Interactive effect of aging and local muscle heating on renal vasoconstriction during isometric handgrip

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Kuiipers NT, Sauder CL, Kearney ML, Ray CA. Interactive effect of aging and local muscle heating on renal vasoconstriction during isometric handgrip. Am J Physiol Renal Physiol 297:F327–F332, 2009. First published June 10, 2009; doi:10.1152/ajprenal.00165.2009.—The purpose of the study was to determine the interactive effect of aging and forearm muscle heating on renal vascular conductance and muscle sympathetic nerve activity (MSNA) during ischemic isometric handgrip. A tubelined, water-perfused sleeve was used to heat the forearm in 12 young (27 ± 1 yr) and 9 older (63 ± 1 yr) subjects. Ischemic isometric handgrip was performed before and after heating. Muscle temperature (intramuscular thermistor) was 34.3 ± 0.2 and 38.7 ± 0.1°C during normothermia and heating, respectively. At rest, heating had no effect on renal blood velocity (Doppler ultrasound) or renal vascular conductance in either group (young, n = 12; older, n = 8). Heating compared with normothermia caused a significantly greater increase in renal vasoconstriction during exercise and postexercise muscle ischemia (PEMI) in both groups. However, the increase in renal vasoconstriction during heating was greater in the older compared with the young subjects (18 ± 3 vs. 8 ± 3%). During handgrip, heating elicited greater increases in MSNA responses in the older group (young, n = 12; older, n = 6), whereas no statistical difference was observed between groups during PEMI. In summary, aging augments renal vascular responses to ischemic isometric handgrip during heating of the exercising muscle. The greater renal vasoconstriction was associated with augmented MSNA in the older subjects.

Kidney; sympathetic; hyperthermia

EXERCISE CAN GENERATE SIGNIFICANT increases in body and muscle temperature (29, 30) and aging is associated with an increased prevalence of developing heat-related illnesses (21). Moreover, cardiovascular responses to exercise in the heat are altered with age. However, older individuals increase skin blood flow and decrease renal and splanchnic blood flow less during upright dynamic leg exercise (i.e., cycling) in the heat compared with younger individuals (8, 9). The exact mechanisms behind these age-related differences during exercise in the heat remain equivocal.

Thinly myelinated skeletal muscle afferents are responsive to mechanical deformation, metabolic by-products, and changes in muscle temperature. Activation of the muscle afferents contributes to renal vasoconstriction in humans (10, 15, 20) and increases renal sympathetic nerve activity in cats (7, 14, 25). Animal studies clearly demonstrated reductions in renal vascular conductance elicited by the muscle metaboreflex (1, 16, 17). Older individuals constrict the renal vasculature greater during isometric handgrip than younger individuals (19). Momen et al. (19) concluded increased renal vasoconstriction in older individuals was mediated by increased sensitivity of mechanically sensitive muscle afferents because responses were observed at the onset of exercise and not during postexercise muscle ischemia (PEMI). Muscle heating has been observed to augment muscle sympathetic nerve activity (MSNA) during isometric exercise via greater activation of muscle afferents (26). Currently, it remains unknown how aging and local muscle heating interact on renal vascular responses to isometric exercise. Because aging augments mechanoreceptor-mediated renal vasoconstriction during normothermic isometric handgrip (19) and local heating augments muscle mechanoreceptor sensitivity and renal vasoconstriction in younger individuals (10, 26), we hypothesized that aging would augment renal vasoconstriction during isometric handgrip with local muscle heating.

Second, we hypothesized greater renal vasoconstriction with muscle heating would be associated with greater MSNA during isometric exercise. The current study supports these hypotheses.

METHODS

Twelve young (7 men and 5 women; 27 ± 1 yr, 175.0 ± 3.1 cm height, 73.0 ± 4.4 kg body wt) and nine older volunteers (3 men and 6 women; 63 ± 1 yr, 170.3 ± 1.8 cm, 70.5 ± 2.8 kg) participated in the study. We previously reported renal responses of the young subjects (10); however, their MSNA responses were not reported. All subjects were normotensive, nonobese, nonsmokers, not taking any medications, and had no autonomic dysfunction or cardiovascular diseases. Subjects who were endurance or resistance trained were excluded from the study. Subjects arrived at the laboratory fasted and having abstained from caffeine, alcohol, and exercise for 12 h. 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.

Experimental Design

To serve as their own controls, subjects performed a normothermic and heating trial during the same visit but in random order. To regulate forearm temperature, subjects wore a water-perfused sleeve (Med-Eng Systems, Ottawa, ON, Canada) over the exercising forearm (dominant arm was used). During the heating trial, water at 35°C was circulated through the sleeve for 30 min. At the end of 30 min, the water temperature was reduced to 50°C for the exercise protocol. The alteration in water temperature was necessary to maintain muscle temperature at ~39°C during exercise. For the normothermic trial, water in the sleeve was regulated so that muscle temperature in the forearm equaled initial measurements recorded during insertion of the muscle temperature probe (~34°C). A minimum of 40 min separated the normothermic and heating trials to allow all measures to return to baseline. Ambient temperature in the laboratory during testing was 21–23°C.
Experimental Protocol

The experimental protocol for both temperature conditions was as follows: 3-min baseline, 1 min of ischemia, ischemic isometric hand-grip to fatigue, 1 min of PEMI, and 3-min recovery. During exercise, subjects squeezed a hand dynamometer at 30% of their maximal voluntary contraction. Maximal voluntary contraction was determined before the experimental protocol and before muscle temperature probe insertion. The same workload was used for each exercise trial. Measurements during each trial included muscle temperature, arterial blood pressure, heart rate, MSNA, renal artery blood velocity, and ratings of perceived exertion.

Measurements

Muscle temperature was measured using a 22-gauge hypodermic intramuscular thermistor (YSI 552, 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 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 every minute during baseline and at 30-s intervals for the remainder of the experimental protocol. Continuous skin temperature of the exercising limb was measured via two thermocouples attached to the 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) to monitor possible changes in core body temperature during the protocol.

Multifiber recordings of MSNA were made by inserting a tungsten microelectrode into the peroneal nerve at the head of the fibula. A reference electrode was inserted subcutaneously 2–3 cm from the recording electrode. Both electrodes were connected to a differential preamplifier and then to an amplifier (total gain between 40,000–80,000, where the nerve signal was band-pass filtered (700–2,000 Hz) and integrated (time constant, 0.1 s) to obtain a mean voltage display of nerve activity. A satisfactory recording of MSNA was defined as spontaneous, pulse-synchronous bursts that increased during end-expiratory apnea and did not change during stroking of the skin or auditory stimulation (yell).

Doppler ultrasound (HDI 5000, ATL Ultrasound, Bothell, WA) was used to measure renal artery blood velocity. The renal artery was scanned using the anterior abdominal approach. To scan the renal artery, a curve-array transducer (2–5 MHz) with a 2.5-MHz pulsed Doppler frequency was used. 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 tracings during each trial. Often the renal artery moves with respect to the abdominal wall during the various phases of respiration not permitting high-quality recordings during both phases of respiration. Thus, the data were obtained in the same phase of the respiratory cycle for each subject at all recorded time points during the protocol. Doppler tracings were analyzed using the software of the ATL to obtain renal artery blood velocity for each cardiac cycle. At least six cardiac cycles were averaged together for each reported time point; however, often there were more than six acceptable velocity tracings for each time point. Because of technological limitations, it is not possible to accurately measure renal artery diameter using the ATL Doppler ultrasound machine, therefore an index of renal vascular conductance was calculated by dividing renal artery blood velocity by mean arterial blood pressure. One older subject’s renal artery could not be imaged thus renal artery blood velocities could not be recorded for that subject.

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

Data Analysis

Data, except renal blood velocities, were analyzed offline using Chart 5.4.2 software (ADI Instruments, Newcastle, Australia). Sympathetic bursts were identified from inspection of mean voltage neurograms. MSNA was quantified as bursts per 30 s. Sympathetic nerve recordings were obtained in 12 young and 6 older individuals.

Resting variables for each trial were compared using a paired t-test within age groups and an unpaired t-test across age groups. Because exercise time differed between temperature conditions, data were compared at fatigue. Individual group responses were compared within themselves using a two-within repeated-measures ANOVA (temperature × exercise). To compare responses between the young and older subjects, effect differences were calculated by subtracting measures during normothermia from measures during the heating trial. Effect differences during fatigue and during PEMI were compared using a one-factor ANOVA (age). Significance was considered at P < 0.05. Results are expressed as means ± SE.

RESULTS

Baseline

Measurements at baseline for the older group are presented in Table 1. The baseline data for the younger subjects have been reported previously (10) with the exception of MSNA. Baseline MSNA for the young group was 4 ± 1 and 5 ± 1 bursts/30 s for normothermia and heating trials, respectively. At rest, renal artery blood velocity, renal vascular conductance, and MSNA were higher in the older group compared with the young at both temperatures. However, forearm muscle heating did not affect any of the variables at rest. Heating significantly increased forearm skin and muscle temperature in both groups. Forearm heating did not alter tympanic temperature in either group. All other measured variables were the same between age groups and temperature conditions.

Exercise Workload, Time, and Ratings of Perceived Exertion

Exercise workload was not significantly different between the young and the older group (10.5 ± 1 and 9.5 ± 0.4 kg, respectively). Exercise time was not different between the young and older groups during normothermia (179 ± 10 vs. 186 ± 7 s, respectively). Both groups gripped for a shorter duration during heating compared with normothermia (young,
128 ± 9 s; older, 154 ± 6 s). The older group gripped significantly longer than the young during heating. No differences in ratings of perceived exertion were observed at fatigue between trials in the either group. Rating of perceived exertion reached similar values in both groups (19 ± 1).

**Cardiovascular and Sympathetic Responses to Exercise and PEMI**

**Mean arterial blood pressure.** Forearm heating augmented mean arterial blood pressure increases in both groups during exercise, but there was no difference in responses between age groups (Fig. 1; \( P = 0.72 \)). During PEMI, mean arterial blood pressure was significantly higher in the young subjects during heating compared with normothermia. However, changes in mean arterial blood pressure from normothermia to heating did not differ between age groups (Fig. 1; \( P = 0.47 \)).

**Heart rate.** Heart rate increased with exercise but responses to exercise did not differ between heating and normothermia in either the young or older group (Fig. 1). Heating did not alter heart rate responses during PEMI in either the young or older group.

**Renal vascular responses.** Forearm heating significantly augmented decreases in renal vascular conductance during exercise in both the young and older subjects (Fig. 1). However, the decreases were significantly greater in the older group (\( \Delta \text{renal vascular conductance}, P = 0.01 \)) compared with the young (Fig. 2). Renal vascular conductance was significantly lower during PEMI in both groups during heating compared with normothermia. However, heat-induced changes in renal vascular conductance during PEMI did not differ between age groups (Fig. 2; \( P = 0.26 \)).

**MSNA.** Exercise elicited increases in MSNA in both groups (Fig. 1). However, heating the forearm elicited greater increases in MSNA during exercise in the older subjects compared with young subjects (Fig. 2). Forearm heating resulted in higher MSNA during PEMI in both groups (Fig. 1). However, there were no statistical differences between groups (Fig. 2; \( P = 0.20 \)).

**DISCUSSION**

The purpose of this study was to test the following two hypotheses: 1) aging augments renal vasoconstriction during isometric handgrip with local muscle heating and 2) greater renal vasoconstriction with muscle heating would be associated with greater MSNA during isometric exercise. The results of the study indicate that the interaction between forearm heating and aging elicits greater renal vasoconstriction during isometric handgrip in older subjects than young subjects. Moreover, the greater renal vasoconstriction observed during isometric handgrip with forearm heating was associated with greater MSNA in the older subjects.

Exercise can generate marked increases in muscle temperature up to 40°C (29, 30). The heat-induced increases in muscle temperature elicited by the experimental intervention (\( \sim 39°C \)) are commonly observed during exercise and are thus physiological relevant. The current study is the first study to demonstrate that local forearm heating further augments renal vasoconstriction during isometric handgrip. These greater renal vascular responses were associated with augmented MSNA, suggesting greater sympathetic outflow to the kidney. Because renal vasoconstriction was increased during PEMI with heating compared with normothermia, greater activation of the muscle metaboreflex could have mediated the larger renal vasoconstriction in both age groups. However, based on previous studies heating may also sensitize the muscle mechanoreflex (10, 26). The muscle mechanoreflex has been reported to mediate renal vasoconstriction in humans (10, 20).
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 MSNA did not differ between normothermia and heating. 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 were below those reported for causing pain in the arm (22). For these reasons, we do not believe that afferents in the skin contributed to the observed differences between temperature conditions.

We also observed greater effect of muscle heating on renal vasoconstriction during exercise in the older subjects. During the normothermia trial, renal vascular conductance decreased similarly in both the young and older groups. In contrast, during the heating trial, the decrease in renal vascular conductance was significantly greater in the older than young subjects. This augmented response in the older subjects was associated with a greater augmentation of MSNA in the older subjects during the heating trial. This finding suggests that greater sympathetic outflow to the kidneys likely occurred in the older group than younger group. Previous work indicated that renal sympathetic activity is the primary regulator of renal blood flow during isometric exercise (18). The results of the current study and Momen et al. (19) suggest that renal sympathetic outflow during isometric handgrip is higher in older individuals than in the young or that renal vascular responsiveness to vasoconstrictors is augmented with age.

As observed previously, MSNA was elevated in the older subjects at rest (4, 23, 27, 31). Previous studies found that during exercise in normothermic conditions absolute changes in MSNA in older individuals are either similar (24) or less (12) than in younger individuals. Likewise, we observed that aging did not alter changes in MSNA during isometric handgrip under normal conditions. However, this was not the case with muscle heating. Isometric handgrip in older subjects elicited a greater augmentation of MSNA compared with younger subjects with muscle heating. The exact mechanism for this difference is not clear.

Ray and Gracey (26) reported that local forearm heating increased blood pressure and MSNA responses to exercise. Our results agree with Ray and Gracey (26) because mean arterial blood pressure and MSNA were greater with forearm heating during exercise compared with normothermic exercise. Moreover, the current study expands on these findings because it is the first study to demonstrate that blood pressure and MSNA responses to isometric exercise are affected by muscle temperature in older humans as well.

Increases in arterial blood pressure during heating in the young are related to augmented peripheral and renal vasoconstriction (10). In the current study, aging did not alter changes in mean arterial blood pressure during isometric exercise with local muscle heating. However, renal vasoconstriction was
greater during exercise in the older subjects, indicating that the contribution of renal vasoconstriction to the overall blood pressure response to exercise is different between young and older subjects. One possible mechanism that would produce comparable blood pressure responses to exercise despite lower renal vasoconstriction is higher cardiac output in the young during the heating trial compared with the older group. In agreement with this concept, exercise-induced increases in myocardial contractility have been shown to decline with age (5). Another possible mechanism is that younger subjects may have greater vasoconstriction in another vascular bed not measured in the current study (e.g., limb, splanchnic). Finally, ratings of perceived exertion during exercise were not altered in the older group during the heating compared with the normothermia trial. This finding suggests that central command did not contribute to age-related differences in renal vasoconstriction in the current study.

The renal responses to isometric handgrip with a heated forearm in the current study differ from vascular responses to dynamic upright exercise in the heat in older individuals. During upright cycling in the heat (∼36°C), older individuals decrease renal and splanchnic blood flow and increase skin blood flow less than younger individuals (8, 9). This response during dynamic cycling is different than that observed during forearm isometric exercise. In the current study, isometric exercise in the heat elicited greater renal vasoconstriction in the old. The mechanism(s) responsible for eliciting these contrasting results is unknown. Augmented sympathetic outflow would be observed during both exercise paradigms in the heat (26, 28). However, the extent of the thermal stress on the body is markedly different between local heating of the forearm vs. whole body heating. The cardiovascular consequences of this difference in thermal load might precipitate the difference in renal vascular responses.

This study has several limitations. First, to limit changes in muscle temperature during exercise because of exercise-induced hyperemia, subjects performed handgrip while ischemic. Changes in blood flow during contraction may alter the effect of thermal stress on the exercise pressor reflex (3). However, isometric contractions greater than 15% of maximal voluntary contraction do not raise muscle blood flow and are therefore largely ischemic (6). Second, in the current study Doppler ultrasound cannot accurately measure renal artery diameter. Therefore, changes in renal artery diameter could account for age-related changes in renal vascular responses to normothermic and forearm-heated handgrip. There is some evidence to suggest that pharmacological-mediated renal vasoconstriction (13) and vasodilation (11) 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. However, if differences in renal diameter do occur between age groups to heating this could explain some of the differences between groups.

In summary, we compared renal and sympathetic responses to isometric handgrip before and after local forearm heating in young and older subjects. Despite similar renal vascular responses to isometric handgrip during the control trial, muscle heating elicited greater renal vasoconstriction and MSNA responses in older subjects than younger subjects. The present study indicates that the augmentation of the exercise pressor reflex during heating may be a mechanism that promotes vasoconstriction of the visceral tissue in older subjects to help maintain blood pressure.

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