Repeated Bouts of Moderate-Intensity Aerobic Exercise Reduce Airway Reactivity in a Murine Asthma Model

Matt Hewitt¹, Kim Estell¹, Ian C. Davis², and Lisa M. Schwiebert¹

¹Department of Physiology and Biophysics, University of Alabama at Birmingham, Birmingham, Alabama; and ²Department of Veterinary Biosciences, The Ohio State University, Columbus, Ohio

We have reported that moderate-intensity aerobic exercise training attenuates airway inflammation in mice sensitized/challenged with ovalbumin (OVA). The current study determined the effects of repeated bouts of aerobic exercise at a moderate intensity on airway hyperresponsiveness (AHR) in these mice. Mice were sensitized/challenged with OVA or saline and exercised at a moderate intensity 3 times/week for 4 weeks. At protocol completion, mice were analyzed for changes in AHR via mechanical ventilation. Results show that exercise decreased total lung resistance 60% in OVA-treated mice as compared with controls; exercise also decreased airway smooth muscle (ASM) thickness. In contrast, exercise increased circulating epinephrine levels 3-fold in saline- and OVA-treated mice. Because epinephrine binds β_2 -adrenergic receptors (AR), which facilitate bronchodilation, the role of β_2 -AR in exercise-mediated improvements in AHR was examined. Application of the β_2 -AR antagonist butoxamine HCl blocked the effects of exercise on lung resistance in OVA-treated mice. In parallel, ASM cells were examined for changes in the protein expression of β_2 -AR and G-protein receptor kinase-2 (GRK-2); GRK-2 promotes β_2 -AR desensitization. Exercise had no effect on β_2 -AR expression in ASM cells of OVA-treated mice; however, exercise decreased GRK-2 expression by 50% as compared with controls. Exercise also decreased prostaglandin E_2 (PGE₂) production 5-fold, but had no effect on E prostanoide-1 (EP1) receptor expression within the lungs of OVA-treated mice; both PGE₂ and the EP1 receptor have been implicated in β_2 -AR desensitization. Together, these data indicate that moderate-intensity aerobic exercise training attenuates AHR via a mechanism that involves β_2 -AR.

Keywords: asthma; airway hyperresponsiveness; exercise; β_2 -adrenergic receptor

Allergic or atopic asthma, the most common form of asthma, is identified by the presence of characteristic clinical symptoms of wheezing, chest tightness, dyspnea and cough, and by the presence of reversible airway narrowing and/or airway hyperresponsiveness (AHR) to a variety of inhaled bronchoconstrictor stimuli. For the treatment of asthma-associated AHR, patients are typically administered inhaled, long-acting β_2 -agonists. β_2 -agonists bind to and activate β_2 -adrenergic receptors (β_2 -AR), which are expressed on a variety of cell types, including airway smooth muscle (ASM) cells, airway epithelial cells, and mast cells (1).

Several clinical studies suggest that continued aerobic exercise training improves the overall physical fitness and health of

CLINICAL RELEVANCE

This is the first report to demonstrate that aerobic exercise at a moderate intensity attenuates airway hyperresponsiveness via a mechanism that involves the β_2 -adrenergic receptor. Because increased airway hyperresponsiveness is a hallmark of the asthmatic response, this study supports the use of moderate-intensity aerobic exercise as an adjunct therapy for the treatment of this chronic disease.

individuals with asthma and reduces their disease-related hospital admissions (2–4); these studies also observed improvements in exercise-induced bronchoconstriction after physical training. We have reported previously that moderate intensity aerobic exercise training reduces lung inflammatory responses in an ovalbumin (OVA)-driven mouse model of pulmonary inflammation (5, 6).

Repetitive exercise increases the levels of circulating catecholamines, including epinephrine (7). Epinephrine binds β_2 -AR in the airways to facilitate bronchodilation and, thereby, functions as a β_2 -agonist. Upon binding β_2 -agonist, the β_2 -AR couples with the stimulatory G protein (Gs) to stimulate adenylate cyclase, cAMP production, and subsequent protein kinase A (PKA) activation. Activated PKA stimulates myosin light chain kinase and myosin light chain phosphatase; these events lead to decreased myosin light chain activity and smooth muscle tone (1). Desensitization occurs upon G-protein receptor kinase-2 (GRK-2)-mediated phosphorylation of β_2 -AR, which uncouples the receptor from the Gs-adenylated cyclase complex (8). The prostaglandin PGE₂ may also regulate β_2 -AR desensitization indirectly via activation of the E prostanoide-1 (EP1) receptor. Upon activation, the EP1 receptor heterodimerizes with β_2 -AR, which impairs β_2 -AR coupling to the Gs-adenylated cyclase complex and, thereby, reduces β_2 -AR-stimulated cAMP production (9).

In the current study, we hypothesized that repetitive moderate-intensity aerobic exercise would attenuate asthma-associated AHR. To test this hypothesis, mice were sensitized and challenged with OVA or control saline and exercised on a motorized treadmill repetitively at a moderate intensity as reported previously (5, 6). At the conclusion of the protocol, changes in AHR were monitored upon exposure to increasing doses of methacholine, a bronchoconstrictor stimulus. In parallel, changes in circulating epinephrine levels, β_2 -AR expression, GRK-2 expression, ASM thickness, PGE₂ content, and EP1 receptor expression were measured. Collectively, results presented herein indicate that moderate-intensity aerobic exercise attenuates AHR via a mechanism that involves β_2 -AR.

MATERIALS AND METHODS

Animals

Female BALB/cJ mice (3–5 wk old; The Jackson Laboratory, Bar Harbor, ME), a strain susceptible to OVA-induced IgE responses (10, 11), were used. Mice were housed in a pathogen-free containment

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Correspondence and requests for reprints should be addressed to Lisa M. Schwiebert, Ph.D., Department of Physiology and Biophysics, MCLM, Room 955, University of Alabama at Birmingham, 1918 University Boulevard, Birmingham, AL 35294-0005. E-mail: lschwieb@uab.edu

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facility and maintained in autoclaved Microisolator cages (Lab Products, Maywood, NJ). Mice were provided with food (Teklad, Madison, WI) and water *ad libitum*. Mice were allowed to acclimate to housing condition for 1 week before experimental manipulation. All animal treatments were approved by the University of Alabama at Birmingham (UAB) Institutional Animal Care and Use Committee (IACUC) and were in accordance with the National Institutes of Health recommended guidelines.

OVA Sensitization and Exercise Protocol

OVA sensitization and moderate-intensity aerobic exercise training were performed as described previously (5, 6). Briefly, sensitization (Days 0 and 14, intraperitoneally) and challenge (Days 21–25 and Day 28, via aerosolization) with OVA (Sigma Chemical, St. Louis, MO) or saline was performed (5). We have shown previously that the utilization of this OVA sensitization scheme in BALB/cJ mice initiates an airway inflammatory response and immunoglobulin profile that is consistent with those observed in asthma (5). Exercised mice underwent bouts of moderately-intense aerobic exercise on a motorized treadmill (Exer 6M; Columbus Instruments, Columbus, OH) at 1 hour after OVA treatment. Exercise bouts were performed three times per week for a total of 10 bouts; mice were exercised for 45 minutes at 13.5 m/minute (0% grade). As noted in our earlier study, previous reports have defined moderate-intensity aerobic exercise as brief (15- to 60-min) bouts of treadmill running at 50 to 75% maximum O_2 consumption or approximately 15 to 22 m/minute (5, 12). All exercise bouts included brief warm-up and cool-down periods so that the total treadmill time was about 60 minutes.

Mechanical Ventilation

Mice were mechanically ventilated and challenged with increasing concentrations of methacholine as described previously (13). Briefly, 48 hours after the last exercise bout, mice were anesthetized with diazepam (17.5 mg/kg) and ketamine (450 mg/kg), and a tracheotomy tube (18G) was inserted and connected to the inspiratory and expiratory ports of a ventilator (Flexivent; SCIREQ, Montreal, PQ, Canada). Mice were ventilated at a rate of 160 breaths per minute at a tidal volume of 0.2 ml with a positive end-expiratory pressure of 2 to 4 cm H_2O . Increasing concentrations of methacholine (0–50 mg/ml) were administered via aerosolization. From 20 seconds up to 3 minutes after each aerosol challenge, resistance (R) was recorded continuously; an average value of R was taken to express changes in murine airway function. For studies involving the β_2 -AR antagonist butoxamine HCL (Sigma), mice received a nebulization of either butoxamine HCL (200 mg/ml) or saline before each methacholine concentration as just described; R was recorded continuously.

Lung Fixation and ASM Thickness Measurements

Lungs were extracted and prepared for analysis as described previously (5, 6). Briefly, mice were injected with a lethal dose of ketamine/xyzazine. The mice were tracheotomized using an 18G catheter and the lungs were filled with 10% paraformaldehyde. Once the lungs were filled the trachea was tied off to prevent leakage of the paraformaldehyde using suture silk and lungs were removed from the body cavity. All excess tissue was clipped off and the lungs were suspended in a tube of 10% paraformaldehyde, embedded in paraffin, and then was sliced into 10- μ m sections for staining. Paraffin tissue sections were rehydrated as described (5, 6). Rehydrated tissue was stained with a biotinylated primary antibody against α -smooth muscle actin (Dako, Carpinteria, CA) and subsequently probed with streptavidin-peroxidase. Staining was completed with incubation with 3,3'-diaminobenzidine (DAB) + substrate, resulting in a brown-colored precipitate at the antigen site. Slides were then visualized under a light field microscope.

For ASM thickness measurements, airways with a longitudinal diameter between 150 and 200 μ m were identified and ASM thickness was measured in a blinded fashion. Specifically, a minimum of 10 airways with the required diameter per mouse were selected randomly and ASM thickness was measured on each airway at two sites 90 degrees apart using the imaging program Metamorph. Measuring two sites on each airway analyzed accounted for any differences in the plane of sectioning between airways.

Epinephrine Measurements

For measurement of circulating epinephrine levels, mice underwent cardiac puncture and blood was collected into EDTA-coated tubes (Becton Dickinson, Franklin Lakes, NJ) as described previously (5). Care was taken to ensure that the same individual handled all the mice in the study in the same manner to minimize the impact of killing on basal epinephrine measurements. From these blood samples, plasma was isolated and then stored at -20°C . Epinephrine levels were assayed using an epinephrine-specific ELISA kit (R&D Systems and Research Diagnostics, Minneapolis, MN, performed according to manufacturer's instructions). All plasma sampling was performed between 7:30 A.M. and 11:00 A.M.

ASM Isolation and Western Blot Analysis

Tracheas were removed, and the trachealis muscle was isolated and digested in a digestion buffer containing collagenase, soybean trypsin inhibitor, and elastase as described previously (14). The resulting cell suspension was filtered to remove debris and resuspended in Ham's F-12 medium (supplemented with 10% FBS). Isolated *ex vivo* ASM cells were immediately lysed and prepared for Western blot analysis as described previously (15). Equivalent amounts of protein lysates (20 μ g/lane) were electrophoresed and transferred to PVDF membrane blots. Blots were blocked with 5% BSA-containing buffer and then stained with either a rabbit anti-mouse anti- β_2 -AR antibody (Abcam, Cambridge, MA), anti-GRK-2 antibody, or anti-EP1 receptor antibody (Santa Cruz Biotechnology, Santa Cruz, CA) followed by a goat-anti-rabbit IgG antibody conjugated to horseradish peroxidase (HRP). Immunoblots were developed using chemiluminescence and imaged using an Chemidoc XRS Imaging Station (Bio-Rad).

To verify ASM phenotype and to account for differences in lane loading, blots were stripped and re-probed with HRP-conjugated antibodies directed against the ASM marker α -smooth muscle actin (Santa Cruz). Re-probed blots were developed via chemiluminescence and analyzed as just described. Results were quantitated via densitometry.

Lavage Protein Analysis

Mice were lavaged as described previously (5, 6). Briefly, mice were lavaged with 1.0 ml of 0.9% saline, and bronchoalveolar lavage fluid (BALF) supernatant samples were analyzed for differences in PGE₂ content via ELISA (R&D Systems).

Statistical Analysis

Data were analyzed using SPSS Version 11.0. Results are reported as group means $\pm$ SD. A one-way ANOVA determined differences among the group means and the Bonferroni *post hoc* analysis determined which group means differed significantly (at a level of $P \leq 0.05$).

RESULTS

Moderate-Intensity Aerobic Exercise Decreases Airway Resistance in OVA-Treated Mice

To determine the effect of a moderate-intensity aerobic exercise training on AHR in OVA-treated mice, mice were mechanically ventilated and challenged with increasing concentrations of methacholine (0–50 mg/ml); changes in resistance (R) were recorded continuously. Results shown in Figure 1 demonstrate that, at the highest methacholine dose, OVA-treated, sedentary (SO) mice exhibited a total lung resistance that was approximately 4-fold greater than saline-treated sedentary (S) and exercise (E) controls. In contrast, mice that were OVA-treated and exercised (EO) displayed a 65% reduction in total lung resistance as compared with OVA-treated, sedentary mice (SO); this level of resistance was equivalent to that observed in S and E control groups. Significant decreases in overall lung resistance were also observed in OVA-treated and exercised (EO) mice at intermediate methacholine doses (20 mg/ml, 40 mg/ml) as compared with sedentary controls (Figure 1).

Aerobic Exercise at a Moderate Intensity Decreases ASM Thickness in OVA-Treated Mice

Increases in airway wall thickness have been implicated as an underlying mechanism in the initiation and/or exacerbation of

Figure 1. Moderate-intensity aerobic exercise attenuates airway hyperresponsiveness (AHR) in ovalbumin (OVA)-treated mice. Mice were OVA treated and exercised as described in MATERIALS AND METHODS (open squares: sedentary, saline-treated; open circles: exercised, saline-treated; open triangles: sedentary, OVA-treated; solid triangles: exercised, OVA-treated). At the conclusion of the protocol, mice were assessed for changes in AHR via mechanical ventilation with methacholine challenge at increasing concentrations. Results are presented as total lung resistance (cm H₂O/mL/s; **P* < 0.02, as compared with sedentary, OVA-treated mice; *n* = 6–8 mice per group).

AHR (16, 17). Because increases in ASM thickness contribute most to the increased airway wall thickness in individuals with asthma (17), the effect of moderate-intensity aerobic exercise on ASM thickness in OVA-treated mice was determined. To this end, lung tissues were harvested and stained with a biotinylated α -smooth muscle actin antibody and subsequently probed with streptavidin–peroxidase; tissues were then analyzed for changes in ASM thickness via Metamorph analysis. Data presented in Figure 2 demonstrate that OVA treatment of sedentary (SO) mice increased ASM thickness 2-fold as compared with saline-treated controls (S, E; Figures 2A, 2B, and 2E). Mice that were OVA-treated and exercised (EO), however, displayed a 35% reduction in ASM thickness as compared with sedentary controls (Figures 2C, 2D, and 2E); this level of thickness was equivalent to that observed in saline-treated control mice (Figures 2A, 2C, and 2E).

Repeated Bouts of Aerobic Exercise at a Moderate Intensity Increase Circulating Epinephrine Levels

Because circulating epinephrine has been implicated in bronchodilation (18), the effects of exercise on epinephrine production in OVA-treated mice was determined. At the conclusion of the exercise and OVA treatment protocol, mice were cardiac punctured and plasma was isolated; epinephrine levels were assessed via ELISA. As shown in Figure 3, epinephrine production was enhanced approximately 3-fold in both saline- (E) and OVA-treated, exercised (EO) mice as compared with sedentary controls (S, SO).

Exercise-Mediated Improvement of AHR in OVA-Treated Mice Is Dependent upon β_2 -AR

β_2 -AR expressed within the airways facilitates bronchodilation (1, 8); therefore, the role of β_2 -AR in exercise-mediated attenuation of AHR in OVA-treated mice was examined. Mice were ventilated and challenged with methacholine at increasing concentrations as described above. Before each methacholine challenge, however, mice received a nebulization of either saline or the specific β_2 -AR antagonist butoxamine HCl. At the highest methacholine doses (30–50 mg/mL), treatment with the β_2 -AR antagonist increased total lung resistance significantly in mice that were OVA-treated and exercised (EO) as compared with controls (Figure 4A); increases in total lung resistance were also observed in exercised (E) mice that were

Figure 2. Moderate-intensity aerobic exercise decreases airway smooth muscle (ASM) thickness in OVA-treated mice. Mice were OVA treated and exercised as described in MATERIALS AND METHODS. At the conclusion of the protocol, whole lung tissues were harvested and prepared for peroxidase staining and ASM thickness measurements with MetaMorph software. (A–D) Representative images of lung tissue stained for α -smooth muscle actin and probed with peroxidase ($\times 40$ magnification). Arrows indicate ASM layer; asterisks indicate airway lumen. (E) Results of Metamorph analysis are presented as ASM thickness in microns (**P* < 0.02, as compared with sedentary, saline-treated mice; **P* < 0.02, as compared with sedentary, OVA-treated mice; *n* = 4–8 mice per group). Open bars, sedentary mice; solid bars, exercised mice.

treated with saline (Figure 4B). In contrast, treatment with the β_2 -AR antagonist had no significant effect on total lung resistance in sedentary (S, SO) mice (Figure 4).

Moderate-Intensity Aerobic Exercise Alters GRK-2, but Not β_2 -AR, Protein Expression in the Lungs of OVA-Treated Mice

β_2 -AR are expressed on a variety of cell types, including ASM cells, and have been shown to facilitate bronchodilation (1). To determine the effect of moderate-intensity aerobic exercise training on β_2 -AR protein expression, ASM cells were harvested and prepared for Western blot analysis. Data presented in Figure 5A suggests that moderate-intensity aerobic exercise training does not alter β_2 -AR protein expression on ASM cells. OVA treatment also did not affect β_2 -AR expression in these cells (Figure 5A).

In parallel with analyses of β_2 -AR expression, studies also determined the effect of moderate-intensity aerobic exercise training on GRK-2 protein expression. GRK-2 phosphorylates

Figure 3. Moderate-intensity aerobic exercise increases circulating epinephrine levels in saline- and OVA-treated mice. Mice were OVA treated and exercised as described in MATERIALS AND METHODS. At the conclusion of the protocol, plasma was isolated from blood collected via cardiac puncture and then analyzed via ELISA for changes in epinephrine levels. Results are reported as pg/ml of

epinephrine (* $P < 0.05$ as compared with sedentary, OVA-treated mice; $n = 10$ per group). Open bars, sedentary mice; solid bars, exercised mice.

β_2 -AR, which promotes desensitization of the receptor and, thereby, reduces its bronchodilatory actions (1, 8). Results shown in Figure 5B indicate that OVA treatment of sedentary (SO) mice increased GRK-2 expression approximately 4-fold above that of saline-treated (S, E) controls. In contrast, mice that were OVA-treated and exercised (EO) exhibited a 50% reduction in GRK-2 expression as compared with OVA-treated, sedentary mice; this level of GRK-2 expression was equivalent to that observed in saline-treated controls (S, E).

Moderate-Intensity Aerobic Exercise Attenuated PGE₂ Levels but Not EP1 Receptor Expression within the Lungs of OVA-Treated Mice

Increases in PGE₂ levels within the lung have been implicated in the initiation and/or exacerbation of AHR (19, 20). To determine the effect of moderate-intensity aerobic exercise on PGE₂ levels within the lungs of OVA-treated mice, mice were subjected to bronchoalveolar lavage and the resulting BALF was analyzed for changes in PGE₂ content. Results presented in Figure 6A demonstrate that OVA treatment of sedentary mice increased BALF PGE₂ levels approximately 250-fold as compared with saline-treated sedentary or exercised controls. In contrast, mice that were OVA-treated and exercised displayed an 80% reduction in BALF PGE₂ levels as compared with OVA-treated, sedentary mice (Figure 6A); however, these levels were significantly greater than those observed in saline-treated sedentary or exercised (S, E) controls.

PGE₂ binds and activates the E prostanoide (EP) receptor-1, which is expressed on ASM cells. As described above, activation of the EP1 receptor permits its heterodimerization with β_2 -AR, which impairs β_2 -AR coupling to the Gs-adenylated cyclase complex and, thereby, reduces β_2 -AR-stimulated cAMP production (9). To determine the effect of exercise on EP1 receptor expression, ASM cells were harvested and prepared for Western blot analysis. Data presented in Figure 5 indicate that moderate-intensity aerobic exercise training does not alter EP1 receptor protein expression on ASM cells. OVA treatment also did not affect EP1 receptor expression in these cells (Figure 5).

DISCUSSION

We have reported previously that repeated bouts of aerobic exercise at a moderate intensity attenuate airway inflammation and remodeling in OVA-treated mice (5); our current findings extend and complement this original study. Specifically, in the current study, we report that repeated bouts of aerobic exercise at a moderate intensity decreased the following parameters as compared with sedentary controls: (1) total lung resistance, (2) ASM thickness, (3) GRK-2 protein expression in ASM cells,

Figure 4. β_2 -AR antagonist blocks the effect of moderate intensity aerobic exercise on AHR in OVA-treated mice. Mice were OVA-treated and exercised as described in MATERIALS AND METHODS. At the conclusion of the protocol, mice were assessed for changes in AHR upon challenge with methacholine at increasing concentrations. Before each methacholine challenge, mice received a nebulization of either butoxamine HCL or saline. Results for (A) OVA-treated and (B) saline-treated mice are presented as total lung resistance (cm H₂O/ml/s; * $P < 0.02$ as compared with exercised, saline-treated [E]; § $P < 0.02$ as compared with EO, saline-treated; $n = 6$ –8 mice per group). OVA- and saline-treated mice were prepared and analyzed in a paired fashion; results from paired experiments are presented on separate graphs for the sake of clarity. (A) Open squares: sedentary, OVA-treated—saline; open circles: sedentary, OVA-treated— β_2 -AR antagonist; solid squares: exercised, OVA-treated—saline; solid circles: exercised, OVA-treated— β_2 -AR antagonist. (B) Open squares: sedentary, saline-treated—saline; open circles: sedentary, saline-treated— β_2 -AR antagonist; solid squares: exercised, saline-treated—saline; solid circles: exercised, saline-treated— β_2 -AR antagonist.

and (4) PGE₂ production. In contrast, repeated bouts of exercise increased circulating levels of epinephrine in OVA-treated mice. Exercise had no effect on β_2 -AR or EP1 receptor expression in ASM cells. Treatment with a β_2 -AR antagonist, however, blocked the effects of exercise on total lung resistance in OVA-treated mice. Together, these results suggest that repeated bouts of moderate-intensity aerobic exercise improve AHR in OVA-treated mice via a mechanism that involves β_2 -AR.

In a separate study, we have demonstrated that a single bout of moderate-intensity aerobic exercise decreased airway inflammatory responses, but had no effect on airway remodeling or AHR (13). These seemingly contrasting results between our studies may be explained, in part, by the differences in the length of the exercise protocols. The effects of exercise on physiologic responses are dependent upon several variables, including the total duration of the exercise protocol (21–26). The mechanism that underlies such a disparity likely involves changes in the levels of circulating hormones, including catecholamines, that are released from the hypothalamic–pituitary–adrenal axis (HPA) upon exercise. In the current study, we demonstrate that repeated exercise bouts increase circulating epinephrine levels in both saline- and OVA-treated mice; epinephrine levels were elevated at 48 hours after exercise.

Figure 5. Moderate-intensity aerobic exercise decreases protein expression of GRK-2, but not β_2 -AR or EP1 receptor, in the ASM of OVA-treated mice. Mice were OVA-treated and exercised as described in MATERIALS AND METHODS (S, sedentary, saline-treated; E, exercised, saline-treated; SO, sedentary, OVA-treated; EO, exercised, OVA-treated). At the conclusion of the protocol, ASM cells were harvested and prepared for Western blot analyses of (A) β_2 -AR (65 kD), GRK-2 (80 kD), and EP1 receptor (EP1 R; 32 kD) protein expression. (B) Densitometric analyses were performed; differences in lane loading were normalized via measurement of α -smooth muscle actin levels. Results are reported as fold difference in protein expression (†*P* < 0.02, as compared with sedentary, saline-treated controls; **P* < 0.02, as compared with sedentary, OVA-treated mice; *n* = 4–7 mice per group). Open bars, sedentary mice; solid bars, exercised mice.

Previous reports suggest that aerobic exercise training increases plasma concentrations of epinephrine and, moreover, that such increases are sustained at rest (7). Further, these reports indicate that post-exercise epinephrine concentrations are a consequence of increased secretion rather than accumulation (7). Importantly, Gilbert and colleagues have shown that repetitive exercise correlates positively with increased epinephrine levels and enhanced bronchodilation in adults with asthma (18); epinephrine can function as a β_2 -AR agonist, which promotes bronchodilation. In light of these previous reports, we conclude that repeated bouts of exercise attenuated AHR in OVA-treated mice via a mechanism that may involve sustained increases in circulating epinephrine.

Because epinephrine can function as a β_2 -AR agonist, the role of β_2 -AR in exercise-mediated attenuation of AHR was determined. Data presented herein demonstrate that, in both saline- and OVA-treated mice, acute exposure to the specific β_2 -AR antagonist butoxamine HCl blocked the effects of exercise on total lung resistance in response to methacholine challenge. This observation argues for a role of β_2 -AR in exercise-mediated attenuation of AHR; however, no changes in β_2 -AR protein expression were observed in ASM. These results contrast with those of Lin and coworkers, which demonstrated that chronic administration of the β_2 -AR-selective

Figure 6. Moderate-intensity aerobic exercise attenuates PGE₂ levels but not EP1 receptor expression in the lungs of OVA-treated mice. Mice were OVA-treated and exercised as described in MATERIALS AND METHODS. At the conclusion of the protocol, mice were subjected to bronchoalveolar lavage (BAL); BAL fluid was analyzed for changes in PGE₂ content via PGE₂-specific ELISA (†*P* < 0.01 as compared with sedentary, saline-treated controls; **P* < 0.01 as compared with sedentary, OVA-treated; *n* = 6–8 mice per group). Open bars, sedentary mice; solid bars, exercised mice.

antagonist ICI 118551 produced a bronchoprotective effect and an increase in β_2 -AR protein expression within the airways of OVA-treated mice (27). As such, the differences in the effect of β_2 -AR antagonist exposure on AHR between these studies are likely due to the level of β_2 -AR expression within the airways at the time of AHR measurements.

Upon activation, β_2 -AR facilitates bronchodilation through coupling to the Gs-protein complex and subsequent decreases in myosin light chain activity and smooth muscle tone (1). Associated with β_2 -AR activation is the autoregulatory process of receptor desensitization that involves phosphorylation of β_2 -AR via GRK-2 and its subsequent uncoupling from the Gs-protein complex. GRK-mediated phosphorylation of β_2 -AR also facilitates the binding of β_2 -AR to β -arrestins, including β arr-1 and β arr-2, which promote its subsequent desensitization (28). In particular, Deshpande and colleagues have shown recently that β arr-2 serves as a selective regulator of β_2 -AR signaling and function in ASM (29). Interestingly, repeated bouts of exercise decreased GRK-2 protein expression in ASM. Mak and coworkers have indicated that decreasing the expression of GRK-2 within the airways may lessen β_2 -AR desensitization (30). This observation is supported by a recent study from Kong and colleagues, which reported that inhibition of endogenous GRK2 and GRK3 in ASM is associated with increases in β_2 -AR acute signaling (31). Together, these results indicate that repeated bouts of moderate-intensity aerobic exercise may attenuate AHR by decreasing GRK-2-mediated desensitization of β_2 -AR.

Thickening of the airway wall is an indicator of asthma severity (17). Factors that contribute to increased airway wall thickness in the asthmatic lung include epithelial thickening, reticular basement membrane thickening, increased extracellular matrix, and fibrosis; however, ASM hypertrophy and/or hyperplasia contributes most to overall airway wall thickening (17). Data presented herein demonstrate that, in OVA-treated mice, repeated bouts of aerobic exercise at a moderate intensity decreased ASM thickness to control levels; however, it is unclear whether exercise lessened ASM cell size and/or proliferation. Within the OVA-treated lung, exercise-mediated decreases in ASM thickness correlated positively with exercise-mediated attenuation of AHR. The relationship between increased airway wall/ASM thickness and AHR has been explored in both clinical and animal model studies. Several studies suggest that increases in total wall thickness are associated with increased AHR. For example, Boulet and coworkers reported a positive correlation between airway wall thickening and hyperresponsiveness to methacholine challenge in patients with asthma with airway obstruction (32). The findings presented in

the current study support this observation. In contrast, Niimi and coworkers reported that there exists a negative correlation between airway wall thickness and AHR (33). These authors suggested that increased airway stiffness due to increased airway thickening may prevent ASM shortening and subsequent narrowing of the airway lumen.

Within the asthmatic lung and airways, PGE₂ has been considered protective against allergen-induced AHR (reviewed in Ref. 20). In contrast, results presented herein suggest that increased PGE₂ levels in the lungs of sedentary, OVA-treated mice do not protect against AHR. Specifically, results presented in Figure 6 show that exercise-mediated decreases in BALF PGE₂ levels correlate with decreased total lung resistance in response to methacholine challenge. Our results, in part, support the observations of Mathe and Hedqvist, who reported that administration of PGE₂ to individuals with asthma caused either bronchodilation or bronchoconstriction (34). Our results also support the findings of Pang and colleagues, who demonstrated that blockade of PGE₂ production leads to partial reversal of β_2 -AR desensitization in the context of inflammation (35). PGE₂ exerts its biological effects via binding and activating EP receptors, including the pro-bronchoconstrictive EP1 receptor (9). McGraw and colleagues have reported that activation of the EP1 receptor causes its heterodimerization with β_2 -AR, which impairs β_2 -AR coupling to the Gs-adenylated cyclase complex and, thereby, reduces β_2 -AR-stimulated cAMP production (9); therefore, we examined the effect of moderate-intensity aerobic exercise on EP1 receptor expression in these cells. Results presented in Figure 5 indicate that exercise has no effect on EP1 receptor expression in the ASM of saline- or OVA-treated mice. In light of these results, we conclude that decreases in PGE₂ within the asthmatic airways may lessen PGE₂-mediated EP1 receptor activation and subsequent decreases in β_2 -AR signaling.

Because of the potential for exercise-induced bronchospasm, we would like to note that none of the exercised mice exhibited labored or difficult breathing. Interestingly, these observations parallel the findings of previous clinical studies, which documented improvements in exercise-induced bronchoconstriction after physical training (36–40). Specifically, reports from Emtner and coworkers and from Henriksen and Nielsen each demonstrated that adults with mild–moderate asthma who participated in an aerobic exercise training program exhibited decreased exercise-induced bronchospasm (36, 37).

As noted above, the results of the current study demonstrate that repeated bouts of aerobic exercise at a moderate intensity attenuate asthma-associated AHR via a mechanism that involves β_2 -AR. Exercise-mediated decreases in GRK-2 protein expression, ASM thickness, and PGE₂ levels within the OVA-treated lung combined with exercise-mediated increases in circulating epinephrine levels support this conclusion. We believe that this study builds upon our previous work and further demonstrates the potential of moderate-intensity aerobic exercise as a therapeutic intervention for the treatment of asthma-associated airway inflammation, attenuate disease progression, and decrease asthmatic symptomatology.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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References

- Yang I, Ng T, Molenaar P, Fong K. β_2 -adrenoreceptor polymorphisms and obstructive airway diseases: Important issues of study design. *Clin Exp Pharmacol Physiol* 2007;34:1029–1036.
- Satta A. Exercise training in asthma. *J Sports Med Phys Fitness* 2000;40:277–283.
- Lucas S, Platts-Mills TA. Physical activity and exercise with asthma: relevance to etiology and treatment. *J Allergy Clin Immunol* 2005;115:928–934.
- Lucas S, Platts-Mills TA. Paediatric asthma and obesity. *Paediatr Respir Rev* 2006;7:233–238.
- Pastva A, Estell K, Schoeb TR, Atkinson TP, Schieberr LM. Aerobic exercise attenuates airway inflammation in a mouse model of atopic asthma. *J Immunol* 2004;172:4520–4526.
- Pastva A, Estell K, Schoeb TR, Schieberr L. RU486 blocks the anti-inflammatory effects of exercise in a murine model of allergen-induced pulmonary inflammation. *Brain Behav Immun* 2005;19:415–422.
- Zouhal H, Jacob C, Delamarche P, Gratas-Delamarche A. Catecholamines and the effects of exercise, training and gender. *Sports Med* 2008;38:401–423.
- Mutlu G, Koch W, Factor P. Alveolar epithelial β_2 -adrenergic receptors. *Am J Respir Crit Care Med* 2004;170:1270–1275.
- McGraw DW, Muhlbacker K, Schwarb M, Rahman F, Small K, Almoosa K, Liggett SB. Airway smooth muscle prostaglandin-EP1 receptors directly modulate β_2 -adrenergic receptors within a unique heterodimeric complex. *J Clin Invest* 2006;116:1400–1409.
- Duguet A, Biyah K, Minshall E, Gomes R, Wang C, Taoudi-benchekroun M, Bates JHT, Eidelman DH. Bronchial responsiveness among inbred mouse strains. *Am J Respir Crit Care Med* 2000;161:839–848.
- Herz U, Lumpp U, Daser A, Gelfand EW, Renz H. Murine animal models to study the central role of T cells in immediate-type hypersensitivity responses. *Adv Exp Med Biol* 1996;409:25–32.
- Cooper CB. Exercise in chronic pulmonary disease: limitations and rehabilitation. *Med Sci Sports Exerc* 2001;33:S643–S646.
- Hewitt M, Creel A, Estell K, Davis I, Schieberr L. Acute exercise decreases airway inflammation, but not responsiveness, in an allergic asthma model. *Am J Respir Cell Mol Biol* 2009;40:83–89.
- Amrani Y, Ammit AJ, Panettieri RA. Tumor necrosis factor receptor (TNFR) 1, but not TNFR2, mediates tumor necrosis factor- α -induced interleukin-6 and RANTES in human airway smooth muscle cells: role of p38 and p42/44 mitogen-activated protein kinases. *Mol Pharmacol* 2001;60:646–655.
- Propst SM, Estell K, Schieberr LM. CD40-mediated activation of NF- κ B in airway epithelial cells. *J Biol Chem* 2002;277:37054–37063.
- Cockcroft D, Davis B. Mechanisms of airway hyperresponsiveness. *J Allergy Clin Immunol* 2006;118:551–559.
- James A, Wenzel S. Clinical relevance of airway remodeling in airway diseases. *Eur Respir J* 2007;30:134–155.
- Gilbert I, Lenner K, McFadden E. Sympathoadrenal response to repetitive exercise in normal and asthmatic subjects. *J Appl Physiol* 1988;64:2667–2674.
- McGraw DW, Elwing JM, Foget KM, Wang W, Glinka C, Muhlbacker KA, Rothernberg ME, Liggett SB. Crosstalk between G1 and Gq/Gs pathways in airway smooth muscle regulates bronchial contractility and relaxation. *J Clin Invest* 2007;117:1391–1398.
- Vancheri C, Mastruzzo C, Sortino M, Crimi N. The lung as a privileged site for the beneficial actions of PGE₂. *Trends Immunol* 2004;25:40–46.
- Ceddia MA, Voss EW, Woods JA. Intracellular mechanisms are responsible for the exhaustive exercise-induced suppression of macrophage antigen presentation. *J Appl Physiol* 2000;88:804–810.
- Nieman DC, Nehlsen-Cannarella SL. The immune response to exercise. *Semin Hematol* 1994;31:166–179.
- Pedersen BK, Hoffman-Goetz L. Exercise and the immune system: regulation, integration, and adaptation. *Physiol Rev* 2000;80:1055–1081.
- Smith LL, Anwar A, Fragen M, Rananto C, Johnson R, Holbert D. Cytokines and cell adhesion molecules associated with high intensity eccentric exercise. *Eur J Appl Physiol* 2000;82:61–67.
- Su SH, Chen H, Jen CJ. Severe exercise enhances phagocytosis by murine bronchoalveolar macrophages. *J Leuk Biol* 2001;69:75–80.
- Woods JA, Lu Q, Ceddia MA, Lowder T. Exercise-induced modulation of macrophage function. *Immunol Cell Biol* 2000;78:545–553.
- Lin R, Peng H, Nguyen L, Dudekula N, Shardonofsky F, Knoll B, Parra S, Bond R. Changes in β_2 -adrenoreceptor and other signaling proteins produced by chronic administration of 'b-blockers' in a murine asthma model. *Pulm Pharm Therapeut*. 2008;21:115–124.
- Penn R, Pascual R, Kim Y, Mundell S, Krymskaya V, Panettieri R, Benovic J. Arrestin specificity for G protein-coupled receptors in human airway smooth muscle. *J Biol Chem* 2001;276:32648–32656.

29. Deshpande DA, Theriot B, Penn RB, Walker J. β -arrestins specifically constrain β_2 -adrenergic receptor signaling and function in airway smooth muscle. *FASEB J* 2008;22:2134–2141.
30. Mak J, Hisada T, Salmon M, Barnes PJ, Chung K. Glucocorticoids reverse IL-1 β -induced impairment of β -adrenoreceptor-mediated relaxation and up-regulation of G-protein-coupled receptor kinases. *Br J Pharmacol* 2002;135:987–996.
31. Kong K, Gandhi U, Martin T, Anz C, Yan H, Misior A, Pascual R, Deshpande D, Penn R. Endogenous Gs-coupled receptors in smooth muscle exhibit differential susceptibility to GRK2/3-mediated desensitization. *Biochemistry* 2008;47:9279–9288.
32. Boulet L, Turcotte H, Hudon C, Carier G, Maltais F. Clinical, physiological, and radiological features of asthma with incomplete reversibility of airflow obstruction compared with those of COPD. *Can Respir J* 1998;5:270–277.
33. Niimi A, Matsumoto H, Takemura M, Ueda T, Chin K, Mishima M. Relationship of airway wall thickness to airway sensitivity and airway reactivity in asthma. *Am J Respir Crit Care Med* 2003;168:983–988.
34. Mathe A, Hedqvist P. Effect of prostaglandins F₂ α and E₂ on airway conductance in healthy subjects and asthmatic patients. *Am Rev Respir Dis* 1975;111:313–320.
35. Pang L, Holland E, Know A. Role of cyclo-oxygenase-2 induction in interleukin-1 β induced attenuation of cultured human airway smooth muscle cell cyclic AMP generation in response to isoprenaline. *Br J Pharmacol* 1998;125:1320–1328.
36. Emtner M, Herala M, Stalenheim G. High-intensity physical training in adults with asthma: a 10-week rehabilitation program. *Chest* 1996;109:323–330.
37. Henriksen JM, Nielsen TT. Effect of physical training on exercise-induced bronchoconstriction. *Acta Paediatr Scand* 1983;72:31–36.
38. Matsumoto I, Araki H, Tsuda K, Odajima H, Nishima S, Higaki Y, Tanaka H, Tanaka M, Shindo M. Effects of swimming training on aerobic capacity and exercise induced bronchoconstriction in children with bronchial asthma. *Thorax* 1999;54:196–201.
39. Shaaban R, Leynaert B, Soussan D, Anto J, Chinn S, de Marco R, Garcia-Aymerich J, Heinrich J, Janson C, Jarvis D, *et al.* Physical activity and bronchial hyperresponsiveness: European Community Respiratory Health Survey II. *Thorax* 2007;62:403–410.
40. Hallstrand TS, Bates PW, Schoene RB. Aerobic conditioning in mild asthma decreases the hyperpnea of exercise and improves exercise and ventilatory capacity. *Chest* 2000;118:1460–1469.