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*J Immunol* 2019; 203:3013-3022; Prepublished online 25 October 2019; doi: 10.4049/jimmunol.1900144 http://www.jimmunol.org/content/203/11/3013

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Exercise Promotes Resolution of Acute Inflammation by Catecholamine-Mediated Stimulation of Resolvin D1 Biosynthesis

Jing-Juan Zheng, Ernesto Pena Calderin, Bradford G. Hill, Aruni Bhatnagar, and Jason Hellmann

The mechanisms by which regular exercise prevents the development and progression of chronic inflammatory diseases are largely unknown. We find that exercise enhances resolution of acute inflammation by augmenting resolvin D1 (RvD1) levels and by promoting macrophage phagocytosis. When compared with sedentary controls, mice that performed a four-week treadmill exercise regimen displayed higher macrophage phagocytic activity, enhanced RvD1 levels, and earlier neutrophil clearance following an acute inflammatory challenge. In acute inflammatory cell extracts from exercised mice, we found elevated expression of Alox15 and Alox5 and higher RvD1 levels. Because exercise stimulates release of epinephrine, which has immunomodulatory effects, we questioned whether epinephrine exerts proresolving actions on macrophages. Epinephrine-treated macrophages displayed higher RvD1 levels and 15-lipoxygenase-1 protein abundance, which were prevented by incubation with the α1 adrenergic receptor (α1-AR) antagonist prazosin. Likewise, stimulation of the α1-AR with phenylephrine enhanced macrophage phagocytosis and RvD1 production. During acute inflammation, prazosin abrogated exercise-enhanced neutrophil clearance, macrophage phagocytosis, and RvD1 biosynthesis. These results suggest that exercise-stimulated epinephrine enhances resolution of acute inflammation in an α1-AR-dependent manner. To our knowledge, our findings provide new mechanistic insights into the proresolving effects of exercise that could lead to the identification of novel pathways to stimulate resolution. The Journal of Immunology, 2019, 203: 3013–3022.

Exercise or regular physical activity prolongs lifespan and enhances resilience against chronic diseases. Conversely, physical inactivity and a sedentary lifestyle are major risk factors for several major noncommunicable diseases, including type 2 diabetes, breast and colon cancers, dementia, depression, and cardiovascular disease (CVD) (1). The World Health Organization ranks physical inactivity as the fourth-leading cause of mortality, linked to 6% of deaths worldwide (2). Although many of the health benefits of exercise could be attributed to increased cardiorespiratory fitness and an improved CVD risk profile, the beneficial effects of exercise extend beyond reduction in CVD risk factors.
exercise-trained mice was associated with increased biosynthesis of the endogenous SPM, RvD1, and increased macrophage phagocytosis. Mechanistically, our findings suggest that exercise-induced enhancement of resolution is due to catecholamine-induced activation of the α1 adrenergic receptor (α1-AR). These findings provide new insights into how exercise regulates acute inflammatory processes and offer new ideas into how exercise may prevent or mitigate disease.

Materials and Methods

Animals and reagents

Male FVB/NJ mice were purchased from The Jackson Laboratory at 8 wk of age. At 9–10 wk of age, they were either subjected to exercise training or maintained in a normal, sedentary state. Food and water were provided ad libitum, and the animals were maintained on a 12h:12h light–dark schedule. Adrenergic receptor (AR) agonists and antagonists were purchased from Sigma-Aldrich. All animal procedures were approved by the University of Louisville Institutional Animal Care and Use Committee. Primary conjugated Abs used for flow cytometry were purchased from eBioscience.

Exercise training

Mice were subjected to treadmill exercise as previously described (8). Animals were randomly assigned to sedentary and exercise groups and familiarized for 20 min with the rodent exercise treadmill (Columbus Instruments) and its environment for 2 d. Following familiarization, a baseline exercise capacity test (ECT) was performed on exercise-trained mice. For ECT, mice were placed on the treadmill for 10 min at 0 m/min and 0° incline with the shock grids on. Mice were then given an initial familiarization/warmup period of 9 min at 8.5 m/min (0° incline). Subsequently, the speed was increased by 2.5 m/min after each 3 min interval, thereby subjecting the mice to a linear increase in speed. Their workload was increased by raising the vertical incline of the treadmill by 5° every 9 min up to a maximum of 15° (all angles with respect to horizontal). During exercise training, if mice spent more than half the time on the shock grid rather than running on the treadmill, the time, distance and speed were recorded, and the animals were removed from the treadmill. To set the initial training regimen for each group, we exercised mice at 75% of the maximum average speed attained during the baseline ECT at a 1° inclination. Mice in the exercise group exercised 5 d a week [Monday through Friday] for 4 wk. The regular exercise routine consisted of a 10 min warmup period at 10 m/min (10° incline), followed by 40 min runs during week 1, 50 min runs during week 2, and 60 min runs during weeks 3 and 4. The following week (week 5), the mice exercised on Monday, and were then allowed to recover for 24 h prior to additional experiments (e.g., acute peritonitis) or tissue harvest.

Acute peritonitis and flow cytometry

Self-resolving acute peritonitis was induced by i.p. administration of zymosan A (0.04 mg/g; Sigma-Aldrich). Inflammatory and resolving peritoneal exudates were collected from sedentary and exercise-trained mice at 0, 4, 12, 24 and 48 h or when indicated after zymosan challenge. In select experiments, mice received the α1-AR antagonist prazosin (2.5 mg/kg; i.p.) 30 min prior and 12 h after zymosan injection. Exudates were then treated with fluorescein isothiocyanate–conjugated CD45-bated with Fc block (anti-mouse CD16/CD32) for 10 min on ice. Cells were then treated with deoxythymine primers were used to prepare cDNA by PCR. Real-time amplification was performed using PerfeCta SYBR Green FastMix with ROX (Quantabio) and a 9700HT Fast Real-Time PCR (Applied Biosystems). Commercially available, validated PCR primers for Alox5, Alox5, and Fpr2 were purchased from SABiosciences. Housekeeping gene Hprt was considered the control, and relative expression was calculated using the 2-ΔΔCT method. For immunoblotting, bone marrow–derived macrophages were plated (1 x 10⁶ cells per well) in six-well plates in DMEM supplemented with 10% FBS, 1% HEPES, and 1% penicillin/streptomycin and incubated overnight at 37°C with 5% CO₂. After 24 h, the media was removed, and the cells were treated with ephrinephine at the indicated dose in fresh culture media for 2 h. For AR experiments, the mice were pretreated with specific receptor antagonists (prazosin 1 µM; betaxolol 0.3 µM; ICI 118,551 0.3 µM; Sigma-Aldrich) for 30 min before the addition of ephrinephine (1 µM). After removal of the media, the cells were washed once with ice-cold PBS and resuspended with the addition of RIPA buffer (Sigma–Aldrich) containing protease and phosphatase inhibitors. Samples were briefly sonicated on ice, and protein levels were measured on a NanoDrop (Thermo Fisher Scientific) by following a modified Lowry method (Bio-Rad Laboratories). Equal amounts of reduced sample lysates were separated using 10% SDS-polyacrylamide gels (Bio-Rad Laboratories) and transferred to a 0.2-µm polyvinylidene difluoride membrane. Membranes were blocked at room temperature for 1 h in 5% powdered milk suspended in 0.1% Tween 20 (TBS-T), and then incubated overnight at 4°C in TBS-T containing anti-mouse primary Abs to detect 15-LOX-1, 5-LOX (AbCam), or GAPDH (Cell Signaling Technology). (AbCam) and GAPDH (Cell Signaling Technology). The following day, membranes, were washed for 1 h with TBS-T and then incubated with HRP-conjugated secondary Abs for 1 h. Binding was visualized with the addition of Luminata Forte ECL chemiluminescence reagent (Millipore). GAPDH was used as a loading control in all samples.

Macrophage phagocytosis

To assess in vivo phagocytic capacity, exercise-adapted mice were injected (i.p.) with PE-labeled latex beads (in 500 µl of sterile saline; Cayman Chemical) 24 h after their final exercise bout. The animals were euthanized after 2 h, and peritoneal lavage exudates were collected in 3 ml of DPBS+/+. Sample aliquots were isolated from sedentary and exercise-trained mice on Ab on ice, as described above, for 10 min on ice followed by 30-min incubation in the dark with anti-F4/80 Ab (allophycocyanin-conjugated). Using flow cytometry, the mean fluorescence intensity of PE (i.e., latex beads) was measured in macrophage (F4/80+) cells. In select experiments, mice received α1-AR antagonist treatment (prazosin, 2.5 mg/kg i.p.) 30 min prior to latex bead injection. Macrophage phagocytosis was also measured ex vivo in cells isolated from sedentary and exercise-trained mice 2 h following 4 wk of training. For this measurement, resident peritoneal macrophages were isolated by lavaging the peritoneal cavity with 5 ml of DPBS+. Peritoneal macrophages were then seeded in duplicate and allowed to adhere to a 96-well plate at 4 x 10⁶ cells per well in 100 µl of DMEM with 10% FBS. After 1 h, nonadherent cells were removed, and the plate was washed with washing medium (DMEM plus 10% FBS). Cells were then probed with IgG-opsonized FITC–zymosan A (ratio 10:1; Life Technologies) particles was added. After incubation for 1 h, the cells were washed with DPBS++ to remove nonphagocytosed zymosan and incubated for 1 min with trypan blue to quench extracellular fluorescence. Phagocytosis was quantified by measuring fluorescence intensity on a spectrophotometer (PerkinElmer BioTek plate reader). In select experiments, bone marrow–derived macrophages were plated overnight in a 96-well plate and treated without or with phenylephrine for 30 min in DMEM supplemented with 10% FBS, 1% HEPES, and 1% penicillin/streptomycin. The medium was then replaced, and the cells were incubated for 1 h with DMEM supplemented with 10% FBS, 1% HEPES, and 1% penicillin/streptomycin containing FITC–zymosan A (10:1) and assessed for phagocytosis using a spectrophotometer, as described above. To test the contribution of 15-LOX, bone marrow–derived macrophages were incubated without or with 10 μM of ML351 (15-LOX inhibitor) for 1 h prior to phenylephrine treatment.

Macrophage α1-AR expression

Bone marrow–derived macrophages were plated at 1 x 10⁴ cells per well in a 96-well plate in DMEM supplemented with 10% FBS, 1% HEPES, and 1% penicillin/streptomycin. After 24 h, cell media was replaced with fresh media without or with BODIPY FL–labeled prazosin (2 µM; Life Technologies). After 24 h, cells were washed with DPBS++ and imaged using an EVOS FL Digital Inverted Microscope (Advanced Microscopy Group). For peritoneal macrophages, mice were injected (i.p.) with BODIPY FL–labeled prazosin (2.5 mg/kg) or sterile
saline. Following euthanization, peritoneal cells were isolated using 5 ml of DPBS
lavage. Following centrifugation and removal of supernatant, the cells were
incubated on ice in the dark with Fc block for 10 min and then incubated for
30 min in the dark with anti-F4/80 Ab (allophycocyanin-conjugated) and
collected using a BD LSRII flow cytometer equipped with FACSDiva V6.0 software.

Quantification of resolvin D1 by targeted liquid chromatography–tandem mass spectrometry

Cell-free peritoneal lavage fluid was collected from sedentary and exercised animals during the resolution phase (48 h after zymosan injection) and immediately stored at −80°C. On the day of extraction, the samples were thawed on ice and incubated for 45 min with two volumes of ice-cold methanol. Protein precipitate was pelleted by centrifugation (1000 × g, at 4°C for 10 min), and the supernatant was collected into 10-mL glass conical tubes. Samples were diluted to ~400 μL under a gentle steady stream of nitrogen gas. Before solid-phase extraction, deuterium-labeled, internal standards (i.e., d5-RvD2, d5-LXA4, d4-LTB4, d8-S(6)-HETE and d4-PGE2) were added to determine extraction recovery. Solid-phase extraction was performed using C18 columns (Waters). Columns were first equilibrated with 3 ml of methanol and 3 ml of H2O. Samples were acidified by adding 9 ml of pH 3.5 High pressure liquid chromatography–grade H2O and rapidly loaded onto columns. Following sample loading, the columns were neutralized with 5 ml of H2O. Columns were then treated with 5 ml of hexane and 5 ml of methanol. After solvent products were removed and collection of 5 ml of methyl formate. Samples were completely dried using a steady stream of N2 gas and immediately resuspended in methanol–water (50:50 v/v) for liquid chromatography–tandem mass spectrometry (LC-MS/MS). For quantification, a Shimadzu LC-20AD HPLC and an SIL-20AC autoinjector (Shimadzu) coupled to an API 2000 (AB Scieix) was used. Targeted LC-MS/MS was carried out using an Eclipse Plus C18 column (100 mm × 4.6 mm × 1.8 μm; Agilent Technologies) by eluting with mobile phase consisting of methanol:water:acetic acid at a flow rate of 0.4 ml/min throughout. The API 2000 was operated in negative ionization mode and RvD1 was detected using multiple reaction monitoring (MRM) with retention time matching. Using authentic RvD1 synthetic standards (Cayman Chemical), a linear calibration curve was generated under the exact same conditions and used to determine RvD1 abundance. Final pg/ml values were determined following sample recovery determination, based on internal deuterium-labeled standards and starting sample volumes.

Analytical catecholamine measurements

For ultra-performance LC–MS/MS (UPLC–MS/MS) analysis of catecholamines, mouse urine samples were collected following exercise training at the indicated time points and frozen immediately. As described above (9), samples were thawed on ice, vortexed, diluted with 0.2% formic acid, and mixed with certified iso-tope–labeled internal standards ([1-13C]-epinephrine-d6, [±]-norepinephrine-d6, [±]-metanephrine-d6, dopamine-d6, 3-methoxytyramine-d6, [±]-4-hydroxy-3-methoxymandelic acid-d6, [±]-normetanephrine-d6, 3-hydroxyindole-4,6,7-d3-acetic acid, 5-hydroxy-3-methoxyphenyl-d3-acetic-d3 acid (Cerilliant); Scerotonin-o,a,b,d (CDN Isotopes)). Samples containing internal standards were then analyzed using an UPLC–MS/MS instrument (Acquity H-Class UPLC and Xevo TQ-S Micro Triple Quadrupole Mass Spectrometer with an electrospray ionization source; all from Waters). Sample constituents were separated on an Acquity UPLC HSS PFP column (150 mm × 2.1 mm, 1.8 μm; from Waters) using a binary gradient composed of 0.2% formic acid (solvent A) and methanol (solvent B). The gradient was started with 0.5% solvent B at a flow rate of 0.4 ml/min for 1 min and was then ramped up to 95% solvent B at a flow rate of 0.35 ml/min over a 3 min period, at which it was maintained for 0.5 min before recycling back to solvent B, 0.5% in 0.1 min. The solvent gradient was then held at 0.5% solvent B at a flow rate of 0.4 ml/min for 5.4 min. The column temperature was maintained throughout the run at 40°C. Samples were injected at a volume of 1 μL. Optimized cone voltage and collision energy were used for each individual analytes. For each sample, three MRM transitions were set up: one for quantification, one for confirmation, and one for labeled internal standard. These MRMs were scheduled around the retention time of the analytes and no less than 12 data points were collected for each peak. Analytes in urine samples were quantified using peak area ratio, based on eight-point standard curves, which were run before and after urine samples. TargetLynx Application Manager (Version 4.1; from Waters) was used for peak integration, calibration, and quantification.

Statistical analysis

Data are presented as means ± SEM. For small sample sizes, nonparametric statistical tests were used. For direct comparisons, nonparametric two-tailed Mann–Whitney tests were performed. Kruskal–Wallis tests were performed for multiple comparisons, followed by a Dunn posttest correction. For direct comparisons with larger sample sizes, normality was tested using the D’Augostino–Pearson omnibus test, followed by unpaired two-tailed Student t tests. Parametric one-way or two-way ANOVA followed by Bonferroni or Tukey posttests were used for unpaired comparisons. In all cases, a value of p < 0.05 was considered statistically significant and is reported in all figures and figure legends. Statistical analysis was performed using GraphPad Prism 6.0.

Results

Exercise training increases RvD1 biosynthesis and promotes resolution of acute inflammation

To examine whether exercise training affects innate immune responses during the resolution of acute inflammation, we compared the kinetics of leukocyte trafficking between sedentary mice and mice subjected to a 4-wk treadmill training protocol (Fig. 1A). As shown in Fig. 1, C10, polymorphonuclear neutrophil (PMN) recruitment peaked 12 h after zymosan injection and was near control levels by 48 h. In both sedentary mice and mice subjected to 4 wk of exercise training, similar numbers of total leukocytes were recruited in response to zymosan challenge (Supplemental Fig. 1A). The total number of Gr-1+ PMNs was significantly reduced 48 h after zymosan injection in exercised animals (Fig. 1C).

To quantify differences in resolution in sedentary and exercised mice, we used previously defined indices (10) to calculate changes in resolution-phase kinetics. Consistent with previous publications showing enhanced PMN recruitment following exercise (11), exercised mice displayed a robust PMN infiltration that peaked (ϕmax = 22.1 ± 3.2 cells per exudate) at 12 h (Tmax) (Fig. 1C, 1D). In response to an acute inflammatory challenge, sedentary mice also showed PMN infiltration that peaked (ϕmax = 13.6 ± 3.9 cells per exudate) at 12 h (Tmax) (Fig. 1C, 1D). Following peak PMN infiltration, the resolution interval (R) is defined as the time point at which 50% of maximum PMN are removed. Despite an average increase in PMN recruitment, exercised mice displayed a shorter Rmax (17 h) when compared with sedentary mice (24 h) (Fig. 1D). Of note, the percentage of apoptotic (annexin V+) PMNs at the peak of infiltration (12 h) were not different between sedentary and exercised mice (Supplemental Fig. 1B). Furthermore, linear regression analysis revealed heightened PMN removal in exercised mice (Fig. 1E), consistent with enhanced resolution of acute inflammation. Because macrophage-mediated effecytosis of apoptotic PMNs is critical for resolution of inflammation (12), we assessed the trafficking kinetics of peritoneal macrophages in both sedentary and exercise-trained mice following zymosan challenge. We found that the total number of F4/80+ macrophages in the peritoneal cavity was not different between sedentary and exercised mice throughout the acute inflammatory response (Fig. 1F).

We next investigated whether exercise augments RvD1 biosynthesis. Our LC-MS/MS analysis showed significantly elevated levels of RvD1 in resolving lavage exudates of exercised animals compared with sedentary controls (Fig. 1G). Moreover, cell pellets collected during the resolution phase from exercised mice demonstrated an increase in the mRNA expression of the RvD1 biosynthetic enzymes, 15-LOX (Alox15) and 5-LOX (Alox5) (Fig. 1H, 1I). The expression of the RvD1 receptor Fpr2 was not different between sedentary and exercise-trained mice (Fig. 1J).

The fact that exercised mice subjected to an acute inflammatory challenge had similar macrophage numbers in the peritoneum yet displayed enhanced PMN removal and higher RvD1 levels suggests that exercise may promote resolution by increasing macrophage...
FIGURE 1. Exercise training enhances RvD1 biosynthesis and resolution of acute inflammation. (A) Schematic of 4-wk exercise training and peritonitis protocol. (B) Representative flow cytometry dot plots of peritoneal lavage PMN (CD45^+Ly-6G^+) and macrophages (CD45^+F4/80^+). (C) Peritoneal PMN kinetics at 0-, 4-, 12-, 24-, and 48-h after zymosan injection in sedentary and exercised animals (n = 3–5 per time point). (D) Resolution indices calculations based on PMN kinetics in sedentary and exercised animals (n = 3–5 per time point). (E) Linear regression analysis of PMN removal during resolution phase in sedentary and exercised animals (n = 3–5 per time point). (F) Peritoneal macrophage kinetics at 0-, 4-, 12-, 24-, and 48-h after zymosan injection in sedentary and exercised animals (n = 3–5 per time point). (G) Resolving peritoneal lavage exudate RvD1 levels in sedentary and exercised animals (48 h; n = 6). (H) Resolving peritoneal exudate cellular expression of Alox15 (n = 8–9), Alox5 (n = 4–5), and RvD1 receptor, Fpr2 (48 h; n = 4–5) in sedentary and exercised animals. (K) Peritoneal macrophage phagocytosis of opsonized FITC–zymosan (n = 3) with representative image (scale bar, 100 μm). (L) Baseline peritoneal RvD1 levels and Fpr2 expression in sedentary and exercised animals (n = 3–4). (M) Phagocytosis of FITC–zymosan by naive bone marrow–derived macrophages treated with either control media or in media with FBS replaced with plasma isolated from sedentary or exercised animals (n = 5–8). *p < 0.05, ****p < 0.0001.
phagocytosis. To test this hypothesis, we isolated peritoneal macrophages from exercised and sedentary mice, and measured their capacity to phagocitize opsonized zymosan particles. As shown in Fig. 1K, macrophages from exercised mice displayed enhanced phagocytic capacity. This improvement in phagocytic capacity manifested by 1 wk of exercise training (Supplemental Fig. 1C). We next questioned whether basal macrophage phagocytosis could be enhanced in exercised mice due to higher RvD1 abundance. In support of this idea, basal RvD1 concentrations were higher in peritoneal lavage fluid collected from exercised mice compared with that from sedentary animals (Fig. 1L). The basal mRNA expression of Fpr2 in peritoneal cell extracts was not different between exercised and sedentary animals (Fig. 1L). Furthermore, we found that exposure of naïve bone marrow–derived macrophages to plasma isolated from exercised mice increased their phagocytic capacity; surprisingly, plasma from sedentary mice significantly diminished phagocytosis (Fig. 1M). Collectively, these findings suggest that exercise training enhances proresolving pathways (e.g., RvD1) and promotes the resolution of acute inflammation by stimulating macrophage phagocytosis.

Exercise training stimulates epinephrine release and elevates leukocytic AR expression

Previous work shows that exercise activates the sympathoadrenal system and increases the production of catecholamines (13), which have direct actions on macrophages (14–16). Therefore, we examined the effects of our exercise training protocol on catecholamine levels. For this, we measured urinary levels of several catecholamines and their metabolites in sedentary and exercised mice. We found that exercise training increased the levels of both

![Graphs showing epinephrine, norepinephrine, dopamine, metanephrine, normetanephrine, dopamine, 3-methoxytyramine, homovanillic acid, and vanillylmandelic acid levels in sedentary and exercised animals.](http://www.jimmunol.org/)
epinephrine and its direct metabolite, metanephrine (Fig. 2A); however, the urinary levels of norepinephrine and its metabolite normetanephrine were similar between sedentary and exercised mice (Fig. 2B). Interestingly, exercise training modestly decreased urinary dopamine and serotonin levels (Fig. 2C, Supplemental Fig. 2A). Exercise training was also associated with a decrease in the urinary levels of vanillylmandelic acid; however, because this metabolite is derived from monoamine oxidase-catalyzed metabolism of both metanephrine and normetanephrine, no clear reason for its decline could be added.

Given the changes in urinary catecholamines and metabolites following exercise training, we next measured the expression of catecholamine receptors during resolution. For this, we isolated cell pellets from exercised and sedentary mice during the resolution phase (at 48 h) of an acute inflammatory response and measured AR mRNA expression. As shown in Fig. 2D, the expression of Adra1a (α1a), Adra1b (α1b), Adra2a (α2a), and Adrab2 (β2) were higher in cells from exercised mice. Moreover, the cell pellets isolated during resolution from exercised mice displayed higher levels of the catecholamine-metabolizing enzymes catecholamine methyl transferase (Comt) and monoamine oxidase a (Maoa), but not monoamine oxidase b (Maob; Fig. 2E). These data suggest that exercise training increases both the release of epinephrine and the expression of ARs in leukocyte-rich resolving exudates.

Epinephrine-induced RvD1 production in macrophages is dependent on the α1-AR

Next, we examined whether stimulation of bone marrow–derived macrophages by catecholamines affects RvD1 synthesis. Treatment of bone marrow–derived macrophages, which express the RvD1 biosynthetic enzymes 15-LOX-1 and 5-LOX (Fig. 3A), with epinephrine increased RvD1 production (Fig. 3B). Interestingly, stimulation of bone marrow–derived macrophages with epinephrine for 6 h also modestly increased 15-LOX-1 protein expression (Supplemental Fig. 3A, 3B). To test whether the increase in macrophage 15-LOX-1 upon epinephrine treatment is AR specific, we incubated bone marrow–derived macrophages with prazosin (α1 antagonist), ICI 118,551 (β2 antagonist), or betaxolol (β1 antagonist) prior to epinephrine treatment. Treatment with prazosin but not ICI 118,551 or betaxolol abrogated epinephrine-dependent induction of 15-LOX-1 (Fig. 3C, 3D). Prazosin pretreatment also inhibited epinephrine-stimulated production of RvD1 in bone marrow–derived macrophages (Fig. 3E). Moreover, direct stimulation of the α1-AR by phenylephrine increased bone marrow–derived macrophage phagocytosis and enhanced RvD1 production (Fig. 3F, 3G). We next tested whether 15-LOX-1 is involved in α1-AR–stimulated phagocytosis. For this test, we treated bone marrow–derived macrophages with the 15-LOX antagonist ML351 prior to phenylephrine stimulation and detected an abrogation of phenylephrine-induced phagocytosis (Fig. 3H).

Using immunofluorescence and flow cytometry, we confirmed the protein expression of α1-AR on the surface of bone marrow–derived macrophages (Fig. 3I) and peritoneal macrophages (Fig. 3J). Collectively, these data suggest that epinephrine induces RvD1 biosynthesis and phagocytosis in bone marrow–derived macrophages in an α1-AR–dependent manner.

Exercise-enhanced resolution of inflammation is dependent on the α1-AR

Given that epinephrine-induced RvD1 biosynthesis is dependent on α1-AR signaling in bone marrow–derived macrophages, we questioned whether exercise-enhanced resolution of acute inflammation could also be mediated by the α1-AR. We found that during the resolution phase of acute inflammation, exercised mice treated with prazosin displayed a 4 h delay in the RI and a significant reduction in PMN removal (Fig. 4A). Moreover, exercised mice treated with prazosin had higher levels of total leukocytes and PMN during the resolution phase of acute inflammation (Fig. 4B). Of note, prazosin treatment had no effect on total leukocyte or PMN levels in sedentary mice (Fig. 4B). To determine whether prazosin-induced abrogation of exercise-enhanced resolution was due to inhibition of RvD1 production, we measured RvD1 in resolving exudates from sedentary and exercised mice pretreated with vehicle or prazosin. As shown in Fig. 4C, resolving exudates from exercised mice contained higher RvD1 levels, and pretreatment with prazosin completely blocked exercise-mediated induction of RvD1 (Fig. 4C). Although prazosin did not affect the phagocytosis of IgG-coated latex beads by macrophages in sedentary mice, it remarkably diminished macrophage phagocytosis in exercised mice (Fig. 4D). Collectively, these results suggest that α1-AR–dependent signaling contributes to exercise-enhanced resolution of acute inflammation.

Discussion

The results of this study demonstrate that exercise training accelerates the resolution of inflammation in mice. We found that exercise training increases the basal levels of the resolution agonist RvD1, which contributes to exercise-enhanced resolution of acute inflammation by increasing macrophage-mediated effectorcytosis of PMN. Our results also show that epinephrine increases RvD1 production in macrophages in an α1-AR–dependent manner. Likewise, α1-AR blockade with prazosin in vivo abrogates exercise enhancement of PMN clearance, macrophage phagocytosis, and RvD1 production. Collectively, these results suggest that exercise training enhances the resolution of acute inflammation in an α1-AR–dependent manner, which supports the notion that the beneficial effects of exercise on innate immune function may relate to enhancement of proresolving pathways.

Although acute inflammation in response to exercise is an area of intense inquiry, few studies address how exercise affects the resolution of inflammation. Some studies have identified augmented levels of SPMs after exercise. Strenuous exercise in humans has been shown to increase urine levels of lipoxin A4, an arachidonic acid–derived SPM (17). Similarly, Markworth et al. (7) reported that in humans postexercise recovery following a single bout of resistance exercise is characterized by an increase in circulating levels of the SPMs RvD1, RvE1, and the protectin D1 isomer PDx. An increase in n-3 DPA–derived resolvins has also been reported following intense exercise training (18). These reports, which are consistent with our results showing increased RvD1 production and upregulation of its biosynthetic enzymes, support the view that acute exercise training stimulates SPM production. Our results, using a well-controlled animal model of acute inflammation, further assess the biological significance of enhanced SPM production following exercise training and show that the increases in SPM production are accompanied by increased macrophage phagocytosis and resolution of acute inflammation.

Our studies suggest that the proresolving effects of exercise are, at least in part, because of catecholamines, which increase transiently during exercise (19). Catecholamine release during exercise is responsible for altering vascular tone, promoting lipolysis in adipose tissue, increasing glucose output from the liver, and diminishing glucose entry into skeletal muscle (20). In addition, exercise-induced increases in catecholamines prime the immune system and promote leukocytosis through a β-AR–mediated mechanism (21–25). Catecholamines also exert receptor-specific immunomodulatory effects on macrophages. Activation of the
FIGURE 3. Epinephrine-induced RvD1 production is α1-AR dependent. (A) Western blot targeting 15-LOX-1 and 5-LOX in bone marrow–derived macrophages (n = 3). (B) RvD1 levels in bone marrow–derived macrophage supernatants following epinephrine incubation (n = 6–13). (C and D) Representative Western blot and quantitation for 15-LOX-1 normalized to GAPDH in bone marrow–derived macrophages treated without or with epinephrine and prazosin (α1-AR antagonist), ICI 118,551 (β2-AR antagonist), or betaxolol (β1-AR antagonist) (n = 3–5). (E) Fold change of RvD1 levels in bone marrow–derived macrophage supernatants treated without or with epinephrine and α1-AR antagonist prazosin (n = 3–4). (F) Bone marrow–derived macrophage phagocytosis of opsonized FITC–zymosan without or with phenylephrine (α1-AR agonist) at indicated concentration (n = 11). (G) RvD1 levels in bone marrow–derived macrophage supernatants treated with phenylephrine (α1-AR agonist) at indicated concentration (n = 3–8). (H) Bone marrow–derived macrophage phagocytosis of FITC–zymosan following phenylephrine (α1-AR agonist) without or with ML351 (15-LOX inhibitor) (n = 5). (I) Representative fluorescence microscopy image of bone marrow–derived macrophages treated without or with BODIPY FL–prazosin (scale bar, 100 µm). (J) Representative flow cytometry dot plots of peritoneal macrophages (F4/80+) treated without or with BODIPY FL–prazosin. *p < 0.05, **p < 0.01, ***p < 0.001.
β2-AR on macrophages promotes alternatively activated M2 polarization with dampened TNF-α and IL-1β cytokine production (26). Conversely, β1 activation enhances LPS stimulation and inflammatory cytokine production (27, 28). Limited data exist regarding the role of α1-AR on macrophage function; however, the α1-AR agonist phenylephrine has been reported to stimulate rat peritoneal macrophage phagocytosis, whereas coculturing rat microglial cells with LPS and phenylephrine decreases TNF-α and IL-6 expression (29, 30). These findings are consistent with our data showing enhanced phagocytic capacity in α1-AR–stimulated bone marrow–derived macrophages. Additional studies on mouse peritoneal macrophages have shown that norepinephrine reduces LPS-stimulated TNF-α production in an α2-dependent manner (14), suggesting that α-ARs exert an anti-inflammatory effect on macrophages.

Our study adds to this body of knowledge by showing that epinephrine increases 15-LOX-1 expression and RvD1 biosynthesis in macrophages in an α1-AR–dependent manner. Because RvD1 has been shown to repress TNF-α production (31), our results appear consistent with the previously observed anti-inflammatory effects of epinephrine and α1-AR signaling. Our results further suggest that the phagocytic enhancement of α1-AR activation in macrophages is associated with elevated RvD1 production and that enhanced resolution in exercised animals is dependent on α1-AR signaling. These results suggest that resolution enhancement with exercise depends on the adrenergic system. Although we report that exercise increases the mRNA expression of leukocytic Alox15 (15-LOX-1) and that macrophages treated with epinephrine display increased protein expression of 15-LOX-1 and RvD1 biosynthesis, the extent to which exercise-stimulated 15-LOX-1 expression enhances resolution of inflammation remains unclear. Increases in 15-LOX-1 in other conditions can be associated with pathological processes such as the atherogenic oxidation of low-density lipoproteins (32, 33). Nevertheless, our data showing enhanced macrophage phagocytosis with α1-AR activation is dependent upon 15-LOX-1 activation are consistent
with the hypothesis that exercise-induced expression of 15-LOX-1 promotes resolution of inflammation through enhanced RvD1 biosynthesis.

The novel link between exercise and the resolution of inflammation suggests that exercise may be an effective intervention not only in promoting the resolution of acute inflammation but also in resolving chronic inflammation as well. Studies from our group and others (34) have shown that nonresolved inflammation is associated with the accumulation of apoptotic PMN and defective SPM biosynthesis (35–37). Such nonresolved inflammation has been linked to diminished macrophage phagocytosis and the development of chronic disease (37). For example, defective macrophage phagocytosis and delayed resolution is a common feature of atherosclerotic lesions and type 2 diabetes, and nonresolving inflammation in these conditions is associated with defective biosynthesis of SPMs, including RvD1 (34, 35, 38). Importantly, we found that treatment with RvD1 restores macrophage phagocytosis and reduces apoptotic PMN burden in diabetic wounds and reduces lesion size in atherosclerosis-prone mice (35, 38). Taken together, this work supports the view that delayed resolution, because of defects in SPM production, is linked to nonresolving inflammation in chronic diseases. Given that physical activity and exercise are associated with a decrease in chronic inflammation in both nondiseased and diseased conditions, we propose that exercise-enhanced improvements in resolution may, in part, be responsible for the salutary changes in inflammation that mediate the beneficial cardiovascular effects of exercise (3). Our results show that exercise training increases the basal levels of RvD1, a potent SPM that is decreased in states of nonresolving inflammation such as diabetes and atherosclerosis. We also found that exercise stimulates the removal of PMN, which led to a reduction in the resolution interval following zymosan-induced peritonitis. This is consistent with extensive previous work showing that acute exercise promotes recovery from infections and that long-term physical activity and exercise can diminish mortality risk associated with CVD and diabetes (39–41). Although additional work is required to assess the extent to which exercise-induced macrophage α1-AR signaling and resolution of inflammation improve cardiovascular health and insulin sensitivity, our current findings strengthen the rationale for studying the effects of exercise on acute inflammatory responses and on the chronic low-grade inflammation associated with chronic disease.

In summary, our findings suggest that the beneficial effects of exercise on inflammatory processes are due to α1-AR–dependent activation of resolution. The extent to which different SPM species contribute to exercise-induced resolution of inflammation in different tissues or in chronic diseases remains to be determined; however, the findings of the current study suggest that RvD1 is a critical mediator of exercise-induced resolution of acute inflammation. These findings provide critical insights into the health-promoting effect of exercise and could inform future studies to delineate how exercise mitigates chronic inflammation and disease.

Acknowledgments
We thank the University of Louisville Diabetes and Obesity Center Flow Cytometry and Bioanalytical Core for technical assistance with leukocyte differentials and urinary catecholamine measurements, respectively.

Disclosures
The authors have no financial conflicts of interest.

References


