Does gender influence the strength and mechanisms of the muscle metaboreflex during dynamic exercise in dogs?

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Laprad, Susanne L., Robert A. Augustyniak, Robert L. Hammond, and Donal S. O’Leary. Does gender influence the strength and mechanisms of the muscle metaboreflex during dynamic exercise in dogs? Am. J. Physiol. 276 (Regulatory Integrative Comp. Physiol. 45): R1203–R1208, 1999.—I. Ischemia of active skeletal muscle stimulates neuronal afferents within the muscle, which elicits a reflex increase in sympathetic nerve activity, systemic arterial pressure (SAP), and heart rate (HR), termed the muscle metaboreflex. We retrospectively investigated whether gender influences the activation of the muscle metaboreflex and the primary mechanisms used by this reflex, augmentation of cardiac output (CO) and peripheral vasconstriction, using 15 female and 13 male chronically instrumented dogs exercising on a treadmill (3.2 km/h, 0% grade). Metaboreflex activation was achieved via progressive partial occlusion of the terminal aorta during exercise. In both females and males, hindlimb ischemia elicited similar substantial increases in SAP (56.1 ± 3.0 and 55.1 ± 4.2 mmHg, respectively), HR (25.8 ± 4.8 and 33.9 ± 2.8 beats/min, respectively), and CO (1.39 ± 0.3 and 1.64 ± 0.2 liters, respectively) and a similar substantial decrease in renal vascular conductance (RVC; 42.7 ± 4.9 and 42.9 ± 5.3%, respectively). Both groups also demonstrated similar metaboreflex thresholds and sensitivities of SAP, HR, CO, and RVC. We conclude that the strength and mechanisms mediating the metaboreflex responses during dynamic exercise in dogs are not affected by gender.

skeletal muscle ischemia; muscle afferents; skeletal muscle blood flow; peripheral vasoconstriction

A number of investigators have studied the effects of gender on the cardiovascular adaptations to exercise and the potential mediators of these reflex responses. Previous studies have shown that gender affects the heart rate (HR) response to dynamic exercise, baroreflex regulation of HR, and cardiac autonomic function in humans (1, 5, 21). It has been discovered in studies with rats that gender influences baroreflex control of HR, cardiac autonomic responses, and control of vaso-motor tone (2, 3, 22). In addition to the above mechanisms of cardiovascular control, several studies have shown that the muscle metaboreflex may contribute significantly to the cardiovascular adjustments to exercise (9, 15, 19, 23–25, 28, 30). When oxygen delivery to active skeletal muscle becomes insufficient for the prevailing metabolic demands, metabolites (including diprotonated phosphate, lactic acid, and H+ ) accumulate and stimulate group III and IV afferent neurons within the active muscle. Activation of these afferents evokes reflex increases in efferent sympathetic nerve activity, systemic arterial pressure (SAP), HR, and cardiac output (CO) (6, 8, 13, 16–19, 26–28, 30), termed the muscle metaboreflex. The two mechanisms responsible for generating the reflex pressor response are an increase in CO and peripheral vasoconstriction (10, 11, 15, 30). During mild dynamic exercise, dogs depend primarily on the increase in CO to raise SAP (15, 30). The role gender plays in regulating the muscle metaboreflex remains unclear.

Ettinger et al. (4) observed a gender effect on the cardiovascular responses to static handgrip (SHG) in humans. When SHG was performed at 30% maximal voluntary contraction (MVC) for 2 min followed by a 2-min posthandgrip circulatory arrest (PHGCA), females exhibited a smaller reflex increase in blood pressure and muscle sympathetic nerve activity (MSNA) compared with males. Furthermore, during 60% MVC static adductor pollicis exercise, MSNA responses were less for females. The attenuated MSNA responses were not demonstrated during ischemic rhythmic handgrip exercise, yet the males did show a significantly larger response in mean arterial pressure (MAP) during the last minute of handgrip and PHGCA. In the same study, Ettinger et al. (4) observed a lower production of diprotonated phosphate and development of acidosis within the active muscle in females during SHG at 30% MVC. They proposed the gender effect to be dependent on blood flow and/or oxygen delivery because it was not fully expressed during ischemic conditions. Whereas the mechanisms mediating the hemodynamic responses to static contraction are complex, involving the action and possible interaction between central command, baroreflexes, and activation of both mechanosensitive and metabosensitive skeletal muscle afferents, PHGCA has been used in many studies to isolate the effects of the muscle metaboreflex. The findings of Ettinger et al. (4) indicate that gender affects the strength of the muscle metaboreflex. However, during

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PHGCA, metaboreflex responses are observed during the recovery from exercise rather than during exercise per se. This may be an important issue inasmuch as the mechanisms mediating the metaboreflex pressor responses may be different in these two settings. For example, during PHGCA, HR declines toward baseline with a time course similar to that observed during the recovery from static exercise without circulatory occlusion (4, 9, 29). In contrast, when the metaboreflex is activated during dynamic exercise, substantial increases in HR and CO occur (13–15, 23, 30). The effects of gender on the strength and mechanisms of the muscle metaboreflex when activated during dynamic exercise are unknown.

For approximately the past 10 years, our laboratory has investigated various aspects of the muscle metaboreflex during dynamic exercise using conscious, chronically instrumented dogs of either gender (13, 15–17, 23). The objective of this study is to provide a retrospective analysis of these data to determine whether gender affects the strength and efferent mechanisms of this reflex. The majority of these data have been published previously (13, 15–17, 23).

METHODS

We retrospectively analyzed experiments that were performed using 28 conscious chronically instrumented mongrel dogs (15 females, 13 males) selected for their willingness to run on a motor-driven treadmill. None of the animals was neutered or spayed. With respect to the female dogs, this study included only those experiments performed during either the anestrous or diestrous (metestrous) cycle of the dog. All procedures were reviewed and approved by the Institutional Animal Care Committee and conformed to Public Health Service guidelines.

Surgical Preparation

All dogs were prepared in a series of surgical sessions with at least a week of recovery between surgeries and between the last surgery and the first experiment. For all procedures, anesthesia was induced with thiamylal (Surital) or pentothal sodium and maintained with isoflurane. The animals were treated with cefazolin (500 mg iv) immediately pre- and postoperatively and then with cephalixin (30 mg/kg po, tid) for at least 1 wk postoperatively. During recovery from surgery, buprenorphine (0.015 mg/kg iv) and acepromazine (0.1 mg/kg im) were administered for analgesia and sedation, respectively, whenever it was deemed necessary.

At the fourth intercostal space, a right thoracotomy was performed on 11 females and 7 males, in which either an electromagnetic (Zepeda Instruments) or an ultrasonic (Transonic Instruments) blood flow transducer was implanted on the ascending aorta to monitor CO. For subsequent ventricular pacing not related to this study, stainless steel electrodes were sutured to the apex of the left ventricle. The pericardium was reapproximated, and the chest was closed in layers. Through a midventral abdominal (4 males) or a retroperitoneal (9 males, 15 females) approach, an electromagnetic (Zepeda Instruments) or an ultrasonic (Transonic Instruments) blood flow transducer was placed on the terminal aorta. Distal to the flow probe, a vascular occluder (In Vivo Metrics) was also placed on the aorta. All side branches between the iliac arteries and the flow probe were ligated and severed. The blood flow transducer was used to monitor terminal aortic blood flow (TAQ). A catheter was placed in a side branch of the aorta above the flow probe to monitor SAP. Furthermore, in 11 females and 7 males, a flow probe (either pulsed Doppler or Transonic) was placed on a renal artery to monitor renal blood flow (RBF).

In another procedure, arterial and venous catheters were inserted into small side branches of the femoral artery and vein to monitor femoral arterial pressure (FAP) and for infusion of drugs for studies unrelated to the present investigation, respectively. In 11 females and 7 males (those animals that underwent thoracotomy), an additional catheter was inserted into the right jugular vein and advanced to the atrial-caval junction to monitor central venous pressure (CVP).

For studies unrelated to the present investigation, two additional surgeries were performed. In five males and two females, vascular occluders were implanted on both carotid arteries. In one male and five females, a flow probe and vascular occluder were implanted on the right axillary artery. For all surgeries, flow probe wires, catheters, occluder tubings, and ventricular pacing electrodes were tunneled subcutaneously and exteriorized between the scapulae.

Experimental Protocols

All experiments were performed after the animals had fully recovered from surgery and were active, afebrile, and of good appetite. The animal was brought to the laboratory, allowed time to familiarize itself with the surroundings until comfortable, and then directed to the treadmill. The blood flow transducers were connected to a flowmeter (either model SWF-SRD, Zepeda Instruments or Transonic Instruments). The FAP, SAP, and CVP catheters were connected to pressure transducers (Spectromed P-10 EZ). HR was monitored via a cardiotachometer triggered by the CO signal or the SAP if the animal was not instrumented with a CO flow probe. Renal vascular conductance (RVC) was calculated as RBF/(SAP – CVP). All data were sampled by a laboratory computer at 1,000 Hz, and mean values for each cardiac cycle were saved on hard disk for subsequent analysis.

Female estrous cycle consideration. When a female dog demonstrated vulvar bleeding (which normally occurs only 2 times/year), it was noted on the animal’s chart. The proestrus period of the estrous cycle is considered to begin when vulvar bleeding is first observed and lasts, on average, 9 days (12). The estrous period, which includes ovulation, follows the proestrus period and continues, on average, 9 days (12). Diestrous (metestrous) and anestrous complete the estrous cycle. From this information, we determined approximately when the dog was in the proestrus and estrous periods (18 days from bleeding onset) and disregarded those experiments performed during that time.

Metaboreflex activation during exercise. The muscle metaboreflex was activated during mild treadmill exercise (3.2 km/h, 0% grade) as described previously (13, 16, 17). Briefly, the treadmill was started, and after 3–5 min steady-state levels of SAP, FAP, CVP, HR, CO, TAQ, and RBF were achieved. Through graded, partial inflation of the vascular occluder implanted on the abdominal aorta, hindlimb perfusion was progressively decreased. Each level of reduction in hindlimb perfusion was maintained until all variables reached steady state (3–5 min).

Statistical Analysis

One-minute averages of all variables were taken during steady-state exercise and at each level of partial vascular occlusion. The data were analyzed as described by Wyss et al.
At the mild workload of 3.2 km/h, 0% grade, the metaboreflex is not activated until hindlimb perfusion is reduced below a threshold level. When the threshold is attained, substantial increases in SAP, HR, and CO and a decrease in RVC occur with further hindlimb ischemia. Therefore, the relationship between hindlimb perfusion and the efferent responses is "dog leg" in shape for SAP, HR, and CO, and an inverse dog leg is seen with RVC. Hence, the data were approximated to two linear regressions, an initial response line and a pressor response line. The initial response line contains data points wherein no significant hemodynamic changes occurred with initial reductions in TAQ, and the pressor response line denotes when further reductions in TAQ elicit substantial increases in SAP, HR, and CO or decreases in RVC. Therefore, the data were approximated to two linear regressions, an initial response line and a pressor response line. The initial response line contains data points wherein no significant hemodynamic changes occurred with initial reductions in TAQ, and the pressor response line denotes when further reductions in TAQ elicit substantial increases in SAP, HR, and CO or decreases in RVC. The intersection of these two lines is designated as the threshold for the reflex. The sensitivities of the muscle metaboreflex were calculated as the slope of the pressor response line. Because RBF was measured using either pulsed Doppler (kHz Doppler shift) or Transonic (ml/min) blood flow transducers, the data were combined by expressing RBF and RVC as a percentage of the resting value for each dog, and the results were averaged to obtain the mean values for that animal. These mean values were averaged across animals of the same gender to obtain a mean value for the females and males separately. For each gender, paired t-tests were performed for all variables between the free-flow exercise and the values attained at the maximum imposed level of hindlimb ischemia. For comparison between females and males, the data were analyzed using unpaired t-tests. For all studies, $P < 0.05$ was considered statistically significant. All values are presented as means ± SE.

RESULTS

Table 1 shows the average weight and hemodynamic values at rest and during free-flow (no occlusion) mild exercise for females and males. At rest, there is no significant difference between genders in SAP, HR, TAQ, or CO. Furthermore, during free-flow exercise, there is not a significant gender effect in SAP, HR, TAQ, CO, and RVC.

Figure 1 shows the mean steady-state values for females and males of SAP, HR, CO, and RVC as a function of FAP during free-flow exercise, at reflex threshold, and the maximal level attained with the largest reduction in hindlimb perfusion. Once beyond threshold, further reductions in hindlimb perfusion caused large, highly significant increases in SAP, HR, and CO and decreases in RVC in both females and males. When we compared females and males, the threshold level and maximal levels of SAP (n = 14 females, 13 males), HR (n = 14 females, 11 males), CO (n = 8 females, 7 males), and RVC (n = 8 females, 7 males) were not significantly different.

Figure 2 shows the mean steady-state values for females and males in SAP, HR, CO, and RVC as a function of TAQ at the same three levels of exercise stated above. Again, females and males have similar responses at the threshold level and maximal levels of
DISCUSSION

The major new finding in this study is that gender does not influence the activation of the muscle metaboreflex in conscious dogs during dynamic exercise. Furthermore, gender does not affect the primary mechanisms attributed to eliciting the reflex responses of the metaboreflex during mild exercise. The efferent responses in SAP, HR, CO, and RVC were very similar and not statistically different between males and females. Moreover, the thresholds and gains of the metaboreflex were not significantly different between the genders.

Our results are consistent with the findings by Ettinger et al. (4), which concluded there was no significant difference between human females and males in the change in MSNA (ΔMSNA) and HR responses during 20% MVC ischemic rhythmic handgrip. Compared with typical static exercise with maintained muscular contraction, rhythmic isometric exercise is more comparable to dynamic exercise, because the short-lasting contractions are intermittent with periods of relaxation and ischemic rhythmic handgrip would be expected to elicit a strong metaboreflex response. However, Ettinger et al. (4) noted a significant dissimilarity in the MAP responses during end handgrip and PHGCA, in which human males demonstrated a greater response, whereas the present study in dogs saw no difference in the hemodynamic responses to mild dynamic exercise and with metaboreflex activation. Ettinger et al. (4) also found significant differences between males and females in ΔMSNA and MAP for 30% MVC SHG but only in ΔMSNA during 60% MVC static adductor pollicis exercise to fatigue. They attributed the gender effect to blood flow and/or oxygen delivery, because the effect was only apparent during nonocclusive static exercise (4). In support of the proposition that the gender effect is due to oxygen delivery, Sheriff et al. (25) discovered that pressor responses are generated when oxygen delivery falls below a critical threshold, not when blood flow is reduced per se. They proposed that a pressor substance is released at a rate inversely proportional to oxygen delivery. Probable substrates linked to the metaboreflex are diprotonated phosphate and lactic acid (18, 26), and Ettinger et al. (4) discovered a reduced production of diprotonated phosphate and lactic acid (18, 26), and Ettinger et al. (4) also discovered a reduced production of diprotonated phosphate and muscle acidosis in the active muscle in females, possibly contributing to the attenuated pressor response to the static contraction and PHGCA.

There are several plausible explanations to why gender did not influence the metaboreflex during dynamic exercise in the present study or during ischemic rhythmic isometric exercise (4) (for the most part), yet gender affected the reflex responses to static muscular

Table 2. Metaboreflex sensitivities

<table>
<thead>
<tr>
<th>Metaboreflex Sensitivities</th>
<th>Female</th>
<th>Male</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAP, mmHg/mmHg</td>
<td>−2.9 ± 0.3</td>
<td>−2.8 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>HR, beats·min⁻¹·mmHg⁻¹</td>
<td>−2.2 ± 0.3</td>
<td>−2.4 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>CO, ml·min⁻¹·mmHg⁻¹</td>
<td>−113.3 ± 17.0</td>
<td>−133.8 ± 17.6</td>
<td>NS</td>
</tr>
<tr>
<td>RVC, %rest·mmHg</td>
<td>1.9 ± 0.4</td>
<td>2.5 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>TAQ</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAP, mmHg·l⁻¹·min⁻¹</td>
<td>−0.13 ± 0.02</td>
<td>−0.15 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>HR, beats·min⁻¹·l⁻¹·min⁻¹</td>
<td>−0.11 ± 0.02</td>
<td>−0.13 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>CO, l·min⁻¹·l⁻¹·min⁻¹</td>
<td>−4.3 ± 0.6</td>
<td>−5.3 ± 0.8</td>
<td>NS</td>
</tr>
<tr>
<td>RVC, %rest·ml⁻¹·min⁻¹</td>
<td>0.08 ± 0.01</td>
<td>0.11 ± 0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. FAP, femoral arterial pressure.
contraction and PHGCA (4). First, the study by Ettinger et al. (4) used humans, whereas our study involved dogs. Major differences in muscle fiber type and cardiac reserve exist between these two species (7, 19) that may affect the magnitude and mechanisms of metaboreflex responses. In addition, the frequency of substantial changes in the plasma levels of sex hormones is quite different between dogs and humans (~2 times/year vs. ~12 times/year).

A second explanation involves the differences in exercise paradigms performed in each study. As described above, the mechanisms mediating the hemodynamic responses to SHG (as well as those during dynamic exercise) are not well understood and involve the action and interaction of multiple reflex systems (20), and thus a gender effect on responses to static exercise does not necessarily imply a gender effect on the muscle metaboreflex. Ettinger et al. (4) did observe a gender effect on the responses to PHGCA (a commonly used method to isolate effects of muscle metaboreflex) both in terms of the hemodynamic responses and muscle concentration of putative metabolites generating the reflex. However, in this setting, the cardiovascular responses are observed during the recovery from exercise rather than during exercise per se, which could affect the mechanisms mediating the pressor responses (14). Indeed, with metaboreflex activation during mild dynamic exercise in dogs, the overwhelming majority of the pressor response is due to the reflex tachycardia coupled with a maintained stroke volume (e.g., increased CO (15, 23, 30)), whereas during PHGCA, HR declines toward resting levels with a time course similar to normal recovery from the contraction (4, 6, 19, 28). In addition, Ettinger et al. (4) did not observe a gender effect in the responses to ischemic rhythmic handgrip, a setting likely to generate a strong metaboreflex response during the exercise. Finally, it should also be noted that in comparison to SHG or PHGCA in humans, moderate hindlimb ischemia in dogs during dynamic exercise elicits quite robust increases in arterial blood pressure (~50 mmHg). The large magnitude of this pressor response may mask observation of a small gender effect as seen in humans (4).

Perspectives

Previous studies from this and other laboratories have used dogs of either gender. However, as described above, studies in other species have uncovered a gender difference in cardiovascular function. This retrospective analysis provides compelling evidence that in dogs, gender does not play a large role in determining the cardiovascular responses to mild exercise or to metaboreflex activation, indicating that dogs of either gender can be used in these experiments without marked biasing of the results. It should be noted, however, that female dogs undergo menstrual cycling and the associated large changes in the plasma concentration of sex hormones with a much lower frequency than observed in other species, which may contribute to the effects of gender (or lack thereof). Whether a similar effect of gender exists in dogs in the responses to heavier workloads and/or responses to activation of other cardiovascular reflexes remains unknown.

In conclusion, gender does not influence the strength of the metaboreflex when it is activated during mild dynamic exercise in dogs. This retrospective investigation revealed similarities between female and male dogs for the responses in SAP, HR, CO, and RVC to mild exercise. This study also showed similar metaboreflex thresholds and sensitivities in SAP, HR, CO, and RVC between males and females.

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