Human cardiovascular and humoral responses to moderate muscle activation during dynamic exercise

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Nishiyasu, Takeshi, Kei Nagashima, Ethan R. Nadel, and Gary W. Mack. Human cardiovascular and humoral responses to moderate muscle activation during dynamic exercise. J. Appl. Physiol. 88: 300–307, 2000.—We examined the hypothesis that activation of the muscle metaboreflex during dynamic exercise would augment influences tending to cause a rise in arginine vasopressin, plasma renin activity, and catecholamines during dynamic exercise in humans. Ten healthy adults performed 30 min of supine cycle ergometer exercise at ~50% of peak oxygen consumption with or without moderate muscle metaboreflex activation by application of 35 mmHg lower body positive pressure (LBPP). Application of LBPP during the first 15 or last 15 min of exercise increased mean arterial blood pressure, plasma lactate concentration, and minute ventilation, indicating an activation of the muscle metaboreflex. These changes were rapidly reversed when LBPP was removed. During exercise at this intensity, LBPP augmented the release of arginine vasopressin and catecholamines but not of plasma renin activity. These results suggest that, although in humans hormonal responses are induced by moderate activation of the muscle metaboreflex during dynamic exercise, the thresholds for these responses may not be uniform among the various glands and hormones.

arginine vasopressin; lower body positive pressure; exercise; mean arterial blood pressure; muscle metaboreflex; plasma renin activity

DURING DYNAMIC EXERCISE, heart rate (HR) and mean arterial blood pressure (MAP) are increased in a graded manner. The cardiovascular responses to dynamic exercise are thought to be regulated by a number of factors: 1) central command (26), 2) peripheral reflexes evoked by activation of afferent nerve endings (mechanical/metabolic) in the working skeletal muscles (14, 25), and 3) arterial and cardiopulmonary baroreflexes (26). Moderate-to-heavy dynamic exercise activates the muscle metaboreflex through the accumulation of metabolites, and this is thought to contribute to cardiovascular regulation. At such exercise intensities, several hormonal responses are also induced, such as increases in plasma renin activity (PRA), plasma vasopressin (AVP), and catecholamines (4, 9, 20). The muscle metaboreflex is implicated in modulating the hormonal response to moderate-to-heavy dynamic exercise (22); however, this interaction is poorly understood.

Yamashita et al. (34) showed that activation of group III and IV afferents from skeletal muscle elicits an increase in the activity of neurons in the supraoptic nucleus in cats, suggesting that the muscle metaboreflex may stimulate the posterior pituitary gland to secrete AVP. However, O’Leary et al. (22) found that during dynamic exercise, activation of the muscle metaboreflex (by decreasing blood flow to the active muscle) did not increase AVP or PRA in dogs. In fact, only when they attenuated the pressor response during the activation of the muscle metaboreflex did AVP release occur. O’Hagan et al. (21) reported that renal sympathetic nerve activity was augmented when activation of the muscle metaboreflex was induced by hind-limb ischemia during dynamic exercise in rabbits. An increase in renal sympathetic nervous activity would promote renin release (5, 11), although PRA was not monitored in the above study (21). In humans, Nishiyasu et al. (18) reported that sustained activation of the muscle metaboreflex induced significant increases in PRA as well as in plasma AVP, ACTH, and catecholamines during intermittent isometric handgrip exercise. However, it is unclear whether similar hormonal responses take place during dynamic exercise in humans as a result of activation of the metaboreflex. The purpose of this study was to examine the hypothesis that moderate activation of the muscle metaboreflex augments the neurohumoral response to dynamic exercise in humans and, if possible, to determine whether secretion of the various hormones is increased in a uniform or nonuniform manner.

METHODS

We studied 10 healthy volunteers (8 men and 2 women) with a mean age of 26 ± 2 yr, a body weight of 68.3 ± 2.8 kg, and height of 174.6 ± 3.0 cm. The subjects were nonsmokers, and none was taking any medication. The female subjects’ experiments were conducted during the first 10 days of their menstrual cycle. Each subject gave written informed consent before participating in this study. The experimental protocol was approved by the Yale University School of Medicine Human Investigation Committee. All subjects came to the laboratory for orientation before the experiment and were familiarized with all procedures and measurement devices.
Each subject underwent a standard test of peak oxygen consumption ($V_{\text{O}}_{2}\text{peak}$) on a supine bicycle ergometer. $V_{\text{O}}_{2}\text{peak}$ averaged 46.3 ± 2.1 ml·kg$^{-1}$·min$^{-1}$. Each subject performed two supine exercise protocols on separate days at an ambient temperature of 27°C (<35% relative humidity, minimal air speed).

**Procedures.** Subjects were instructed to drink 1 liter of water the night before the experiment and to refrain from beverages containing caffeine or alcohol. On arriving at the laboratory at 7 AM, the subjects drank 8 ml/kg tap water, ate a light breakfast, and sat quietly for 2 h before beginning the experiment.

Each subject then entered the environmental chamber (27 ± 0.1°C, <35% relative humidity), swallowed an esophageal thermocouple, and assumed the supine position, with the lower torso up to the iliac crest enclosed in the lower body negative pressure (LBPP) box. A seal was established in the waist region at the level of the iliac crest with the aid of flexible neoprene shorts. The LBPP box was designed to allow supine exercise on a Collins electronically braked cycle ergometer placed within the box. The cycle ergometer was located on an adjustable slide to accommodate variations in leg length, and the box was constructed in such a way that during each cycle of the pedaling the fully extended leg was never more than 20° above or below the horizontal plane of the hip. A saddle and a hip belt were provided to limit movement of the body during the application of LBPP. A Teflon catheter was inserted into a large antecubital vein for blood sampling.

Each experiment consisted of 60 min of supine rest, 3 min of preexercise control data collection, and 30 min of continuous supine exercise at an intensity of 110 ± 4 W. This exercise intensity was identified during the graded maximal supine exercise at an intensity of 110 W. This exercise intensity was selected to meet the following criteria: 1) the supine exercise itself (without LBPP) should not cause any accumulation of lactate (La) during a 30-min exercise period, and 2) the subjects should be able to complete 30 min of total exercise when LBPP was applied for one-half of the exercise period. LBPP was applied 3 min before the onset of exercise, maintained for the first 15 min of exercise, and removed during the final 15 min of exercise ("on-off" protocol) or applied throughout the last 15 min of exercise ("off-on" protocol). The order of LBPP application was determined by a balanced crossover design.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) and heart rate were measured with a Colin blood pressure monitor (model STBP-780B, Aichi, Japan) every 60 s. MAP was calculated as (SBP + 2DBP)/3. Body core temperature was measured every 30 s from a thermocouple inserted through the nose and into the esophagus to a depth of one-fourth the subject's height. During exercise, oxygen consumption ($V_{\text{O}}_{2}$), minute ventilation rate ($V_{\text{E}}$), and respiratory exchange ratio (RER) were measured once per minute by using a SensorMedics metabolic cart. Toracic impedance ($Z_t$) was measured once per minute by using a Minnesota impedance plethysmograph. For this purpose, a tetrapolar electrode system was used, with two pairs of Mylar-tape ring electrodes being employed, one around the neck and one around the trunk. Ratings of perceived exertion (RPE) were recorded at 14 and 29 min of exercise.

Blood samples (15 ml) were obtained at rest (before the application of LBPP or the start of exercise); at 5, 14, 20, and 29 min of exercise; and 3 min after the end of exercise. Blood for hematocrit (Hct), Hb concentration, and total protein (TP) concentration measurements was immediately separated from each sample. The Hct was measured by using a microcentrifuge, Hb concentration by the cyanomethemoglobin method, and TP in the plasma by refractometry. Blood for the measurement of plasma osmolality (Posm) was transferred to a lithium-heparin-treated tube and centrifuged (4°C), and the osmolality of the separated plasma was immediately measured by the freezing point depression method (model 3D11, Advance Instruments). Plasma La was measured in duplicate by using an automatic glucose and La analyzer (model 2300STAT, Yellow Springs Instruments, Yellow Springs, OH). Blood samples for the measurement of AVP concentrations were collected in EDTA tubes and centrifuged at 4°C. Plasma was stored at −70°C until the assays were performed. Plasma AVP was determined by radioimmunoassay (Mitsubishi Yuka AVP assay kit). The intra-assay coefficients of variation for 2.39 and 5.89 pg/ml AVP were 4.7 ± 1.1 and 5.7 ± 1.5%, respectively. The interassay coefficients of variation were 13.3 and 11.0%, respectively. All samples from a given subject were determined within the same assay batch. The extraction recovery of AVP was 66%.

PRA was determined by radioimmunoassay of generated angiotensin I after incubation for 1 h at 37°C, by using a commercial kit. The intra- and interassay coefficients of variation for the assay of PRA were 2.3% and 4.5%, respectively. Plasma levels of norepinephrine (NE) and epinephrine (Epi) were determined by high-performance liquid chromatography with electrochemical detection (Coulochem, ESA, Chelmsford, MA) after alumina adsorption and extraction with acetic acid. Dihydroxybenzylamine (Sigma Chemical, St. Louis, MO) was used as an internal standard in the determinations of NE and Epi levels. The coefficients of variation for the plasma NE and Epi determinations carried out in our laboratory are 3 and 5%, respectively.

**Statistical analysis.** Values are means ± SE for all 10 subjects, except in the case of the catecholamine data, which was for 8 subjects (6 men and 2 women). Statistical analysis was assessed by a repeated-measures analysis of variance followed by a protected least significant difference post hoc test. A P value of < 0.05 was considered statistically significant.

**Results.**

The ability of LBPP application to activate muscle metaboreflex was assessed by examining the changes in the metabolic and respiratory parameters illustrated in Fig. 1. Application of LBPP during the first 15 min of exercise had little impact on $V_{\text{O}}_{2}$, whereas application of LBPP during the last 15 min of exercise increased $V_{\text{O}}_{2}$. The removal of LBPP at 15 min into the exercise resulted in a slight decline in $V_{\text{O}}_{2}$, $V_{\text{E}}$ and RER were always higher during exercise with LBPP than without. Plasma La averaged 0.7 ± 0.1 mM at rest (Table 1), and it did not increase significantly during exercise without LBPP. Application of LBPP at 15 min into the exercise increased plasma La to 2.5 ± 0.2 and 3.8 ± 0.4 mM at 20 and 29 min, respectively. When LBPP was applied during the first 15 min of exercise, plasma La increased to 2.1 ± 0.3 and 3.9 ± 0.6 mM at 5 and 14 min, respectively. After the release of LBPP at 15 min into the exercise, plasma La declined to 2.6 ± 0.5 mM by 29 min of exercise. When the first 15 min of exercise was performed without LBPP, the subjects' own RPE averaged 12 ± 1 (14 min), and this increased to 14 ± 1 (29 min) after the application of LBPP (P < 0.05). When the first 15 min of exercise was performed
with LBPP, their RPE was 16 ± 1 (14 min), and this decreased to 11.1 ± 1 (29 min; P < 0.05) after the removal of LBPP.

The cardiovascular responses to activation of muscle metaboreflex are shown in Fig. 2. Exercise without LBPP increased MAP from 91 ± 1 at rest to 107 ± 3 mmHg by 3 min into the exercise, and it was maintained at this level throughout the first 15 min of the exercise. Subsequent application of LBPP at 15 min increased MAP by 10 ± 3 mmHg within 1 min, and MAP reached 123 ± 3 mmHg by 19 min and then remained at this level for the rest of the 30 min exercise. When applied at rest, LBPP increased MAP from 91 ± 2 to 97 ± 3 mmHg. During the first 15 min of exercise with LBPP, MAP increased to 120 ± 3 mmHg at 15 min. The subsequent removal of LBPP at 15 min decreased MAP by 11 ± 3 mmHg within 1 min, and it declined to 96 ± 3 mmHg by the end of the exercise. Exercise without LBPP increased HR to 124 ± 4 beats/min by 15 min, a rate lower than the 137 ± 4 beats/min recorded during exercise with LBPP. Application of LBPP at 15 min increased HR to 144 ± 6 beats/min, whereas release of LBPP caused HR to decrease to 128 ± 4 beats/min by the end of the exercise.

Table 1 shows the impact of LBPP on plasma constituents at rest; at 5, 14, 20, and 29 min of exercise; and at 3 min into the recovery. During the first 14 min of exercise, Posm increased to 290 ± 1 mosmol/kgH2O without LBPP and to 293 ± 1 mosmol/kgH2O (P < 0.05) with LBPP. Posm increased further to 293 ± 1 mosmol/kgH2O within 5 min after the application of LBPP, and it increased further to 295 ± 1 mosmol/kgH2O by the end of the exercise. When LBPP was removed at 15 min into the exercise, Posm decreased to 291 ± 1 mosmol/kgH2O within 5 min, and it remained at this level for the remainder of the exercise. Hct, Hb, and TP concentration were always higher during exercise with LBPP than without.

Exercise with LBPP elevated AVP compared with exercise without LBPP (Fig. 3A). The increase in AVP was reversed when LBPP was removed. Similarly, exercise with LBPP elevated NE and Epi compared with exercise without LBPP (Fig. 4). The increase in NE and Epi were reversed when LBPP was removed. In contrast, the application of LBPP had little or no impact on PRA during exercise (Fig. 3B).

Resting esophageal temperature was similar in both protocols, averaging 36.71 ± 0.05 and 36.73 ± 0.05°C. During exercise, esophageal temperature increased to 37.35 ± 0.06 and 37.24 ± 0.05°C at 14 min with and without LBPP, respectively (P < 0.05). During the next 15 min of exercise, esophageal temperature increased to 37.61 ± 0.05 and 37.44 ± 0.06°C with and without LBPP, respectively. Z0 increased from 22.6 ± 1.2 Ω at rest to 23.2 ± 1.2 Ω at 14 min without LBPP and to 23.0 ± 1.1 at 29 min with LBPP. Z0 increased from 22.5 ± 1.2 Ω at rest to 23.1 ± 1.1 Ω at 14 min with LBPP and to 23.2 ± 1.2 Ω at 29 min without LBPP. Thus Z0 tended to be lower at 14 min and 29 min with LBPP than without LBPP; however, the differences were not significant.

**DISCUSSION**

The application of positive pressure to the lower one-half of the body increases tissue pressure and reduces the transmural pressure gradient across the vasculature (15, 23). This leads to increases in both central venous pressure (CVP) and MAP (2, 6, 16, 2, 29). When LBPP is applied at rest, the increase in MAP (6 mmHg in the present study) would be due to a combination of the direct mechanical effects of increased tissue pressure on circulatory hemodynamics and the activation of muscle mechanoreflexes (16, 28, 33). During dynamic leg exercise, in addition to the effects mentioned above, the imposition of LBPP reduces muscle blood flow and induces an accumulation of metabolic by-products; these are thought to activate...
Table 1. Plasma lactate, plasma osmolality, hematocrit, hemoglobin, and total protein at rest; at 5, 14, and 29 min of exercise and at 3 min into recovery

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>5 min</th>
<th>14 min</th>
<th>20 min</th>
<th>29 min</th>
<th>Recovery</th>
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<td><strong>La, mM</strong></td>
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<tr>
<td>Off-on</td>
<td>0.7 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>2.5 ± 0.2*</td>
<td>3.8 ± 0.4*</td>
<td>4.3 ± 0.6*</td>
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<tr>
<td>On-off</td>
<td>0.7 ± 0.1</td>
<td>2.1 ± 0.3*</td>
<td>3.9 ± 0.5†</td>
<td>3.8 ± 0.6†</td>
<td>2.6 ± 0.5†</td>
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<td><strong>Posm, mosmol/kgH2O</strong></td>
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<td>Off-on</td>
<td>286.0 ± 0.7</td>
<td>287.9 ± 1.0*</td>
<td>290.3 ± 1.0*</td>
<td>292.9 ± 1.1*</td>
<td>295.3 ± 1.0*</td>
<td>291.1 ± 1.1*</td>
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<tr>
<td>On-off</td>
<td>285.8 ± 0.3</td>
<td>289.1 ± 0.6*</td>
<td>292.5 ± 0.9†</td>
<td>290.9 ± 0.7†</td>
<td>290.8 ± 1.0†</td>
<td>287.7 ± 1.1†</td>
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<td><strong>Hct, %</strong></td>
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<tr>
<td>Off-on</td>
<td>42.0 ± 1.0</td>
<td>43.0 ± 1.0*</td>
<td>43.8 ± 1.0*</td>
<td>44.5 ± 1.0*</td>
<td>45.4 ± 1.1*</td>
<td>44.8 ± 1.1*</td>
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<tr>
<td>On-off</td>
<td>41.2 ± 1.3</td>
<td>43.5 ± 1.1*</td>
<td>44.8 ± 1.0†</td>
<td>44.1 ± 1.0*</td>
<td>43.7 ± 1.0†</td>
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<td><strong>Hb, g/dl</strong></td>
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<td>Off-on</td>
<td>14.4 ± 0.4</td>
<td>14.8 ± 0.8*</td>
<td>15.3 ± 0.4*</td>
<td>15.7 ± 0.4*</td>
<td>16.0 ± 0.4*</td>
<td>15.6 ± 0.4*</td>
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<tr>
<td>On-off</td>
<td>14.3 ± 0.4</td>
<td>15.0 ± 0.4*</td>
<td>15.7 ± 0.3†</td>
<td>15.5 ± 0.3*</td>
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<td><strong>TP, g/dl</strong></td>
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<td>Off-on</td>
<td>6.6 ± 0.1</td>
<td>6.9 ± 0.1*</td>
<td>7.2 ± 0.2*</td>
<td>7.4 ± 0.2*</td>
<td>7.7 ± 0.2*</td>
<td>7.5 ± 0.2*</td>
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<tr>
<td>On-off</td>
<td>6.6 ± 0.2</td>
<td>7.1 ± 0.2†</td>
<td>7.5 ± 0.2†</td>
<td>7.3 ± 0.2*</td>
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Values are means ± SE. La, lactate; Posm, plasma osmolality; Hct, hematocrit; TP, total protein. In “off-on” protocol, lower body negative pressure (LBPP) was applied 15 min into the period of exercise. In “on-off” protocol, LBPP was applied 3 min before the start of the exercise and then removed at 15 min into period of exercise. *Significant difference from corresponding value at rest, P < 0.05. †Significant difference with LBPP vs. without LBPP, P < 0.05.

Muscle metaboreceptors and thus induce a reflex rise in MAP (1, 6, 19, 27, 30). In the present study, the application of LBPP during dynamic exercise induced changes in plasma La and respiratory parameters (Ve and RER), which support the idea that it has a positive influence on the accumulation of metabolic by-products. In addition, MAP increased by 16–26 mmHg after the application of LBPP during exercise, an increase some 10–20 mmHg higher than that observed at rest. This additional increase in MAP during dynamic leg exercise would reflect a moderate activation of the muscle metaboreflex. Furthermore, the increases in MAP, plasma La concentration, Ve, and RER seen during dynamic exercise with LBPP were all reversed when the LBPP was released. Thus our experimental protocol allows fairly rapid modulation of the muscle metaboreflex during dynamic exercise in humans. The principal new finding of this study is that moderate activation of the muscle metaboreflex during dynamic exercise at ~50% of VO2peak in humans augments the secretion of AVP, NE, and Epi but not of PRA. These findings indicate that, although hormonal responses are induced by activation of the muscle metaboreflex during dynamic exercise in humans, the thresholds for these responses may not be uniform among the various glands and hormones.

Yamashita et al. (34) showed that activation of group III and IV afferents from skeletal muscle elicits an increase in the activity of neurons in the suprapontic nucleus in cats, suggesting that the muscle metaboreflex may stimulate the posterior pituitary gland to secrete AVP. In our experiment, dynamic exercise with LBPP was associated with an increase in AVP, and the release of LBPP during exercise caused AVP to return to the control levels. In dogs, O’Leary et al. (22) reported that the muscle metaboreflex provoked during dynamic exercise by decreasing blood flow to the active muscles did not increase AVP. It is likely that the large pressor response seen in the dog may also have influenced AVP secretion. In support of this hypothesis, O’Leary et al. showed that when the pressor response was attenuated by ganglion blockade during the activation of the muscle metaboreflex, AVP secretion was indeed augmented. In our experiment, the increase in MAP due to activation of the metaboreflex ranged from 10 to 20 mmHg; this is similar to (or lower than) the magnitude of blood pressure response at which O’Leary et al. saw AVP secretion. Thus, taken together, our results and those of O’Leary et al. (22) are consistent with the idea that an activation of the muscle metaboreflex that results in a moderate increase in MAP will augment AVP secretion.

Nishiyasu et al. (18) found that muscle metaboreflex activation in humans, produced by combined repeated isometric handgrip exercise and forearm vascular occlusion, increased AVP, PRA, ACTH, NE, and Epi. They suggested that a sustained activation of the muscle metaboreflex produced a general augmentation of the neurally mediated secretion of pressor hormones. Thus the consistent finding seems to be that activation of the muscle metaboreflex in humans causes secretion of AVP, NE, and Epi, both in a near-resting state (post-handgrip ischemia) and during dynamic exercise.

Several factors stimulate the secretion of AVP, with central osmoreceptor stimulation providing the primary signal derived from a monitoring of Posm (5, 24). Takamata et al. (31) reported that there is a linear relationship between Posm and AVP at rest. A linear relationship with a threshold (295 mosmol/kgH2O) is also seen during dynamic exercise (20). During exercise in the present study, application of LBPP increased Posm by 2 mosmol/kgH2O, which would be expected to increase AVP by less than 1 pg/ml (by using the published data). However, the actual increase in AVP after LBPP application was >4 pg/ml (see Fig. 3A). An increase in body core temperature is also known to augment osmotically stimulated AVP secretion (8, 31). At a Posm of 292 mosmol/kgH2O, a 1°C increase in body core temperature caused a 2 pg/ml increase in plasma AVP (31). On this basis, the esophageal temperature...
increase of 0.1°C seen here with LBPP should account for only a 0.2 pg/ml increase in AVP. Thus the increase in AVP seen with LBPP during dynamic exercise in the present study cannot be fully explained by the observed increases in Posm or body core temperature. This conclusion is valid if we assume that these two factors do not potentiate each other’s effects during dynamic exercise.

The second factor that might affect AVP secretion is CVP. The application of LBPP during dynamic exercise might increase CVP by shifting blood to the upper part of the body. Z0, which is thought to be an indicator of changes in central blood volume (12), tended to decrease when LBPP was applied during exercise, suggesting an increase in CVP during LBPP. An increase in CVP would evoke a cardiopulmonary baroreflex, and this should, if anything, reduce the secretion of AVP. On this basis, the effects of such a blood shift cannot explain the increase in AVP seen during LBPP. The final factor we need to consider is emotional stress. Our subjects experienced some discomfort during LBPP, as shown by the elevation in RPE. Although it is known that an increase in AVP secretion occurs in association with significant mental stress or pain (10), it is not clear to what extent the small yet significant rise in RPE in this study might have influenced AVP secretion. Maxiner et al. (13) found that sustained long-term vascular occlusion produced pain equivalent to exercise with vascular occlusion, yet it did not increase MAP. Although we suspect that the mental stress experienced by our subjects was small, we cannot exclude the possibility that it may have contributed to the observed increase in AVP secretion.

O’Hagan et al. (21) reported that renal sympathetic nerve activity was augmented as a result of the activation of the muscle metaboreflex by hindlimb ischemia during dynamic exercise in rabbits. An increase in renal sympathetic nervous activity would be expected to promote renin release (5, 11), although PRA was not increased.

Fig. 2. Levels of mean arterial blood pressure (A) and heart rate (B) with or without LBPP at rest, during dynamic supine exercise, and during recovery. LBPP was applied 3 min before onset of exercise, maintained for first 15 min of exercise, and removed during final 15 min of exercise in on-off protocol or applied only throughout the last 15 min of exercise in off-on protocol. Arrow, application (for off-on protocol) or removal (for on-off protocol) of LBPP after 15 min of exercise. †Significant difference with LBPP vs. without LBPP, P < 0.05.

Fig. 3. Levels of arginine vasopressin (AVP; A) and plasma renin activity (PRA; B) with or without LBPP: at rest, after 14 and 29 min of exercise, and 3 min into recovery. LBPP was applied 3 min before onset of exercise, maintained for first 15 min of exercise, and removed during final 15 min of exercise in on-off protocol or applied only throughout last 15 min of exercise in off-on protocol. Arrow, application (for off-on protocol) or removal (for on-off protocol) of LBPP after 15 min of exercise. *Significant difference from corresponding value at rest, P < 0.05. †Significant difference with LBPP vs. without LBPP, P < 0.05.
monitored in the above study (21). However, O’Leary et al. (22) reported that activation of the muscle metaboreflex in running dogs, by decreasing blood flow to the active muscles, did not increase PRA. Nishiyasu et al. (18) found that muscle metaboreflex activation in humans, by forearm occlusion after isometric handgrip exercise, increased MAP by 25 mmHg and also significantly increased PRA. In the present study, PRA levels during exercise were not changed by the application of LBPP. There are at least two possible explanations for this lack of response. First, it may be that the intensity with which the muscle metaboreflex was activated by LBPP during dynamic exercise (at the level performed by our subjects) was not sufficient to cause an increase in PRA. The second possibility is that the increase in MAP during this moderate activation of the muscle metaboreflex (range of 10–20 mmHg in the present study) produced sufficient loading of arterial and/or renal baroreceptors to limit the increases in renal sympathetic nerve activity and PRA (11, 22). Most likely, both the intensity of activation of muscle metaboreflexes and the pressor-induced inhibition of renal sympathetic nerve activity interact to dictate the actual level of PRA.

It is known that the skeletal muscles and kidneys are the main sources of plasma NE at rest (7) and that the active muscles are the main source during dynamic exercise (3). Because the elevated level of MAP, which is mainly due to the increase in total peripheral resistance evoked by the muscle metaboreflex (16), was sustained during the LBPP, we can assume that sympathetic activity was enhanced to resistance vessels in the muscles and other organs. The sustained enhancement of sympathetic activity to those regions may also have caused greater NE release from these tissues and organs during the LBPP and so account for the elevated plasma NE seen during exercise with LBPP. Epi, on the other hand, is known to be mainly secreted by the adrenal glands in association with increased sympathetic efferent activity. The greater increases in both NE and Epi seen when LBPP was applied during exercise than during exercise alone would support the idea that the metaboreflex activated the efferent sympathetic pathways when LBPP was applied during dynamic exercise.

Limitations. In this study, the interpretation of the data is limited by a few methodological considerations. First, it is questionable whether the hormonal responses seen in this study are characteristic only of the actual combination of exercise intensity and LBPP used in our experiments. We used an exercise intensity found during a graded maximal supine exercise protocol to involve a power requirement sufficient to elicit, 80% of the ventilation threshold (80% of V̇O₂peak), and we combined this with 35 mmHg LBPP to induce a sustained activation of the muscle metaboreflex when LBPP was applied during dynamic exercise. Previous studies have shown that the threshold for increases in PRA or AVP during dynamic bicycle exercise is between 30 and 60% of maximum V̇O₂ (9, 32). Our exercise intensity (50% of V̇O₂peak) was near the threshold identified in previous studies, and it was set below the ventilation threshold. In this study, it can be concluded that the physiological responses evoked during the application of LBPP were due to activation of the metaboreflex during dynamic exercise. Furthermore, by using our on-off and off-on protocols we were able to show rapid and reversible effects of the muscle metaboreflex. The exercise intensity was chosen so as to be just below the anaerobic threshold. If the intensity of the exercise had been above the anaerobic threshold, the physiological response during exercise would not have reached a steady state, thus making it difficult to determine whether the responses occurring during the LBPP were due to activation of the metaboreflex or to time-dependent effects induced by the accumulation of La (or to other factors such as fatigue). Conversely, if the intensity of the exercise had been well below the anaerobic threshold, an increase in La and a physiological response related to the muscle metaboreflex probably would not have occurred during LBPP.
The criteria for LBPP intensity were that 1) 5 min of LBPP during dynamic exercise should be sufficient to increase plasma La by greater than threefold to activate the muscle metaboreflex, and 2) subjects should be able to perform 15 min of exercise with LBPP as well as the whole 30-min exercise protocol. We chose the lowest LBPP level that met the above two criteria, because too high an LBPP level would cause a rapid accumulation of metabolic by-products, and lead to exhaustion. These criteria were evaluated in numerous pilot studies on a separate group of individuals, and the exercise parameters used in this study represent the optimal conditions identified during those pilot experiments. In the present experiments, plasma La did not increase significantly during exercise without LBPP, whereas exercise with LBPP produced a fivefold increase in La. These data indicate that the exercise protocol met our criteria in the sense that only exercise with LBPP promoted a substantial accumulation of the metabolic by-product lactate. In the actual experiments, plasma La increased during exercise with LBPP and decreased after the removal of LBPP. All subjects completed the protocol without excessive effort, as indicated by their RPE ratings of 14–16.

An influence of the muscle mechanoreflex on blood pressure regulation in humans at rest was reported by Williamson et al. (33). However, Nishiyasu et al. (17) recently showed that the periodic application of 50 mmHg LBPP (by using rapidly inflatable leg cuffs to stimulate the muscle mechanoreflex without decreasing muscle blood flow) did not elicit any reflex blood pressure response during dynamic leg exercise. In general, the influence of the muscle mechanoreflex on humoral factors during dynamic exercise is not well understood in humans, and we cannot at present evaluate the possibility that a moderate level of LBPP (such as the 35 mmHg used here) might have affected humoral factors via the muscle mechanoreflex.

The overall physiological responses induced by LBPP (i.e., increases in plasma La and MAP) were moderate in this study, because we adopted an exercise intensity and level of LBPP to meet the specific criteria discussed above. It is possible that changes in PRA might have occurred if a greater activation of the muscle metaboreflex had been employed (by applying a greater level of LBPP). However, the application of higher levels of LBPP might have elicited a greater reflex response from the muscle mechanoreceptors (28, 33), thus complicating the interpretation of the results and leading to subjects being unable to maintain the exercise even after the release of the LBPP, as a result of exhaustion. Further studies will be necessary to test the relationship between the intensity of muscle metaborelex activation and evoked hormonal responses.

In the present experiment, AVP increased from 2.5 pg/ml at rest to 6.3 pg/ml during LBPP plus exercise. Although nonhuman species (e.g., rats and dogs) would show large pressor responses to such an increase in AVP, human subjects are known to exhibit a much smaller pressor response than do other species (5). Thus, in the present experiment, the pressor effect secondary to the increase in AVP would be expected to be small.

In conclusion, during dynamic supine leg exercise in humans moderate muscle metaboreflex activation, induced by the application of LBPP, produced significant increases in AVP, NE, and Epi but not in PRA. With the proviso that our exact results may be true only of the levels of exercise and metaboreflex activation used in this study, the present findings suggest that, although hormonal responses are induced by moderate activation of the muscle metaboreflex during dynamic exercise in humans, the thresholds for these responses may not be uniform among the various glands and hormones.

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