Interaction of gender and exercise training: vasomotor reactivity of porcine skeletal muscle arteries

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Laughlin, M. Harold, William G. Schrage, Richard M. McAllister, H. A. Garverick, and A. W. Jones. Interaction of gender and exercise training: vasomotor reactivity of porcine skeletal muscle arteries. J Appl Physiol 90: 216–227, 2001.—The purpose of the present study was to test the hypothesis that gender influences exercise training-induced adaptations of vascular reactivity of porcine arteries that provide blood flow to skeletal muscle and femoral and brachial arteries. Male and female Yucatan miniature swine were exercise trained on a motor-driven treadmill or cage confined for 16–20 wk. Contractile responses of arterial rings were evaluated in vitro by determining concentration-response curves for endothelin-1 (ET-1; 10⁻¹⁰ to 10⁻⁷ M) and norepinephrine (NE; 10⁻¹⁰ to 10⁻⁴ M). Relaxation responses of arteries precontracted with 30 μM PGF₂α, were examined for endothelium-dependent agents [bradykinin (BK; 10⁻¹¹ to 10⁻⁶ M), ACh (10⁻¹⁰ to 10⁻⁴ M), and a Ca²⁺ ionophore, A-23187 (10⁻⁶ M)] and a endothelium-independent agent [sodium nitroprusside (10⁻¹⁰ to 10⁻⁴ M)]. Arteries from female pigs developed greater contractile force in response to ET-1 than arteries from male pigs, whereas contractile responses to NE and KCl were similar in arteries from both genders. Femoral arteries from females exhibited greater endothelium-mediated vasorelaxation (BK and ACh) than did those from males. In contrast, brachial arteries of males were more responsive to BK and ACh than brachial arteries of females. Exercise training increased ET-1-induced contractions in arteries from males (without endothelium) but not in arteries from females. Training had no effect on endothelium-dependent relaxation in arteries from males but increased relaxation responses in brachial arteries from females. We conclude that both gender and anatomic origin of the artery influence exercise training-induced adaptations of vascular reactivity of porcine skeletal muscle conduit arteries.

endothelium; acetylcholine; skeletal muscle blood flow; vascular smooth muscle; NG-nitro-L-arginine methyl ester; endothelin-derived relaxing factor; indomethacin; nitric oxide; prostacyclin; endothelin; A-23187; nitroprusside

The incidence of atherosclerosis and coronary heart disease (CHD) in men exceeds that of premenopausal women of similar age (32). This gender difference disappears after menopause, when incidence of cardiovascular disease in women increases (20, 34). The slowed development of cardiovascular disease in women appears to be at least partially due to effects of estrogen because the incidence of CHD is less in postmenopausal women who are on estrogen replacement therapy than in age-matched men (3, 32). Although the mechanisms whereby female hormones mediate these beneficial effects have not been established, altered reactivity of arteries may be involved (24). For example, Barber et al. (2) reported that coronary arteries from female pigs generate more forceful contractions in response to endothelin-1 (ET-1) compared with male pigs and that this difference was mediated by increased affinity of the endothelin receptors in coronary vascular smooth muscle of female pigs. Also, Jones et al. (14) reported that chronic exercise training in pigs produced a decrease in ET-1 sensitivity of branches of left circumflex coronary arteries from male pigs but had no effect on ET-1 sensitivity of these arteries from female pigs.

If the effects of gender and interactions of gender with exercise training on coronary vascular reactivity are mediated by circulating hormones [including estrogen, gonadotropins (leutinating hormone and follicle-stimulating hormone), progesterone, testosterone, prolactin and cortisol], similar interactive effects should be apparent in peripheral arteries. We have a long-standing interest in the effects of exercise training on vasomotor responsiveness of arteries that perfuse skeletal muscle. Driven by this interest, the primary purpose of the study reported herein was to test the hypothesis that gender influences exercise training-induced adaptations of vascular reactivity of arteries that provide blood flow to skeletal muscle of pigs. Specifically, we hypothesized that skeletal muscle arteries show gender differences in ET-1 sensitivity that interact with exercise training in a manner similar to observations in coronary arteries (2, 14). We reasoned that, if gender differences in coronary vasomotor reactivity are mediated by circulating gender hormones, then effects of these hormones will also be apparent in arteries perfusing skeletal muscle.

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Although the effects of female gender hormones on vascular function appear to be partially mediated by actions of these hormones on vascular smooth muscle cells, as would be the case for ET-1, there is evidence that these hormones also have important effects on endothelial and adventitial cells in the arteries as well (24). Accordingly, it was proposed that the protective effects of female gender on CHD result from the influence of estrogens on endothelial function (13, 32, 39). There is evidence that female hormones increase endothelial cell nitric oxide synthase (eNOS) gene expression (21, 44), basal release of nitric oxide (NO) by vascular cells (12, 13), and endothelium-mediated vasodilation (5, 13). Exercise training and gender may also have interactive effects on endothelium because exercise training has been reported to increase expression of eNOS protein and to improve endothelium-mediated function in the coronary circulation (17, 26). Therefore, the second purpose of this study was to test the hypothesis that exercise training interacts with gender-specific effects on endothelium-mediated vasodilation of skeletal muscle arteries. We reasoned that exercise training may induce greater enhancement of endothelium-mediated dilation in arteries of male pigs than in those of females because endothelium-mediated dilation is less in arteries of normal males.

We first determined whether there are gender differences in contraction and relaxation responses of femoral and brachial arteries isolated from sedentary pigs. We then tested the hypothesis that gender-specific effects on vascular smooth muscle and endothelium-mediated vasodilation interact with exercise training-induced adaptations of reactivity of these arteries.

METHODS

Experimental Animals

Experiments were completed on adult male (n = 38) and female (n = 38) miniature swine (Charles River) weighing 25–40 kg that were obtained from the breeder. Males were not castrated and were genderually mature boars. Pigs were procured in lots of 8 or 16 animals (3 lots of female pigs and 4 lots of male pigs). Pigs were familiarized with treadmill exercise over a 1–2 wk period of time. Treadmill performance tests were administered to each animal to evaluate exercise tolerance. Each lot of pigs was then randomly divided into two treatment groups: 8 Sed and 8 Ex pigs. One group [exercise-trained (Ex)] underwent a progressive treadmill training program, lasting 13–21 wk, similar to that described for dogs (40). In our hands, this training program produces adaptations in miniature swine that are generally associated with an endurance exercise-trained state (22, 26, 31). The remaining pigs [sedentary control (Sed)] were restricted to their pens (6 × 12 ft) for 13–21 wk.

Training Procedures

Training program. During the first week, Ex pigs ran on a treadmill at 3 miles/h (mph) and 0% grade for 20 to 30 min (endurance) and at 5 mph for 15 min (sprint). The speed and duration of running were progressively increased at a rate dependent on the tolerance of each pig. During the 12th wk of training, a typical training session consisted of the following 85-min workout: 1) 5-min warm-up run at 2.5 mph, 2) 15-min sprint at speeds of 5–8 mph, 3) 60-min endurance run at 4–5 mph, and 4) 5-min warm-down run at 2 mph. Ranges of running speed are presented because the exercise training program was customized to each pig’s exercise ability. The Ex pigs were given positive reinforcement for exercise by feeding them after each training bout. Treadmill performance tests were administered to the Sed and Ex pigs before initiation of exercise training and at the completion of the pen confinement or exercise training periods.

Treadmill performance test. The performance test consisted of 4 stages of exercise (17). During stage 1, pigs ran at 3.1 mph and 0% grade for 5 min. Pigs ran for 10 min at stage 2 (speed = 3.1 mph and grade = 10%) and then for 10 min at stage 3 (speed = 4.3 mph and grade = 10%). Finally, pigs ran at stage 4 (speed = 6 mph, grade = 10%) until exhaustion.

Efficacy of training. The effectiveness of the training program was determined by comparing the exercise tolerance (as reflected in the treadmill performance test), heart weight-to-body weight ratio, and skeletal muscle oxidative capacity of Ex and Sed groups. At the time of death, samples were taken from the middle of the lateral and long head of triceps brachii and the deltoid muscles and were stored at −70°C until processed. Citrate synthase activity was measured from whole muscle homogenate by using the spectrophotometric method of Sjøren (37).

Estrous Cycle

Although it seemed unlikely that these intensities of exercise would alter female hormone levels, we were concerned that the stress of treadmill training might influence female hormones and cycling frequency of the female pigs. To test for this, we made two sets of measurements. First, in one group of Sed and Ex female pigs, we measured estrous cycling duration and frequency for 3 mo before the pigs were started on the protocol and throughout the training (or cage confinement) period. Second, we measured 17β-estradiol and progesterone in blood samples from Sed and Ex, male and female pigs.

Estrous cycles were measured by heat checking 16 female pigs (gilts) on a daily basis with standard procedures (4) using a male pig to determine when gilts were receptive. Gilts were exposed individually to a Yucatan boar daily starting 90 days before the initiation of treadmill training (or cage confinement) and for 6 mo throughout training or cage confinement to establish occurrence and duration of estrous cycles. Gilts were considered in heat when they responded to the presence of the boar (4). After the 90 days of establishing estrous cycle duration in each of the 16 gilts, gilts were divided into two treatment groups: 8 Sed and 8 Ex, as described in Experimental Animals.

Blood sampling regimen and hormone assays. At the conclusion of training or cage confinement, jugular vein blood samples were obtained via venipuncture and collected in vacutainers containing EDTA. Samples were collected after a 12-h fast. Plasma was separated by centrifugation (model TJ-6R centrifuge, Beckman, Palo Alto, CA) at 4°C for 15 min at 3,750 rpm. Plasma was stored at −70°C until concentrations of estradiol-17β and progesterone were determined by validated radioimmunoassays (6, 15). Concentrations of both hormones were measured in one assay. The intra-assay coefficients of variation were 13.4 and 4.8% for 17β-estradiol and progesterone, respectively. Sensitivity of the 17β-estradiol assay was 0.5 pg/ml, and recovery from a spectrum of differing amounts of 17β-estradiol was 88%. Parallelism between the standard curve and different volumes of porcine
plasma was not different (slopes = $-1.99 \pm 0.07$ for standard curve and $-2.22 \pm 0.34$ for porcine plasma). Sensitivity of the progesterone assay was 0.5 ng/mL. Parallelism between the standard curve and different volumes of porcine plasma was not different (slopes = $-1.77 \pm 0.03$ for standard curve and $-1.82 \pm 0.04$ for porcine plasma).

Preparation of Femoral and Brachial Arteries

After completion of exercise training or sedentary confinement and $\sim 24$ h after the last exercise bout, pigs were sedated with ketamine (35 mg/kg; Fort Dodge) and Rompun (2.25 mg/kg; Bayer), anesthetized with thiopental (10 mg/kg; Abbott Laboratories), and euthanized by removal of the heart. Segments of femoral and brachial arteries of $\sim 2$- to 3-mm outer diameter (OD) were carefully removed and trimmed of fat and connective tissue. Artery samples were taken from the same locations of Sed and Ex pigs. Each artery was cut into rings of 2- to 3-mm axial length; OD, inside diameter (ID), and length of each artery ring were measured with a Filar calibrated micrometer eye piece. For studies of rings devoid of endothelium, the endothelium was removed by gentle rubbing of the luminal surface with forceps. Adequate denudation was tested by examining responses to bradykinin. A vessel was considered denuded if bradykinin ($10^{-6}$ M) produced $<5\%$ relaxation of a 30 $\mu$M PGF$_{2\alpha}$

Length-tension relationship. Arterial rings were mounted on two stainless steel wires passed through the vessel lumen. One wire was attached to a force transducer (model FTOS, Grass) and the other to a micrometer microdrive (Stoelting) to allow the vessel to be stretched by known increments. Each vessel apparatus was placed in an individual 20-ml tissue bath containing Krebs bicarbonate buffer equilibrated at $37^\circ$C with 95% O$_2$-5% CO$_2$. Isometric contractions and relaxations were continuously monitored using a computerized data acquisition system (MacLab/Macintosh Computer). Rings were individually stretched to the maximum of the length-developed tension relationship ($L_{\text{max}}$) by repeated test exposures to KCl (30 mM) at increasing vessel diameters. All subsequent pharmacological responsiveness studies were performed with arteries at $L_{\text{max}}$. The arteries were allowed 30 min of stabilization at $L_{\text{max}}$ before further study.

Experimental Design

To allow completion of needed experiments with minimal numbers of animals, we conducted experiments on matched groups of Ex and Sed pigs. Gender effects were determined by comparing results from Sed males with those from Sed females. Effects of exercise training were determined by comparing Ex and Sed groups within each gender group. Experiments were conducted on eight arterial rings (4 femoral and 4 brachial) from each pig. Contractile responses and relaxation responses were examined in arteries from different pigs. The protocols for these experiments are described in Contraction experiments and Relaxation experiments.

Contraction experiments. We selected three vasoconstrictor agents for these experiments: endothelin-1 (ET-1; $10^{-10}$ to $10^{-7}$ M), norepinephrine (NE; $10^{-10}$ to $10^{-4}$ M), and KCl. ET-1 was selected because previous reports indicate a gender difference for ET-1-induced responses of porcine coronary arteries (2, 25) and Jones et al. (14) reported that training decreased sensitivity to ET-1 of coronary arteries from males but not those from females. Rings were tested with 80 mM KCl to establish a reference contracture. Then concentration-response curves were developed for NE or ET-1. Only one concentration-response curve was done on each ring (either NE or ET-1). Concentration-response relationships were evaluated by adding cumulatively increasing concentrations of the appropriate drug to the organ bath and measuring changes in force. The vessel chambers were siliconized to hold solutions that contained the cumulative additions of ET-1 (14).

Relaxation experiments. We selected 30 $\mu$M of PGF$_{2\alpha}$ to contract arterial rings for examination of vasorelaxation responses. Endothelium-mediated vasodilator mechanisms were evaluated by examining vasodilator effectiveness of three different endothelium-dependent vasodilator agents: bradykinin (BK; $10^{-11}$ to $10^{-6}$ M) and ACh ($10^{-10}$ to $10^{-4}$ M) to evaluate endothelium-mediated vasodilation via receptor-mediated pathways and a receptor-independent endothelium-mediated vasodilator (A-23187). Concentration-response relationships were measured for BK and ACh by adding cumulatively increasing concentrations of the appropriate drug to the organ bath and measuring changes in force while responses to only one dose of A-23187 ($10^{-6}$ M) was measured.

Endothelium-dependent relaxation was evaluated with the notion that there are at least three receptor signal transduction pathways in endothelial cells of porcine arteries that signal the release of endothelium-derivied vasodilators: NO, PGL$_2$, and/or endothelium-derived hyperpolarizing factor (28). We previously reported that blockade of nitric oxide synthase (NOS) activity with N$^\omega$-nitro-L-arginine methyl ester (L-NAME) and cyclooxygenase (COX) with indomethacin only blocked a small portion of BK-induced relaxation in femoral and brachial arteries from females, suggesting that other endothelium-derived mediators are involved (23). In the present study, the relative role of these different endothelium-derived mediators was evaluated in arteries from male pigs. L-NAME and indomethacin were used to evaluate the relative importance of the pathways in the observed responses. In each case, responses in the presence of blockers or endothelium removal were paired with measurement of responses of intact, untreated rings.

The contribution of NO to endothelium-mediated responses was evaluated by blocking NOS with 0.3 mM L-NAME. The contribution of PGL$_2$ was evaluated by blocking COX activity with 5 $\mu$M indomethacin (27, 28). Vasodilator responses were examined using paired rings in which one ring was treated with indomethacin and/or L-NAME and responses were compared between treated and untreated rings. Finally, we examined responses to a direct vascular smooth vasodilator agent sodium nitroprusside (SNP). SNP was selected to examine cGMP-mediated vasodilator responses.

Solutions and drugs. Krebs solution contained 131.5 mM NaCl, 5 mM KCl, 1.2 mM NaH$_2$PO$_4$, 1.2 mM MgCl$_2$, 2.5 mM CaCl$_2$, 11.2 mM glucose, and 23.5 mM NaHCO$_3$. All solutions contained propranolol (3 $\mu$M) and 0.025 mM EDTA. Solutions were aerated with 95% O$_2$-5% CO$_2$ (pH 7.4) and maintained at $37^\circ$C. ET-1 was purchased from Calbiochem. All other drugs and chemicals were purchased from Sigma Chemical (St. Louis, MO).

Data Analyses

Concentration-response curves were evaluated using a two-way analysis of variance for repeated measures (SuperANOVA). When this analysis revealed differences in the curves, the multiple-comparison contrast test and Duncan's multiple-range test were used to identify for which doses responses were different between groups. Data were analyzed with each animal counted as one observation for comparisons between groups with respect to each vasoactive
Statistical analysis of EC50 and IC50 were calculated as concentrations that produced responses just below and above each arterial ring with linear interpolation between the log PGF2 concentration that produced 50% inhibition of the response to ET-1 than brachial arteries from males (Fig. 1, bottom) or brachial arterial segments between female and male animals. There were also no differences between contractions of arteries from males and females to NE when results were expressed as percentage of maximal force. For example, EC50 values were not different between groups [femoral EC50 (log M): female = –6.8 ± 0.1, male = –6.7 ± 0.3; brachial EC50 (log M): female = –6.2 ± 0.1, male = –6.6 ± 0.4]. Also, femoral and brachial arteries from male and female pigs exhibited similar contractions in response to 80

### RESULTS

#### Gender Effects

**Structural characteristics of arteries.** Femoral arteries were generally of larger ID and OD than brachial arteries in both male and female pigs (Table 1). Under zero tension, arteries isolated from female and male pigs had similar dimensions for OD, ID, and wall thickness (Table 1). Per experimental design, arterial segments also had similar axial lengths. Arteries from male and female pigs also had similar relative amounts of stretch necessary to attain $L_{max}$ and resting tension at $L_{max}$ was not different in respective arteries from male and female pigs (Table 1).

**Contractile responses: gender effects.** Femoral arteries from female pigs exhibited greater ET-1-induced maximal tension than femoral arteries from males but had similar sensitivity to ET-1 as femoral arteries from males (Fig. 1, top). Brachial arteries from female pigs developed maximal tension at a lower dose of ET-1 than did arteries from male pigs and exhibited greater sensitivity to ET-1 than brachial arteries from males (data not shown). ANOVA for repeated measures revealed significant gender effects for ET-1 responses in both femoral (Fig. 1, top) and brachial arteries. The EC50 for ET-1 of brachial arteries from females (2.7 ± 0.8 × 10^{-8} M) was significantly less than for brachial arteries from males (8.0 ± 2.4 × 10^{-9} M). Endothelium removal did not significantly alter responses to ET-1.

In contrast to the enhanced ET-1-induced contractile response of arteries from female pigs, NE concentration response curves were not different in femoral (Fig. 1, bottom) or brachial arterial segments between female and male animals. There were also no differences between contractions of arteries from males and females to NE when results were expressed as percentage of maximal force. For example, EC50 values were not different between groups [femoral EC50 (log M): female = –6.8 ± 0.1, male = –6.7 ± 0.3; brachial EC50 (log M): female = –6.2 ± 0.1, male = –6.6 ± 0.4]. Also, femoral and brachial arteries from male and female pigs exhibited similar contractions in response to 80

### Table 1. Characteristics of femoral and brachial arteries

<table>
<thead>
<tr>
<th></th>
<th>OD, mm</th>
<th>ID, mm</th>
<th>Resting tension, g</th>
<th>%Stretch</th>
<th>KCl force, g</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Femoral arteries</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Sed</td>
<td>2.6 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>6.7 ± 1.3</td>
<td>204 ± 9</td>
<td>12 ± 1</td>
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<tr>
<td>Ex</td>
<td>2.8 ± 0.2</td>
<td>1.6 ± 0.1</td>
<td>5.2 ± 1.0</td>
<td>198 ± 8</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sed</td>
<td>2.8 ± 0.1</td>
<td>1.5 ± 0.1</td>
<td>6.4 ± 1.2</td>
<td>187 ± 4</td>
<td>15 ± 1</td>
</tr>
<tr>
<td>Ex</td>
<td>2.9 ± 0.3</td>
<td>1.7 ± 0.1</td>
<td>4.7 ± 0.4</td>
<td>190 ± 3</td>
<td>18 ± 1</td>
</tr>
<tr>
<td><strong>Brachial arteries</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sed</td>
<td>2.2 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>5.1 ± 2.0</td>
<td>193 ± 5</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>Ex</td>
<td>2.1 ± 0.3</td>
<td>0.9 ± 0.1</td>
<td>1.8 ± 0.9</td>
<td>189 ± 7</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sed</td>
<td>1.9 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>1.4 ± 0.6</td>
<td>178 ± 3</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Ex</td>
<td>2.2 ± 0.1</td>
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<td>2.8 ± 0.5</td>
<td>180 ± 3</td>
<td>16 ± 2</td>
</tr>
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</table>

Values are means ± SE. OD, outer diameter; ID, inner diameter. %Stretch, %increase (expressed as %unstressed OD) in outer diameter at which optimal contractions to 30 mM KCl were observed. KCl force, increase in force stimulated by 80 mm KCl; Sed, sedentary; Ex, exercise trained.

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**Fig. 1. Contractile responses of femoral arteries (intact, with endothelium) to endothelin-1 (ET-1; top) and norepinephrine (bottom) from sedentary (Sed) male ($n = 9$) and female pigs ($n = 6$). Values are means ± SE. Square brackets denote concentration. ANOVA for repeated measures revealed significant gender effects on endothelin-1 responses of these arteries, and the Student-Newman-Keuls post hoc test revealed that the differences between responses of arteries from males and females to endothelin-1 were significant at the doses indicated with * on the graph. ANOVA for repeated measures revealed no significant gender effects on norepinephrine responses of these arteries.**
mM KCl (Table 1). Finally, there was no gender-related difference between the developed tension produced in response to 30 μM PGF2α (data not shown).

Endothelium-dependent relaxation responses: gender effects. Femoral arteries from female pigs exhibited greater relaxations to both BK and ACh than femoral arteries from male pigs (Fig. 2). Relaxations to BK and ACh were abolished after endothelial removal, independent of gender (denuded in Fig. 2).

Gender effects on BK- and ACh-induced relaxations differed in brachial and femoral arteries. Thus brachial arteries from male pigs relaxed more in response to BK and ACh than did brachial arteries from female pigs (Fig. 3). As was true in femoral arteries, relaxation responses to BK and ACh were abolished after endothelial removal, independent of gender (Fig. 3).

We used the Ca2+ ionophore A-23187 to assess receptor-independent, endothelium-mediated relaxation responses. A-23187 (10⁻⁶ M) produced similar amounts of relaxation in femoral (male = 51 ± 13%, female = 58 ± 17%) and brachial (male = 54 ± 11%, female = 46 ± 13%) arteries with no effect of gender.

Neither L-NAME nor indomethacin plus L-NAME altered resting tension when administered 20 min before preconstriction with PGF2α. Also, indomethacin treatment had no effect on BK-induced or ACh-induced relaxation in femoral arteries isolated from male pigs.

![Femoral artery relaxation](image1)

**Femoral**

- Female intact
- Female denuded
- Male intact
- Male denuded

![Log [Bradykinin], M](image2)

**Log [Acetylcholine], M**

![Brachial artery relaxation](image3)

**Brachial**

- Female intact
- Female denuded
- Male intact
- Male denuded

![Log [Acetylcholine], M](image4)

**Log [Bradykinin], M**

Fig. 2. Relaxation responses of femoral arteries from Sed male (n = 11) and female pigs (n = 13) to bradykinin (top) and acetylcholine (bottom). Arteries were contracted with 30 μM PGF2α, and arteries from male and female pigs had similar developed force (male = 15.5 ± 1.9 g and female = 16.8 ± 1.2 g). Values are means ± SE. ANOVA for repeated measures revealed significant gender effects for both drugs, and the Student-Newman-Keuls post hoc test revealed that the differences between arteries from males and females were significant at the doses indicated with * on the graph. When bradykinin results were expressed as percent maximal, ANOVA for repeated measures revealed no significant gender effects on sensitivity (vasodilator concentration that produced 50% inhibition of the response to PGF2α preconstriction [IC50] (log M): female = −8.6 ± 0.2, male = −8.9 ± 0.1). When ACh results were expressed as percent maximal, ANOVA for repeated measures revealed no significant gender effects on sensitivity (IC50 (log M): female = −7.5 ± 0.1, male = −7.8 ± 0.2).

*Different with P < 0.05.

Fig. 3. Relaxation responses of brachial arteries from Sed male (n = 11) and female pigs (n = 13) to bradykinin (top) and acetylcholine (bottom). Arteries were contracted with 30 μM PGF2α, and arteries from male and female pigs had similar developed force (male = 15.0 ± 1.9 g and female = 17.7 ± 2.2 g). Values are means ± SE. ANOVA for repeated measures revealed significant gender effects, and the Student-Newman-Keuls post hoc test revealed that the differences between intact arteries from males and females were significant at the doses indicated with * on the graph. When bradykinin results were expressed as percent maximal, ANOVA for repeated measures revealed no significant gender effects on sensitivity (IC50 (log M): female = −8.9 ± 0.2, male = −9.0 ± 0.1). When acetylcholine results were expressed as percent maximal, ANOVA for repeated measures revealed no significant gender effects on sensitivity (IC50 (log M): female = −7.1 ± 0.4, male = −7.9 ± 0.2).

*Different with P < 0.05.
Treatment with l-NAME produced a 40% decrease in maximal BK-induced and ACh-induced relaxation in femoral arteries from male pigs (Fig. 4). Combination of indomethacin and l-NAME treatment had the same effect as l-NAME treatment alone on both BK- and ACh-induced relaxation (40%) of femoral arteries (Fig. 4). Results from experiments using treatment with indomethacin, l-NAME, and l-NAME plus indomethacin were similar in male brachial arteries to those shown in Fig. 4 for femoral arteries. However, the decrease in maximal relaxation produced by treatment with l-NAME and l-NAME plus indomethacin was 40% in femoral arteries (Fig. 4) but only 20% in brachial arteries (data not shown).

Qualitatively, the influence of indomethacin, l-NAME, and indomethacin plus l-NAME treatment on these vasorelaxation responses were similar in arteries from both genders except that blockade of NOS had a relatively greater effect in brachial arteries from female pigs. In brachial arteries from male pigs, l-NAME treatment blocked 25–40% of the total relaxation response-induced by BK and ACh. In contrast, l-NAME treatment blocked 50–60% of BK- and ACh-induced relaxation in female brachial arteries. Because the percentage of maximal relaxation blocked by l-NAME treatment reflects the relative contribution of NOS to BK- and ACh-induced relaxation in these arteries, these results suggest that NOS contributes more to endothelium-dependent relaxation in brachial arteries from female pigs than in brachial arteries from males.

Endothelium-independent relaxation: no gender effects.

SNP produced concentration-dependent decreases in contractile force and induced complete relaxation in femoral (Fig. 4) and brachial arteries (data not shown) constricted with PGF2α. There were no differences between SNP-induced relaxation of male and female arteries (data not shown).

Exercise Training Effects

Efficacy of exercise training program. Exercise training produced the expected adaptations to a similar extent in pigs of both genders. Thus, in both male and female pigs, average heart weight and heart weight-to-body weight ratio were greater in Ex than in Sed pigs (Table 2). Male pigs used in this study tended to have lower body and heart weights than female pigs (Table 2). The heart weight-to-body weight ratio of Sed male pigs was greater than that for Sed female pigs. Treadmill performance tests revealed that endurance times were significantly longer in Ex pigs (Table 2). Citrate synthase activity of the long and lateral heads of the triceps brachii muscle and the deltoid muscle of Ex pigs was 20–40% greater than Sed values in male and female pigs, confirming an increase in skeletal muscle oxidative capacity that characterizes effective endurance exercise training (18, 19, 22, 26). Male pigs had significantly higher plasma levels of 17β-estradiol and lower plasma levels of progesterone than female pigs (Table 2). Exercise training did not appear to alter plasma levels of 17β-estradiol or progesterone in female pigs. Furthermore, as shown in Fig. 5, the duration and frequency of estrous cycling were not altered by treatment in either Sed or Ex female pigs.

Training effects on contractile responses. Exercise training did not alter ET-1 responses of male brachial arteries with intact endothelium (Fig. 6). Interestingly, after removal of the endothelium, brachial arteries from Ex male pigs exhibited greater maximal contractile force than arteries from Sed male pigs (Fig. 6, top right). Results from femoral arteries of Sed and Ex
male pigs were similar to those of brachial arteries shown in Fig. 6. In contrast to the effects of training on male brachial artery response to ET-1, brachial arteries from female Ex pigs developed more force than brachial arteries from Sed, and endothelium removal decreased ET-1-induced contraction in brachial arteries of female Ex pigs so that there was no longer a difference between contractions of Sed and Ex arteries (Fig. 6, bottom). In femoral arteries from female pigs, there was no difference in ET-1 contractions between Sed and Ex when the endothelium was intact, but endothelium removal increased ET-1-induced contractions in femoral arteries of female Sed pigs so that they developed significantly more force than denuded femoral arteries from female Ex pigs (data not shown). Exercise training had no effect on NE- (data not shown) or KCl- (Table 1) induced contractions of femoral or brachial arteries from males or females.

Endothelium-dependent relaxations: exercise training effects. There were no significant effects of exercise training on BK- or ACh-induced responses of brachial arteries isolated from male pigs (Fig. 7, left). In contrast, brachial arteries from Ex female pigs exhibited enhanced BK- and ACh-induced relaxation compared with brachial arteries from Sed females (Fig. 7, right).

Exercise training did not alter endothelium-dependent relaxation in femoral arteries of male or female pigs (data not shown). When results were expressed as maximal percentage, ANOVA for repeated measures revealed no significant differences between sensitivity (IC50) of arteries from male or female, Ex or Sed pigs. Exercise training did not alter the relative contribution of endothelium-derived mediators of relaxation in femoral or brachial arteries from male pigs because L-NAME, indomethacin, and L-NAME plus indomethacin had similar effects on BK- and ACh-induced relaxation of arteries from Ex and Sed pigs (data not shown).

Exercise training did not significantly alter A-23187-induced relaxation in either artery from male or female pigs [femoral arteries: female Sed = 58 ± 17%, female Ex = 86 ± 5% (P = 0.08); male Sed = 51 ± 13%, male Ex = 42 ± 10%; brachial arteries: female Sed = 46 ± 12%, female Ex = 48 ± 12%; male Sed = 54 ± 11%, male Ex = 49 ± 13%]. Endothelium removal abolished relaxations to BK, ACh, and A-23187 in arteries from Sed and Ex pigs of both genders. There were no differences between SNP-induced relaxation between arteries from Sed and Ex male pigs or between arteries from Sed and Ex female pigs.

DISCUSSION

The significant new findings of this study are as follows. 1) Femoral and brachial arteries from female pigs developed greater contractions in response to ET-1 than arteries from male pigs. 2) Femoral and brachial arteries exhibited different effects of gender on endothelium-dependent relaxation. Femoral arteries from female pigs were more responsive to BK and ACh than femoral arteries from male pigs. In contrast, brachial arteries from female pigs were less responsive to BK and ACh than brachial arteries from male pigs. 3) Gender also influenced exercise training-induced adaptations in the reactivity of femoral and brachial arteries but not equally. Current literature, combined with these results, supports the concept that exercise training produces differential effects on vasomotor function depending on species, gender, and tissue of origin of the artery studied.
Effects of Gender on Vasomotor Responses of Skeletal Muscle Arteries

Contractile responses. Based largely on the recent work of Miller and colleagues (2, 25), our hypothesis was that femoral and brachial arteries from female swine would exhibit greater ET-1-induced contractile force and greater ET-1 sensitivity than arteries from male swine. We reasoned that, if these gender differences in reactivity of coronary arteries are mediated by circulating gender hormones, then effects of these hormones would also be apparent in arteries perfusing skeletal muscle, such as the femoral and brachial arteries. Our results support this hypothesis because both femoral and brachial arteries from the female pigs exhibited enhanced responses to ET-1 compared with arteries from males. Also, consistent with the report of Barber et al. (2), contractions produced in response to NE and KCl did not exhibit gender differences, indicating that gender specifically alters responsiveness to ET-1. Gender differences in ET-1 responses were not altered by endothelium removal, treatment with indomethacin, or treatment with arginine analogs, consistent with the coronary responses reported by Barber et al. (2). We conclude that our results support the hypothesis that circulating gender hormones are involved in the greater ET-1-evoked responses in smooth muscle of female porcine arteries so that similar gender-related differences are present in femoral, brachial, and coronary conduit arteries of pigs.

Relaxation responses. Barber and Miller (1) examined endothelium-dependent relaxations in coronary arteries of male and female pigs and found that relaxations to BK and UK-14304 ($\alpha_2$-adrenergic agonist) were greater and/or shifted leftward in coronary arterial rings from female pigs. On the basis of these results, our hypothesis was that femoral and brachial arteries from female pigs would also exhibit greater relaxation and/or sensitivity to receptor-mediated, endothelium-dependent vasorelaxing agents. In contrast to our hypothesis, female gender was not consistently associated with increased endothelium-dependent relaxation. Results for femoral arteries support our hypothesis in that femoral arteries of female pigs exhibited greater endothelium-mediated relaxation (BK and ACh induced) than femoral arteries from male pigs. Surprisingly, brachial arteries from males exhibited greater BK- and ACh-induced relaxation than did brachial arteries from female pigs. The reasons that gender did not have a consistent effect on endothelium-mediated responses in these experiments are not clear.
at this time. Our results with blockade of the COX pathway and NOS pathways indicate that gender may influence the relative contribution of the NOS pathway to endothelium-mediated dilation. COX seems to be relatively unimportant, but the NOS pathway appears to contribute more to BK- and ACh-induced relaxation in brachial arteries from females. These effects appear to be specific to agonist-stimulated endothelium-dependent relaxations because A-23187 caused similar amounts of relaxation in arteries from males and females, again similar to results reported by Barber and Miller (1) in coronary arteries.

Barber and Miller (1) reported that the gender differences in porcine coronary endothelium-dependent relaxation may not be related to estrogen levels because male pigs had higher plasma concentrations of estrogen than female pigs, perhaps due to metabolism of testosterone by aromatase in the adipose tissue. Our results (Table 2) confirm these observations because plasma concentrations of 17β-estradiol were much higher in male pigs than in female pigs, and the plasma levels of 17β-estradiol are in the range of values reported by Miller and colleagues (1, 2, 25, 43). These results suggest that other gender hormones and/or other gender-related processes may be responsible for these gender differences in vasomotor reactivity.

There is a growing body of evidence that shows that estrogen can cause increased endothelium-mediated vasodilation in females (5, 13), improved endothelium-mediated relaxation in postmenopausal women (9, 35), increased ecNOS gene expression (21, 44), and increased basal release of NO (11–13). It is possible that the brachial arteries from our male pigs exhibited greater endothelium-dependent relaxation responses than brachial arteries from female pigs because of the relatively high level of plasma 17β-estradiol in these pigs. These levels of plasma 17β-estradiol in our male pigs may suggest that these pigs are similar to human male transsexuals on estrogen therapy who were reported to have greater ACh-induced vasodilation than normal age-matched men (29, 30). However, male pigs
have normal testosterone levels, whereas human male transsexuals have low testosterone levels (1, 29, 30).

If elevated plasma 17β-estradiol levels of our male pigs explain why endothelium-mediated relaxations are greater in brachial arteries of male pigs than in those of females, then it is not clear why coronary and femoral arteries of female pigs have greater endothelium-mediated responses than coronary and femoral arteries of males. It is possible that these differences are mediated by other gender hormones or that arteries perfusing different tissues have different sensitivity to the effects of 17β-estradiol. For example, 17β-estradiol was recently reported to signal an increased expression of ecNOS protein in uterine, but not in systemic, arteries of sheep (41). Our results contribute to a growing body of evidence showing that the mechanisms responsible for gender differences in endothelium-dependent relaxations differ by agonist, species, and anatomic origin of the artery (1).

**Effects of Exercise Training on Vasomotor Responses**

Contractions. Jones et al. (14) reported that exercise training decreased ET-1 sensitivity of branches of left circumflex coronary arteries from male pigs but had no effect on ET-1 sensitivity of these arteries from female pigs. On the basis of these results, we hypothesized that exercise training would also decrease the sensitivity of femoral and brachial arteries from male pigs and that this training effect would be greater in males than female pigs. Contrary to our hypothesis, the ET-1-evoked contractile responses of vascular smooth muscle in denuded femoral and brachial arteries from Ex male pigs were greater than responses of arteries from Sed male pigs. Exercise training did not have the same effect on female arteries.

Results indicate that the endothelium modulates ET-1 responses in these arteries in both genders, but the interaction of the effects of the endothelium and exercise training differ between males and females (Fig. 6). There were no significant differences between Sed and Ex responses of intact brachial arteries from male pigs (Fig. 6, top left). The increased force development of arteries from Ex male pigs apparent after endothelium removal (Fig. 6, top right) may result from removal of greater ET-1-stimulated release of endothelium-derived relaxing substances signaled by endothelial ETB receptors. The effect of the endothelium on ET-1 responses appears different in female arteries. Intact Sed and Ex femoral arteries from females exhibited similar responses to ET-1, whereas intact brachial arteries from Ex female pigs exhibited greater force than arteries from Sed pigs (Fig. 6, bottom left). After removal of the endothelium, brachial arteries from female Ex and Sed pigs exhibited similar ET-1-induced responses (Fig. 6, bottom right). If, as these results suggest, exercise has different effects on endothelial modulation of ET-1 responses in arteries of male and female pigs, the mechanism responsible for this gender effect is not apparent at this time.

Exercise training did not alter NE-induced responses of femoral or brachial arteries from males or females. These results are consistent with a previous report that NE-induced and KCl-induced contractions of femoral and brachial arteries from female pigs were not altered by exercise training (22). Thus ET-1-induced contractions were the only contractions that exhibited an interaction between gender and exercise.

**Endothelium-dependent relaxation.** The hypothesis that exercise training would produce greater increases in endothelium-dependent relaxation in arteries from males than from females is not supported by our results. In fact, exercise training did not alter BK-induced, ACh-induced, or A-23187-induced relaxation in male femoral or brachial arteries. Furthermore, the relative contribution of NOS to BK- and ACh-induced relaxation was not altered by exercise training in male pigs. In contrast, brachial arteries from Ex female pigs relaxed more in response to BK and ACh than did brachial arteries from Sed female pigs. This result was surprising to us because we previously reported no change in BK-induced relaxations in femoral and brachial arteries from Ex female pigs that underwent the same training program used in the present study (22). These previous results indicated that brachial arteries of the Ex female pigs showed 10% greater relaxation of KCl-induced contractions, but this difference was not statistically significant. Also, McAllister and Laughlin (23) reported that short-term exercise training (7 days) produced an increased sensitivity of brachial arteries of female pigs to BK-induced relaxation. Therefore, currently available results indicate that exercise training does not alter endothelium-dependent relaxation of femoral or brachial arteries from males or of femoral arteries from females but increases endothelium-dependent relaxation of brachial arteries from females.

Exercise training has been reported to result in enhanced endothelium-dependent relaxation in rat aorta (7, 8), in rat skeletal muscle arteries (16), in brachial (23) and coronary arteries of female pigs (26, 45), in peripheral arteries of humans (10), and in dog aorta and coronary arteries (36, 42). It is generally believed that one important signal for these endothelial adaptations in response to exercise is increased shear stress associated with increased blood flow during exercise. However, there are also reports in which the increased blood flow during exercise training bouts did not produce enhanced endothelium-mediated vasodilation, i.e., rat coronary arteries (33) and porcine conduit coronary arteries (31). If shear stress during exercise bouts contributes to the differential responses of endothelium to exercise training reported herein, these results suggest that exercise bouts produce a greater shear stress signal in brachial arteries of female pigs than in femoral arteries of female pigs and in femoral and brachial arteries of male pigs. We are not aware of data to support this interpretation, and we have not noticed differences in the gait used by male and female pigs as they run on the treadmill that might cause such differences in regional blood flow during exercise.
SNP-induced vasodilation was measured to directly evaluate cGMP-dependent vasodilation in vascular smooth muscle. The results indicate that SNP-induced responses of vessels contracted with PGF$_{2\alpha}$ were not altered by gender or exercise training. Also, L-NAME and/or removal of endothelial cells had no effects on SNP-induced relaxation.

Conclusion
Female gender is associated with increased responses of vascular smooth muscle in femoral and brachial arteries to the agonist ET-1 but not NE or depolarization, similar to gender effects reported in porcine conduit coronary arteries (2, 25, 43). Female gender also influenced endothelial function as reflected in enhanced endothelium-dependent relaxation of femoral arteries but not brachial. These results add to the growing body of evidence that gender differences in endothelium-dependent relaxation vary with agonist, species, and different arteries within species (1, 24, 41).

Because pigs are quadrupeds, we expected exercise training to have similar effects on vascular smooth muscle and/or endothelium in the brachial and femoral arteries of both genders. However, the interactions of gender and anatomic origin of arteries with the effects of exercise training are not that simple. Exercise training increased the magnitude of ET-1-induced contractions in arteries from males but not from females. Furthermore, training only increased BK- and ACh-induced endothelium-mediated relaxation in female brachial arteries. Thus we conclude that gender and anatomic origin of the artery interact with exercise training in setting vasomotor responses of porcine arteries. The mechanisms producing gender differences in vascular reactivity and of exercise training-induced adaptations of vascular reactivity do not appear to be uniform across agonists, species, or anatomic origin of the artery, even among conduit arteries that provide blood flow to striated muscle: coronary, femoral, and brachial arteries.

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