Aging and exercise training reduce testes microvascular O₂ and alter vasoconstrictor responsiveness in testicular arterioles

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Aging and exercise training reduce testes microvascular O₂ and alter vasoconstrictor responsiveness in testicular arterioles. Am J Physiol Regul Integr Comp Physiol 301: R801–R810, 2011. First published June 15, 2011; doi:10.1152/ajpregu.00203.2011.—Testicular function and associated testosterone concentration decline with advancing age, and an impaired O₂ supply may contribute, in part, to this reduction. We hypothesized that there would be a reduced microvascular O₂ (PO₂m) in the testes from aged rats, and this reduced PO₂m would be associated with impaired vasomotor control in isolated resistance arterioles. In addition, given the positive effect of exercise on microvascular PO₂ and arteriolar function, we further hypothesized that there would be an enhanced PO₂m in the testes from aged animals after aerobic exercise training. Testicular PO₂m was measured in vivo via phosphorescence quenching in young and aged sedentary (SED) and exercise-trained (ET; 15 min/mind treadmill walking, 15-degree incline, 5 days/ wk for 10 wk) male Fischer-344 rats. Vasoconstriction to α-adrenergic [norepinephrine (NE) and phenylephrine (PE)] and myogenic stimuli in testicular arterioles was assessed in vitro. In the SED animals, testicular PO₂m was reduced by ~50% with old age (aged SED 11.8 ± 1.9 vs. young SED 22.1 ± 1.1 mmHg; P = 0.0001). Contrary to our hypothesis, exercise training did not alter PO₂m in the aged group and reduced testicular PO₂m in the young animals, abolishing age-related differences (young ET 10.0 ± 0.8 vs. aged ET, 10.7 ± 0.9 mmHg; P = 0.37). Vasoconstrictor responsiveness to NE and PE was diminished in aged compared with young (NE: young SED, 58 ± 2 vs. aged SED, 47 ± 2%; P = 0.001) (PE: young SED, 51 ± 3 vs. aged SED, 36 ± 5%; P = 0.008). Exercise training did not alter maximal vasoconstriction in NE in young or aged groups. In summary, advancing age is associated with a reduced testicular PO₂m and impaired adrenergic vasoconstriction. The diminished testicular microvascular driving pressure of O₂ and associated vascular dysfunction provides mechanistic insight into the old age-related decrease in testicular function, and a reduced PO₂m may contribute, in part, to reduced fertility markers after exercise training.

vasoconstriction; fertility

CIRCULATING TESTOSTERONE CONCENTRATION decreases progressively with advancing age, resulting in an estimated 2–4 million hypogonadal men in the United States (59). This reduction of endogenous testosterone production with advancing age occurs in all male mammalian species, including humans (19, 28, 32) and rats (10, 53). Furthermore, testosterone has an inverse association with many deleterious symptoms of aging, e.g., low testosterone concentration in older men is correlated with higher body mass index and waist circumference (70), increased incidence of the metabolic syndrome (34, 39, 45), and increased prevalence of osteoporosis and fracture (20, 43) compared with men with normal or higher testosterone concentration.

Several possible local mechanisms exist that may contribute to the diminished steroidogenic function of the testes with advancing age, including 1) alterations in testes composition (i.e., seminiferous tubular narrowing, vacuolization of Sertoli cells, etc.) (for a review see Ref. 51); 2) impaired Leydig cell responsiveness to luteinizing hormone stimulation (46); 3) mitochondrial dysfunction (for a review see Ref. 1); and 4) atherosclerotic alterations in testicular arteries (63), the latter of which may affect perfusion. Furthermore, alterations in testicular blood flow or heterogeneities in perfusion with old age could critically alter the composition of the intratesticular fluid, thereby contributing to reduced testicular function (71, 72, 74). However, with advancing age, testicular perfusion has been shown to be either reduced (53, 86) or unchanged (56, 57) compared with younger counterparts.

Age-associated alterations within the testes resistance arteries (e.g., impaired vasoconstriction and/or reduced luminal diameter) or within the microvasculature [e.g., collapse of peritubular capillary networks (73)] may perturb the ability to match O₂ delivery (QO₂) to O₂ uptake (VO₂) within the testes. Based upon Fick’s law, an old age-related reduced QO₂-to-VO₂ ratio would reduce the microvascular PO₂ [PO₂m, which represents the driving force for transcapillary O₂ flux; (7)] in the testes, exacerbate perturbations to the intracellular milieu, and possibly contribute to a reduced steroidogenic function in the elderly population. It has been demonstrated that advancing age elicits QO₂-to-VO₂ mismatching within skeletal muscle, resulting in a reduced PO₂m (4, 26). Whether similar perturbations (i.e., reduced PO₂m and possible hypoxic regions) occur within the testes with old age remains to be determined.

Exercise training has numerous beneficial effects in improving cardiovascular function; however, there is debate regarding the effects of exercise training on testicular function. We have recently demonstrated that exercise training enhances PO₂m and improves arteriolar function in nonrecruited muscle (i.e., shows no net hyperemic response during exercise) from aged animals (41). Therefore, it is possible that exercise training manifests similar adaptations in PO₂m and vascular function in the testes of aged individuals. Using phosphorescence quenching and microscopy, we tested the hypotheses that advancing age will 1) reduce the matching of QO₂-to-VO₂ (demonstrated via diminished PO₂m in the testis and 2) alter vasomotor function (i.e., vasoconstriction) within the testicular resistance vasculature. Based upon the results of the first study, we conducted a second set of experiments to 1) determine whether chronic endurance exercise enhances testis PO₂m in the aged group and 2) characterize testicular vasoconstrictor responses
associated with exercise training in both young and aged rats. Results from the present investigation have the potential to elucidate mechanisms that contribute to the age-associated decline in testosterone and fertility.

MATERIALS AND METHODS

All procedures performed herein were approved by the University of Florida Institutional Animal Care and Use Committee.

Animals

Forty-one young (6-mo-old) and 38 aged (24-mo-old) male Fischer-344 rats were obtained from the National Institute on Aging colony. All rats were housed in a temperature-controlled (23 ± 2°C) room with a 12:12-h light-dark cycle. Water and rat chow were provided ad libitum.

Endurance Exercise Training

Young and aged rats were randomly assigned to either a sedentary control (SED) group or an exercise-trained (ET) group. ET rats were habituated to treadmill exercise, during which each rat walked on a motor-driven treadmill at 15 m/min (0-degree incline), 5 min/day for 3 days. After the habituation period, the incline was raised to 15-degrees for the duration of the training period, while the 15 m/min speed was maintained. During the first 5 wk of training, the time of exercise was increased by 10 min/wk, until 60 min duration was reached by the 6th wk. The ET rats continued to exercise 5-days/wk for 60 min/day for the remainder of the 10- to 12-wk training period as previously described (17). Due to the time needed to complete data collection on the isolated microvessels, two animals were used each day for these studies. Therefore, the initiation of the training protocol was offset to ensure that each animal completed the same amount of exercise training prior to data collection. Vascular responses were determined at least 24 h after the last exercise bout in ET rats. To determine the efficacy of the training protocol, citrate synthesize activity, a measure of muscle oxidative capacity (15), was measured in the soleus muscle.

Phosphorescence Quenching

Phosphorescence quenching was used to measure microvascular PO2 in young SED (n = 9), young ET (n = 6), aged SED (n = 6), and aged ET (n = 6) animals. Rats were anesthetized with pentobarbital sodium (40 mg/kg ip, supplemented as needed) and the right carotid artery was isolated. The artery was cannulated with a fluid-filled polyethylene catheter (PE-50) to monitor arterial blood pressure and heart rate (model 400a Digi-Med BPA; Micro Med, Louisville, KY) for the duration of the experiment. This fluid-filled catheter was also used for the infusion of the phosphorescent probe. Rectal temperature was monitored and maintained at 37–38°C with a heating pad.

The rat was secured in a supine position, and the testes were exposed. Specifically, the scrotal sack was palpated to locate the testes. A longitudinal incision (~2 cm) was made through the scrotum. The skin and fascia not intimately connected to the testes was retracted to expose the ventral aspect of the testis. The phosphorimeter probe was positioned ~2 mm above the testis, along the midline, so as to avoid the involvement of the surrounding glandular and epididymal structures during PO2 measurements. The testes were kept moist using a Krebs-Henseleit, bicarbonate-buffered solution equilibrated with 5% CO2-95% N2 at 37°C during a 10-min stabilization period following exposure and prior to infusion of the phosphorescent probe. The phosphor, palladium meso-tetra-(4-carboxyphenyl)-porphin dendrimer (R2; Oxygen Enterprises, Philadelphia, PA) was infused at a dose of 15 mg/kg through the arterial cannula, and PO2m measurements were recorded every 2 s for 60 s for an average baseline PO2m and completed in < 5 min after infusion of the R2. All PO2m measurements were performed in a dark room to avoid ambient light contamination. Upon completion of the experiment, each rat was killed with an overdose of anesthesia (pentobarbital sodium, >80 mg/kg ia), and a thoracotomy was performed to visually verify cardiac arrest.

PO2m Measurements and Calculations

The PMOD 5000 Frequency Domain Phosphorimeter (Oxygen Enterprises) light guide contained within the probe focused excitation light (524 nm) on the medial region of the exposed testis (~2.0 mm diameter, to ~500 μm deep). The PMOD 5000 uses a sinusoidal modulation of the excitation light (524 nm) at frequencies between 100 Hz and 20 kHz, which allows phosphorescence lifetime measurements from 10 μs to ~2.5 ms. In the single frequency mode, 10 scans (100 ms) were used to acquire the resultant lifetime of the phosphorescence (700 nm) and repeated every 2 s (for a review see Ref. 81). Phosphorescence lifetime was computationally obtained based on the decomposition of data vectors to a linearly independent set of exponentials (82).

The Stern-Volmer relationship allows the calculation of PO2m, from a measured phosphorescence lifetime using the following equation (61): PO2m = [(τ0/τ)−1](k0/τp), where k0 is the quenching constant (mmHg/s) and τ0 and τ are the phosphorescence lifetimes in the absence of O2, and at the ambient PO2, respectively. As the testes temperature averages 34 ± 1°C, for R2 in vitro conditions similar to those found in the blood of the testis, k0 is 345 mmHg/s and τ0 is 640 μs (77). R2 is tightly bound to albumin in the plasma and is negatively charged. These properties, in combination with the short time between R2 infusion and measurement (~5 min), ensure that the PO2 measurements emanate from the plasma within the microvasculature rather than the surrounding muscle tissue (55). Phosphorescence lifetime is insensitive to probe concentration, excitation light intensity, and absorbance by other chromophores in the tissue (61). Effects of pH and temperature are negligible within the normal physiological range, which was maintained herein (50, 77).

Testis Microvessel Preparation

Vasomotor responses were determined in the testis from young SED (n = 15), young ET (n = 11), aged SED (n = 19), and aged ET (n = 7) rats. All rats were anesthetized with isoflurane (5%/oxygen) and euthanized by incising the heart. Testes were removed and placed in cold (4°C) physiological saline solution (PSS). By using a dissecting microscope (Olympus SVH10), the dorsal aspect of the testicular tunica albuginea was cut longitudinally to expose the testicular parenchyma. Arteries with a length of ~1 mm were selected for vasomotor assessment after satisfying the following criteria: 1) downstream of the subcapsular artery, 2) within the testicular parenchyma, and 3) ~150 μm in diameter. Additionally, in aged animals, arterioles were only taken from testis that displayed a normal phenotype (e.g., devoid of adenomas). Arterioles were cleared of surrounding tissue and transferred to a Lucite chamber containing PSS equilibrated with room air as described previously (44). Each end of the vessel was cannulated with a glass micropipette (30- to 50-μm diameter tip) and secured with 11-0 nylon monofilament suture (Alcon Laboratories; Fort Worth, TX). The microvessel chamber was transferred to the stage of an inverted microscope (Olympus IX70) equipped with a video camera (Panasonic BP310), video caliper (model 307A; Colorado Video, Boulder, CO), and data acquisition system (PowerLab; ADInstruments, Australia) for the measurement and recording of intraluminal diameter. Vessels were pressurized to 70 cmH2O and verified to be free of leaks by closing both reservoirs, ensuring the maintenance of steady diameter. Arterioles were then warmed to 34°C and allowed to develop spontaneous tone during a 60 min equilibration period.

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Myogenic Vasoconstrictor Responses

Active diameter changes were evaluated as follows: intraluminal pressure was lowered to 0 cmH2O for 10 min and subsequently increased in 15 cmH2O increments at 5-min intervals up to 135 cmH2O. Intraluminal diameter was recorded for each pressure at the end of each 5-min period. Buffer solution was then replaced with a Ca2+-free PSS solution, and following a 60-min equilibration period, passive diameter changes were obtained using the same pressure intervals described above.

Adrenergic Vasoconstrictor Responses

To determine the effects of age and exercise training on vasomotor function in the testis, arteriole responses were evaluated following the cumulative addition of the adrenergic receptor agonists norepinephrine (NE) and phenylephrine (PE). Because adrenergic stimulation can result in both α-receptor-mediated vasoconstriction and vasodilatation, as well as β-receptor-mediated vasodilatation, we further delineated signaling pathways after a 30-min incubation with propranolol (10-5 M), a nonspecific β-adrenergic inhibitor, and Nω-nitro-L-arginine methyl ester (L-NAME; 10-5 M), a nonspecific nitric oxide (NO) synthase (NOS) inhibitor, followed by the cumulative addition of NE. Preliminary studies were conducted to ensure that vascular responses to NE were not altered by multiple dose-response tests. At the end of each experiment, maximal diameter was determined in Ca2+-free PSS.

Citrate Synthase Activity

To confirm the efficacy of the exercise training protocol, the soleus muscle from each rat was used to determine citrate synthase activity, a measure of muscle oxidative capacity, as described by Srere (69). Briefly, homogenized samples in 300 µl aliquots at 30°C were measured spectrophotometrically in duplicate using a Spectramax M5 microplate reader ( Molecular Devices; Sunnyvale, CA) in 300 µl aliquots at 30°C. Citrate synthase activity was expressed as micromoles per minute per gram wet weight.

Chemicals

All chemicals were purchased from Sigma-Aldrich (St. Louis, MO). PSS buffer contained (in mM): 145 NaCl, 4.7 KCl, 1.2 NaH2PO4, 1.17 MgSO4, 2.0 CaCl2, 5.0 glucose, 2.0 pyruvate, 0.02 EDTA, and 3.0 MOPS at a pH of 7.4. Ca2+-free PSS buffer was similar to the PSS buffer, except it contained 2 mM EDTA, and CaCl2 was replaced with 2.0 mM NaCl.

Data Presentation and Analysis

Measured intraluminal diameters are expressed as a percentage of maximal possible vasoconstriction according to the following formula: vasoconstriction (% maximal response) = (Dm - Ds)/Dm × 100, where Dm is maximal inner diameter obtained in Ca2+-free PSS.

Active and passive diameter changes to altered intraluminal pressure were normalized according to the formula: normalized diameter = (Ds/Dm), where Ds is the steady-state diameter measured after each incremental pressure change. The data are expressed as normalized diameter to account for any differences in vessel size between experimental groups.

The agonist concentration that produced 50% of the maximal vasoconstrictor response is referred to as EC50. All EC50 values were converted to log values for statistical comparison. A statistical power analysis (Sigma Stat 4.0; Systat Software) was performed to estimate the sample size needed with the given statistical analyses. P0.2m and vessel characteristics were analyzed with a two-way ANOVA to test for differences. Repeated-measures ANOVA was used to determine differences among dose-diameter responses in isolated vessels to detect differences within (dose) and between (age and exercise training) factors. Post hoc analysis was performed by Duncan’s multiple range test to determine the significance of differences among means. All data are presented as mean ± SE. Significance was set at P ≤ 0.05.

RESULTS

Animal Characteristics

Advancing age was associated with increased body mass and atrophy of the testes, and exercise training reduced body mass in aged animals versus SED counterparts (Table 1). The efficacy of the training protocol was confirmed via elevated citrate synthase activity of the soleus muscle from both young and aged ET rats compared with their SED counterparts [young SED, 20 ± 1; young ET, 29 ± 1 (P = 0.003) and aged SED, 18 ± 1; aged, ET 23 ± 1 (P = 0.02) µmol-min−1·g wet weight−1].

Testis Microvascular Po2

Mean arterial pressure did not differ between groups (young SED, 100 ± 14; young ET, 103 ± 11; aged SED, 95 ± 13; aged ET, 96 ± 12 mmHg; P > 0.05) and remained stable throughout the data collection period. Fig. 1 illustrates representative Po2m profiles for the groups, and the mean Po2m data is demonstrated in Fig. 2A. Oscillations in Po2m, were observed in both young groups and in the aged ET animals, whereas the aged SED group did not demonstrate Po2m oscillations. The frequency of the oscillations was significantly different between age groups (Fig. 2B). Resting Po2m varied substantially between young and aged SED animals as well as between SED and ET animals in the young but not aged group (Figs. 1 and 2A). In the SED groups, testis Po2m was reduced with aging by ~50% versus young counterparts. In the young group, exercise training significantly reduced Po2m by ~55% versus SED counterparts (Fig. 2A). Exercise training did not affect the

Table 1. Characteristics of testicular arterioles from young and aged, sedentary (SED) and exercise trained (ET) rats

<table>
<thead>
<tr>
<th>No. Rats</th>
<th>Body Weight, g</th>
<th>Testis Mass, mg</th>
<th>Maximum Diameter, µm</th>
<th>Tone, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young SED</td>
<td>15</td>
<td>369 ± 5</td>
<td>1490 ± 30</td>
<td>170 ± 10</td>
</tr>
<tr>
<td>Young ET</td>
<td>11</td>
<td>378 ± 9</td>
<td>1480 ± 100</td>
<td>161 ± 7</td>
</tr>
<tr>
<td>Aged SED</td>
<td>19</td>
<td>462 ± 14#</td>
<td>950 ± 100#</td>
<td>153 ± 7†</td>
</tr>
<tr>
<td>Aged ET</td>
<td>7</td>
<td>434 ± 9*#</td>
<td>920 ± 100#</td>
<td>133 ± 8*#</td>
</tr>
</tbody>
</table>

*#P ≤ 0.05 vs. corresponding young group; *P ≤ 0.05 vs. aged-matched sedentary group; †P < 0.10 vs. corresponding young group.
average PO$_2$m in the aged group (Fig. 2A). Age-related differences in testis PO$_2$m were abolished after exercise training.

**Isolated Microvessel Data**

Vessel characteristics are detailed in Table 1. In the aged SED group, there was a trend for a reduced maximal luminal diameter ($P = 0.07$) and an enhanced spontaneous tone ($P = 0.08$) versus young SED. Exercise training did not affect maximal diameter or spontaneous tone in the young group. In the aged group, exercise training resulted in a significantly smaller maximal diameter as well as a greater amount of spontaneous tone (Table 1).

**Myogenic vasoconstriction.** Fig. 3 illustrates active and passive diameter changes as a function of intraluminal pressure. Both young and aged rats displayed active myogenic vasoconstriction and no differences were observed between young and aged SED groups for active or passive diameter changes.

**Vasoconstrictor Responses to Adrenergic Stimulation**

**SED group.** There was a diminished testicular arteriolar vasoconstriction in the aged SED compared with young SED (Fig. 4A) to NE. Inhibition of β-adrenergic and NOS signaling exacerbated the age-associated vasoconstrictor diminution (Fig. 5) and reduced the sensitivity to NE in aged compared with young animals (i.e., increased EC$_{50}$ young SED, 2.06E$^{-7} \pm 4E^{-8}$; aged SED, 9.67E$^{-7} \pm 2E^{-7}$, $P = 0.003$). In young SED animals, simultaneous inhibition of NOS and β-adrenergic signaling increased vasoconstriction in response to NE (Fig. 5). In aged SED animals, combined inhibition of NOS and β-adrenergic signaling did not affect vasoconstriction in response to NE (Fig. 5). Vasoconstrictor responsiveness to the α$_1$-receptor agonist PE was greater in young SED compared with aged SED (Fig. 4B).

**ET group.** The age-associated diminished vasoconstriction in response to NE persisted after ET (Fig. 6). Contrary to that observed in the young SED group, combined inhibition of β-adrenergic and NOS signaling did not affect maximal vasoconstriction in the young ET group (Fig. 7). In addition, combined NOS and β-adrenergic inhibition did not affect vasoconstriction in response to NE in the aged group after exercise training (Fig. 7). The vasoconstrictor diminution in response to PE with advancing age remained after ET (data not shown).

**DISCUSSION**

To our knowledge this is the first study to demonstrate that old age reduces testis microvascular PO$_2$ and diminishes adrenergic vasoconstrictor responsiveness of the resistance vasculature. Specifically, with advancing age, testis PO$_2$m was reduced by ~50% versus that measured in the young group. Furthermore, there was a blunted vasoconstriction with old age to both NE and PE in isolated resistance arterioles from the testis. The greater adrenergic vasoconstriction in the young group suggests an enhanced sympathetic control of the resistance vasculature that may result in a better ability to precisely
set precapillary resistance and match O₂ delivery to metabolic demand. Since we have previously demonstrated that exercise training can enhance PO₂m in aged muscle (41), we conducted a second set of experiments to investigate the effects of aerobic exercise training on testis PO₂m and vascular reactivity with advancing age. Contrary to our hypothesis, exercise training did not enhance PO₂m nor affect adrenergic vasoconstriction in the aged group. In addition, exercise training significantly reduced PO₂m in the young group and abolished age-related differences. The low PO₂m observed in the testis of the old age group (as well as the young ET group) could result in a diffusion limitation of O₂ transport from the microvasculature to the testis mitochondria and potentially hypoxic regions of the testis, which may affect testicular function. These results provide further insight into mechanisms of age-associated decreases in testosterone production and fertility.

**Aging and Testis PO₂**

It has been demonstrated that structural (8) and functional (3, 44) arteriolar adaptations within skeletal muscle with advancing age compromise the ability to match O₂ delivery to O₂ uptake, resulting in a resting PO₂m, ~25% less than measured in young healthy muscle (4, 5). In the present study, aging similarly decreased resting PO₂m in the testis versus that measured in the young group (Figs. 1 and 2A). A significant reduction in the testis was more substantial (~50%) than observed in aged skeletal muscle. Whereas few studies have examined testis PO₂m in young subjects, our knowledge on the effects of advancing age on either testis microvascular or interstitial PO₂m.

In young subjects, the interstitial PO₂ of the testis ranges from 11 to 15 mmHg (22, 37, 40), which, given the PO₂m of the young group in the present study (22.1 ± 1.1 mmHg), would...
result in an adequate microvascular-to-interstitial diffusion gradient for transcapillary O2 flux. However, with old age, PO2m was reduced to 11.8 ± 1.9 mmHg (Fig. 2A), which is comparable to (or below) interstitial PO2 values reported for young testis and would be disadvantageous to red blood cell-mitochondrial O2 transfer according to Fick’s law. Specifically, as O2 flux from the microvasculature to the interstitium requires a pressure gradient for diffusion, the interstitial PO2 in the aged animals would have to be considerably lower (and possibly anoxic in some regions) than that within the microcirculatory exchange vessels to facilitate the diffusion of O2. Pertinent to this issue, hypoxia has been demonstrated to reduce fertility in male rats (23, 35), monkeys (64), and men (47), and both luteinizing hormone (60, 66) and serum testosterone (65) concentrations are lower in patients exposed to hypoxia as well as in aged individuals (19). It should be noted that a reduced O2 supply to young testicular tissue elicits similar changes in steroid patterns as that observed normally with old age (54), suggesting a diminished O2 supply as a possible mechanism for the age-related decline in testicular function.

In the present study, oscillations were observed in the PO2m profile from the young SED group (Fig. 1A), with a mean frequency of 11.3 ± 1.2 cycles/min (Fig. 2B) and an amplitude of 2.6 ± 0.9 mmHg (~11% of the mean). Lysiak et al. (37) reported similar oscillations in rat testes interstitial PO2 with a temporal variation of ~12 cycles/min, resulting from oscillations in microvascular blood flow presumably due to vasomotion. In the aged SED animals, oscillations in PO2m were abolished (Fig. 1B), and exercise training reestablished PO2m oscillations in the aged group, although with a temporal pattern (~4 cycles/min) much slower than either young SED or exercise training responses (Fig. 2B). In addition to an exceedingly low PO2m, the young ET group demonstrated several oscillations of greater relative magnitude (~40% of the mean), resulting in very low PO2m values (i.e., 5–6 mmHg) (Fig. 1A). Age-related alterations in testis vascular function (Figs. 4 and 5) likely contribute to these aberrant PO2m oscillations. Indeed, pathological conditions that elicit arteriolar dysfunction (e.g., type I and II diabetes) result in similar deviations in PO2m oscillations, i.e., transient reductions in PO2m of greater magnitude (6, 48). Vasomotion within the testis is thought to be essential for normal function; however, the precise mechanisms for the vasomotion are not entirely understood. In addition to hormonal (12, 84) and developmental (13) factors, the vasomotion may reflect the latency period between production of metabolic signals in the large extravascular space of the testis and the resultant response (i.e., vasoconstriction or dilation) of the resistance vasculature. Given the juxtaposition of blood vessels to the seminiferous tubules, it is possible that alterations in vascular function with aging (Figs. 4 and 5) and/or exercise training (Figs. 6 and 7) may contribute to the lack of, or discrepancies in, PO2m oscillations observed in the present study.

Regulation of QO2 and VO2 in the Testis with Advancing Age

Phosphorescence quenching (61) is a powerful tool that permits quantification of the local QO2-to-VO2 relationship through the measurement of PO2 in microcirculatory exchange vessels (42). So why is testis PO2m reduced to such a degree with advancing age? The lower PO2m can result from either a reduced O2 supply or an increased O2 uptake in the aged testis. The latter seems unlikely, as aging decreases the number and volume of mitochondria in Leydig cells (49) and decreases testicular mitochondrial function (80) due, in part, to enhanced free radical generation (79). Nonetheless, if the VO2 is estimated according to the methods of Behnke et al. (2) in the young and aged sedentary testis using the PO2m values measured herein and published resting testis blood flow in the rat (16) there is a slightly elevated VO2 in the aged testis. Specifically, the aged SED group has an estimated testis VO2 of 3.75 ml O2·min⁻¹·100 g⁻¹ vs. 3.40 ml O2·min⁻¹·100 g⁻¹ in the young SED group. This slight increase in testis VO2 with
age is a surprising finding as the primary metabolic function of the testis is spermatogenesis, which is reduced with advancing age (49). In the aged testis the increased VO2 may reflect mitochondrial dysfunction (e.g., increased H+ leak) and/or a reduced NO inhibition of VO2 versus an enhanced spermatogenesis, which seems unlikely.

There is considerable evidence for a reduced testis O2 supply with advancing age, including structural changes to the testis vasculature, such as arteriolar lumen intima fibrosis (29), 2) hyalinosi (25), 3 sclerosis (58), and 4) vasomotor dysfunction (Figs. 4 and 5), as well as the collapse and regression of the peritubular capillary network (73), which likely culminate in an enhanced vascular resistance. Indeed, laser Doppler flow measurements indicate increased vascular resistance in the testis of aged compared with young men (86) and Pirke et al. (53) demonstrate a reduced capillary blood flow in aged rats compared with young (see Ref. 56 for an exception). Furthermore, it has been speculated that even in young healthy individuals the testis operate on the verge of anoxia as interstitial P02 is dependent upon blood flow and the testis have little capacity to augment total blood flow (67). Therefore, age-related increases in testis vascular resistance (86) and reduced P02m (Fig. 2A) may have important consequences as the decreased O2 supply may not be adequate to support the metabolic processes of spermatogenesis.

Vasomotor Control in the Testis Resistance Vasculature

Testicular blood flow is predominately controlled through local (e.g., myogenic) and systemic (e.g., autonomic) mechanisms to regulate the transport of key nutrients. With advancing age there appear to be functional alterations within either local or systemic mechanisms regulating testis vascular tone as Ramsey et al. (57) demonstrated an old age-associated inability to increase testicular vascular resistance during an orthostatic challenge. The findings of Ramsey et al. (57) suggest an impaired myogenic autoregulation and/or adrenergic vasoconstriction within the testis resistance vasculature. In the present study, we did not observe a difference in myogenic vasoconstriction of the testis arterioles with advancing age (Fig. 3). We are unaware of any study that measured myogenic autoregulation in the testis arterioles; however, Davis (14) investigated pressure-diameter responses in larger testicular subcapsular arteries (~450 μm diameter) of young rats. It is difficult to compare the magnitude of responses between the present (Fig. 3) and the former study, as Davis (14) reported neither normalized diameter nor maximal diameter (in the presence of a Ca2+-free medium).

We did observe a diminished α-adrenergic vasoconstriction in the testis arterioles with advancing age (Figs. 4, A and B). In an effort to normalize arterial pressure in the face of increased sympathetic outflow, our findings that arterial vasoconstrictor responses to α-adrenergic stimulation are attenuated in old compared with young males (Figs. 4–7) are compatible with previous reports (27). Despite elevated levels of sympathetic nerve activity with age (2, 31, 52), previous studies have identified an age-associated desensitization of arterial vasoconstriction to adrenergic stimulation in both cardiac (52) and skeletal muscle (27) as well as a reduced sympathetic innervation of the spleen (9). It is not known whether similar compensatory mechanisms occur in the testicular vasculature, and future studies are required to further address whether an impaired adrenergic responsiveness in the testicular vasculature contributes to a reduced steroidogenic function in the elderly.

Simultaneous NOS and β-adrenergic inhibition increased responsiveness to NE compared with NE in the absence of inhibitors in young SED but not aged SED rats (Fig. 5). As NO has been implicated in inhibiting adrenergic vasoconstriction in animals (11, 33) and humans (18, 68), a reduced bioavailability of NO in the aged animals would have a diminished modulatory role on adrenergic vasoconstriction. Therefore, the lack of change in maximal vasoconstriction with simultaneous NOS and β-adrenergic inhibition is likely evidence of an impaired NOS-mediated endothelial α2-adrenergic receptor pathway in aged animals (78). Consonant with the results from Fig. 4, young SED rats displayed greater vasoconstriction to the α1-adrenergic agonist PE. Thus, the etiology of the vasoconstrictor defect with advancing age in testicular arterioles appears to manifest from the α1-adrenergic receptor pathway.

Effects of Exercise Training on Testis P02m and Vasomotor Control

In the present study, aerobic exercise training was used as an intervention that we hypothesized would enhance P02m and improve arteriolar function in the aged testis. Contrary to our hypothesis, chronic exercise did not enhance P02m nor arteriolar function in the aged testis. Furthermore, exercise training significantly reduced P02m (Fig. 2A) and the relative contribution of α- and β-adrenergic vasodilator mechanisms (Fig. 7) to the NE response in the young testis. During exercise testis blood flow is reduced by ~60% versus resting values with an approximately threefold increase in vascular resistance (21), which would result in a large decrease in intraluminal shear stress and may contribute to the reduced contribution of adrenergic vasodilator mechanisms to vascular tone after training (Fig. 7). Furthermore, a reduction in testis blood flow (and thus O2 supply) of this magnitude during exercise may result in reduced testicular function. Indeed, Hu et al. (30) have demonstrated that serum testosterone is reduced during exercise whether performed in normoxic or hypobaric hypoxia environments. The latter suggests exercise may decrease O2 supply to the testes below a given threshold that affects testicular function and further exposure to hypoxia does not result in additional declines in testosterone production. Contrary to a diminished O2 delivery, an elevated V02 may result in an increased O2 extraction (and therefore lower P02m) in the young group after exercise training. Although this seems unlikely as the main role of the testis mitochondria is spermatogenesis, which is depressed following exercise training (62, 76, 77), it does warrant further investigation.

Experimental Considerations

In the present study, we utilized the technique of phosphorescence quenching, which, depending on the duration of the protocol and the specific vascular permeability of the measured tissue, can measure either microvascular or interstitial PO2. In skeletal muscle, the PO2 measured with phosphorescence quenching techniques reflects that from the microvasculature (55). However, within tissues that have a low albumin reflection coefficient, the compartmentalization of the phosphores-
cent probe is more variable. To minimize any signal that may arise from extravasation of the probe in the present study, PO2 measurements were completed within 5 min of systemic infusion of the phosphorescent probe. If there was a significant extravasation of the probe, PO2 values would decrease over the measurement period with an increasing proportion of the signal emanating from the interstitium. In addition, considering the sparse vascular space of the testis, a significant extravasation would be visually detectable (i.e., a red tint), which was not observed. Indeed, both the signal amplitude from the PMOD 5000 and the PO2 were stable throughout the measurement period, which indicates that the PO2 signal emanated from the microvasculature.

In the present study, we did not measure plasma testosterone or reproductive function in these animals. It should be noted that accumulating evidence suggests chronic exercise results in lower testosterone (24, 30, 75, 83, 85) and cortisol (77) concentrations, with testosterone concentration being inversely related to training volume (38). Furthermore, endurance exercise deleteriously affects seminal parameters (i.e., reduced sperm count/concentration and motility) (62, 76, 77) (for an exception see Ref. 36). However, it remains to be determined how long-term aerobic exercise training affects reproductive function or testosterone concentration with advancing age.

Conclusion

This study is the first to demonstrate an old age-associated reduction in testis PO2m, and a diminished vasomotor responsiveness of the resistance vasculature. These data suggest that, with advancing age, there is an inherent inability to modulate precapillary resistance to match precisely QO2-to-VO2. Furthermore, we anticipated that the age-related impairments in the QO2-to-VO2 relationship would be ameliorated following aerobic exercise training. However, aerobic exercise training did not improve PO2m in aged testes and actually decreased PO2m in young testes. The age-associated reduction in testicular PO2m and the associated vascular dysfunction may facilitate the generation of an anoxic environment and a consequent reduction in testicular health and function. Whether exercise-induced reductions in testicular PO2m have the potential to further influence steroidogenic function and reduced fertility in the testes, remains to be determined.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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