Changes in forearm muscle temperature alter renal vascular responses to isometric handgrip

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Kuipers NT, Sauder CL, Kearney ML, Ray CA. Changes in forearm muscle temperature alter renal vascular responses to isometric handgrip. Am J Physiol Heart Circ Physiol 293: H3432–H3439, 2007. First published October 5, 2007; doi:10.1152/ajpheart.00822.2007.—The purpose of the present study was to examine the effect of heating and cooling the forearm muscles on renal vascular responses to ischemic handgrip (IHG). It was hypothesized that heating and cooling the forearm would augment and attenuate, respectively, renal vascular responses to IHG. Renal vascular responses to IHG were measured during forearm heating at 39°C (n = 15, 26 ± 1 yr) and cooling at 26°C (n = 12, 26 ± 1 yr). For a control trial, subjects performed the experimental protocol while the forearm was normothermic (~34°C). Muscle temperature (measured by intramuscular probe) was controlled by changing the temperature of water cycling through a water-perfused sleeve. The experimental protocol was as follows: 3 min at baseline, 1 min of ischemia, ischemic IHG to fatigue, and 2 min of postexercise muscle ischemia. At rest, renal vascular conductance (RVC) was greater during cooling than in normothermia. During ischemic IHG, there were greater decreases in RBV and RVC during cooling than in normothermia. RBV and RVC were similar during postexercise muscle ischemia during heating and normothermia. These results indicate that heating augments mechanoreceptor-mediated renal vasoconstriction whereas cooling blunts metaboreceptor-mediated renal vasoconstriction.

Vasoconstriction of the renal vascular bed is an important mechanism used to meet the pressure and flow demands necessary to perfuse the muscle and skin during exercise and heat stress (1, 24, 25). During exercise, renal vasoconstriction appears to be mediated by activation of the exercise pressor reflex (4, 15, 29). This reflex is controlled by mechanically and metabolically sensitive afferents in the working muscle. These afferents, aside from being sensitive to mechanical and metabolic changes, are responsive to temperature changes in the muscle (5, 11, 19, 20). Little is known about how changing temperature of the exercising muscle may alter exercise pressor reflex-mediated renal vasoconstriction.

Precedently, we found that altering muscle temperature can alter cardiovascular and autonomic responses to exercise in humans. For example, heating the forearm increased mean arterial blood pressure and muscle sympathetic nerve activity at the beginning of ischemic isometric handgrip (IHG) (19), and cooling the forearm muscles delayed increases in muscle sympathetic nerve activity during ischemic IHG (20). Ray and Gracey (19) concluded that the greater increase in mean arterial blood pressure and muscle sympathetic nerve activity during handgrip with a heated forearm was mediated by an increase in sensitivity of mechanically sensitive muscle afferents. This conclusion was based on the observation of the blood pressure and muscle sympathetic nerve activity responses near the onset of exercise, when mechanoreceptor-mediated responses would be greatest, but not during postexercise muscle ischemia, when there would be no mechanoreceptor-mediated responses. In contrast, the attenuation of sympathetic responses during muscle cooling appeared to be mediated by a delay in activation of metabolically sensitive muscle afferents (20). This conclusion was based on the fact that muscle sympathetic nerve activity increased less during exercise in the cold but did not differ at fatigue or during postexercise muscle ischemia.

Because, in humans, activation of the exercise pressor reflex can alter renal vascular conductance (14, 15) and because muscle heating and cooling augment and attenuate muscle sympathetic nerve activity, respectively (20, 21), the following two hypotheses were tested in the present study: 1) forearm heating would augment renal and calf vasoconstriction during ischemic IHG via sensitization of mechanically sensitive afferents, and 2) forearm cooling would delay renal and calf vasoconstriction during ischemic IHG via delayed activation of metabolically active muscle afferents.

METHODS

The study consisted of two experimental groups (heating and cooling groups): 15 subjects (8 men and 7 women; 26 ± 1 yr of age, 174.4 ± 3.0 cm height, 70.7 ± 4.2 kg body wt) participated in the heating study, and 12 subjects (7 men and 5 women; 25 ± 1 yr of age, 175.7 ± 3.3 cm height, 73.6 ± 4.3 kg body wt) participated in the cooling study. All subjects were normotensive, nonobese nonsmokers who did not take any medications and had no autonomic dysfunction or cardiovascular diseases. Endurance- or resistance-trained individuals were excluded from the study. Subjects arrived at the laboratory fasted and having abstained from caffeine, alcohol, and exercise for 12 h. All testing procedures, except measurement of forearm temperature during each respective thermal stress trial, were the same for the two groups. To serve as their own controls, subjects performed a normothermic trial and the thermal stress trial during the same visit. The experimental protocol was approved by the Institutional Review Board at the Pennsylvania State University College of Medicine, and all subjects gave written informed consent before participating.

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Forearm muscle temperature was regulated by a water-perfused sleeve (Med-Eng Systems, Ottawa, ON, Canada) worn over the subject’s dominant arm. For the heating group, 55°C water was circulated through the sleeve for 30 min. At the end of this 30-min period, the water was cooled to 50°C for the experimental protocol. In the cooling group, a bag of ice was placed over the sleeve, and 1°C water was circulated through the sleeve for ~1 h. At the end of this 1-h period, the temperature of the circulating water was increased to 10°C. The order of the normothermic and thermal stress trials was randomized. The normothermia and thermal stress trials were separated by 40 min to allow all measures to return to baseline, and the subsequent trial did not begin until baseline measures were reached. When the normothermic trial was performed after the thermal trial, the forearm muscle temperature was adjusted by varying the temperature of the circulating water until the forearm temperature equaled that measured before heating or cooling the forearm. Ambient temperature in the laboratory during testing was 21–23°C.

The experimental protocol for all temperature conditions was as follows: 3 min at baseline, 1 min of ischemia, ischemic handgrip to fatigue, 1 min of postexercise muscle ischemia, and 3 min of recovery. During exercise, subjects squeezed a hand dynamometer at 30% of their maximal voluntary contraction. The 30% workload was determined before the experimental trials. Measurements during each trial included muscle temperature, skin temperature, arterial blood pressure, heart rate, renal artery blood velocity, rating of perceived exertion (RPE), and calf blood flow.

Measurements

Muscle temperature was measured using a 22-gauge hypodermic intramuscular thermistor (model 552, Yellow Springs Instruments, Yellow Springs, OH). The thermistor was placed 2–3 cm below the skin into the flexor muscles of the forearm. To limit the possibility that heating or cooling the probe at the surface of the skin altered temperature readings in the muscle, the top of the probe was insulated from direct contact with the water-perfused sleeve. Measurements were taken at 1-min intervals during baseline and at 30-s intervals for the remainder of the experimental protocol. Skin temperature of the exercising limb was measured continuously via two thermocouples attached to the dorsal forearm skin and routed through a thermocouple meter (model TC-1000, Sabel Systems, Henderson, NV). Tympanic temperatures were recorded using a First Temp Genius Tympanic Thermometer (Sherwood Medical, St. Louis, MO) after change of forearm muscle temperature to monitor possible changes in body temperature.

Doppler ultrasound (model HDI 5000, ATL Ultrasound, Bothell, WA) was used to measure renal artery blood velocity. The renal artery was scanned using the anterior abdominal approach. A curved-array transducer (2–5 MHz) with a 2.5-MHz pulsed Doppler frequency was used to scan the renal artery. The probe insonation angle to the artery was <60°. The focal zone was set at the depth of the artery. The transducer was held in the same place to record velocity traces during each trial, and the data were obtained in the same phase of the respiratory cycle. Doppler traces were analyzed using software from ATL Ultrasound to obtain renal artery blood velocity measurements for each cardiac cycle. The ratio of renal artery blood velocity to mean arterial blood pressure was used as an index of renal artery conductance.

Calf blood flow was measured using venous occlusion plethysmography. A mercury-in-Silastic strain gauge (Hokanson, Bellevue, WA) was placed around the maximal circumference of the calf. The calf was positioned above the heart. Blood flow to the foot was occluded by inflation of an ankle cuff to 220 mmHg. A thigh cuff (model CC 17, Hokanson) was placed around the thigh and inflated to 50 mmHg to occlude venous outflow for 7.5 s every 15 s. Venous congestion caused by the thigh cuff increased calf volume, which caused the mercury-in-Silastic strain gauge to stretch. The ratio of change in electrical resistance in the mercury-in-Silastic strain gauge as it stretched is directly proportional to calf blood. The ratio of calf blood flow to mean arterial blood pressure was used to calculate calf vascular conductance.

Heart rate and arterial blood pressure were continuously recorded during all trials using a Finometer (Finapres Medical Systems, Amsterdam, The Netherlands). Before all trials, resting brachial artery blood pressure (Dinamap, General Electric, Waukesha, WI) was recorded. Subjects were asked to give RPEs every 30 s during exercise and at fatigue (2).

Data Analysis

Data, except renal blood flow velocities, were analyzed offline using Chart 5.4.2 software (ADI Instruments, Newcastle, Australia). Resting variables at each temperature were compared using a paired t-test. Baseline and postexercise muscle ischemia data were averaged over their respective time periods. Because exercise time differed between temperature conditions, data are expressed as a percentage of time to fatigue. The following exercise time periods were averaged: 0–20%, 20–40%, 40–60%, 60–80%, and 80–100%. All data were analyzed using a two-within repeated-measures analysis of variance (temperature × time). Significance was considered at $P < 0.05$. Values are means ± SE.

RESULTS

Heating Study

Baseline. Baseline measurements during normothermia and heating are presented in Table 1. Heating significantly in-

### Table 1. Baseline measurement during normothermia, heating, and cooling

<table>
<thead>
<tr>
<th>Variable</th>
<th>Heating Study (n = 15)</th>
<th>Cooling Study (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature, °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle</td>
<td>33.9 ± 0.3</td>
<td>38.5 ± 0.2</td>
</tr>
<tr>
<td>Tympanic</td>
<td>36.8 ± 0.1</td>
<td>36.8 ± 0.1</td>
</tr>
<tr>
<td>Skin</td>
<td>31.0 ± 0.4</td>
<td>40.2 ± 0.2*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>87 ± 2</td>
<td>89 ± 2</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>59 ± 2</td>
<td>62 ± 3</td>
</tr>
<tr>
<td>RBV, cm/s</td>
<td>52.9 ± 2.2</td>
<td>53.6 ± 2.1</td>
</tr>
<tr>
<td>RVC, cm·s⁻¹·mmHg⁻¹</td>
<td>0.57 ± 0.04</td>
<td>0.60 ± 0.04</td>
</tr>
<tr>
<td>CBF, ml·min⁻¹·dl⁻¹</td>
<td>1.8 ± 0.2</td>
<td>1.9 ± 0.2</td>
</tr>
<tr>
<td>CVC, ml·min⁻¹·dl⁻¹·mmHg⁻¹</td>
<td>0.018 ± 0.002</td>
<td>0.021 ± 0.002</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial blood pressure; HR, heart rate; RBV, renal blood flow velocity; RVC, renal vascular conductance; CBF, calf blood flow; CVC, calf vascular conductance. *Significantly different from respective normothermia value ($P < 0.05$).
creased forearm muscle and skin temperatures but did not alter tympanic temperature, indicating that local heating did not alter core body temperature. All other hemodynamic measurements were the same at baseline for the two trials.

Exercise responses. Mean arterial blood pressure increased significantly more during heating than in normothermia (Fig. 1). The decreases in renal and calf vascular conductance were greater during heating than in normothermia (Figs. 2 and 3, respectively). RPEs were slightly higher throughout the first 2 min of exercise during heating than in normothermia (Δ1 RPE unit) but did not differ at fatigue. Exercise time was significantly longer in normothermia than...
during heating (174 ± 15 vs. 128 ± 15 s). Skin temperatures did not change during normothermia and heating and averaged 30.8 ± 0.5 and 40.5 ± 0.3°C, respectively, throughout exercise.

**Postexercise muscle ischemia.** Mean arterial blood pressure and heart rate decreased from fatigue but were elevated above baseline for both temperatures. Mean arterial blood pressure was significantly higher during heating than in normothermia. Renal and calf vascular conductance values were not different between the two temperature conditions (Figs. 2 and 3, respectively).

**Cooling Study**

**Baseline.** Baseline measurements during forearm cooling and normothermia are presented in Table 1. Cooling significantly decreased forearm muscle and skin temperatures but did not alter tympanic temperature. All other hemodynamic measurements were the same at baseline for the two trials.

**Exercise responses.** Mean arterial blood pressure and heart rate increased during exercise at both forearm temperatures (Fig. 4). The increase in mean arterial blood pressure was greater in normothermia than during cooling. Renal artery blood velocity tended to be higher during cooling (P = 0.06; Fig. 5). Renal vascular conductance was significantly higher during cooling than in normothermia (Fig. 5). Changes in calf blood flow and vascular conductance (Fig. 6) were not different between normothermia and cooling. RPEs were not different between normothermia and cooling throughout exercise and at fatigue. Exercise time was not significantly different between normothermia and cooling (184 ± 11 and 156 ± 16 s, respectively). Skin temperatures did not change in normothermia.
Mia and during cooling and averaged 31.8 ± 0.5 and 18.8 ± 0.6°C, respectively, throughout exercise.

Postexercise muscle ischemia. During postexercise muscle ischemia, the increase in mean arterial blood pressure was greater in normothermia than during cooling (Fig. 4). The increase in heart rate did not differ between temperatures. Renal artery blood velocity and renal vascular conductance were higher during cooling than in normothermia (Fig. 5). Calf vascular conductance did not differ between normothermia and cooling (Fig. 6).

Fig. 5. Changes from baseline in RBV and RVC during exercise and PEMI during forearm cooling and in normothermia. Points are averages of 20% of exercise time before each point. Cooling the forearm attenuated the decrease in RVC during exercise. During PEMI, RBV and RVC were higher in the cooling trial. Values are means ± SE. *Significantly different from normothermia ($P < 0.05$).

Fig. 6. Changes from baseline in CVC during exercise and PEMI during forearm cooling and in normothermia. Points are averages of 20% of exercise time before each point. Cooling did not alter CVC compared with normothermia at any time. Values are means ± SE.
DISCUSSION

The major new findings of these studies are as follows: 1) varying forearm muscle temperature did not alter resting renal or calf vascular conductance; 2) heating the forearm muscles augmented renal and calf vasoconstriction during ischemic IHG via an increase in sensitivity of muscle mechanoreceptors; and 3) cooling the forearm muscles attenuated renal vasoconstriction during exercise as a result of blunted activation of the muscle metaboreflex.

Our laboratory previously found that forearm heating augments increases in muscle sympathetic nerve activity during the first few minutes of IHG but does not alter activity at fatigue or during postexercise muscle ischemia (19). Ray and Gracey (19) concluded that this increase in muscle sympathetic nerve activity was mediated by increased sensitivity of the mechanoreceptors. This conclusion was drawn because the augmented response was observed early in exercise and because no differences were observed during postexercise muscle ischemia. The results of the present study indicate that, in humans, heating the forearm muscles elicits a similar influence on mechanoreceptor-mediated renal vasoconstriction, because the differences in renal vasoconstriction occurred only during exercise but not when the muscle metaboreceptors were engaged in isolation during postexercise muscle ischemia. These findings are in agreement with other studies that have found that mechanoreceptors contribute to decreases in renal conductance in humans (14, 15) and decreases in renal conductance and sympathetic nerve activity in animals (9, 10, 29). The greater decrease in calf vascular conductance with heating corresponds to greater increases in muscle sympathetic nerve activity, which we observed previously using the same protocol (19).

Ray et al. (20) observed that forearm cooling delayed muscle metaboreceptor-mediated increases in muscle sympathetic nerve activity during ischemic IHG. This conclusion was based on the fact that the differences in muscle sympathetic nerve activity between muscle cooling and normothermia occurred later during exercise, when metaboreceptor-mediated increases in muscle sympathetic nerve activity would be greatest. In accordance with previous findings (20), the results of the present study may be due to a decrease in sympathetic nerve activity to the renal vasculature. In the present study, during postexercise muscle ischemia, which selectively engages the metaboreflex only, renal vascular conductance was higher in the cooling trial. If cooling did not attenuate metaboreflex-mediated renal vasoconstriction, renal vascular conductance should have been similar during postexercise muscle ischemia during cooling and normothermia. Although cooling did not significantly blunt calf vasoconstriction during exercise, there was a definite trend for calf vascular conductance to be lower during cooling than in normothermia.

In the present study, the arterial baroreflexes and central command could have contributed to measured changes in renal vasoconstriction during thermal stress and exercise. In the present study, we observed that mean arterial blood pressure was greater during heating than in normothermia and in normothermia than during cooling. Increased arterial blood pressure and loading of the arterial baroreflexes decrease sympathetic outflow and vascular tone and, thereby, increase vascular conductance (16). In the present study, if the baroreflexes were contributing to the measured differences in arterial blood pressure and blood flow between temperature conditions, we would have expected greater renal and peripheral vasodilation when comparing heating with normothermia and normothermia with cooling. We observed the opposite response between thermal conditions; therefore, we believe that the arterial baroreflexes may not contribute to the differences between thermal stimuli and normothermia. Consistent with this notion, we found that muscle sympathetic nerve activity and mean arterial blood pressure during ischemic IHG were higher in heated than in normothermic muscle and higher in normothermic than in cooled muscle (19, 20). The contribution of central command to increases in muscle sympathetic nerve activity and arterial blood pressure is thought to occur mainly during intense exercise and at fatigue when volitional effort is greatest (21, 30). In the present study, RPEs were slightly higher during exercise and heating but were not different at fatigue. During the cooling trial, RPEs were not different during exercise or at fatigue. The finding that perceived exertion was the same at fatigue in both trials, when volitional effort was greatest, suggests that central command did not contribute to measured differences in renal vasoconstriction. Further support that central command did not contribute to exercise-induced renal vasoconstriction is that, in humans, electrical stimulation of the bicep muscles and postexercise muscle ischemia, two ways to selectively activate muscle afferents without central command input, both increase renal vasoconstriction (14, 15).

Changing visceral blood flow is an important mechanism the body uses to control core temperature during thermal stress and to increase perfusion of blood into metabolically active tissues during exercise (24). Combined physical and heat stress pose a severe challenge to maintaining blood pressure, because the demands of these two vascular beds can outstrip available cardiac output. Therefore, the cardiovascular system must reduce blood flow to these tissue beds or direct blood flow from other tissues to deter decreases in performance and decreases in heat loss. Muscle blood flow has been found to not change during exercise in the heat (18, 26), and skin blood flow remains relatively unchanged as internal temperature increases over 38°C during exercise (7). Therefore, the body must limit blood flow to other vascular beds such as the renal or splanchnic vascular beds. The results of the present study indicate that the augmentation of the exercise pressor reflex during heating may be a mechanism that promotes vasoconstriction of the visceral tissues and inactive skeletal muscle to help maintain blood pressure. The reason for changes in the exercise pressor reflex during cooling may be different from that during heating. During cold stress, the body decreases peripheral blood flow to increase insulation to maintain internal temperature (8, 28). By delaying increases in renal vasoconstriction during exercise and limiting perfusion of the exercising muscle, higher peripheral insulation may be maintained and decreases in core temperature delayed.

The mechanisms behind temperature-induced changes in sensitivity of the exercise pressor reflex remain equivocal. Mechanoreceptor sensitivity can be altered by a variety of factors, including prostaglandins, bradykinin, and lactic acid (6, 27). Arachidonic acid derivatives have been found to selectively excite mechanoreceptors and not metaboreceptors (22, 23). Therefore, it is possible that in the heating trials the...
increased muscle temperature may have altered the chemical milieu of the muscle and increased the concentration of a neurologically active substance that could sensitize the mechanoreceptors. Several mechanisms may explain the responses during the cooling trials. 1) Cooling the muscle has been found to decrease firing of muscle afferents themselves (5, 11). 2) Cooling the muscle may have decreased the metabolic rate of the muscle, which could lower the production of various exercise metabolites that are known to activate the metaboreceptors (e.g., lactic acid and hydrogen ions). Future investigations are needed to elucidate temperature-induced changes in sensitivity of the muscle pressor reflex.

Skin afferents could have contributed to the differences between thermal conditions; however, there are several reasons to suggest this is unlikely. 1) At rest when only temperature of the exercising limb was altered, baseline hemodynamic measurements and muscle sympathetic nerve activity (19, 20) did not differ between normothermia and heating and between normothermia and cooling. 2) During exercise there was no change in skin temperature for any of the thermal conditions. 3) Subjects did not complain of pain related to the skin, and the temperatures of the skin recorded during heating and cooling were below and above those reported for causing pain in the arm, respectively (17, 31). 4) Two subjects performed normothermic handgrip while the skin of the contralateral arm was altered to equal skin temperatures measured in the normothermic, heating, and cooling trials using the water-perfused sleeve. Changes in mean arterial blood pressure and renal and calf vascular conductance did not differ between any of the temperatures. For these reasons, we do not believe that afferents in the skin contributed to the observed differences between temperature conditions.

The goal of the present study was to examine whether changing local muscle temperature altered muscle afferent control of renal blood flow. To limit the influence of the effect of changes in muscle temperature and metabolism because of exercise-induced hyperemia, subjects performed handgrip during ischemia. It is possible that changes in blood flow during contraction may alter the influence of thermal stress on the exercise pressor reflex. However, isometric contractions >15% of maximal voluntary contraction do not raise muscle blood flow and are, therefore, largely ischemic (3).

In the present study, the temperature of only a small muscle mass was altered. The present study contributes to the understanding of the cardiovascular responses to whole body exercise and thermal stress by isolating possible contributions to cardiovascular changes from a peripheral limb. However, during whole body exercise and thermal stress, the challenges placed on the cardiovascular system and sensory input will differ from that of the present study. Therefore, the results of the present study may not be synonymous with whole body exercise and thermal stress.

Because of resolution limitations, it is not possible to accurately measure renal artery diameters using Doppler ultrasound. Consequently, we used blood velocity as a surrogate for flow. It is possible that diameters of the renal arteries changed during the study and, thus, could alter our interpretation of renal blood flow. There is some evidence to suggest that pharmacologically mediated renal vasoconstriction (13) and vasodilation (12) do not alter diameter of the renal artery. Furthermore, the vessel we examined was a conduit vessel and not a resistance vessel. Therefore, it is unlikely that changes in renal artery diameter influenced the results of the study.

In summary, heating the muscle augmented renal and calf vasoconstriction during forearm exercise, and cooling the muscle attenuated renal vasoconstriction at fatigue and during postexercise muscle ischemia. The augmentation of renal vasoconstriction during exercise with a heated muscle appears to be associated with an increase in sensitivity of the muscle mechanoreceptors, whereas the attenuation of renal vasoconstriction during exercise with a cooled muscle was related to a blunting of the muscle metaboreflex.

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