Short-term regular aerobic exercise reduces oxidative stress produced by acute in the adipose microvasculature

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CARDIOVASCULAR DISEASE (CVD) is the number one cause of death in the United States (35). Hypertension is the number one risk factor for CVD (35), and endothelial dysfunction also precedes the development of CVD (23). However, the temporal relation between high blood pressure and endothelial dysfunction has not been not fully elucidated (7, 38, 42). Regular aerobic exercise lowers the risk of CVD and is beneficial for blood pressure and endothelial cell health (45). However, acute aerobic exercise bodes paradoxically increase the risk of cardiovascular events in sedentary individuals, in part through a transient increase in blood pressure and endothelial dysfunction (2, 9, 15). Extensive work characterizing subcutaneous adipose resistance arteries has been done in humans (22, 24, 40), demonstrating that endothelium-dependent vasodilation in these arteries correlates with clinical measures of peripheral vascular function (6), that high intraluminal pressure elicits endothelial dysfunction in these arteries (9, 40), and that regular exercise may be protective against said endothelial dysfunction (16, 40). High blood pressure is associated with excessive oxidative stress in the endothelium. Primary sources of oxidative stress include NADPH oxidase II (NOX II) and mitochondrial ROS. The local renin-angiotensin system (RAS) plays a role in both of these pathways (8, 11, 46, 47). In adipose resistance arteries, blockade of the local RAS through angiotensin type 1 receptor (AT1R) blockade or angiotensin-converting enzyme (ACE) inhibition prevents both increased superoxide (O2−) levels and impairment of endothelium-dependent dilation after high pressure (11). Uncoupled endothelial nitric oxide synthase (eNOS) in the presence of tetrahydrobiopterin oxidation is also a source of superoxide (28). Recent findings suggest that exercise leads to an increase in antioxidant mechanisms that prevent oxidative stress and endothelial dysfunction in the face of high pressure (9, 40).
is viscosity (0.015 g·cm⁻¹·s⁻¹). Q is the volumetric flow rate \([\Delta P \times r^4]/(8\pi \times l)\], where \(l\) is length, \(r\) is the internal radius of the vessel (in \(\mu\)m converted to \(cm\)), and \(\Delta P\) is the change in the pressure gradient (in \(cm\)H₂O converted to \(Pa\)).

**Methods**

**Exercise protocol.** Twelve- to sixteen-week-old male C57BL/6J mice (obtained from Jackson Laboratory, Bar Harbor, ME) were used for this study. Mice ran on an exercise wheel (12.7-cm diameter) placed inside their cage for 2 wk. The wheels were instrumented with a tachometer, composed of a ring magnet on the wheel axle and a Hall effect sensor (magnetic flux sensor) on the wheel apparatus base to count the number of revolutions. The analog signal from the Hall effect sensor was acquired continuously using an analog-to-digital converter and data logger (DL-710-ULS, Dataq Instruments, Akron, OH). WinDaq Data Acquisition and Playback Software was used to determine distance ran based on wheel revolutions and wheel diameter. One revolution corresponds to 40 cm; thus, the revolution count was converted to running distance by multiplying by 40 cm.

Mice ran an average of 6 km every 24 h, similar to previously reported values (5, 29). Mice were acclimatized to the running wheels for 48 h before being given permanent access to the unlocked wheels. Mice ran on the wheels during the nocturne (2000 to 0800 hours) and were on a 12:12-h light-dark light cycle. Mice were fed normal mouse chow ad libitum. Mice were anesthetized with isoflurane (5%) and, except for where otherwise stated, euthanized for experimentation within 2 h of their last exercise bout. Mice were not fasted at the time of death. Control mice weighed 29.4 ± 0.54 g, whereas exercised mice weighed 28.0 ± 0.71 g. Animal experiments were conducted in accordance with the University of Illinois at Chicago’s Animal Care and Use Committee (ACC no. 15-037) and National Institutes of Health (NIH) guidelines.

**Resistance artery FID.** Resistance artery FID was performed as previously described (24, 40). For further detail, refer to Fig. 1. Briefly, bilateral hindlimb subcutaneous (inguinal) fat pads were removed from mice and placed in cold (4°C) HEPES buffer. After dissection of vessel arcades, single resistance arteries (internal diameter: 86 ± 5.45 \(\mu\)m) were cannulated on glass micropipettes (internal
diameters of ~20 to 40 μm) in an organ chamber filled with Krebs solution. The remaining arteries were snap frozen in liquid nitrogen for molecular biology experiments described below. Resistance artery diameter was monitored by placing the organ chamber on the stage of an inverted microscope attached to a real time video-measuring apparatus (model VIA-100, Boeckeler). Arteries were maintained at 37°C, continuously perfused with warm Krebs buffer, and aerated with a gas mixture of 21% O₂ and 5% CO₂.

After an intraluminal pressure of 60 cmH₂O (44 mmHg) was maintained for 30 min, arteries were constricted 40–60% with endothelin-1 (ET-1; 125 pmol for control mice and 115 ± 3 pmol for exercised mice, not significant, means ± SE). FID was produced using two Krebs solution-filled reservoirs to generate pressure gradients of Δ10, Δ20, Δ40, Δ60, and Δ100 cmH₂O. Pressure gradients are generated by moving the reservoirs in equal and opposite directions. Diameters were taken after 3 min of exposure to each pressure gradient and then moved to create the next pressure gradient. For the high pressure model, high intraluminal pressure (HILP: 150 cmH₂O or 110 mmHg) was administered for 45 min followed by 15 min of reequilibration at 60 cmH₂O, similar to previously published protocols (2, 9, 11). FID was measured in the absence and presence of the NOS inhibitor L-NAME (100 μM), H₂O₂ scavenger polyethylene glycol-catalase (PEG-Cat; 500 U/ml), AT₁ blocker losartan (50 μM), NOX II inhibitors VAS2870 (2 μM) and NSC23766 (10 μM), and SOD inhibitor znic-diethyldithiocarbamate (Zn-DDC; 1 mM), which were added in randomized order either 30 min before preconstriction with ET-1 or coincubated during HILP. The maximal diameter of every vessel (endothelium-independent vasodilation) was determined with papaverine (100 μM) at the end of each round of FID. FID was calculated as the percent change from ET-1-constricted diameter relative to the maximal diameter measured at rest before ET-1 constriction. Three control male C57BL/6J mice were used for experiments to determine if impaired FID occurred in another vascular bed. Six resistance arteries (n = 6) were obtained, one from each gracilis muscle of the three mice. The skeletal muscle resistance arteries were 63.9 ± 9.7 μm (means ± SD). In agreement with our previous work, we noted that skeletal muscle resistance arteries (32, 37) were smaller than adipose resistance arteries (11, 24, 40). VAS2870 was obtained from Enzo Life Sciences (East Farmingdale, NY). NSC23766 was obtained from Tocris Bioscience (Bristol, UK). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO).

**Resistance artery ANG II dose responses.** Resistance artery vasoconstriction to ANG II doses was performed as previously described (37). Resistance arteries maintained at an intraluminal pressure of 60 cmH₂O (44 mmHg) were exposed to incremental doses of ANG II (10⁻¹⁰–10⁻⁶ M) for 3 min each. Minimal internal diameters were recorded for each dose. After the dose response, vessels were maximally dilated using papaverine (100 μM).

**Resistance artery fluorescence.** Superoxide and H₂O₂ levels were measured as previously described (24, 40). Briefly, arteries were maintained at 37°C in 20-ml aerated organ chambers bathed in Krebs solution at an equilibrium pressure of 60 cmH₂O (44 mmHg) for 30 min or exposed to HILP (110 mmHg), as described above. Superoxide levels were determined using 5 μM dihydroethidium (DHE). H₂O₂ levels were assessed using 1 μM 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA). Both dyes were purchased from Thermo Fisher Scientific (Waltham, MA). After incubation with dyes, vessels were exposed to flow (460 cmH₂O) 30 min. Prepared vessels were then excised from the glass micropipettes and subsequently mounted on slides with Dako fluorescent mounting medium (Dako, Carpinteria, CA). Mounted vessels were then immediately examined via fluorescent microscopy (Eclipse 80i, Nikon). Acquired images were analyzed for fluorescence intensity in arbitrary units using NIH ImageJ software. Fluorescence was measured three times along different lengths of each vessel. Background intensities were subtracted, and fluorescence was normalized to vessel size to account for autofluorescence (34). Buthionine sulfoxamine (BOS; 1 mM) was used as a positive control to induce O₂⁻ and H₂O₂ production in resistance arteries. A combination treatment of the SOD mimetic tiron (1 mM) and PEG-Cat (500 U/ml) was used for the negative control.

**Human adipose microvascular endothelial cells.** Experiments were performed on isolated human adipose microvascular endothelial cells (HAMECs) obtained from ScienCell (Cedro, Carlsbad, CA). HAMECs were grown in ScienCell endothelial cell growth medium.

![Fig. 2. High intraluminal pressure (HILP) impairs FID in resistance arteries from control mice but not exercised mice. A: high pressure (HILP) significantly reduced resistance artery FID compared with baseline (BSL) in control mouse arteries. *P < 0.01 at Δ10, 20, 40, 60, and 100 cmH₂O. B: there was no difference in resistance artery FID after HILP compared with BSL in exercise mouse arteries. Vasodilation to papaverine was similar between resistance arteries from control and exercised mice. C: there was no influence of HILP on the papaverine response. Data are presented as means ± SE.](http://ajpheart.physiology.org/Download/10.1152/ajpheart.00684.2016)
(ECM-1007, PRF) supplemented with 5% FBS, EC growth supplement, 100 U/ml penicillin, and 100 μg/ml streptomycin at 37°C in a humidified incubator (5% CO₂).

*Shear stress experiments.* HAMECs were grown to ~80–90% confluence. High physiological laminar shear stress (20 dyn/cm² for 24 h) was generated using a previously described modified cone and plate design (3, 39). This level of shear stress has been used in several exercise mimetic models (14, 26). The cone used in these studies is designed to fit into a 20-mm tissue culture dish. Magnetism to rotate the cone is provided by magnets within a modified Thermo Scientific Super-Nuova multiplace stirrer (Thermo Fisher Scientific). The angle between the cone and plate is negligible (~0.5°), thus facilitating uniform flow over all HAMECs on the culture dish. HAMECs were switched to serum-free media before shear stress experiments to prevent bubbling. Control (static) HAMECs were also switched to serum-free media. All shear experiments were conducted under sterile conditions. *Passages 5–8* were used for all experiments.

*Cellular fluorescence.* HAMECs were grown to ~90% confluence on coverslips pretreated for 24 h with 2% bovine plasma fibronectin. Once the desired confluence was met, cells were incubated with the O₂ indicator DHE (5 μM) and cotreated with or without losartan (50 μM), NOX II inhibitors VAS2870 (2 μM) and NSC23766 (10 μM), or the SOD mimetic tiron (1 mM) for 30 min. ANG II with VAS2870 (HILP) restored FID in control mouse arteries. *P < 0.01 at 10, 20, 40, 60, and 100 cmH₂O. B: blockade of the angiotensin type 1 receptor (AT₁R) with losartan (HILP + losartan) restored FID in control mouse arteries. *P < 0.01 at Δ10, 20, 40, 60, and 100 cmH₂O. C: blockade of NOX II with VAS2870 (HILP + VAS2870) restored FID in control mouse arteries. *P < 0.01 at Δ10, 20, 40, 60, and 100 cmH₂O. D: blockade of NOX II though inhibition of RAC1 guanine exchange factor (GEF) with NSC23766 (HILP + NSC23766) restored FID in control mouse arteries. *P < 0.01 at Δ 20, 40, 60, and 100 cmH₂O.

Sample preparation for immunoblot analysis. Resistance arteries were homogenized and sonicated (Qsonica Q55 Sonicator Ultrasonic Processor, Cole Palmer) in 60 μl RIPA buffer (Sigma) containing 3X protease and 3X phosphatase inhibitor cocktails (Thermo Fisher Scientific). After treatment with shear and pharmacological agents, cells were washed twice with cold PBS and lysed with 250 μl of 3X protease and phosphatase inhibitor cocktail. Lysates were then centrifuged at 17,000 rcf at 4°C for 20 min. Protein determination of tissue and cell lysates was determined using a bicinchoninic acid assay (Pierce, Thermo Fisher Scientific). Tissue and cell supernatants were subsequently heated at 95°C for 5 min at a 2:1 with a 5% β-mercaptoethanol 2X Laemmli sample buffer.

**Western blot determination of protein expression.** For Western blot experiments, 20 μg of protein were loaded into each well of Bio-Rad 10% gels. Samples were processed by SDS-PAGE (time varied depending on molecular weight of target proteins at 200 V) and transferred to polyvinylidene difluoride membranes (0.22 μm, Bio-Rad). Proteins were wet transferred at 4°C from 70 to 90 min at 90-110 V depending on the molecular weight of the target protein. Membranes were blocked with Li-Cor blocking buffer (Lincoln, NE) for 2 h at room temperature. Membranes were incubated with primary antibodies for gp91phox and p47phox (1:1,000, Cell Signaling Technologies), GAPDH (1:5000, Cell Signaling Technologies), actin (1:5000, Cell Signaling Technologies), SOD I (1:3,300, R&D Systems), and SOD II (1:1,000, Santa Cruz Biotechnology) in Li-Cor blocking buffer (0.2% Tween 20). Secondary antibodies (1:15,000) were incubated for 2 h at room temperature. Membranes were incubated with primary antibodies for gp91phox and p47phox (1:1,000, Cell Signaling Technologies), GAPDH (1:5000, Cell Signaling Technologies), actin (1:5000, Cell Signaling Technologies), SOD I (1:3,300, R&D Systems), and SOD II (1:1,000, Santa Cruz Biotechnology) in Li-Cor blocking buffer (0.2% Tween 20). Secondary antibodies (1:15,000) were incubated for 2 h in Li-Cor blocking buffer (0.2% Tween 20 and 0.01% SDS). Membranes were washed in Tris-buffered saline-Tween 20 (4 × 5 min) after antibody incubations. Membranes were transferred to Tris-buffered saline after the last wash and imaged within 1 h using a Li-Cor Odyssey scanner. Bands were manually placed around each band of interest, which returned near-infrared fluorescent values of

**Fig. 3.** Impaired FID after HILP in resistance arteries from control mice involves the local renin-angiotensin system (RAS) and NADPH oxidase II (NOX II). A: exercise mouse arteries were significantly less sensitive to ANG II-induced constriction compared with control (CON) mouse arteries. *P < 0.01 at 10–6–10–8 M. B: blockade of the angiotensin type 1 receptor (AT₁R) with losartan (HILP + losartan) restored FID in control mouse arteries. *P < 0.01 at Δ10, 20, 40, 60, and 100 cmH₂O. C: blockade of NOX II with VAS2870 (HILP + VAS2870) restored FID in control mouse arteries. *P < 0.01 at Δ10, 20, 40, 60, and 100 cmH₂O. D: blockade of NOX II though inhibition of RAC1 guanine exchange factor (GEF) with NSC23766 (HILP + NSC23766) restored FID in control mouse arteries. *P < 0.01 at Δ 20, 40, 60, and 100 cmH₂O. Data are presented as means ± SE.
raw intensity with intralane background subtracted using Odyssey analytic software (27, 31).

Statistics. All FID and ANG II dose-response comparisons were analyzed using two-way repeated-measures ANOVA using vessel diameter as a covariate followed by pairwise comparisons using Bonferroni’s adjustment (treatment × time). One-way ANOVA with Tukey’s post hoc test was used to compare HAMEC fluorescent data. Western blot and resistance artery fluorescence data were compared using t-tests. α was set at <0.05. With power (1 − β) set at 0.80 and α at <0.05 and assuming 20% differences in treatment conditions with 10% SD, it was calculated that five samples were needed for each condition allowing for two pairwise comparisons (adjusted α 0.025). In some cases, the effect size was adequately large that only four samples in each condition were needed to satisfy power (1 − β) set at 0.80.

RESULTS

Resistance artery FID and fluorescence. Baseline resistance artery FID was similar in vessels obtained from control and

![Fig. 4](https://example.com/fig4.png)

Fig. 4. Effect of ANG II, NOX II, and SOD blockade with HILP on FID in resistance arteries from exercised mice. A: there was no difference in resistance artery FID after HILP compared with HILP + blockade of AT1Rs (HILP + losartan) in exercise mouse resistance arteries. B: there was no difference in resistance artery FID after HILP compared with HILP + NOX II inhibition via VAS2870 (HILP + VAS2870) in exercise mouse resistance arteries. C: blockade of endogenous SOD with zinc-diethyldithiocarbamate (HILP + Zn-DDC) reduced FID compared with HILP alone in exercise mouse arteries. *P < 0.01 at Δ20, 60, and 100 cmH2O. Data are presented as means ± SE.

![Fig. 5](https://example.com/fig5.png)

Fig. 5. Differential oxidative stress levels after HILP in resistance arteries from control and exercised mice. A: HILP evoked a significant increase in O2− levels compared with BSL (60 cmH2O) in control mouse arteries. *P < 0.05. B: in exercise mouse arteries, HILP exposure did not evoke a significant increase in O2− levels. C: in control mouse arteries, HILP did not evoke a significant increase in H2O2 levels compared with BSL. D: in exercise mouse arteries, HILP elicited a significant increase in H2O2 levels. *P < 0.05. E: data are presented as means ± SE. Buthionine sulfoxamine (BOS; 1 mM) increased and tiron (1 mM) polyethylene glycol-catalase (PEG-Cat; 500 U/ml) decreased dihydroethidium (DHE) and 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) signals, indicating that these fluorophores are sensitive to increased ROS.
exercise mice (Fig. 2, comparison not shown). After exposure to high intraluminal pressure, FID was reduced in control mouse arteries ($P < 0.01$ at 10, 20, 40, 60, and 100 cmH$_2$O; Fig. 2A). FID was preserved after high intraluminal pressure in exercise mouse arteries (Fig. 2B). Vasodilation to papaverine was similar between resistance arteries from control and exercise mice, and there was no influence of high intraluminal pressure on the papaverine response (Fig. 2C).

Vasoreactivity to ANG II was greater in control mouse arteries compared with exercise mouse arteries ($P < 0.01$ at $10^{-6}$; Fig. 3A). Impaired FID in control mouse arteries exposed to HILP was restored by AT$_1$R blockade with losartan ($P < 0.01$ at 10, 20, 40, 60, and 100 cmH$_2$O; Fig. 3B). FID in control mouse arteries exposed to HILP was also restored with NOX II inhibition via VAS2870 ($P < 0.01$ at 10, 20, 40, 60, and 100 cmH$_2$O; Fig. 3C) and NSC23766 ($P < 0.01$ at 20, 40, 60, and 100 cmH$_2$O; Fig. 3D). The addition of losartan or VAS2870 did not influence FID after high pressure exposure in exercise mouse arteries (Fig. 4, A and B). FID after high intraluminal pressure exposure was significantly reduced by the endogenous SOD inhibitor Zn-DDC in resistance arteries obtained from exercised mice ($P < 0.01$ at 20, 60, and 100 cmH$_2$O; Fig. 4C).

High pressure resulted in increased O$_2^-$ levels in control mouse arteries compared with baseline pressure ($P < 0.01$; Fig. 5A). In contrast, exercise mouse arteries exposed to high pressure did not exhibit significantly greater O$_2^-$ levels compared with exercise mouse arteries maintained at baseline pressure (Fig. 5B). H$_2$O$_2$ levels were similar in control mouse arteries exposed to high pressure and control mouse arteries maintained at normal pressure (Fig. 5C). Arteries obtained from exercise mouse arteries exposed to high pressure exhibited significantly greater H$_2$O$_2$ levels compared with exercise mouse arteries maintained at 60 cmH$_2$O ($P < 0.01$; Fig. 5D). The fluorescence control experiments indicated that the positive control BOS increased superoxide and H$_2$O$_2$ production in resistance arteries, whereas the application of tiron and PEG-Cat reduced the signal for superoxide and H$_2$O$_2$ (Fig. 5E).

At baseline, FID in arteries from exercised mice was significantly reduced by both L-NAME and PEG-Cat, but the reduction via L-NAME was significantly greater than that of PEG-Cat ($P < 0.01$ at 20, 40, 60, and 100 cmH$_2$O; Fig. 6A). In contrast, the preserved FID in exercise mouse arteries after exposure to HILP was also significantly reduced by PEG-Cat and L-NAME, but the reduction in the presence of PEG-Cat was significantly greater than that of L-NAME ($P < 0.01$ at 20, 40, 60, and 100 cmH$_2$O; Fig. 6A). In the baseline condition, combination blockade of L-NAME + PEG-Cat resulted in a greater reduction in vasodilation than L-NAME alone (Fig. 6C, main effect for condition $F = 6.64$, $P < 0.01$). After high intraluminal pressure, combination blockade of L-NAME + PEG-Cat resulted in a greater reduction in vasodilation than PEG-Cat alone (Fig. 6D, main effect for condition $F = 6.64$, $P < 0.05$).

Resistance artery protein expression. Resistance arteries obtained from exercised animals expressed lower protein levels of the gp91$^{\text{phox}}$ NOX II subunit compared with arteries from control mice ($P < 0.05$; Fig. 7A). Resistance arteries obtained from exercised animals also expressed lower protein levels of the p47$^{\text{phox}}$ NOX II subunit compared with arteries from control mice ($P < 0.01$; Fig. 7B). In contrast, exercise mouse arteries expressed more SOD I ($P < 0.01$; Fig. 7C), SOD II ($P < 0.01$; Fig. 7D), and SOD III protein ($P < 0.05$; Fig. 7E).

HAMEC fluorescence and protein expression. HAMECs exposed to ANG II exhibited significantly greater O$_2^-$ levels compared with HAMECs maintained in the control condition. AT$_1$R blockade with losartan, NOX II inhibition with VAS2870 or NSC23766, and treatment with exogenous SOD mimetic tiron all prevented increased O$_2^-$ ($P < 0.01$; Fig. 8). In HAMECs exposed to high shear stress, there was less protein...
expression of the gp91\textsuperscript{phox} and p47\textsuperscript{phox} NOX II subunits than in static cells ($P < 0.01$ for both; Fig. 9, A and B). High shear stress did not influence SOD I or SOD II protein expression (Fig. 9, C and D). Endothelial cells do not express SOD III (19, 33). All blots were normalized to \(\beta\)-actin or GAPDH.

In additional FID experiments, skeletal muscle (gracilis) resistance arteries obtained from control mice displayed reduced FID after high pressure exposure ($P < 0.01$ at 20, 40, 60, and 100 cmH\textsubscript{2}O; Fig. 10). FID was preserved after high intraluminal pressure in adipose resistance arteries from a mouse that was regularly exercised but withheld from exercise for 24 h (Fig. 11).

**DISCUSSION**

The main findings from this study are that high intraluminal pressure induced resistance artery vasodilator dysfunction in a RAS/NOX II-dependent fashion in control mice, whereas ar-

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**Fig. 7.** Differential expression of NOX II subunits and SOD isoforms in resistance arteries from control and exercised mice. A: there was a significant reduction in gp91\textsuperscript{phox} expression in exercise mouse arteries compared with control mouse arteries. *$P < 0.05$. B: there was a significant reduction in p47\textsuperscript{phox} expression in exercise mouse arteries compared with control mouse arteries. *$P < 0.01$. C: there was a significant increase in SOD I expression in exercise mouse arteries compared with control mouse arteries. *$P < 0.01$. D: there was a significant increase in SOD II expression in resistance arteries from exercise mouse arteries compared with control mouse arteries. *$P < 0.01$. E: there was a significant increase in SOD III expression in in exercise mouse arteries compared with control mouse arteries. *$P < 0.05$. Data are presented as means \pm SE.

**Fig. 8.** Effects of ANG II on superoxide levels in human adipose microvascular endothelial cells (HAMECs). In HAMECs, treatment with ANG II (400 nM) evoked a significant increase in O$_2^\cdot$ levels. *$P < 0.05$. Combined treatment with ANG II + losartan (AT\textsubscript{1}R blocker), VAS2870 or NSC23766 (NOX II inhibitors), or tiron (SOD mimetic) significantly reduced the ANG II-induced increase in O$_2^\cdot$ in HAMECS. †$P < 0.05$. Data are presented as means \pm SE.
Exercise averts high pressure-induced vascular dysfunction

Fig. 9. Expression of NOX II subunits and SOD isoforms in HAMECs exposed to laminar shear stress. A: expression of high shear stress in HAMECs reduced gp91phox protein expression compared with HAMECs raised under static conditions. * P < 0.05. B: in HAMECs, exposure to high shear stress reduced p47phox expression compared with HAMECs raised under static conditions. * P < 0.01. C and D: there was no effect of high shear stress in HAMECs compared with cells grown under static conditions in regard to SOD I expression (C) or SOD II expression (D). Data are presented as means ± SE.

The role of NOX II-induced oxidative stress was indicated by the reduced FID after H2O2 scavenging with PEG-Cat, by the reduced FID after inhibition of endogenous SOD with Zn-DDC, and, finally, by the increased H2O2 fluorescence after NO·-generation and p47phox expression (Fig. 7), which have been shown to play a critical role in O2•- generation in endothelial cells (21, 30). Collectively, these findings may partially explain the preserved FID after high pressure exposure in exercise mouse arteries. We did not assess NO· due to limited tissue availability. However, the finding that L-NAME reduced vasodilation after high intraluminal pressure, coupled with reduced NOX II expression and O2•- levels, suggests that exercise mouse arteries had greater relative NO· bioavailability. However, NO· was not the predominate vasodilator after high pressure in resistance arteries from exercised mice.

The preserved dilation in resistance arteries from exercised mice was accompanied by a phenotypic switch from primarily NO·-dependent to H2O2-dependent dilation. This was indicated by the reduced FID after H2O2 scavenging with PEG-Cat, by the reduced FID after inhibition of endogenous SOD with Zn-DDC, and, finally, by the increased H2O2 fluorescence after high pressure exposure. While the reduction in preserved dilation with catalase in exercise mouse arteries supports previous findings (9, 30), we show here, for the first time, that the blockade of endogenous SOD also abrogates the preserved dilation in resistance arteries from exercised mouse arteries. An illustrative summary of the primary findings is shown in Fig. 12. We have previously shown that ANG II plays a role in high pressure-induced vascular function (10, 11), and the local RAS has been well described elsewhere (4, 36). Yet, the molecular means through which regular exercise dampens the preserved dilation after HILP remains poorly understood. Illustrating the role of local RAS losartan restored protection against local activation of the RAS, as indicated by the reduced FID after HILP and no impact of AT1R blockade of endogenous SOD also abrogates the preserved dilation after inhibition of endogenous SOD with Zn-DDC, and, finally, by the increased H2O2 fluorescence after high pressure exposure. While the reduction in preserved dilation with catalase in exercise mouse arteries supports previous findings (9, 30), we show here, for the first time, that the blockade of endogenous SOD also abrogates the preserved dilation in resistance arteries from exercised mice.

Exercise mouse arteries did not exhibit increased O2•- (Fig. 6) and were also found to express significantly less protein for NOX II subunits gp91phox and p47phox (Fig. 7), which have been shown to play a critical role in O2•- generation in endothelial cells (21, 30). Collectively, these findings may partially explain the preserved FID after high pressure exposure in exercise mouse arteries. We did not assess NO· due to limited tissue availability. However, the finding that L-NAME reduced vasodilation after high intraluminal pressure, coupled with reduced NOX II expression and O2•- levels, suggests that exercise mouse arteries had greater relative NO· bioavailability. However, NO· was not the predominate vasodilator after high pressure in resistance arteries from exercised mice.

The preserved dilation in resistance arteries from exercised mice was accompanied by a phenotypic switch from primarily NO·-dependent to H2O2-dependent dilation. This was indicated by the reduced FID after H2O2 scavenging with PEG-Cat, by the reduced FID after inhibition of endogenous SOD with Zn-DDC, and, finally, by the increased H2O2 fluorescence after high pressure exposure. While the reduction in preserved dilation with catalase in exercise mouse arteries supports previous findings (9, 30), we show here, for the first time, that the blockade of endogenous SOD also abrogates the preserved dilation in resistance arteries from exercised mice.

Fig. 10. HILP impairs FID in skeletal muscles in resistance arteries from control mice. HILP significantly reduced FID compared with BSL in control mouse arteries. * P < 0.01 at Δ20, 40, 60, and 100 cmH2O. Data are presented as means ± SE.

Fig. 11. HILP does not impair FID in resistance arteries from exercised mice after 24 h of wheel removal. There was no difference in resistance artery FID after HILP compared with BSL in resistance arteries from exercised mice who did not run for 24 h. Data are presented as means ± SE.
FID. These findings suggest that exercise mouse arteries maintained FID after high pressure due to greater SOD activity/expression and a resultant increase in the conversion of O2/H2O2 to H2O2. In agreement, exercise mouse arteries were found to express significantly more SOD protein compared with control mouse arteries. This finding is also similar to previously reported regular exercise-driven adaptations in other microvascular depots (41, 44).

We previously reported PEG-Cat reactivity was largely unaffected, whereas the response to L-NAME was largely reduced, after high pressure exposure in resistance arteries obtained from sedentary humans. These findings suggest impaired dilation in sedentary humans is due to impaired NO· bioavailability without a concomitant phenotypic switch to rescue vasodilation (9, 40). We did not denude vessels in this particular study to determine if our findings were solely attributable to the endothelium, although our group has done this in the past (9). We were also not able to distinguish if the molecular changes made in the vascular wall were specific to the endothelium as isolating the endothelium and quantifying protein expression solely from this vascular tunic was not feasible. As a substitution, we used HAMECs. We hypothesized that ANG II is the primary mediator of high pressure-induced oxidative stress and endothelial dysfunction. To directly assess the effect of ANG II on the endothelium, we used ANG II treatment in HAMECs. Laminar shear stress was used to mimic exercise.

Treatment with ANG II in HAMECs resulted in increased O2 levels. Treatment with losartan prevented the ANG II-mediated increase in O2. These findings provide additional evidence suggesting that the local RAS is an upstream mediator of oxidative stress in response to high pressure. NOX II inhibition also reduced the ANG II-mediated increases in O2, indicating that NOX II is a primary downstream mediator of local RAS. The finding that HAMECs exposed to shear stress expressed significantly lower protein of the gp91phox and p47phox NOX II subunits indicates that increased shear stress during exercise bouts could be the primary stimulus for reduced NOX II-dependent O2. Contrary to our initial hypothesis, SOD protein expression in HAMECs was unaffected by laminar shear stress. This finding could be due to several reasons, including that SOD changes in resistance arteries were primarily due to changes in vascular smooth muscle. Alternatively, paracrine factors released during whole body acute exercise may play a role in regulating endothelial SOD expression in the adipose microvasculature (10, 18, 48). Nonetheless, treatment with exogenous tiron also prevented ANG II-mediated increases in O2 levels in HAMECs, suggesting that exercise-induced increases in SOD could play a role in combating oxidative stress after local activation of the RAS in the adipose microcirculation. A limitation to the HAMEC model was that static cells were used as a comparison with high physiological shear, whereas a low shear stress model may...
have served as a more appropriate control to model in vivo conditions.

Additional experiments (n = 2) were performed on arteries obtained from an exercised mouse whose wheel was removed for 24 h before death. Endothelium-dependent dilation was preserved after high pressure exposure, despite no exercise being performed at least 24 h before the artery was obtained (Fig. 11). These findings are in agreement with our previous human studies (4,18). Taken together, these findings suggest preserved endothelium-dependent dilation after high pressure in exercisers is due to chronic exercise adaptations. The exact time course for this regular exercise-conferred protection remains to be elucidated. Additional experiments performed in skeletal muscle (gracilis m.) resistance arteries suggest impaired FID after high intraluminal pressure may be systemic (Fig. 10). However, our findings are of particular relevance to the adipose tissue microcirculation. Impaired adipose tissue perfusion may contribute to CVD risk by promoting inflammation through the secretion of proinflammatory adipokines (20). Prior studies have shown that inflation modulates endothelial dysfunction in human adipose resistance arteries (12,13). Our findings suggest regular exercise may play a role in preventing this impaired perfusion after high pressure exposure.

Clinical implications. The collective findings presented here elucidate a damaging pathway elicited by high pressure involving local activation of the local RAS and activation of NOX II, which results in excess oxidative stress in the vascular wall. Our results suggest regular exercise protects against high pressure-induced microvascular dysfunction by creating a favorable vascular redox environment in the adipose microcirculation. Regular exercise may protect against high pressure by creating a favorable redox environment, thus lessening the risk for cardiovascular events.

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

AUTHOR CONTRIBUTIONS

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