Published in final edited form as:

Am J Physiol Heart Circ Physiol. 2007 August ; 293(2): H1196–H1205.

Adaptation of coronary microvascular exchange in arterioles and venules to exercise training and a role for sex in determining permeability responses

Virginia H. Huxley^{1,2}, Jian Jie Wang^{1,2}, and Ingrid H. Sarelius³

¹ Department of Medical Pharmacology and Physiology, University of Missouri School of Medicine, Columbia, Missouri

² National Center for Gender Physiology, University of Missouri School of Medicine, Columbia, Missouri

³ Department of Pharmacology and Physiology, University of Rochester, Rochester, New York

Abstract

Studies of physical performance and energy metabolism during and following exercise have shown significant sex-specific musculoskeletal adaptations; less is known of vascular adaptations, particularly with respect to exchange capacity. In response to adenosine (ADO), a metabolite produced during exercise, permeability (P_s) of coronary arterioles from female pigs changed acutely; the magnitude and direction of the change (ΔP_s) were determined by training status. In the present study P_s to albumin was assessed in arterioles ($n = 138$) and venules ($n = 24$) isolated from hearts of male ($N = 27$) and female ($N = 59$) pigs in the exercise training group (EX). We evaluated the hypothesis that coronary microvessel exchange adapts to endurance exercise training not by altering basal P_s , per se, but by elevating P_s on exposure to ADO. In contrast, training resulted in a reduction of basal P_s in all arterioles, and in venules from males, with no change in venules from EX females. Exposure to ADO resulted in the predicted increase in P_s except for venules from EX males where P_s was reduced. ΔP_s responses of arterioles to mediators of adenylyl cyclase (isoproterenol)- and guanylyl cyclase (atrial natriuretic peptide)-signaling pathways were attenuated in EX pigs relative to pigs in the sedentary group. The adaptation of EX arterioles involves an upregulation of a nitric oxide-dependent pathway since nitric oxide synthase inhibition blocks ΔP_s by ADO. Thus adaptation of microvascular exchange capacity to endurance exercise training not only occurs but also involves multiple mechanisms that differ in arterioles and venules with their relative contribution to net flux being a function of sex.

Keywords

α -lactalbumin; albumin; heart; porcine; protein flux; sexual dimorphism; transvascular flux

In response to physical activity, particularly exercise, changes in musculoskeletal structure and function have been observed during and following training bouts and the constellation of changes has been shown to differ between males and females (16,57). Less is known of the relationships among training, sex, and vascular physiology. In a study of vascular adaptation to endurance exercise training, Jones et al. (30) isolated coronary arteries from sedentary cage-confined (SED) and endurance exercise-trained (EX) pigs. They found that exercise training reduced the sensitivity of coronary smooth muscle to endothelin-1, and, interestingly, this

effect was greater in male than in female pigs. In the same animal model, Laughlin et al. (37) assessed vascular reactivity of skeletal muscle conduit arteries. The vascular reactivity responses were found to depend on the anatomic origin of the artery and could also differ in males and females. Sex differences have also been shown to influence coronary microvessel smooth muscle L-type calcium current density in adaptation to exercise (4) in this same model. Whether differences in adaptation to endurance exercise training exist for solute, particularly protein, transport across vessel walls is not known. Adaptation of transport capacity in the heart of EX pigs is the focus of the present study with particular attention being paid to whether the adaptive responses of arterioles and venules are the same and whether the responses are the same in age-matched male and female animals.

The cell membrane permeant by-product of ATP hydrolysis, adenosine (ADO), is produced during exercise, and it is established that ADO acutely alters macromolecule permeability (P_s) of coronary arterioles isolated from the hearts of female pigs (26,28). For these vessels, the magnitude and direction of the change in P_s (ΔP_s) were a function of training status (28). Data from limited studies of pig coronary arterioles (28) and venules (18) implicated both exercise training status and sex as determinants of basal arteriolar P_s for the small protein, α -lactalbumin, but not for the larger plasma protein, porcine serum albumin (PSA). The inference from these data was that the mechanisms regulating basal P_s are complex and that the regulation of basal P_s involves a different set of mechanisms from those determining ΔP_s in response to metabolically relevant vasoactive agents, such as ADO. The purposes of the present study were to determine whether adaptation of coronary microvascular exchange, particularly that of albumin which ferries free fatty acids, to endurance exercise training is similar in arterioles and venules and, importantly, to establish whether adaptational changes in P_s are indeed influenced by the animal's sex. We hypothesized that basal P_s and ΔP_s to ADO would be independent of sex but dependent on exercise training. To test the hypothesis, P_s was assessed in arterioles and venules isolated from the hearts of 16- to 20-wk treadmill-trained (EX) male and female pigs before and following suffusion with 10^{-5} M ADO and the data were compared with data generated using the same protocols on age- and sex-matched cage sedentary (SED) pigs (25,27,28).

In the aggregate, our results demonstrate clearly that the exchange characteristics of coronary arterioles differ from venules. Coronary arterioles were responsive to endurance exercise training, whereas coronary venules were sensitive to the animal's sex. The data were also consistent with the interpretation that multiple mechanisms regulate not only the transvascular flux of protein, but also, in the case of the heart, the transvascular flux of solutes, such as free fatty acids, carried by those proteins. Furthermore, the relative importance of the set of mechanisms to net flux involves sex hormones. Finally, these data demonstrate that differences in adaptation to physical training extend beyond the musculoskeletal system, involve more than blood flow distribution, and likely influence volume distribution and solute flux differently during exercise in males versus females. Preliminary results of these studies have been reported previously (18,24).

MATERIALS AND METHODS

Animals

All animal care and research was conducted in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* under the supervision and approval of the Office of Laboratory Medicine at the University of Missouri.

Endurance Training Protocol

The training and performance testing protocols have been described previously (34). Briefly, during a 1- to 2-wk pretraining period, Yucatan miniature swine (females, 20–40 kg, $N = 81$; and males, 23–45 kg, $N = 38$; ages, 13–16 mo) were exposed to the treadmill (Quinton Fitness Equipment, Bothell, WA; or Warren E. Collins, Braintree, MA) and taught to run. Veterinary students assigned to individual pigs remained with the animals throughout the training period. Following the 2-wk pretraining period, the animals were divided randomly into SED [22 females and 11 males, in addition to the 74 SED animals reported on previously (25)] and EX (59 females and 27 males) groups. The animals were housed with one SED and one EX pig of the same sex.

During the first week of training, the EX group exercised at 8 km/h for 15 min (sprint) and 4.8 km/h for 20–30 min (endurance run). By *week 8* the duration of each exercise training bout lasted for 85 min/day, 5 day/wk. The training regime consisted of a 5-min warm-up at 4 km/h, a 15-min sprint at 9.7 to 12.9 km/h, a 60-min endurance run at 6.4 to 9.7 km/h, and a 5-min warm-down at 3.2 km/h. This intensity of exercise was maintained for the next 12–20 wk. During the workout the animals were kept cool with convection and misted water. After completion of a training session, the animals were fed Purina pig chow; the amount was based on the animal's weight (36).

Training effectiveness was assessed by measuring cardiovascular and metabolic indexes in both EX and SED animals during both baseline and treadmill performance testing. The treadmill performance test consisted of four stages of exercise (34). During *stage 1*, pigs ran at 5 km/h and 0% grade for 5 min. Pigs ran for 10 min at *stage 2* (speed = 5 km/h, and grade = 10%) and then for 10 min at *stage 3* (speed = 6.9 km/h, and grade = 10%). Finally, pigs ran at *stage 4* (speed = 9.7 km/h, and grade = 10%) until exhaustion.

Surgical Preparation

On the day of an experiment, the pig was sedated with ketamine (25 mg/kg im) and xylazine (Rompun; 2.25 mg/kg im), anesthetized with pentobarbital sodium (20 mg/kg iv), intubated, and then ventilated with room air. Following the placement of a catheter into an ear vein, heparin was administered (1,000 U/kg) and a left thoracotomy was performed. The heart was excised, its wet weight determined, and it was immersed into cold (4°C) mammalian Krebs solution (36). Other tissues, blood, and organs (brain, lung, liver, skeletal muscle, fat, skin, and eyes) were harvested for studies in multiple laboratories before final spinal transection.

Oxidative Enzyme Activity

After removal of the heart, samples were taken from the middle of the long, medial, lateral, and accessory heads of triceps brachii and deltoid muscles; frozen in liquid N₂; and stored at –70°C until processed. Citrate synthase activity was measured from these tissues (56) and used to assess training status.

Microvessel Plexus Isolation and Cannulation

The right ventricular wall (5–7 × 2–3 cm) of the excised heart was removed into fresh Krebs solution containing 10 mg/ml PSA (Krebs-PSA) at 4°C (25) for transport and storage (not more than 15 min) before microvessel dissection. For dissection the tissue was submerged in fresh Krebs-PSA and pinned onto a closed-cell foam pad with Minutien pins (Carolina Biological, Burlington, NC) to maintain the tissue at a constant length. When venules were isolated from the tissue, they were removed first because of their anatomical location near the epicardial surface of the ventricle away from the arterial circulation. In the case of the venules, the plexus contains vessels of irregular, noncylindrical shapes described previously by Kassab et al.

(31) as “rootlike” rather than “treelike” in topology. In the case of the arterioles, the plexus consisting of interconnected vessels (32) was then removed from the surrounding myocardium. The arterioles (<100 μm diameter, $\approx 1,000$ μm long) branched from larger vessels (>250 μm diameter), which, in turn, had originated from the right coronary artery or the left anterior descending artery.

The excised arteriolar (which could contain microvessels that spanned, in situ, from the epi- to the endocardium) or venular (primarily epicardial) plexus was secured with Minutien pins (~ 100 μm OD, Carolina Biological) to a 3-mm deep Sylgard (Dow Corning, Midland, MI) pad set on an inverted 5-cm-diameter organ culture dish (Falcon, 1008) at approximately its in vivo length. Finally, a microvessel was cannulated with a beveled glass theta micropipette (WPI, Sarasota, FL), resulting in an immediate perfusion through several branches of the plexus.

Solutions

Mammalian Krebs—All perfusion and suffusion solutions were prepared and used fresh daily. The Krebs base consisted of (in mmol) 141.4 NaCl, 4.7 KCl, 2.0 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.2 MgSO_4 , 1.2 NaH_2PO_4 , 5.0 glucose, 3.0 NaHCO_3 , and 1.5 Na-HEPES. The pH of the solution was 7.37 and 7.41 ± 0.01 at 4° and 37°C , respectively.

Krebs-PSA—PSA (Sigma, St. Louis, MO), used as the colloid in all solutions, was prepared from a stock solution of 60 mg/ml dialyzed previously (in 12–14,000 mol wt cut-off dialysis tubing; SpectroPor, Spectrum, Houston, TX) against 2 liters of Krebs solution in three sessions over 72 h 8°C . The dialysis procedure equilibrated the plasma protein solutions with the Krebs solution, removed small water soluble vasoactive contaminants, set the calcium content, and fixed the pH of the protein solution (3,20). The protein content of the final solution was determined by absorbance spectroscopy and adjusted so that the perfusate and suffusate solutions contained 20 and 10 mg/ml, respectively.

Labeled protein—The primary globular protein used to assess microvessel exchange was PSA (65,000 mol wt). In a selected subset of experiments, the smaller globular protein, α -lactalbumin (14,000 mol wt, Sigma) was used in place of PSA (20,21). In either case, one half of a perfusion theta pipette contained unlabeled protein; the second half contained the same concentration of probe protein labeled with a fluorescent tag [either tetramethylrhodamine isothiocyanate (TRITC) Isomer L (CalBiochem or Molecular Probes) or 2',4,5,6,7,7'-hexafluorofluorescein, Oregon Green 514 (Molecular Probes)] using methods detailed in previous work (20,26). The final protein content of these solutions was 20 mg/ml for PSA and 23.5 (0.56 mM) for α -lactalbumin, respectively. Given that TRITC-PSA was 0.17 mM, the total PSA concentration was 0.33 mM. The net oncotic pressure of these protein solutions ranged between 6 and 8 cmH_2O .

ADO, isoproterenol, atrial natriuretic peptide, and N^G -monomethyl-L-arginine—ADO (Sigma) was dissolved in Krebs solution (10^{-3} M stock) and prepared in a 1:100 dilution into PSA. The final concentration was 10^{-5} M ADO in 10 mg/ml protein. Isoproterenol (ISO; Sigma) was diluted 1:100 into Krebs-PSA and labeled α -lactalbumin from a 10^{-3} M stock in Krebs solution, making a final concentration of 10^{-5} M used in the reported experiments. Human atrial natriuretic peptide (1–28) (hANP; Bachem) with an identical amino acid sequence to porcine ANP was prepared from a stock of 10^{-6} M peptide in Krebs solution into Krebs-PSA and labeled α -lactalbumin to a final concentration of 10^{-8} M. The solutions containing N^G -monomethyl-L-arginine (L-NMMA, CalBiochem) were prepared from a 5×10^{-3} M stock solution in 0.9% (wt/vol) NaCl and diluted into either Krebs solution to a final concentration of 10^{-6} M or into Krebs plus ADO to a final concentration of 10^{-5} M ADO and 10^{-6} M L-NMMA.

Measurement of Microvessel Protein Flux

The method for assessing P_s to proteins in isolated mammalian microvessels and its limitations is described in multiple publications (25–28,53,60,61). Briefly, the microvessels were perfused with either Krebs-PSA and fluorescently labeled solute (dye) or unlabeled solute (washout) solution using a system of manometers connected to hydraulic switches to control the choice of solution and the hydrostatic pressure in the vessel segment. With a switch between the pairs of manometers, perfusate in the vessel could contain just nonfluorescent washout solution, be changed rapidly to the dye solution for a time necessary to measure solute flux, or to the clear washout to reestablish the baseline, all within seconds. The relatively low perfusion pressures (14.9 ± 0.2 in arterioles and 10.3 ± 0.4 cmH₂O in venules) used in these experiments minimized the contributions of convective coupling, or solvent drag, in the estimates of apparent permeability (P_s) (26,53).

The perfused plexus was transilluminated and viewed at $\times 10$ or $\times 20$ magnification (numerical aperture, 0.32; UM 10, Leitz) with a fixed stage inverted microscope (Diavert, Leica, NJ) equipped with an adjustable magnifier (up to $\times 2$, providing up to $\times 20$ real magnification). The light path of the microscope was split 50/50 and projected simultaneously to a video system and to an analog microscope photometer (Leitz, PTI, or Solamere). In this system light intensity at the focal plane of the vessel was 5–11 mW/cm² when using the N2 filter cube (Leitz) for the red fluorescent dye, TRITC, or the H2 filter cube (Leitz) for the green fluorescent dye, Oregon Green 514, in the light path. At these light intensities, fading of the fluorescent signal and/or light-induced changes in function are either absent or minimized (48,50). Vessels were imaged using a black and white CCD camera (Dage-MTI 72, Michigan City, IN) fitted with an image intensifier (Dage-MTI Geni-Sys) or a low-light black and white camera (PTI, Brunswick, NJ) and displayed on two video monitors (projecting a field of view of 0.65×0.78 to 1.30×1.56 mm; Sony).

When labeled protein solution filled the lumen, fluorescence intensity (I_f) emanated from the perfused microvessel. A rectangular window (width ≥ 3 vessel diameters), located in the light path between the vessel and the photometer, was used to limit and define the area over which the solute flux (J_s , in mmol/s) was measured. Apparent solute permeability (P_s) was determined from the relation J_s per unit surface area (S , in cm²) and constant concentration gradient (ΔC , in mmol/ml): $P_s = J_s(S\Delta C)^{-1} = 0.25 D(dI_f/dt)_i \cdot \Delta I_0^{-1} (Eq. 1)$, where ΔI_0 was the step change of fluorescence when the dye replaced the nonfluorescent washout solution in the lumen, $(dI_f/dt)_i$ was the initial change in fluorescence intensity as solute moved across the vessel wall, and D was the internal diameter of the microvessel (in μ m). As discussed later, the vessels were assumed to be circular in cross section and have a volume-to-surface area ratio of $0.25 D$. All experimental protocols were performed at 15°C to minimize changes in diameter (25–28,61) during the measurement of flux.

Experimental Protocols

Flux was measured at least twice in all vessels (arterioles and venules). From J_s , P_s was assessed first to determine whether the exchange properties of the microvascular barrier differed with vessel type, endurance exercise training, and/or sex. The microvessels were then exposed to ADO in the suffusate, and P_s was assessed a second time to test whether a metabolic vasodilator known to increase during exercise influenced macromolecule exchange and whether the responses to the adenine nucleoside differed with training, vessel type, and/or sex.

Measures of J_s commenced after a minimum of 5-min suffusion with 10^{-5} M ADO and continued for up to 90 min, depending on the rate of probe flux from the microvessels (e.g., “high” permeability vessels were exposed to basal and ADO perfusions for shorter times than “low” permeability vessels). Diameter was measured under both conditions.

Five additional protocols were carried out, requiring multiple measurements of flux following the determination of the basal P_s .

In *protocol 1*, the concentration response of P_s to ADO was assessed in arterioles isolated from the hearts of EX ($N = 7$) and SED [$N = 7$, data published previously in Wang et al. (61)] age-matched female pigs first in the absence (basal) and then following exposure to concentrations of ADO from 10^{-9} to 10^{-5} M. Up to five measures of P_s at increasing concentrations of ADO were made in individual arterioles.

In *protocols 2 and 3*, the adaptation of adenylyl cyclase (AC)- or guanylyl cyclase (GC)-dependent pathways in trained animals were probed using ISO (to activate AC-dependent signaling) or ANP (to activate particulate GC signaling). P_s was measured during perfusion of ISO (10^{-7} M) on arterioles of 16 (7 SED and 9 EX) pigs and during perfusion of ANP (10^{-9} M) in coronary arterioles from 12 age-matched (7 SED and 5 EX) pigs.

In *protocol 4*, to determine whether the response to nitric oxide (NO) synthase (NOS) activity was itself adapted to endurance exercise training, P_s in response to L-NMMA suffusion alone was assessed in arterioles from 18 age-matched female (9 EX and 9 SED) pigs.

In *protocol 5*, to determine whether the increase in P_s of EX arterioles, as opposed to the decrease in SED arterioles on exposure to ADO (25), involved a NO-dependent pathway, P_s was measured in arterioles from 23 age-matched females pigs (10 EX and 13 SED) that were suffused with 10^{-5} M ADO; each vessel was then suffused with 10^{-5} M L-NMMA plus 10^{-5} M ADO, and the P_s measurement was repeated.

In all the above protocols, the measures were paired, basal P_s was determined during perfusion with pipettes containing the labeled protein in Krebs-PSA, the test solution was then added to the solution suffused over the arteriole, and the P_s measurement was repeated. In the case of ISO or ANP, the perfusion pipette was withdrawn and the vessel was recannulated with a new pipette containing ISO or ANP plus the fluor-labeled protein before measures of flux were repeated.

Statistical Analyses

At the time of experimentation, the investigators were blind to the training status but not to the vessel type or sex of the pigs. Training status was revealed on completion of experiments on groups of 8 to 16 animals. Data from some of the age- and sex-matched SED pigs were reported recently in a companion article (25); the heart weight (HW)/body weight (BW) data as well as the concentration-response studies, NOS blockade, responses to ISO and ANP, or basal venular permeability to α -lactalbumin have not been reported previously. Over a 7-yr period, data were obtained from 6 groups of 8–16 male and 12 groups of 8–16 female pigs. Only data from EX pigs, in which measures of basal coronary arteriole P_s and P_s following exposure to ADO, L-NMMA, ISO, or ANP were obtained, analyzed, and reported in this study. The effectiveness of training, for the animals from which P_s data are reported in this study, was determined by comparing data (means $\pm$ SE) from HW and BW, HW-to-BW ratios, and values for skeletal muscle oxidative enzyme activity between the SED and EX groups.

An average of a minimum of five measures of $J_s/S\Delta C$ at a single hydrostatic pressure was used to represent P_s for an individual arteriole or venule. Data from individual microvessels (n) were averaged to represent a single value for each heart (N). In the majority, only a single arteriole and/or venule was sampled per heart. Although the distributions of values for P_s to α -lactalbumin of arterioles tended to be skewed, the means $\pm$ SE are reported for clarity, and nonparametric statistics were used in the statistical comparisons (StatView SE + Graphics, Abacus Concepts, Berkeley, CA). Repeated-measures two-way ANOVA with Games/Howell

(nonparametric) post hoc test (5,6,10) was used to test for differences in values of P_s with exercise training and sex. The response to ADO was calculated as the ratio of P_s^{ADO} (test) to P_s^{Control} (basal). The same approach was used when ADO was replaced by ISO, ANP, L-NMMA, or ADO + L-NMMA. In the figures showing ΔP_s , the scales are $\log_{10}$ to represent appropriately the magnitude of decreases and increases in permeability [i.e., a twofold decrease (a ratio of 0.5) has the same magnitude as a twofold-increase (a ratio of 2) with regard to a change in P_s from the prestimulation levels]. A significant change from basal levels was determined using paired Student's *t*-test or Wilcoxon's signed-rank test, as appropriate. ΔP_s with ADO was analyzed using repeated-measures ANOVA and using a Bonferroni/Dunn correction when there were more than two groups.

Power analyses (44) indicated that seven animals were required per solute to minimize both type I and type II errors. Consequently, for the venular data using α -lactalbumin as the solute, only the mixed data (males and females) are reported since the sample size was too small to test whether the pig's sex influenced the results. A significance level of $P < 0.05$ was set before calculating the power analysis and performing the experiments.

RESULTS

Efficacy of Training: the Role of Sex

Body weights did not differ with training status or sex on the day of euthanasia (Table 1). The hearts from the EX animals, however, weighed more than those from SED pigs without regard to sex ($P < 0.01$). Consistent with the HW and BW results, the HW-to-BW ratio was increased with training ($P < 0.01$). Interestingly, the HW-to-BW ratio was lower in SED females compared with that in SED male pigs (4.6 ± 0.1 vs. 5.2 ± 0.2 for SED females and males, respectively, $P < 0.05$), but this sex difference was no longer evident after training (5.5 ± 0.1 vs. 5.7 ± 0.2 for EX females and males, respectively).

With respect to citrate synthase activity, tissue samples from the lateral head of muscularis tibialis and the deltoid demonstrated increases in activity with training that were independent of sex (Table 1).

As illustrated in Fig. 1A, resting heart rate (HR) was higher in female than in the male pigs in both experimental groups before (Pre) training. This difference was maintained, as expected, between female and male SED pigs after 16 wk. In contrast, HR was reduced for both sexes, and posttraining and the female/male difference were maintained. Both the male and female pigs improved their duration of exercise in the performance test; duration and magnitude of improvement did not differ with sex (Fig. 1B). Changes in HR in response to acute exercise in the stress test ($\text{HR}_{\text{Pretraining}} - \text{HR}_{\text{Posttraining}}$, Fig. 1C) were observed following endurance exercise training before the performance test (rest) and at most stages of the exercise testing protocol. Of interest, the training-induced reduction in HR was most evident for the female pigs at all stages of work. Posttraining bradycardia was greater for males than for females at rest, and the change in HR levels in EX males was not significant beyond the S2 workload of the performance test. For the EX females significant reductions in HR, relative to their pretest levels, were observed at all four levels of work.

Basal Permeability to Protein in Microvessels from EX Pigs

In EX pigs, P_s was measured under basal conditions on 138 coronary arterioles having an internal diameter of $40.4 \pm 1.5 \mu\text{m}$ (mean $\pm$ SE; range 6–126 μm) and on 24 venules with an average diameter of $76 \pm 9 \mu\text{m}$ (mean $\pm$ SE; range 26 to 126 μm , major axis). The majority of these arterioles would be classified as A4 (32), and the predominant venule classification would be V4 (31).

Overall, the EX group resulted in a reduction of P_s^{PSA} compared with SED pigs. Basal arteriole P_s^{PSA} was $4.8 \pm 0.4 \times 10^{-7}$ cm/s (mean $\pm$ SE; $n = 56$) in EX pigs, which is significantly lower ($P < 0.01$) than P_s^{PSA} in SED animals [$6.3 \pm 0.6 \times 10^{-7}$ cm/s; $n = 61$ (25)]. Arteriolar basal permeability did not differ between males and females; $P = 0.9$ (Fig. 2). Similarly, in EX males, basal venule P_s^{PSA} was significantly lower ($8.1 \pm 3.0 \times 10^{-7}$ cm/s; $n = 7$) than in SED animals [$22.2 \pm 3.5 \times 10^{-7}$ cm/s; $n = 24$ (25)]. In contrast, for venules from EX females, P_s^{PSA} was $21.6 \pm 6.1 \times 10^{-7}$ cm/s ($n = 11$), a value that was not different from venules of SED animals' P_s^{PSA} [$22.2 \pm 3.5 \times 10^{-7}$ cm/s; $n = 24$ (25)]. Thus venular P_s^{PSA} in EX pigs differed significantly with sex ($P < 0.001$). Although basal arteriole P_s^{PSA} was significantly less than venule P_s^{PSA} ($P < 0.001$) for EX females, the arteriovenous difference for P_s^{PSA} was not demonstrable ($P = 0.42$) in coronary microvessels from EX males.

In a separate set of microvessels, P_s to α -lactalbumin, a smaller, monomeric protein possessing a shape and charge similar to albumin at physiological pH, was determined. α -Lactalbumin has been used in multiple studies and shown to be without apparent influence on exchange barrier function (19–21,25–28). As with PSA, basal arteriole $P_s^{\alpha\text{-lactalbumin}}$ ($10.7 \pm 0.9 \times 10^{-7}$ cm/s; $n = 82$) did not differ with sex ($P = 0.29$). As anticipated, $P_s^{\alpha\text{-lactalbumin}}$, for the smaller solute, was significantly greater than that of the larger protein PSA ($P < 0.001$), and similar to the results with PSA $P_s^{\alpha\text{-lactalbumin}}$ in arterioles from EX pigs was significantly less than that of SED pigs [$12.1 \pm 1.4 \times 10^{-7}$ cm/s; $n = 76$ (25)].

In the venules basal $P_s^{\alpha\text{-lactalbumin}}$ was $30.2 \pm 8.9 \times 10^{-7}$ cm/s (4 females and 2 males). Although the small sample size precluded drawing conclusions concerning sex-related differences, $P_s^{\alpha\text{-lactalbumin}}$ of venules was greater than $P_s^{\alpha\text{-lactalbumin}}$ of arterioles ($P < 0.01$).

Barrier Properties Following ADO Exposure in EX Pigs

The cumulative concentration-response curve (Fig. 3) illustrates that P_s^{PSA} in coronary arterioles from EX pigs (3 females and 4 males) increases from basal levels at concentrations of ADO $> 10^{-9}$ M. For this sample size, the increase was significant at 10^{-7} M, the EC_{50} was 2.3×10^{-8} M, and a maximal response (70% over basal levels) was reached at 10^{-6} M. For all of the subsequent studies, ΔP_s was tested at a concentration of 10^{-5} M ADO. For comparison, the concentration-response for coronary arterioles (4 females and 3 males) isolated from SED pigs (61) is given as the broken gray line in Fig. 3.

Following 5 min of suffusion of the arteriolar plexus from EX hearts with ADO, the diameter increased by $15 \pm 4\%$ ($P < 0.01$). The magnitude of response was identical in both sexes. For venules, ADO was without a significant effect on vessel diameter ($-3 \pm 4\%$, $P = 0.47$), and this result was again independent of sex. ADO increased P_s^{PSA} in three of the four experimental groups (all EX arterioles and venules from EX females) (Fig. 4).

In the coronary arterioles from EX pigs, ADO elicited a twofold increase in P_s in contrast to the 22% decrease observed in corresponding vessels from SED animals (25). The difference in the magnitude of the ΔP_s response of arterioles from EX females (2.27 ± 0.34 -fold, $n = 35$, $P < 0.01$) and EX males (1.65 ± 0.29 -fold, $n = 18$, $P < 0.05$) failed to reach significance ($P < 0.17$). Venules from the hearts of EX females also responded to ADO with an increase in P_s^{PSA} (1.31 ± 0.09 -fold, $n = 7$, $P < 0.05$), whereas the same treatment of venules from EX males resulted in a decrease in P_s^{PSA} (-0.15 ± 0.10 -fold, $n = 7$, $P < 0.01$). This sex difference was previously observed in venules from SED pigs (25) where there was an increase in SED female

venule P_s^{PSA} (1.4 ± 0.2 -fold, $n = 15$, $P < 0.05$) and a decrease in SED male venule P_s^{PSA} [-0.21 ± 0.06 , $n = 13$, $P < 0.01$, (25)]. Importantly, the responses of venules differed from those of arterioles, whether from EX ($P < 0.05$) or SED ($P < 0.05$) pigs (25), indicating that some aspect of the training-induced adaptation of coronary venules, but not arterioles, is sex dependent.

Does Adaptation to EX Involve Different Second Messenger Pathways?

Most of the evidence indicates that vascular ADO receptor activation is mediated by activation of AC following binding to ADO A_{2A} receptors (8). Our working hypothesis is that AC-mediated signaling mechanisms reduce P_s , whereas those mediated by GC will increase P_s (22,40–42). Therefore, if in the coronary microvasculature ADO regulates exchange via exclusive activation of A_{2A} receptors, then we predicted that P_s in EX animals would fall from basal levels in response to ADO as occurred in arterioles from SED animals (25,27). Instead, in arterioles from the EX pigs, P_s increased following exposure to ADO. Previous studies in this laboratory have demonstrated both message and protein expression for three of the four ADO receptor subtypes (A_1 , A_{2A} , and A_{2B}) in both coronary arterioles and venules (61). The signaling mechanisms for the A_1 and A_{2B} receptor subtypes result in decreases and/or increases in AC, respectively (9,54), via their respective G_i - and G_s coupling (8). Consequently, the net change in P_s that we observe could be a mixed response reflecting the interplay of the different second messenger systems, depending on the relative abundance and activities of the ADO receptor subtypes (54). We speculate that their affinity, abundance, and/or efficacy is modified in the EX adaptation.

As a first step to investigating whether the cyclase system activities were indeed modified by EX per se, we chose two agents thought to work exclusively by a single cyclase: ISO, which on binding to the β -adrenergic receptor activates AC (2,33,43,47), and ANP, the receptor of which is a particulate GC (15,29). In previous studies, we and others have shown that ΔP_s of intact vessels can decrease in response to ISO (1,17,24) and increase in response to ANP (23, 40,51). Given that the basal P_s levels and the responses of the arterioles of the SED and EX pigs were independent of sex, the data from female and male pigs were pooled according to training status.

In arterioles isolated from 16 (7 SED and 9 EX) pigs, basal $P_s^{\alpha\text{-lactalbumin}}$ was assessed, and the microvessels were then recannulated with pipettes containing 10^{-5} M ISO. Exposure to ISO, as expected, reduced P_s in all microvessels (Fig. 5A). The magnitude of the response was greater ($P < 0.05$) in those from SED pigs than those from the age-matched EX counterparts [0.39 ± 0.08 -fold ($P < 0.01$) vs. 0.59 ± 0.05 -fold ($P < 0.01$) for SED and EX pigs, respectively], suggesting that the attenuation of AC-mediated responses may constitute an additional adaptation to endurance training.

Exposure to ANP (10^{-9} M), as predicted, resulted in an overall increase in $P_s^{\alpha\text{-lactalbumin}}$ from basal levels (7 SED and 5 EX pigs, Fig. 5B). Again, there was a significant difference in the magnitude of the response with training in that the response of arterioles from SED pigs (2.08 ± 0.62 -fold; $P < 0.01$) significantly ($P = 0.02$) exceeded that from EX animals (1.31 ± 0.08 -fold; $P < 0.01$). Thus the arteriolar permeability response in trained animals appears consistent with an attenuation in GC-dependent signaling.

Our data thus show that the overall response of adaptation to exercise is complex, involving alterations in the contributions of both of these signaling pathways; the resulting effect on P_s will reflect their relative balance but is likely to include an overall lower permeability phenotype.

Contribution of NO to the ADO-Induced Increase in Arteriole P_s

To further explore possible adaptations in signaling pathways modulating P_s in exercise-adapted animals, we asked whether the increase in arteriole P_s produced by ADO involved an NO-dependent component (7,45), as we and others (24,49) have shown that the ADO-induced increase in P_s has an NO-sensitive component, and it has also been shown that exercise adaptation includes an increase in endothelial NOS expression (34,35,37,38,62).

In arterioles from EX animals, inhibition of NOS by an addition of 10^{-5} M L-NMMA to the suffusate produced a significant $25 \pm 9\%$ reduction in arteriole $P_s^{\alpha\text{-lactalbumin}}$ ($P < 0.05$, $N = 9$, Fig. 6A), confirming that there is a NO-dependent component to the basal P_s in these vessels. In a second set of EX arterioles, suffusion with 10^{-5} M ADO first resulted, as expected, in a $32 \pm 13\%$ increase ($P < 0.05$) in P_s ; on subsequent exposure to 10^{-5} M L-NMMA in combination with 10^{-5} M ADO, P_s fell to $86 \pm 11\%$ of control levels ($N = 10$, Fig. 6B). The reduction in P_s from basal levels did not differ ($P = 0.78$) between L-NMMA alone (Fig. 6A) and L-NMMA + ADO (Fig. 6B), indicating that ADO affects P_s via a NO-dependent pathway.

DISCUSSION

The present study represents an evaluation of the hypothesis that coronary microvessel exchange capacity adapts to endurance exercise training (EX) not by altering basal P_s , per se, but by elevating P_s on exposure to metabolites, such as ADO, that are produced by working muscle during the exercise bout. Consequently, we anticipated that the basal permeability properties of coronary exchange microvessels to macromolecules of EX pigs would be the same as their SED littermates and would not differ between males and females. Contrary to those predictions, we observed that coronary microvascular exchange adapted to EX pigs with a reduction in basal P_s in three of the four groups, and in venules from EX females, it was unchanged by training. As predicted, the overall response to ADO in EX microvessels was an increase in P_s to both the free fatty acid carrier, albumin, and the smaller protein, α -lactalbumin. The single exception was the venules from EX males where P_s^{PSA} was reduced. In addition, the sex-dependent responses of the venules of SED pigs persisted after and were not modified by EX training.

Overall, our data demonstrate that coronary microvascular exchange capacity is altered in adaptation to 16 wk of endurance exercise training. The mechanisms responsible for this adaptation depend, to varying extents, on microvessel location (arterioles vs. venules), as well as the sex of the adult animals used in this study.

Method Limitations and Assumptions

An assumption fundamental to the calculations of solute flux is that the isolated coronary microvessels are, during perfusion and determination of flux, circular in cross section and have a volume-to-surface area ratio of $0.25 D$ (Eq. 1). Preliminary work in this study, and detailed to a greater degree by studies of rat and mouse skeletal muscle preparations (53), suggests that the assumption of a circular cross section under the conditions of these experiments is valid for coronary arterioles. Venules, especially those in the coronary circulation, are less likely to have a circular cross section and instead are relatively flat such that the minor axis, b , is not equal to the measured major axis, D (31,58). The consequence of the noncircularity will be in the reporting of the absolute value of P_s rather than in the direction and/or the magnitude of the responses to test substances since the geometrical terms, in both the numerator and denominator, will be cancelled out (58). Thus it is possible, given the wider availability of techniques (e.g., confocal microscopy), to determine both axes in vivo with greater accuracy; hence, the absolute values of venule P_s will be lower than reported in this or previous studies (18,24,25,27,49,61).

Influence of Training Status and Sex on Basal Coronary Microvascular P_s to Macromolecules

Basal P_s^{PSA} of both arterioles and venules changed in adaptation to endurance exercise training, in a manner that was independent of the animal's sex. Basal P_s of arterioles from both males and females and of venules from males was reduced following 16 wk of EX relative to their SED counterparts. Contrary to our hypothesis, basal P_s of the arterioles from EX pigs was reduced relative to their SED counterparts. Similarly, in the venous coronary microvasculature, endurance exercise training resulted in a reduction in P_s (pooled data from the males and females) relative to comparable vessels from SED pigs. Thus, overall, coronary microvessels are less leaky in trained animals and represent an adaptation that clearly supports the maintenance of vascular volume in performance-adapted subjects.

Consistent with the conclusion that it is the venular elements of the coronary exchange microcirculation that are sex sensitive, we found that basal P_s^{PSA} of venules from the EX females was >80% higher than comparable venules taken from the hearts of males ($P < 0.05$). We found that venules from male EX animals were less than a third as leaky as all the other vessel groups: P_s of venules from females remained unchanged by training in contrast to the decrease in P_s in the venules from males. No other apparent features, including the diameters ($P = 0.73$), of the venules differed between groups. Although the mechanism(s) underlying the adaptation in P_s of venules of males remains to be determined, there are clear functional implications of these findings. Assuming that there are no sex-dependent differences in coronary network architecture, it is clear from the measured P_s values that net macromolecule flux will be greater in exercise-trained females compared with males under basal, nonexercising conditions. Given that there is literature (55,59) indicating that hydrostatic pressures are lower in the exchange microvasculature of females compared with age-matched males, the net effect may thus be to minimize differences in macromolecular flux between the sexes under resting conditions.

Training Status and Sex Alter the Coronary Microvascular Permeability Gradient to Macromolecules

In characterizing the permeability properties of vessels making up the exchange microvascular network, it is generally assumed that there is a gradient of permeability properties such that the vessels at the arterial, high-pressure end of the vascular network are tighter (less permeable) than those at the venous, low-pressure end of the network. In the present studies, this was true for three of the four experimental groups (SED females and males and EX females, independently of whether permeability increased or decreased on exposure to ADO). EX males, to our surprise, did not fit this paradigm. Two aspects of the data were notable. First, the basal atrioventricular (AV) gradient in the EX males did not reach significance before exposure to ADO ($P_s^{PSA} = 5 \pm 1$ in arterioles vs. $8.1 \pm 3 \times 10^{-7}$ cm/s in venules; $P = 0.08$). Second, the gradient was abolished, if not reversed, following ADO application ($P_s^{PSA} = 9.2 \pm 1$ in arterioles vs. $6.5 \pm 2 \times 10^{-7}$ cm/s in venules; $P = 0.10$). In both cases, the feature that distinguished EX males from the EX females and SED pigs of either sex was the fact that their coronary venules had permeability properties similar to arterioles, and in response to ADO, permeability fell from basal levels. Thus our study shows that, in EX males, it was the venules that adapted to become "tighter" compared with arterioles in the same network or to venules in EX female pigs. Clearly, it will be important to investigate the mechanisms underlying this unexpected adaptational response in the male animals.

A reduction in permeability on exposure to ADO has been observed in a variety of endothelial monolayer model systems (13,46), as well as in the isolated coronary arterioles from SED animals of both sexes (this study and Ref. 25), in isolated coronary venules from SED males (this study and Ref. 25) and also in skeletal muscle arterioles and venules isolated from juvenile female rats (60). We note that the AV gradient observed by Wang and Huxley (60) in the

skeletal muscle microvessels under basal conditions was abolished in the presence of ADO. As in the present study, this was a consequence of the P_s in the venous vessels being lower than expected rather than the P_s in arteriolar vessels being higher than expected. This ability for venous vessels to modulate their permeability by becoming “as tight as arterioles” provides support to the conclusion that mechanisms underlying venular responses differ significantly from those regulating an exchange across the walls of coronary arterioles.

We considered whether the absence of the AV gradient in the EX males might serve to limit solute flux across the intact microvasculature during EX. As was shown in Fig. 1, HR differs between EX females and males and, given that an exchange in the myocardium occurs primarily during diastole, we speculated that a reduction in venous permeability would help to limit net transport in the EX males relative to females. However, the training bradycardia was actually greater in the EX females than in the males during all stages of the performance test (Fig. 1); hence, we would expect overall solute flux to be greater in females also. Thus it remains to be determined whether adaptation to EX with respect to net coronary exchange involves more than the changes we have seen in microvessel barrier properties. Other candidates likely to contribute to the net flux of solute include differences in coronary microvessel anatomy, hence, surface area for exchange; differences in extracellular matrix composition, thereby, influencing both transfer and gradients within the matrix; lymphatic function, again influencing solute gradients in the compartment outside of the vascular space; and differences in microvascular hydrostatic pressures, thereby influencing convective transport of the macromolecules, to mention a few possibilities.

Our use of AC- and GC-dependent pathways to probe their adaptation in regulation of permeability responses indicated that both of these signaling pathways had been modulated in response to exercise training. Exchange capacity mediated by both pathways was attenuated in microvessels from exercise-adapted animals: determining whether this represents a change in expression of these signaling molecules per se, or some other adaptation of other elements in the response pathway, is beyond the scope of the present work. Importantly, our experiments also indicate that exercise adaptation involves an up-regulation of an NO-dependent pathway, which appears to contrast with our conclusion that GC-dependent pathways are downregulated but which further emphasizes that multiple mechanisms are involved in the regulation of barrier properties and have been modulated in a complex way in response to the exercise training regime.

The outcomes of these changes in microvascular exchange capacity on fluid volume distribution and hence cardiovascular homeostasis in intact exercising individuals are likely to be complex. Single bouts of intense exercise in healthy human adults have been shown to result in a retention of albumin in the vascular compartment that cannot be accounted for by changes in albumin metabolism per se (11,12). Plasma volume is reported to decrease in mixed (male and female) groups of exercising dogs (52) and humans (39); direct measurements of interstitial colloid osmotic pressure in the Mack et al. (39) study showed that this did not change, implicating decreased barrier function per se in the response. These studies are consistent with the decrease in P_s to ADO that we report here. In other work, Haskell et al. (14) reported that ANP levels in human subjects increased under the same exercise regime as in their earlier work, and they observed an increased transcapillary escape of albumin. From our results, we speculate that the extent to which ANP release during exercise could be the driving force for albumin flux out of the vasculature will be limited by an exercise-induced diminution in the response to ANP. Although both male and female subjects were included in the study of Mack et al. (39), the data are not broken out according to sex. We suggest that at least part of the variability in the findings of these studies will likely relate to the relative numbers of males and females in the study groups. Furthermore, because our data show differences in exchange function between different regions of the microvasculature (arterioles and venules), overall

fluid volume status such as relative hydration (with consequent effects on venous capacitance) will likely influence the integrative fluid volume distribution response to exercise.

In summary, we show in this study that in response to exercise training there is an overall reduction in basal permeability. In response to the metabolic product ADO, P_s is increased in arterioles, but there is a sex-dependent difference in the response of venules in that P_s in venules from males decreased and P_s in venules from females increased with ADO exposure. Regulation of permeability via both AC- and GC-dependent pathways was reduced, whereas NO-dependent regulation of permeability was upregulated. Overall, our study shows that the adaptation of microvascular exchange capacity to endurance exercise training involves multiple mechanisms that can differ in arterioles versus venules: their relative contribution to net flux is a function of sex.

Acknowledgements

We thank the members of Dr. Laughlin's team, particularly Pam Thorne, and Susan Bingaman and Steve Sieveking in the Huxley laboratory for providing outstanding technical assistance. This project could not have succeeded without their vigilant attention to detail with respect to the animals, assays, and equipment. We also appreciate the extensive discussions of the data and protocols with the members of the "Exercise PPG" led by Dr. Laughlin.

GRANTS

Support was derived over the tenure of these studies from National Heart, Lung, and Blood Institute Grants PO1-HL-52490, R01-HL-075186, R01-HL-078816, and R37-HL-42528 and National Aeronautics and Space Administration Grant NNJ05HF37G.

References

1. Adamson RH, Liu B, Fry GN, Rubin LL, Curry FE. Microvascular permeability and number of tight junctions are modulated by cAMP. *Am J Physiol Heart Circ Physiol* 1998;274:H1885–H1894.
2. Bindewald K, Gunduz D, Hartel F, Peters SC, Rodewald C, Nau S, Schafer M, Neumann J, Piper HM, Noll T. Opposite effect of cAMP signaling in endothelial barriers of different origin. *Am J Physiol Cell Physiol* 2004;287:C1246–C1255. [PubMed: 15475517]
3. Bingaman S, Huxley VH, Rumbaut RE. Fluorescent dyes modify properties of proteins used in microvascular research. *Microcirculation* 2003;10:221–231. [PubMed: 12700589]
4. Bowles DK. Gender influences coronary L-type Ca^{2+} current and adaptation to exercise training in miniature swine. *J Appl Physiol* 2001;91:2503–2510. [PubMed: 11717211]
5. Dunnett CW. Pairwise multiple comparisons in the homogeneous variance, unequal sample size case. *J Am Stat Assoc* 1980;75:789–795.
6. Dunnett CW. Pairwise multiple comparisons in the unequal variance case. *J Am Stat Assoc* 1980;75:796–800.
7. Fahim M, Hussain T, Mustafa SJ. Role of endothelium in adenosine receptor-mediated vasorelaxation in hypertensive rats. *Fundam Clin Pharmacol* 2001;15:325–334. [PubMed: 11903501]
8. Fredholm BB, IJzerman AP, Jacobson KA, Klotz KN, Linden J. International Union of Pharmacology. XXV Nomenclature and classification of adenosine receptors. *Pharmacol Rev* 2001;53:527–552. [PubMed: 11734617]
9. Fredholm BB, Arslan G, Halldner L, Kull B, Schulte G, Wasserman W. Structure and function of adenosine receptors and their genes. *Naunyn Schmiedeberg Arch Pharmacol* 2000;362:364–374. [PubMed: 11111830]
10. Games PA, Howell JF. Pairwise multiple comparison procedures with unequal n's and/or variances: a Monte Carlo study. *J Educ Stat* 1976;1:113–125.
11. Gillen CM, Lee R, Mack GW, Tomaselli CM, Nishiyasu T, Nadel ER. Plasma volume expansion in humans after a single intense exercise protocol. *J Appl Physiol* 1991;71:1914–1920. [PubMed: 1761491]

12. Gillen CM, Nishiyasu T, Langhans G, Weseman C, Mack GW, Nadel ER. Cardiovascular and renal function during exercise-induced blood volume expansion in men. *J Appl Physiol* 1994;76:2602–2610. [PubMed: 7928889]
13. Haselton FR, Alexander JS, Mueller SN. Adenosine decreases permeability of in vitro endothelial monolayers. *J Appl Physiol* 1993;74:1581–1590. [PubMed: 7685754]
14. Haskell A, Nadel ER, Stachenfeld NS, Nagashima K, Mack GW. Transcapillary escape rate of albumin in humans during exercise induced hypervolemia. *J Appl Physiol* 1997;83:407–413. [PubMed: 9262434]
15. Hempel A, Noll T, Muhs A, Piper HM. Functional antagonism between cAMP and cGMP on permeability of coronary endothelial monolayers. *Am J Physiol Heart Circ Physiol* 1996;270:H1264–H1271.
16. Hoyenga KB, Hoyenga KT. Gender and energy balance: sex differences in adaptations for feast and famine. *Physiol Behav* 1982;28:545–563. [PubMed: 7043508]
17. Huxley VH. Physiologic regulation of capillary permeability. *J Reconstr Microsurg* 1988;4:341–346. [PubMed: 2459381]
18. Huxley VH. What do measures of flux tell us about vascular wall biology? *Microcirculation* 1998;5:109–116. [PubMed: 9789252]
19. Huxley VH, Curry FE. Differential actions of albumin and plasma on capillary solute permeability. *Am J Physiol Heart Circ Physiol* 1991;260:H1645–H1654.
20. Huxley VH, Curry FE, Adamson RH. Quantitative fluorescence microscopy on single capillaries: α -lactalbumin transport. *Am J Physiol Heart Circ Physiol* 1987;252:H188–H197.
21. Huxley VH, Curry FE, Powers MR, Thipakorn B. Differential action of plasma and albumin on transcapillary exchange of anionic solute. *Am J Physiol Heart Circ Physiol* 1993;264:H1428–H1437.
22. Huxley VH, McKay MK, Meyer DJ Jr, Williams DA, Zhang RS. Vasoactive hormones and autocrine activation of capillary exchange barrier function. *Blood Cells* 1993;19:309–320. [PubMed: 8312566]
23. Huxley VH, Meyer DJ Jr. Atrial natriuretic peptide (ANP)-induced increase in capillary albumin and water flux. *Adv Exp Med Biol* 1988;242:23–31. [PubMed: 2977524]
24. Huxley VH, Rumbaut RE. The microvasculature as a dynamic regulator of volume and solute exchange. *Clin Exp Pharmacol Physiol* 2000;27:847–854. [PubMed: 11022981]
25. Huxley VH, Wang J, Whitt SP. Sexual dimorphism in the permeability response of coronary microvessels to adenosine. *Am J Physiol Heart Circ Physiol* 2005;288:H2006–H2013. [PubMed: 15563527]
26. Huxley VH, Williams DA. Basal and adenosine-mediated protein flux from isolated coronary arterioles. *Am J Physiol Heart Circ Physiol* 1996;271:H1099–H1108.
27. Huxley VH, Williams DA. Role of a glycocalyx on coronary arteriole permeability to proteins: evidence from enzyme treatments. *Am J Physiol Heart Circ Physiol* 2000;278:H1177–H1185. [PubMed: 10749712]
28. Huxley VH, Williams DA, Meyer DJ Jr, Laughlin MH. Altered basal and adenosine-mediated protein flux from coronary arterioles isolated from exercise-trained pigs. *Acta Physiol Scand* 1997;160:315–325. [PubMed: 9338512]
29. Ijima SC, Challiss RA, Boyle JP. Comparative effects of activation of soluble and particulate guanylyl cyclase on cyclic GMP elevation and relaxation of bovine tracheal smooth muscle. *Br J Pharmacol* 1995;115:723–732. [PubMed: 8548169]
30. Jones AW, Rubin LJ, Magliola L. Endothelin-1 sensitivity of porcine coronary arteries is reduced by exercise training and is gender dependent. *J Appl Physiol* 1999;87:1172–1177. [PubMed: 10484592]
31. Kassab GS, Lin DH, Fung YC. Morphometry of pig coronary venous system. *Am J Physiol Heart Circ Physiol* 1994;267:H2100–H2113.
32. Kassab GS, Rider CA, Tang NJ, Fung YC. Morphometry of pig coronary arterial trees. *Am J Physiol Heart Circ Physiol* 1993;265:H350–H365.
33. Langelier EG, van Hinsbergh VW. Norepinephrine and iloprost improve barrier function of human endothelial cell monolayers: role of cAMP. *Am J Physiol Cell Physiol* 1991;260:C1052–C1059.
34. Laughlin MH. Endothelium-mediated control of coronary vascular tone after chronic exercise training. *Med Sci Sports Exerc* 1995;27:1135–1144. [PubMed: 7476057]

35. Laughlin MH, McAllister RM, Jasperse JL, Crader SE, Williams DA, Huxley VH. Endothelium-mediated control of the coronary circulation. Exercise training-induced vascular adaptations. *Sports Med* 1996;22:228–250. [PubMed: 8898457]
36. Laughlin MH, Overholser KA, Bhatte MJ. Exercise training increases coronary transport reserve in miniature swine. *J Appl Physiol* 1989;67:1140–1149. [PubMed: 2551878]
37. Laughlin MH, Schrage WG, McAllister RM, Garverick HA, Jones AW. Interaction of gender and exercise training: vasomotor reactivity of porcine skeletal muscle arteries. *J Appl Physiol* 2001;90:216–227. [PubMed: 11133913]
38. Laughlin MH, Welshons WV, Sturek M, Rush JW, Turk JR, Taylor JA, Judy BM, Henderson KK, Ganjam VK. Gender, exercise training, and eNOS expression in porcine skeletal muscle arteries. *J Appl Physiol* 2003;95:250–264. [PubMed: 12626483]
39. Mack GW, Yang R, Hargens AR, Nagashima K, Haskell A. Influence of hydrostatic pressure gradients on regulation of plasma volume after exercise. *J Appl Physiol* 1998;85:667–675. [PubMed: 9688745]
40. McKay MK, Huxley VH. ANP increases capillary permeability to protein independent of perfusate protein composition. *Am J Physiol Heart Circ Physiol* 1995;268:H1139–H1148.
41. Meyer DJ Jr, Huxley VH. Capillary hydraulic conductivity is elevated by cGMP-dependent vasodilators. *Circ Res* 1992;70:382–391. [PubMed: 1310449]
42. Meyer DJ Jr, Huxley VH. Differential sensitivity of exchange vessel hydraulic conductivity to atrial natriuretic peptide. *Am J Physiol Heart Circ Physiol* 1990;258:H521–H528.
43. Mongillo M, Tocchetti CG, Terrin A, Lissandron V, Cheung YF, Dostmann WR, Pozzan T, Kass DA, Paolocci N, Houslay MD, Zaccolo M. Compartmentalized phosphodiesterase-2 activity blunts beta-adrenergic cardiac inotropy via an NO/cGMP-dependent pathway. *Circ Res* 2006;98:226–234. [PubMed: 16357307]
44. Neter, J.; Wasserman, W.; Kutner, M. *Applied Linear Statistical Models: Regression, Analysis of Variance, and Experimental Designs*. Homewood, IL: Irwin; 1990.
45. Olanrewaju HA, Mustafa SJ. Adenosine A_{2A} and A_{2B} receptors mediated nitric oxide production in coronary artery endothelial cells. *Gen Pharmacol* 2000;35:171–177. [PubMed: 11744240]
46. Richard LF, Dahms TE, Webster RO. Adenosine prevents permeability increase in oxidant-injured endothelial monolayers. *Am J Physiol Heart Circ Physiol* 1998;274:H35–H42.
47. Ruan Y, Kan H, Malik KU. Beta adrenergic receptor stimulated prostacyclin synthesis in rabbit coronary endothelial cells is mediated by selective activation of phospholipase D: inhibition by adenosine 3'5'-cyclic monophosphate. *J Pharmacol Exp Ther* 1997;281:1038–1046. [PubMed: 9190834]
48. Rumbaut RE, Harris NR, Sial AJ, Huxley VH, Granger DN. Leakage responses to L-NAME differ with the fluorescent dye used to label albumin. *Am J Physiol Heart Circ Physiol* 1999;276:H333–H339.
49. Rumbaut RE, Huxley VH. Similar permeability responses to nitric oxide synthase inhibitors of venules from three animal species. *Microvasc Res* 2002;64:21–31. [PubMed: 12074627]
50. Rumbaut RE, Sial AJ. Differential phototoxicity of fluorescent dye-labeled albumin conjugates. *Microcirculation* 1999;6:205–213. [PubMed: 10501094]
51. Sabrane K, Kruse MN, Fabritz L, Zetsche B, Mitko D, Skryabin BV, Zwiener M, Baba HA, Yanagisawa M, Kuhn M. Vascular endothelium is critically involved in the hypotensive and hypovolemic actions of atrial natriuretic peptide. *J Clin Invest* 2005;115:1666–1674. [PubMed: 15931395]
52. Sarelius IH. Plasma volume shifts during moderate exercise in splenectomized greyhounds. *J Physiol* 1979;292:391–405. [PubMed: 490369]
53. Sarelius IH, Kuebel JM, Wang J, Huxley VH. Macromolecule permeability of in situ and excised rodent skeletal muscle arterioles and venules. *Am J Physiol Heart Circ Physiol* 2006;290:H474–H480. [PubMed: 16126813]
54. Schulte G, Fredholm BB. Signalling from adenosine receptors to mitogen-activated protein kinases. *Cell Signal* 2003;15:813–827. [PubMed: 12834807]
55. Shore AC, Sandeman DD, Tooke JE. Capillary pressure, pulse pressure amplitude, and pressure waveform in healthy volunteers. *Am J Physiol Heart Circ Physiol* 1995;268:H147–H154.
56. Srere PA. Citrate synthase. *Methods Enzymol* 1969;13:3–5.

57. Tate CA, Holtz RW. Gender and fat metabolism during exercise: a review. *Can J Appl Physiol* 1998;23:570–582. [PubMed: 9822793]
58. Thipakorn, B. Defining the Cross-Section of the Fluorescence-Filled Capillaries from Profiles Viewed at Different Angles (PhD thesis). Columbia, MO: Univ. of Missouri; 1996.
59. Valensi P, Behar A, Attalah M, Cohen-Boulakia F, Paries J, Attali JR. Increased capillary filtration of albumin in diabetic patients—relation with gender, hypertension, microangiopathy, and neuropathy. *Metabolism* 1998;47:503–507. [PubMed: 9591738]
60. Wang J, Huxley VH. Adenosine A_{2A} receptor modulation of juvenile female rat skeletal muscle microvessel permeability. *Am J Physiol Heart Circ Physiol* 2006;291:H3094–H3105. [PubMed: 16815983]
61. Wang J, Whitt SP, Rubin LJ, Huxley VH. Differential coronary microvascular exchange responses to adenosine: roles of receptor and microvessel subtypes. *Microcirculation* 2005;12:313–326. [PubMed: 16020078]
62. Woodman CR, Muller JM, Laughlin MH, Price EM. Induction of nitric oxide synthase mRNA in coronary resistance arteries isolated from exercise-trained pigs. *Am J Physiol Heart Circ Physiol* 1997;273:H2575–H2579.

Fig. 1.

Assessment of endurance exercise training (EX) in age-matched Yucatan miniature swine of both sexes. **A:** resting heart rate (HR; beats/min) in naive [pretraining (Pre)] females (F) was higher than males (M) (** $P < 0.05$, EX F significantly different from EX M); following 16 wk endurance exercise training, HR fell [$*P < 0.05$, Pre $\neq$ posttraining (Post)], in both F and M pigs and the sex difference in HR was maintained. **B:** duration of exercise stress test administered to pigs before (Pre) initiation of training regime and following 16 wk of training (Post) for the EX M and F pigs. Although the training regime resulted in a significantly increased duration ($*P < 0.05$), there were no discernible sex differences. **C:** change in HR between the stress test administered prior (HR_{Pre}) and following (HR_{Post}) the 16-wk regime for the pigs at rest and the four stages of work (S1, 5 min at 5 km/h, 0% grade; S2, 10 min at 5 km/h, 10% grade; S3, 10 min at 6.9 km/h, 10% grade; and S4, 9.7 km/h, 10% grade until exhaustion). $*P < 0.05$, significant difference; $**P < 0.05$, EX F significantly different from EX M. SED, sedentary; bpm, beats/min.

Fig. 2.

Basal permeability (Basal P_s) to porcine serum albumin (PSA) is plotted (means $\pm$ SE) for arterioles and venules isolated from the hearts of EX female and male pigs. Basal P_s was lower in venules from the hearts of EX M than in EX F pigs ($*P < 0.05$). P_s of coronary venules was greater than those of the arterioles ($**P < 0.05$) whether the data were combined or separated by sex. The gray-shaded bars represent the mean data from SED littermates (25). Numbers in bars represent number of animals.

Fig. 3.

Concentration-response relationship of the permeability responses, ΔP_s , of isolated coronary arterioles from a mixed population of 7 (3 F and 4 M) EX pigs. The data (means $\pm$ SE) are plotted as the ratio of the P_s during suffusion with adenosine (ADO, in M) (P_s^{ADO}) relative to basal permeability to PSA ($P_s^{Control}$) over the range of 10^{-9} to 10^{-5} M. * $P < 0.05$, significant response ($P_s^{ADO}/P_s^{Control} \neq 1$). Comparison of the concentration-response for the age- and sex-matched SED pigs is given by the broken gray line (Ref. 25). N , number of hearts.

Fig. 4.

ΔP_s of arterioles and venules isolated from the hearts of EX pigs are plotted as the ratio of the P_s during suffusion with 10^{-5} M ADO (P_s^{ADO}) relative to basal permeability to PSA ($P_s^{Control}$). Data are given as means $\pm$ SE. * $P < 0.05$, significant response ($P_s^{ADO}/P_s^{Control} \neq 1$); ** $P < 0.05$, significant differences between females and males; *** $P < 0.05$, significant differences between arterioles and venules. The responses of the age- and sex-matched SED pigs (Ref. 25) are represented by the light gray bars.

Fig. 5.

The influence of exercise adaptation on responses thought to be mediated primarily by adenylyl cyclase (using isoproterenol; *A*) and by guanylyl cyclase [using atrial natriuretic peptide (ANP); *B*] was explored in arterioles isolated from the hearts of age- and sex-matched SED and EX pigs. Data are given as the means $\pm$ SE of the ratio of P_s to α -lactalbumin measured in the presence of the drug (P_s^{Test}) relative to its absence (P_s^{Control}). Ratios > 1 represent increases in P_s ; values < 1 indicate a reduction in P_s , with * $P < 0.05$, significant difference from 1; ** $P < 0.05$, significant difference with training status. *n*, Number of microvessels.

Fig. 6.

A role for a contribution of nitric oxide to the P_s response of female EX pig arterioles to ADO. *A*: a significant reduction ($*P < 0.05$) in P_s from basal levels is observed following blockade of nitric oxide synthase with 10^{-5} M NG -monomethyl-L-arginine (L-NMMA). *B*: in 10 additional arterioles, ADO first induces a significant increase ($*P < 0.05$) in P_s , which was then abolished by the presence of L-NMMA ($**P < 0.05$).

Table 1
Evaluation of training status: heart and body weights and citrate synthase

	Weight		N	Citrate Synthase Activity		N
	Heart, g	Body, kg		Muscularis Tibialis (Lateral Head)	Muscularis Deltoidens	
Females						
SED	162±3 *	35.6±0.7	57	14.3±.5 *	16.0±.5 *	35
EX	187±4	34.4±0.8	55	19.0±.8	21.4±.8	34
Males						
SED	167±6	33.2±2.1	19	13.9±.8	17.5±.8	18
EX	195±8 *	34.5±1.5	19	18.7±1.3 *	21.5±1.2 *	11

Values are means ± SE; N, number of hearts. SED, sedentary group; EX, exercise training group.

* $P < 0.05$, EX ≠ SED.