiological Society
2013-Present	American College of Sports Medicine
2018	American Heart Association

Peer Review

American Journal of Physiology
Journal of Applied Physiology

Professional Service

2017-Present	Kansas State Honor and Integrity System Honor Council