Mechanisms Underlying Exaggerated Metaboreflex Activation in Prehypertensive Men

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ABSTRACT

KIM, K.-A., C. L. STEBBINS, H.-M. CHOI, H. NHO, and J.-K. KIM. Mechanisms Underlying Exaggerated Metaboreflex Activation in Prehypertensive Men. *Med. Sci. Sports Exerc.*, Vol. 47, No. 8, pp. 1605–1612, 2015. **Purpose**: Previously, we found that the pressor response to muscle metaboreflex activation is enhanced in prehypertension and associated with peripheral vasoconstriction. However, mechanisms underlying this exaggerated response are not clear. Therefore, we tested the hypothesis that activation of this reflex is augmented owing to increased production of muscle metabolites (i.e., lactate, K^+, and H^+). **Methods**: Twenty-two men (11 normotensive and 11 prehypertensive) were studied. Changes in cardiac output ($\dot{Q}$), mean arterial pressure (MAP), and total peripheral resistance (TPR) were compared between the two groups during static exercise (SE) and postexercise muscular ischemia (PEMI). Subjects completed 2 min of SE at 50% of maximal voluntary contraction (MVC) followed by 2 min of PEMI. Venous blood samples for determination of metabolites and hormones (catecholamines, vasopressin, and plasma renin activity) were taken from the exercising and nonexercising arm, respectively. **Results**: Mean arterial pressure responses to SE (39 ± 3 vs 31 ± 2 mm Hg) and PEMI (24 ± 3 vs 19 ± 3 mm Hg) were significantly higher in the prehypertensive group. Increases in lactate and decreases in pH during PEMI were seen in both groups. However, changes in these variables were greater in the prehypertensive group (lactate, 50.1 ± 6.2 vs 32.8 ± 7.6 mg·dL^{-1}; pH, −0.06 ± 0.02 vs −0.01 ± 0.01) ($P < 0.05$). Postexercise muscular ischemia did not evoke increases in hormones in either group. **Conclusions**: Compared to the normotensive group, the augmented pressor response to the metaboreflex in the prehypertensive group was associated with greater production of muscle metabolites that activate its afferent arm. The augmented response was not associated with activation of the vasopressin and renin-angiotensin systems and greater activation of the sympathetic nervous system was not apparent. Consequently, additional factors specific to prehypertension, such as arterial stiffness, may have been involved. **Key Words:** POSTEXERCISE MUSCULAR ISCHEMIA, EXERCISE PRESSOR REFLEX, TOTAL PERIPHERAL RESISTANCE, CATECHOLAMINES

BASIC SCIENCES

The cardiovascular response to exercise (e.g., increases in blood pressure (BP), heart rate (HR), and cardiac output ($\dot{Q}$) is evoked, in part, by a reflex originating in skeletal muscle (i.e., the exercise pressor reflex) (8,23). The afferent arm of this reflex arc consists of thinly myelinated group III and nonmyelinated group IV muscle afferents whose nerve endings terminate in the interstitium of skeletal muscle. The exercise pressor reflex is initiated by activation of group III muscle afferents that are primarily responsive to mechanical stimuli, and group IV muscle afferents that primarily respond to metabolic stimuli released from contracting muscles (18,22). Consequently, the exercise pressor response can be viewed in the context of the combined contribution of a mechanoreflex and a metaboreflex.

Recently, we investigated the contribution of these two subreflexes to the exercise pressor reflex in subjects with prehypertension (7). Prehypertension represents a new classification of BP between normal and established hypertension that is not considered a disease state (6). It was created to highlight the possible risk of this BP range for subsequent development of hypertension and is based on observations of marked increases in the risk of cardiovascular events associated with BP values previously considered to be normal (6). We found that reflex-induced increases in BP, evoked by forearm static contraction, were augmented in prehypertensive compared to control subjects and that this augmentation was predominantly due to an exaggerated metaboreflex induced by postexercise muscle ischemia (PEMI) (7). In addition, the enhanced pressor response was associated with elevated vasoconstriction (i.e., increased vascular resistance). Although the role played by metaboreceptors in eliciting this enhanced BP response has been demonstrated, the underlying mechanisms have not been identified.

One possibility is that in prehypertensive individuals, there is a greater production and accumulation of skeletal muscle metabolites that activate group III and IV muscle

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afferent nerve endings to cause the exercise pressor reflex. In particular, it seems that decreases in muscle acidity associated with the production of metabolic acids (e.g., lactate) play an important role in the activation of the muscle metaboreflex. In this regard, decreases in skeletal muscle pH during muscle contraction follow a pattern similar to that seen for increases in BP, sympathetic nerve activity, and vascular resistance; and these responses are sustained during PEMI (i.e., during metaboreflex activation alone) (3,4,29,36).

Another possible metabolic candidate is K^+ which is released from skeletal muscle during contraction (31) and can activate group III and IV muscle afferents to cause reflex increases in BP and HR (26,27). Interestingly, potassium efflux in skeletal muscle is elevated in the spontaneously hypertensive rat (32), which might lead to elevated levels of this ion during contraction and PEMI. However, it is not known if such a scenario may occur in prehypertensive humans.

Activation of the muscle metaboreflex in humans has been shown to induce increases in sympathetic outflow and in hormones such as renin and arginine vasopressin (AVP) (24,34). Increases in these factors have also been reported at rest and/or during exercise in some forms of hypertension (34,41). Thus, they may play a role in metaboreflex-induced augmentations of peripheral vasoconstriction and BP seen in prehypertensive individuals.

Based on these observations, we tested the hypotheses that: (1) compared to normotensive individuals, the elevated pressor response to metaboreflex activation in prehypertensive individuals is associated with enhanced production of skeletal muscle lactate, K^+, and H^+; and (2) this response is associated with augmentations in sympathetic outflow and elevations in AVP and renin activity.

METHODS

Subjects. Eleven prehypertensive and 11 normotensive men age 20–28 yr (23 ± 1 vs 24 ± 1, respectively), participated in this study. Subjects were recruited from the Kyung Hee University campus. Prehypertensive and normotensive subjects were matched by age, weight, height, and body mass index (BMI).

Prehypertensive individuals were selected according to the criteria set forth in The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (systolic BP (SBP), 120–139 mm Hg and/or a diastolic BP (DBP) of 80–89 mm Hg) (6). None of the subjects was taking medications that could affect cardiovascular function or BP. Based on health history questionnaires, subjects were determined to be free of any signs or symptoms of overt cardiovascular disease and were considered to be in good health. Subjects were instructed to abstain from caffeine, alcohol, and exercise for at least 24 h before the screening visit. The Kyung Hee University Human Investigation Committee reviewed and approved this study, and each subject gave informed written consent.

Blood pressure screening. When subjects reported to the laboratory for their screening visit, they rested quietly for 30 min. Subsequently, arterial BP was measured in the nondominant arm with the subject in a seated position. Three BP measurements were taken 5 min apart using a calibrated mercury sphygmomanometer and a cuff size that was appropriate for the arm circumference of each subject. Blood pressure measurements were performed with the arm at the heart level after each subject had been seated for at least 5 min with his back supported in a chair and both feet on the floor. Blood pressure was taken in the same manner before the experimental protocol (i.e., static contraction and metaboreflex activation).

Experimental protocols. Static contraction and skeletal muscle metaboreflex. To determine the relative exercise intensity, maximal voluntary contraction (MVC) of the dominant forearm was performed by having the subjects squeeze a handgrip dynamometer three times at maximal effort for less than 1 s. Subsequently, the workload for the static contraction protocols was calculated as 50% of each subject's MVC. After a rest period of 10 min, the subjects then performed isometric handgrip exercise for 2 min at this work intensity. The target force was displayed on the handgrip device to assist the subjects in achieving and maintaining the prescribed workload.

Before exercise, a rapidly inflatable occlusion cuff was fitted on the upper arm that would be exercised. Ten seconds before the end of the 2-min contraction period, the cuff was inflated to suprasystolic pressures (>200 mm Hg) to occlude the forearm vasculature in the exercising forearm and induce PEMI. Retrograde insertion of a 20-gauge catheter into the antecubital vein of the exercising arm was performed to collect blood samples trapped in the forearm below the occlusion cuff for measurement of K^+, lactate (as index of lactate), and pH (as an index of H^+) during PEMI. This approach allowed us to identify potential metabolites that contribute to the metaboreflex. Antegrade placement of a second 20-gauge catheter in the antecubital vein of the nonexercising arm was also performed to allow for sampling of venous blood for determination of plasma concentrations of catecholamines (an index of general sympathetic activation) (12). From these samples, plasma renin activity (PRA) (an index of activation of the renin-angiotensin system) and AVP (an index of activation of the vasopressin system) were also assessed.

Five milliliters of venous blood were drawn from each catheter at rest and during the last 15 s of PEMI. Two minutes of static handgrip exercise was followed immediately by 2 min of PEMI.

Blood sampling and biochemical analysis. Blood samples were collected into prechilled tubes containing EDTA and centrifuged at 3000 rpm for 10 min. The supernatant was then collected and stored at 5°C for future analysis. Plasma catecholamine concentrations were measured by reverse-phase high-performance liquid chromatography (HPLC). Extraction of norepinephrine and epinephrine was

performed by selective absorption from aluminum oxide (Plasma-Catecholamine-Kit, BIO-RAD, Korea). A 200-μL internal standard solution + 1 mL Tris buffer were added. The suspension was vortex mixed for 12 min followed by centrifugation at 2500 rpm for 5 min. Subsequently, the supernatant (minus the alumina) was aspirated using a vacuum aspirator. The samples were then centrifuged again at 2500 rpm for 2 min. Next, 200-μL of the supernatant was injected onto the HPLC column and eluted by mobile phase. The intra-assay coefficients of variation were 4.0% for norepinephrine and 4.0% for epinephrine. Plasma renin activity was determined in duplicate by radioimmunoassay of generated angiotensin I after incubation by using a commercial kit (Dream Gamma-10 Counter, Japan). The intra-assay coefficient of variation was 4.0%. Arginine vasopressin was determined in duplicate by radioimmunoassay also using a commercial kit (Dream Gamma-10 Counter). The intra-assay coefficient of variation was 6.0%. Lactate was determined by chemiluminescence immunoassay (Cobas Integra-800, Switzerland), K^+ by an ion selective electrode assay (Rapodchem 744-Siemens, Germany), and pH by an ion selective electrode (Easy Blood Gas, Medica Corp, USA).

Cold pressor test. The cold pressor test was performed to determine the BP response to a nonexercise, nonmetabolic sympathoexcitory stimulus. After baseline measurement collection, the dominant hand of each subject was placed into ice water (8°C) at the level of the wrist for 2 min. During this time, SBP and DBP were measured continuously in the contralateral hand.

Measurement of hemodynamic variables. Changes in SBP, DBP, HR, and stroke volume (SV) were assessed continuously in the middle finger of the nonexercising hand at the level of the heart. Measurements were made using servo-controlled finger photoplethysmography (Finometer, Finapres Medical Systems, The Netherlands). This device uses the volume-clamp technique to measure finger arterial pressure and has been validated to assess changes in both SBP and DBP in conditions of hypertension according to the Association for the Advancement of Medical Instrumentation (28).

Beatscope Software, together with a Finapres finger cuff, was used to assess SV from finger arterial pressure by simulating a three-element (aortic impedance, arterial compliance, and peripheral vascular resistance) nonlinear mathematical model of aortic input impedance (the Modelflow technique) that accounts for variations in aortic properties associated with changes in distending pressure (39). Use of finger pressure derived from the Modelflow technique reflects changes in SV that are consistent with changes assessed via brachial artery pressure (19). Integration of the computed flow waveform per beat provides left ventricular stroke volume. This technique has been validated using both thermodilution and Doppler ultrasound (2,35,39).

Sphygmomanometry was used to verify absolute brachial artery BP because values measured from the inflatable finger cuff may differ from those of standard BP measurements. Mean arterial BP (MAP) was calculated according to the following formula: MAP = [(SBP – DBP) × 1/3] + DBP. $\dot{Q}$ was calculated according to the Fick equation ($\dot{Q}$ = SV × HR), and total peripheral resistance (TPR) was calculated as MAP /$\dot{Q}$.

Data analysis. Although the Modelflow technique (via averaging procedures) has been shown to accurately account for absolute changes in SV from baseline, there is evidence that it does not precisely reveal beat-to-beat variations (2). Accordingly, we used the relative changes from baseline to assess the effects of static contraction and metaboreflex activation on SV, $\dot{Q}$, TPR, MAP, and HR.

Absolute changes in HR, SV, $\dot{Q}$, MAP, and TPR are expressed as means ± SEM. Baseline hemodynamic variables were measured immediately before any intervention. A 30-s average of each hemodynamic parameter was determined during this time. During static contraction and metaboreflex activation, the peak 30-s average response for each variable was assessed just before the end of both contraction and PEMI (i.e., during 90–120 s of contraction and 60–90 s of PEMI), and compared between groups and conditions. A two-way analysis of variance (ANOVA) (group × condition) was used to assess differences in hemodynamic parameters and metabolites and hormone concentrations during static exercise and PEMI. If a significant interaction was found, *post hoc* tests were performed via the Tukey test. Physical characteristics and responses to the cold pressor response were compared between the normotensive and prehypertensive groups using an independent *t*-test. Correlation analysis was used to determine relationships between MAP and metabolite production. The criterion for significant differences was $P < 0.05$.

RESULTS

Subjects' characteristics are presented in Table 1. Prehypertensive and normotensive individuals were age matched and had similar body mass indices (Table 1). By study design, the prehypertensive group had a significantly higher resting SBP and DBP compared with the normotensive group (Table 1). Maximal voluntary contraction in the prehypertensive group was greater than in the normotensive group (24.8 ± 1 vs 21.5 ± 1.0 kg).

Figure 1 shows the absolute peak 30-s average changes in the hemodynamic values in response to 2 min of static exercise in both groups. Peak responses were observed at the period of 90–120 s. Static exercise resulted in significant increases in MAP in both groups, but the rise in MAP was

TABLE 1. Physical characteristics of subjects.

Variables	Normotensive Group (n = 11)	Prehypertensive Group (n = 11)
Age, yr	23 ± 1	24 ± 1
Height, cm	174.7 ± 2.3	175.5 ± 0.8
Body mass, kg	73.1 ± 2.6	73.0 ± 1.9
BMI, kg·m^{-2}	23.9 ± 0.6	23.7 ± 0.6
SBP, mm Hg	113 ± 2	131 ± 1*
DBP, mm Hg	69 ± 2	77 ± 2*

Values are mean ± SE.
*$P < 0.05$ vs normotensive group.
BMI, body mass index.

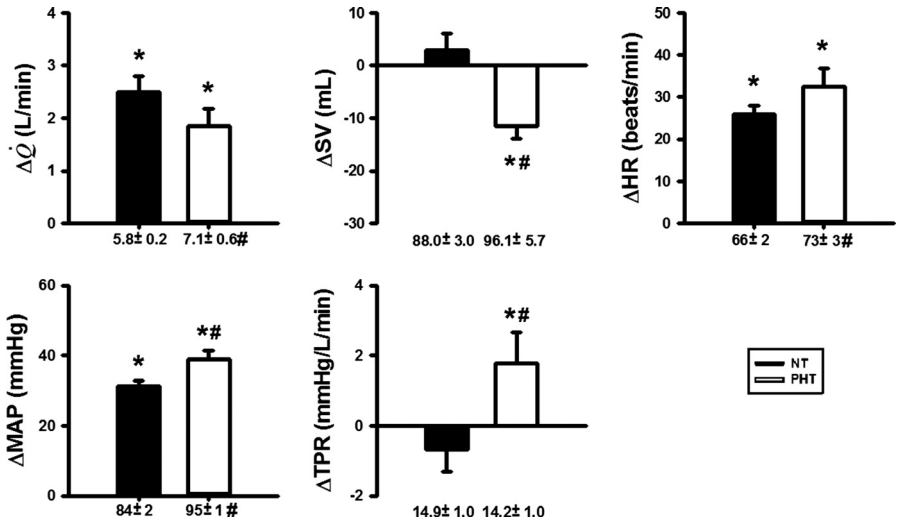

FIGURE 1—Peak 30-s average changes from rest in MAP, HR, $\dot{Q}$, SV, and TPR during static exercise in normotensive (NT) and prehypertensive (PHT) individuals. *$P < 0.05$ vs resting; #$P < 0.05$ vs NT. *Numbers* below the *histograms* represent resting values.

significantly higher in the prehypertensive group than in the normotensive group. $\dot{Q}$ also increased in both groups but tended to increase to a lesser extent in the prehypertensive group. Heart rate was significantly increased in both groups, but there were no group differences in the magnitudes of these increases. There were no significant changes in SV and TPR in response to static exercise in the normotensive group, but SV decreased and TPR increased during contraction in the prehypertensive group.

Absolute peak 30-s average changes in the hemodynamic data from rest in response to 2 min of metaboreflex activation are shown in Figure 2. Metaboreflex activation resulted in significant increases in MAP in both groups, but the rise in MAP was significantly greater in the prehypertensive group compared to the normotensive group. While metaboreflex activation caused increases in $\dot{Q}$, SV and HR ($P < 0.05$) in the normotensive group, no statistically significant changes in these variables were seen in the prehypertensive group. TPR was unchanged in the normotensive group but increased in the prehypertensive group ($P < 0.05$).

Figure 3 shows plasma hormone concentrations in both groups at rest and during PEMI. No significant differences in norepinephrine, epinephrine, or AVP concentrations at rest or during PEMI were found between the groups. There were no group differences in PRA at rest. Plasma renin activity was higher during PEMI in the prehypertensive group but did not increase from resting conditions.

Skeletal muscle venous pH and concentrations of lactate and K^+ at rest and during PEMI and changes in these variables from rest to PEMI are presented in Figure 4, respectively.

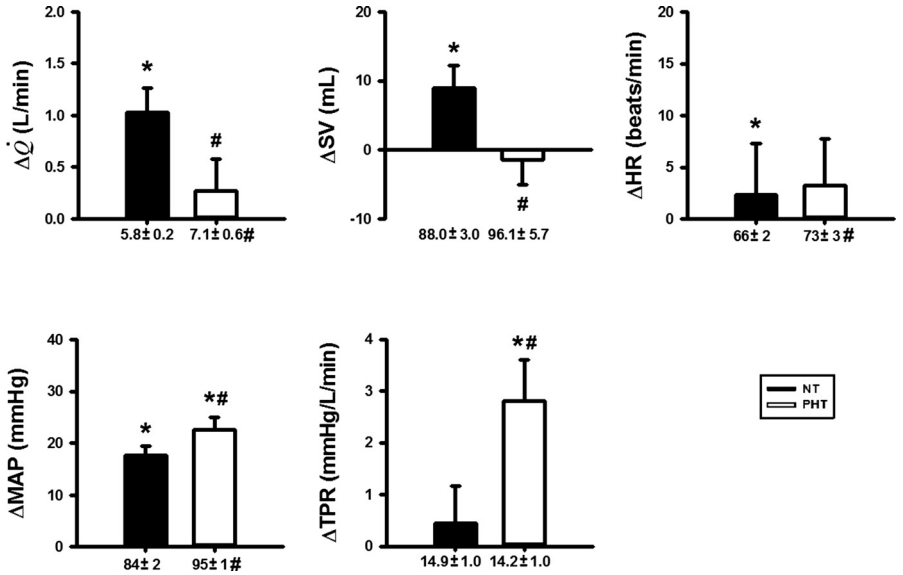

FIGURE 2—Peak 30-s average changes from rest in MAP, HR, $\dot{Q}$, SV, and TPR during PEMI in NT and PHT individuals. *$P < 0.05$ vs resting; #$P < 0.05$ vs NT. *Numbers* below the *histograms* represent resting values.

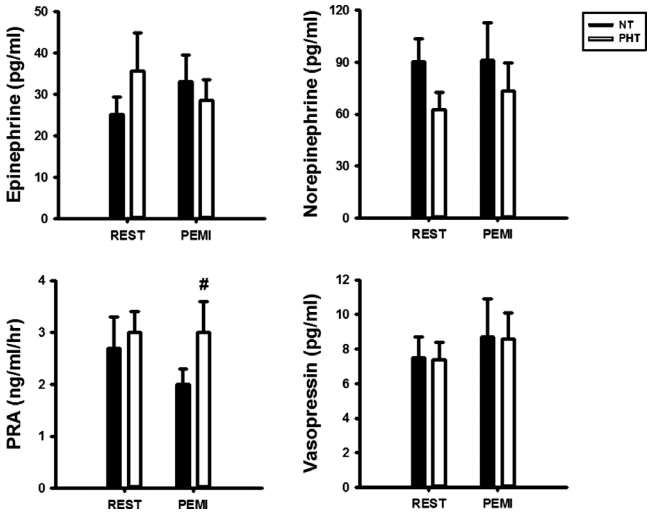

FIGURE 3—Plasma concentrations of norepinephrine, epinephrine, vasopressin, and renin at resting and during PEMI in NT and PHT individuals. #$P < 0.05$ vs NT.

Venous pH at rest was higher in the prehypertensive group but no group differences were seen during PEMI. However, pH decreased significantly from rest during PEMI in the prehypertensive group, whereas no change was seen in the normotensive group (Fig. 4). Lactate concentrations were similar at rest but higher in the prehypertensive group during PEMI (Fig. 4). No group differences between the prehypertensive and normotensive groups were seen in K^+ concentrations at rest or during PEMI (Fig. 4). There were significantly positive correlations between MAP and lactate in the normotensive and prehypertensive groups ($r = 0.49$, $P < 0.05$; $r = 0.69$, $P < 0.05$, respectively). No correlation between MAP and pH was found in the normotensive group, but MAP was negatively correlated with pH in the prehypertensive group ($r = -0.42$; $P < 0.05$). No correlation between MAP and K^+ was found in either group.

Cold pressor test. The cold pressor test resulted in significant increases in MAP from baseline in both groups. However, as observed previously (7), these increases were not different between the normotensive and prehypertensive groups when expressed as absolute increases (normotensive, Δ31 ± 2 mm Hg; prehypertensive, Δ28 ± 3 mm Hg) or relative increases (normotensive, 38% ± 3%; prehypertensive, 29% ± 3%).

DISCUSSION

Our results confirmed our previous observations that the BP response to both the exercise pressor reflex and metaboreflex are augmented in prehypertension and that this augmentation is associated with reductions in SV and elevations in TPR (7). The major new findings are that the exaggerated BP response to activation of the metaboreflex in the prehypertensive individuals is associated with an enhanced production of lactate and an augmented reduction in pH in skeletal muscle compared to the normotensive individuals, and that BP was positively correlated to production of these two metabolites in the prehypertensive group.

These findings are in contrast to those reported in hypertensive subjects where metaboreflex-evoked increases in lactate and decreases in pH were not different from those seen in normotensive individuals (9). This inconsistency may be due to possible differences in skeletal muscle metabolism between prehypertensive and hypertensive individuals. Nevertheless, there were also differences in work intensity between the two studies. Our subjects performed static contraction at 50% of MVC for 2 min before PEMI, whereas in the Delaney et al. study, the highest intensity used was 40% of MVC and contraction lasted for only 1.5 min (inconsistencies that could account for differences in muscle acidity). Moreover, the subjects in the Delaney et al. study were older (60 ± 1 vs 20–28 yr.).

It was surprising that activation of the metaboreflex in the normotensive group did not induce a decrease in skeletal muscle pH despite an increased production of lactate. Since lactate production in the normotensive group was less that that seen in the prehypertensive group, potentially smaller changes in pH may have been offset by buffers in the skeletal muscle or blood (e.g., carnosine, inorganic phosphate, bicarbonate, and creatine phosphate). On the other hand, resting pH in the normotensive group was lower than in the prehypertensive group, which may have influenced the extent to which it could change during the metaboreflex according to the law of initial values (40).

Evidence from animal studies suggests that effects of lactic acid and H^+ ions may be mediated, at least in part, by acid-sensing ion channels (ASIC3) located on the nerve endings of group III and group IV muscle afferents (17). These channels can be activated by changes in pH that are

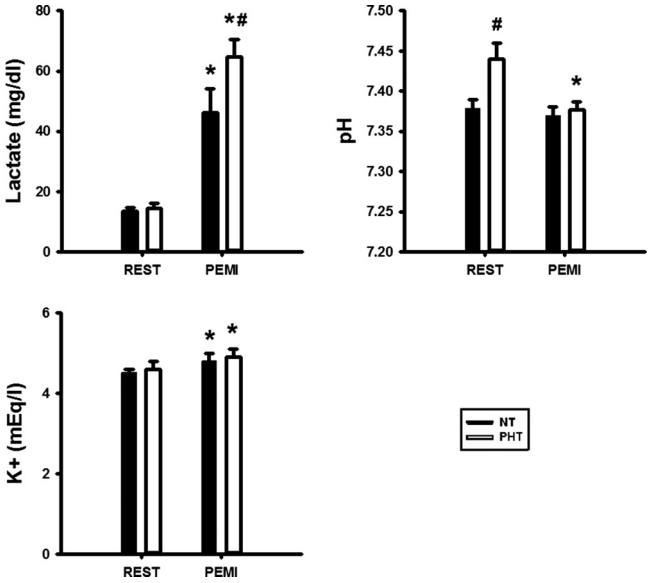

FIGURE 4—Lactate and potassium concentrations and pH in skeletal muscle venous effluent at rest and during PEMI in NT and PHT individuals. *$P < 0.05$ vs resting; #$P < 0.05$ vs NT.

within the range seen in contracting muscle (16). In addition, blockade of ASIC3 attenuates the exercise pressor response in rats, particularly those that have had their hind limb arteries ligated such that muscle contraction would be expected to enhance the production of lactic acid and H^+ (33). Thus, it is worth acknowledging the possibility that enhanced activation of ASIC3 receptors in our prehypertensive humans (via augmentations in contraction-induced increases in lactate and H^+ ions) may have contributed to the simultaneous exaggeration of the resulting metaboreflex response.

One shortcoming of our study was that we only assessed metabolites whose production we believed was most likely to be enhanced by skeletal muscle contraction in the prehypertensive state. Therefore, we acknowledge the likelihood that there may also be an augmented production/release of other metabolites, such as deprotonated phosphate (4,30) and possibly ATP (via activation of P_{2x} receptors) (21).

Although muscle metaboreflex activation may cause peripheral vasoconstriction that is related to increases in muscle sympathetic nerve activity (7,24,29), we did not find evidence of augmented sympathetic outflow in our prehypertensive group. The BP response to the cold pressor test (our nonmetabolic, nonexercise sympathetic index) was not different between our two groups of subjects, which is consistent with what we have reported previously (7). In addition, plasma catecholamine concentrations (our index of general sympathetic nerve activity) were similar between the normotensive and prehypertensive groups at rest and during PEMI. Surprisingly, no increase in catecholamine concentrations from baseline values during PEMI were found in either group, which is in agreement with what has been seen in normotensive individuals (25). However, in studies where muscle sympathetic nerve activity (MSNA) was recorded during static contraction-induced metaboreflex activation, increases in this variable have been reported (14,19). This discrepancy between MSNA and norepinephrine responses probably occurs because the magnitude of increase in norepinephrine concentrations in response to static contraction is considerably smaller than the concomitant increase in MSNA (38) and because its concentrations in the blood represent only a small amount of that which is actually released from postganglionic sympathetic neurons (10). Consequently, catecholamine concentrations are a less sensitive index of overall sympathetic nerve activity MSNA. It is also possible that concentrations of catecholamines in the blood taken from our sampling site (the antecubital vein of the noncontracting forearm) were not representative of concentrations in other circulations where sympathetic activity may have been greater (20). Thus, the use of catecholamines as an index of sympathetic nerve activity represents a limitation to our study such that we cannot dismiss the possibility that augmentations in sympathetic outflow contributed to the exaggerated metaboreflex in our prehypertensive group.

Another factor that may contribute to the enhanced BP responses to metaboreflex activation in prehypertension is vascular stiffness, which has been found to be amplified in prehypertension (11) and can occur in large arteries such as the aorta (5). Increases in vascular stiffness tend to elevate distending pressures (1), an outcome that may contribute to elevations in vascular resistance seen in young prehypertensive individuals (42). It also may have been a contributing factor to the contraction-induced increases in vascular resistance seen in our prehypertensive subjects.

Stimulation of skeletal muscle afferent nerves has been shown to cause reflex-induced increases in AVP and activation of the renin-angiotensin system (15,37). Since enhanced activity of these two pressor systems has been observed in some forms of hypertension (34,41), we compared AVP and PRA responses to metaboreflex activation between our prehypertensive and normotensive groups. Since we observed no differences in AVP concentrations or PRA at rest between the groups and no increases in response to PEMI, we ruled out a role of these two pressor systems in the metaboreflex under our experimental conditions. This lack of response may be due to the short duration of PEMI (i.e., 2 min), as sustained muscle metaboreflex activation (i.e., 14–15 min) has been shown to cause significant increases in both AVP and PRA (24).

Limitations. Although we found positive correlations between BP and production of H^+ and lactate in the prehypertensive group, we did not establish a cause-and-effect relationship. This would have required blockade of receptors for these substances, which are located on group III and IV muscle afferents. We did not conduct such a protocol because we are not aware of any antagonists that can be used effectively in humans without causing nonspecific effects.

Since the muscle tension produced during MVC was higher in the prehypertensive group than in the normotensive group (24.8 ± 1 vs 21.5 ± 1.0 kg), the absolute tension produced during activation of the metaboreflex via static contraction at 50% of MVC would also have been greater in that group. Thus, it could be argued that this difference in absolute tension could have resulted in a greater production of lactate and H^+ during PEMI in the prehypertensive group. If so, then higher K^+ concentrations would also have been expected in this group, but this was not the case. Moreover, expressing work intensity in relative terms (i.e., as a percentage of maximal) normalizes this variable for differences in muscle mass among subjects and is consistent with the manner in which previous studies have expressed work intensity associated with activating of the metaboreflex (3,10,13).

In conclusion, our study has shown that exaggerated metaboreflex-induced increases in BP and vascular resistance in prehypertensive individuals are accompanied by enhanced increases in skeletal muscle lactate and H^+. We found no evidence of augmented activation of the AVP or renin-angiotensin systems. Although increases in the production of these muscle metabolites were not associated with parallel elevations in BP reactivity or circulating catecholamines, we cannot rule out the possibility that activation of the sympathetic nervous system was enhanced because we did not measure sympathetic nerve

activity. Exaggerated effects of PEMI on BP may have been due, in part, to enhanced vascular stiffness that exacerbated vascular resistance in response to a given pressor stimulus.

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None of the authors declares a conflict of interest. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

REFERENCES

1. Arnett DK, Boland LL, Evans GW, et al. Hypertension and arterial stiffness: the atherosclerosis risk in communities study. *Am J Hypertens*. 2000;13:317–23.
2. Bogert LW, Van Lieshout JJ. Non-invasive pulsatile arterial pressure and stroke volume changes from the human finger. *Exp Physiol*. 2005;90(4):437–46.
3. Boushel R. Muscle metaboreflex control of the circulation during exercise. *Acta Physiol Scand*. 2010;199(4):367–83.
4. Boushel R, Madsen P, Nielsen HB, Secher NH. Contribution of pH, deprotonated phosphate and potassium for the reflex increase in blood pressure during handgrip. *Acta Physiol Scand*. 1998; 164(3):269–76.
5. Celik T, Lyisoy A, Kursaklioglu H, et al. Impaired aortic elastic properties in young patients with prehypertension. *Blood Press Monit*. 2006;11(5):251–55.
6. Chobanian AV, Bakris GL, Black HR, et al. Seventh report of the joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension*. 2003;42: 1206–52.
7. Choi HM, Stebbins CL, Lee OT, et al. Augmentation of the exercise pressor reflex in prehypertension: roles of the muscle metaboreflex and mechanoreflex. *Appl Physiol Nutr Metab*. 2013;38(2):209–15.
8. Coote JH, Hilton SM, Perez-Gonzalez JF. The reflex nature of the pressor response to muscular exercise. *J Physiol*. 1971;215(3): 789–804.
9. Delaney EP, Greaney JL, Edwards DG, Rose WC, Fadel PJ, Farquhar WB. Exaggerated sympathetic and pressor responses to handgrip exercise in older hypertensive humans: role of the muscle metaboreflex. *Am J Physiol Heart Circ Physiol*. 2010;299(5): H1318–27.
10. Elser M, Jennings G, Lambert G, Meredith I, Home M, Eisenhofer G. Overflow of catecholamine neurotransmitters to the circulation: source, fate, and functions. *Physiol Rev*. 1990;70(4):963–85.
11. Gedikli O, Kiris A, Ozturk S, Baltaci D, Karaman K, Durmus I. Effects of prehypertension on arterial stiffness and wave reflections. *Clin Exp Hypertens*. 2010;32(2):84–9.
12. Goldstein DS, McCarty R, Polinsky RJ, Kopin IJ. Relationship between plasma norepinephrine and sympathetic neural activity. *Hypertension*. 1983;5(4):552–9.
13. Greaney JL, Schwartz CD, Edwards DG, Fadel PJ, Farquhar WB. The neural interaction between the arterial baroreflex and muscle metboreflex is preserved in older men. *Exp Physiol*. 2013;98(10): 1422–31.
14. Hansen J, Thomas GD, Jacobsen TN, Victor RN. Muscle metaboreflex triggers parallel sympathetic activation in exercising and resting human skeletal muscle. *Am J Physiol Heart Circ Physiol*. 1994;266:H2508–14.
15. Holdaas H, DiBona GF. Stimulatory and inhibitory reflexes from somatic receptors: effect on renin release. *Am J Physiol Reg Integr Comp Physiol*. 1984;246:R1005–10.
16. Immke DC, McCleskey EW. Lactate enhances the acid-sensing Na+ channel on ischemia-sensing neurons. *Nat Neurosci*. 2001;4(9): 869–70.
17. Jankowski MP, Rau KK, Ekmann KM, Anderson CE, Koerber HR. Comprehensive phenotyping of Group III and IV muscle afferents in mouse. *J Neurophysiol*. 2013;109(9):2374–81.
18. Kaufman MP, Longhurst JC, Rybicki KJ, Wallach JH, Mitchell JH. Effects of static muscular contraction on impulse activity of group III and IV afferents in cats. *J Appl Physiol*. 1983;55:105–112.
19. Jellema WT, Imholz BPM, Oosting H, Wesseling KH, van Lieshout JJ. Estimation of beat-to-beat stroke volume from arterial pressure: a comparison of two pressure wave analysis techniques during head-up tilt testing in young, healthy men. *Clin Auton Res*. 1999;9(4):185–92.
20. Koba S, Xing J, Sinoway LI, Li J. Differential sympathetic outflow elicited by active muscle in rats. *Am J Physiol Heart Circ Physiol*. 2007;293(4):H2335–43.
21. McCord JL, Tsuchimochi H, Kaufman MP. P2X2/3 and P2X3 receptors contribute to the metaboreceptor component of the exercise pressor reflex. *J Appl Physiol*. 2010;109(5):1416–23.
22. McCloskey DI, Mitchell JH. Reflex cardiovascular and respiratory responses originating in exercising muscle. *J Physiol*. 1972; 224(1):173–186.
23. Mitchell JH, Kaufman MP, Iwamoto GA. The exercise pressor reflex: its cardiovascular effects, afferent mechanisms, and central pathways. *Annu Rev Physiol*. 1983;45:229–42.
24. Nishiyasu T, Tan N, Morimoto K, Sone R, Murakami N. Cardiovascular and humoral responses to sustained muscle metaboreflex activation in humans. *J Appl Physiol*. 1998;84(1):116–22.
25. Pawelczyk JA, Pawelczyk RA, Warberg J, Mitchell JH, Secher NH. Cardiovascular and catecholamine responses to static exercise in partially curarized humans. *Acta Physiol Scand*. 1997; 160(1):23–8.
26. Rybicki KJ, Kaufman MP, Kenyon JL, Mitchell JH. Arterial pressure responses to increasing interstitial potassium in hindlimb muscle of dogs. *Am J Physiol Regul Integr Comp Physiol*. 1984; 247:R717–21.
27. Rybicki KJ, Waldrop TG, Kaufman MP. Increasing gracilis muscle interstitial potassium concentrations stimulate group III and IV afferents. *J Appl Physiol*. 1985;58(3):936–41.
28. Schutte AE, Huisman HW, Van Rooyen JM, Malan NT, Schutte R. Validation of the Finometer device for measurement of blood pressure in black women. *J Hum Hypertens*. 2004;18(2):79–84.
29. Sinoway L, Prophet S, Gorman I, et al. Muscle acidosis during static exercise is associated with calf vasoconstriction. *J Appl Physiol*. 1989;66(1):429–36.
30. Sinoway LI, Smith MB, Enders B, et al. Role of diprotonated phosphate in evoking muscle reflex responses in cats and humans. *Am J Physiol Heart Circ Physiol*. 1994;267:H770–8.
31. Stebbins C, Longhurst J. Potentiation of the exercise pressor reflex by muscle ischemia. *J Appl Physiol*. 1989;66(3):1046–53.
32. Syme PD, Dixon RM, Aronson JK, Grahame-Smith DG, Radda GK. Evidence for increased in vivo sodium-potassium pump activity and potassium efflux in skeletal muscle of spontaneously hypertensive rats. *J Hypertens*. 1990;8(12):1161–6.
33. Tsuchimochi H, Yamauchi K, McCord JL, Kaufman MP. Blockade of acid sensing ion channels attenuates the augmented exercise pressor reflex in rats with chronic femoral artery occlusion. *J Physiol*. 2011;589:6173–89.
34. Uchida K, Yamamoto I, Ishise S, Morimoto S, Takeda R. Plasma renin activity response to isometric handgrip exercise in normotensive and hypertensive patients. *Nihon Naibunpi Gakkai Azsshi*. 1978;54(6):752–8.

35. Van Lieshout JJ, Toska K, Van Lieshout EJ, Eriksen M, Walloe L, Wesseling KH. Beat to beat non-invasive stroke volume from arterial pressure and Doppler ultrasound. *Eur J Appl Physiol*. 2003; 90(1–2):131–7.
36. Victor RG, Bertocci LA, Pryor SL, Nunnally RL. Sympathetic nerve discharge is coupled to muscle cell pH during exercise in humans. *J Clin Invest*. 1988;82(4):1301–5.
37. Yamashita H, Kannan H, Inenaga K, Koizumi K. The role of cardiovascular and muscle afferent systems in control of body water balance. *J Auton Nerv Syst*. 1984;10:305–16.
38. Wallin BG, Mörlin C, Hjemdahl P. Muscle sympathetic activity and venous plasma noradrenaline concentrations during static exercise in normotensive and hypertensive subjects. *Acta Physiol Scand*. 1987;129(4):489–97.
39. Wesseling KH, Jansen JRC, Settels JJ, Schreuder JJ. Computation of aortic flow from pressure in humans using a nonlinear, three element model. *J Appl Physiol*. 1993;74(5):2566–73.
40. Wilders J. Recent developments in the law of initial values. *Exp Med Surg*. 1962;20:126–31.
41. Zhang X, Hense HW, Riegger GA, Schunkert H. Association of arginine vasopressin and arterial blood pressure in a population-based sample. *J Hypertens*. 1999;17(3):319–24.
42. Zhu H, Weili Y, Ge D, et al. Cardiovascular characteristics in American youth with prehypertension. *Am J Hypertens*. 2007;20(10):1051–7.