Acute exercise is associated with reduced exhaled nitric oxide in physically inactive adults with asthma

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ABSTRACT

Background: Although exercise has multiple health benefits, relatively little attention has been paid to its potential therapeutic effects in those with asthma.

Objectives: To examine the effects of acute exercise on inflammation, in physically inactive and active adults with asthma.

Methods: Fourteen adults with asthma (n=6 physically inactive, n=8 physically active) completed 1) 30 minutes of moderate-intensity exercise on a treadmill, and 2) 30 minutes of rest in random order, with 4 weeks between sessions. Exhaled nitric oxide (eNO) was measured pre- and post-intervention (0, 0.5, 1, 2, 4, 24 hours). Blood inflammatory mediators were measured pre- and post-intervention (0, 2, 24 hours).

Results: Physically inactive participants had a significant decrease in eNO 4 hours post-exercise [-4.8 (-6.4, -0.5)ppb, p=0.028], which was not observed in physically active participants (p=0.362). IL-1ra increased in the physically inactive group 2 hours post-exercise, with this increase strongly correlated with the decrease in eNO both at 4 hours (R=-0.685, p=0.007) and 24 hours (R=-0.659, p=0.014) postexercise. IL-6 was increased significantly 2 hours post-exercise in physically inactive participants. Blood neutrophils and NRF2 gene expression were increased 2 hours post-exercise in the overall cohort.

Conclusion: This study demonstrates that acute moderate-intensity exercise is associated with reduced eNO in physically inactive adults with asthma and suggests that IL-1ra may have a role in mediating this effect. The attenuated response in physically active participants may be due to sustained anti-inflammatory effects of exercise training. Future studies should investigate the impact

INTRODUCTION

Physically active asthmatics experience fewer exacerbations of asthma, reduced airway hyperresponsiveness and improved asthma symptoms compared to those who are physically inactive[1-3]. Unfortunately, half of the Australian adult population fails to undertake sufficient exercise to provide any health benefit and, to amplify this, those with asthma are even less likely to exercise than non-asthmatics[4-6]. Fear of inducing asthma symptoms may be the cause, as exercise can cause exercise-induced bronchoconstriction (EIB) in some people; however, both pre-treatment with short-acting β_2-agonist[7] and regular exercise training[8] will minimize the risk of EIB.

The health benefits of exercise are believed to result in part from modification of systemic inflammation, and there is now an expanding literature on the modulatory effects of so-called '*myokines*': cytokines and growth factors released from exercising muscle that exert systemic effects[9]. IL-6 is the first myokine secreted in response to exercise[9], with the magnitude of secretion dependent on exercise intensity, exercise duration, mass of muscle recruited and host endurance capacity[10-12]. This then leads to activation of an anti-inflammatory response including inhibition of TNF-α, and production of IL-10 and IL-1ra (Interleukin-1 receptor antagonist)[13].

Murine models of asthma suggest that the anti-inflammatory effects of exercise may extend to the airways. For example, Hewitt et al[14] found that 45 minutes of moderate-intensity exercise on a treadmill significantly reduced BAL eosinophils, IL-5, IL-13 and NF-κB activity in a murine asthma model. In a similar model, Vieira et al[15] demonstrated that exercise training reduces epithelial markers of oxidative stress and nitrosative stress. While several studies exist in animals, less attention has been paid

to the effects of exercise in modifying airway inflammation in humans with asthma. Mendes et al[3] demonstrated that three months of aerobic training led to a reduction in both exhaled nitric oxide (eNO) and sputum eosinophils, as well as a reduction in asthma symptoms and asthma exacerbations, in adults with asthma. We previously conducted a 10 week exercise training program in overweight and obese adults with asthma and found a more than fivefold decrease in sputum eosinophils, with a corresponding significant improvement in the asthma-related quality of life score[16].

Mechanistic studies examining the drivers of exercise-induced reductions in airway inflammation are lacking in humans with asthma and, to our knowledge, no studies have examined whether usual physical activity level impacts this inflammatory response. Research in healthy adults indicates that exercise training increases resting muscle glycogen content which, in turn, substantially reduces the increase in IL-6 mRNA in exercising muscle, resulting in a smaller systemic inflammatory response following exercise[17]. That is, regular exercise training dampens the inflammatory response to acute exercise in healthy adults; however, the effect in those with asthma has yet to be investigated. Therefore, this study sought to examine potential mechanisms influencing reductions in airway inflammation in those with asthma and investigate whether the response is modified by habitual activity level. We hypothesised that a single bout of moderate-intensity exercise would acutely reduce eosinophilic airway inflammation, measured by eNO, in the airways of adults with asthma. We also hypothesised that this reduction in airway inflammation would be due to increases in anti-inflammatory myokines and endogenous antioxidants secreted by muscle cells, and that these effects would be more pronounced in those who were not regularly physically active. The aim of this study was to examine the effect of a

single bout of moderate-intensity exercise on airway inflammation in physically inactive and active adults with asthma, and to examine the potential roles of myokines and antioxidants in mediating these effects.

MATERIALS AND METHODS

Participants

Non-smoking asthmatic adults aged 18-65 years were recruited via the Hunter Medical Research Institute, NSW Australia between December 2013 and October 2014. Subjects had physician-diagnosed asthma with current episodic symptoms. Asthma was stable during all clinic visits, defined as no asthma exacerbation, respiratory tract infection or oral corticosteroid use in the month prior and no hospitalization for asthma in the three months prior. Inclusion criteria included body mass index (BMI) $\leq$40kg/m^2, a post-bronchodilator forced expiratory volume in 1 second (FEV_1) $\geq$50% and currently undertaking $\leq$90 minutes of structured moderate or vigorous-intensity exercise each week. Participants were not excluded from participating on the basis of unstructured activities, such as house and garden work. Exclusion criteria included current smokers, a cardiac or musculoskeletal contraindication to exercise, diabetes mellitus, uncontrolled hypertension, active cancer, and pregnancy or breastfeeding. The Hunter New England Human Research Ethics Committee approved this project and all subjects provided written informed consent. The trial was registered with the Australian New Zealand Clinical Trials Registry (http://www.anzctr.org.au), Registration Number: ACTRN12613001014741.

Experimental Protocol

This was a randomised controlled crossover trial where participants completed in random order: 1) 30 minutes of moderate-intensity exercise on a treadmill and 2) 30 minutes of rest in a chair, with a four week washout period between visits. Participants were randomised after baseline data collection was completed. The randomisation codes were computer-generated random sequences, derived and held

by an independent statistician.

Participants were asked to refrain from exercise for 72 hours and caffeine for 6 hours prior to each visit, and to consume a light breakfast 2 hours prior to their scheduled appointment. Upon arrival, participants had a venous blood sample collected and exhaled nitric oxide (eNO) (Ecomedics, Duernten, Switzerland) measured [18]. Spirometry was performed pre- and 15 minutes post- administration of 400ug salbutamol (Medgraphics, CPFS/DTM usb Spirometer, BreezeSuite v7.1, Saint Paul, USA). Twenty minutes post-bronchodilator, participants underwent their allocated intervention. Exhaled nitric oxide was measured 5 minutes, 30 minutes, 1, 2, 4 and 24 hours post-intervention while venous blood samples were collected 5 minutes, 2 hours and 24 hours post-intervention.

For the rest (control) intervention, participants were seated in a chair for 30 minutes. For the exercise intervention, participants completed a single moderate-intensity exercise session, which involved 30 minutes of walking on a treadmill at 4km/hour. After a 2-minute warm-up period, the incline on the treadmill was increased by 2.5% each minute to a maximum incline of 15%, until the target heart rate was achieved [60-80% of the maximal age-predicted heart rate (220 - age bpm)]. After 30 minutes, participants completed a one minute cool-down. Participant's heart rate and oxygen saturation were monitored throughout exercise and until 10 minutes post-exercise, at which point FEV_1 was measured. Spirometry was also performed at 1, 2, 4 and 24 hours post-intervention.

Participant Characterisation

Participants completed the long form of the International Physical Activity Questionnaire (IPAQ) to quantify physical activity levels, with results expressed as METS (Metabolic Equivalent Tasks)[19]. A categorical variable was generated according to IPAQ guidelines, classifying participants as having a low, moderate or high level of physical activity[20]. This questionnaire captures both structured and unstructured physical activities. Participants were then categorised as physically inactive (low physical activity level) and physically active (moderate to high physical activity level) groups. Body weight (Nuweigh LOG 842, NWS, Newcastle Australia) and height were measured. Waist circumference was measured in duplicate at the midpoint of the lowest rib and iliac crest[21] (Lufkin W606PM Executive Diameter Tape 2mx6mm, Lufkin USA). A total body scan was performed by dual-energy x-ray absorptiometry (Lunar Prodigy Series, GE Medical Systems, Madison USA) as described previously[16]. Participants completed the Juniper Asthma Control Questionnaire (ACQ)[22] and Juniper asthma-related quality of life questionnaire (AQLQ)[23]. Atopy was determined by skin prick allergy test.

Inflammatory Markers and Antioxidants

Whole blood was collected into EDTA tubes and centrifuged at 3000 g at 4^{o}C for 10 minutes. The plasma was separated and stored at -80^{o}C until analysis. Commercial ELISAs were used to measure plasma IL-6, IL-1ra and IL-10 (R&D systems, Minneapolis MN, USA). The assay sensitivities were 0.039pg/ml, 6.3pg/ml and 0.09pg/ml, respectively.

For assessment of antioxidant activity, erythrocytes were lysed in ice-cold HPLC-grade water and centrifuged at 10,000 g at 4^{o}C for 15 minutes, and the lysate stored at -80^{o}C until analysis. Erythrocyte total superoxide dismutase (SOD) activity was measured using a Superoxide Dismutase Assay Kit (Cayman, Ann Arbor MI, USA), while erythrocyte glutathione peroxidase (GSH-Px) activity was measured using a Glutathione Peroxidase Assay Kit (Cayman, Ann Arbor MI USA). SOD and GSH-Px were adjusted for erythrocyte hemoglobin (Hb) concentration (Hemoglobin Colorimetric Assay Kit, Cayman, Ann Arbor MI, USA). A full blood count was performed using an automated Coulter count (Beckman Coulter LH 780 Hemotology Analyzer, Brea CA USA).

Inflammatory Gene Expression

After erythrocyte lysis, blood leukocytes were resuspended in RLT buffer, homogenised via vigorous vortexing and stored at -80^{o}C until RNA extraction. RNA was extracted using the QIAamp RNA Blood Mini Kit (QIAgen, Hilden, Germany) and an automated protocol (Qiacube, Hilden, Germany) as per manufacturer's instructions with an on column DNase digestion. RNA concentration was measured using the RiboGreen RNA Quantification Assay [Quant-iT™ Ribogreen® RNA Assay Kit, Life Technologies (Invitrogen), Carlsbad, CA, USA] and 200ng of RNA was reverse transcribed into cDNA using the High-Capacity cDNA Reverse Transcription Kit as per manufacturer's instructions (Life Technologies, Carlsbad, CA, USA). Taqman qPCR primer and probes for *NRF2*, *HO-1*, *GST-P1*, *GPx2*, *SOD2*, *IL1RA*, *IL4*, *IL5*, *IL6*, *IL10* and housekeeping 18s rRNA was purchased in kit form (Life Technologies, Carlsbad, CA, USA). PCR primers and probes were combined with Taqman gene expression master mix and cDNA in duplicate

singleplex qPCRs (ABI 7500 Real Time PCR System). Statistical analysis to compare baseline differences was performed on the change in cycle threshold (ΔCt) between the target gene and the housekeeping gene (18s rRNA). Fold change results were calculated using $2^{-\Delta\Delta Ct}$ relative to both the housekeeping gene (ΔCt; 18s rRNA) and participant's baseline visit (ΔΔCt).

Statistical Analysis

Data were analysed using the statistical software package Intercooled Stata Version 11.1 (Stata Corporation, College Station TX USA) and are reported as median (interquartile-range), mean±standard deviation or the percentage of participants with the specified variable. Group comparisons of proportions were examined by Pearson's chi squared test. Continuous baseline data were compared in physically inactive and physically active participants using an unpaired t-test or Wilcoxon rank sum testing. The effect of acute exercise on airway inflammation, systemic inflammation and RNA expression was examined using a paired t-test or Wilcoxon signed rank testing at each time point. These changes were also examined by comparing usual physical activity level using an unpaired t-test or Wilcoxon rank sum testing. Correlations between usual physical activity levels, and baseline and change in inflammation and antioxidants, were assessed using the Pearson or Spearman correlation test. *P*-values <0.05 were considered statistically significant.

RESULTS

Baseline Characteristics

Fourteen participants (87.5%), six categorised as physically inactive and eight as physically active, completed both the exercise and rest sessions (Figure 1, Table 1). Physically inactive and active participants did not differ with respect to age, sex, body composition, lung function, atopy, smoking history, inhaled corticosteroid use, age of asthma onset or ACQ score (Table 1). They also had similar baseline levels of cytokines, cell counts, antioxidants and gene expression (Table 2). Higher expression of *IL10* (ΔCt) in blood leukocytes at baseline was correlated with a higher usual level of moderate-intensity exercise (r_s=-0.586, p=0.035), however gene expression of *IL1RA*, *HO-1*, *NRF2* and *GSTP1* did not differ with activity level (data not shown). *GPx2*, *IL6*, *IL5* and *IL4* gene expression was not detectable.

Comparison of Rest and Exercise Conditions on Lung Function, Airway Inflammation and Systemic Inflammation

Participants exercised at 70.3±3.1% of their age predicted maximal heart rate, with no difference between physically active and inactive groups (p=0.903). FEV_1 was unchanged from pre-exercise post 400µg salbutamol [2.56 (2.19, 3.23)L] to 10 minutes post-exercise [2.60 (2.20, 3.23)L, p=0.327)]. Furthermore, there were no differences in lung function at 1, 2, 4 and 24 hours following exercise versus rest (data not shown). The effects of the acute bout of exercise on inflammation and gene expression are detailed in Table 3. eNO did not change following the rest or exercise conditions, except for a statistically significant increase 24 hours following the rest condition (Table 3). Compared to rest, IL-6 was increased significantly immediately post-exercise (Figure 2a), and was followed by a significant increase in IL-1ra and IL-

10 at 2 hours (Table 3). Peripheral blood neutrophils were significantly increased immediately post and 2 hours post exercise (Figure 2b), while blood eosinophils were unchanged (Table 3). *NRF2* expression was increased 2 hours post-exercise (Table 3). SOD and GSH-Px activity, and gene expression of *IL1RA*, *IL10*, *SOD2*, *GSTP1* and *HO-1* were unchanged post exercise (Table 3).

Effects of Usual Physical Activity Level on Exercise-Induced Changes to Airway Inflammation, Systemic Inflammation and Antioxidants

In participants who were regularly physically inactive, eNO was significantly reduced 4 hours post-exercise (Figure 3a, Table 4). Conversely, eNO was unchanged in participants who were regularly physically active except for a statistically significant increase at 24 hours post-exercise (Figure 3a, Table 4). Significant differences in the change in eNO were observed between physically inactive and physically active adults at 2 hours (p=0.028), 4 hours (p=0.024) and 24 hours (p=0.040) post-exercise (Table 4).

The increase in plasma IL-6 immediately post and 2 hours post exercise was statistically significant in the physically inactive, but not in the physically active, participants although the median magnitude of the increase was similar (Figure 3b, Table 4). IL-1ra and IL-10 were also significantly increased 2 hours post-exercise in the physically inactive participants, but not in the physically active participants (Figure 3, Table 5). Interestingly, the increase in IL-1ra concentration at 2 hours post-exercise was strongly correlated with the decrease in eNO, both at 4 hours (R=-0.685, p=0.007) and 24 hours (R=-0.659, p=0.014) post-exercise (Figure 4).

Immediately post-exercise, there was a significant increase in blood neutrophils in the physically active group and similar trend in the physically inactive group (p=0.052); at 2 hours post-exercise this increase was significant for both groups (Table 4). There was a trend towards a reduction in blood eosinophils in physically inactive participants 2 hours post-exercise, however this did not reach statistical significance (p=0.086) (Table 4). Erythrocyte SOD and GSH-Px activity were unchanged following exercise in both physically inactive and active participants (Table 4). Relative to inactive participants, *IL10* gene expression was reduced immediately following exercise in active participants (Table 4). *NRF2* expression was increased 2 hours post exercise in active participants, but not inactive participants (Table 4). There was a reduced expression of *GSTP1* 2 hours post exercise in physically inactive participants, while *IL1RA*, *SOD2* and *HO-1* were unchanged in both groups (Table 4).

DISCUSSION

This study demonstrates that the inflammatory responses to a single bout of moderate-intensity exercise are influenced by habitual physical activity status in adults with asthma. Exhaled nitric oxide, a surrogate marker of eosinophilic airway inflammation, was transiently reduced following exercise in participants who were physically inactive, with the reduction in eNO strongly correlated with increases in circulating levels of the anti-inflammatory cytokine, IL-1ra. This suggests for the first time in humans a plausible mechanism mediating the exercise-induced reduction in airway inflammation. As expected, we observed a significant increase in circulating neutrophils, IL-6, IL-1ra and IL-10 in response to exercise; however, when examined by usual physical activity level this increase was only significant in physically inactive participants. There was a small increase in *NRF2* expression, a reduction in *IL10* in active participants and a reduction in *GSTP1* in inactive participants.

We observed a significant 5ppb reduction in eNO in physically inactive adults with asthma four hours post exercise. Similarly, Tufvesson[24] observed a reduction in eNO of 3ppb following an exercise challenge in adults with mild asthma. Clinical guidelines produced by the American Thoracic Society suggest that a change in eNO of 10ppb may indicate a significant response to therapy in those who have a baseline eNO of less than 50ppb[25]. While the response we observed to acute exercise was half this size, it is likely that in a chronic setting, or with an extended exercise duration, this response would increase in magnitude. Indeed, following three months of aerobic training Mendes et al[3] observed a significant reduction in eNO of approximately 8ppb in addition to a decrease in sputum eosinophils. This intervention was associated with reduced asthma symptoms and fewer exacerbations of asthma, suggesting a clinically

important reduction in eNO. Taken together, these studies support our observation that exercise reduces nitric oxide within the airways of adults with asthma, while our study proposes that this decrease is modulated by habitual physical activity level.

We observed an increase in blood neutrophils, IL-6, IL-1ra and IL-10 post-exercise, which is consistent with the literature[12, 26]. However, blood IL1RA RNA expression was unchanged, and IL6 and IL10 mRNA remained undetectable, suggesting that the increased levels of IL-6, IL-1ra and IL-10 in the plasma were not due to increased production from the inflammatory cells in the blood following this acute bout of moderate-intensity exercise. Interestingly, we found a strong correlation between the increase in plasma IL-1ra at 2 hours post-exercise and the decrease in eNO in all participants, both 4 hours and 24 hours post-exercise. IL-1ra is an antagonist of IL-1 receptor type 1, with IL-1 promoting eosinophil infiltration into the airways by increasing production of IL-4, IL-5 and IL-13[27, 28]. In a study of ovalbumin-sensitised guinea pigs, Watson et al[29] found an inhibition of antigen-induced eosinophil accumulation in the BALF after pre-treatment with aerosolised IL-1ra. Additionally, following antigen challenge in IL-1 receptor type 1 deficient mice, Broide et al[30] found significantly reduced BALF eosinophils when compared to wild type mice. These studies support our observation, suggesting that the systemic rise in IL-1ra following exercise could plausibly be driving anti-inflammatory effects in the lungs, resulting in reduced airway eosinophils.

Murine models of asthma have been studied to better understand the mechanisms driving exercise-induced reductions to eosinophilic airway inflammation. These studies demonstrate that moderate-intensity exercise results in an increased expression

of the anti-inflammatory cytokine IL-10, and a reduction in the Th2-derived cytokines IL-4 and IL-5, resulting in a reduced eosinophil influx into the airways[14, 31]. While we were unable to detect IL-4 and IL-5 in this population, we also observed an increase in IL-10 post-exercise; however, this increase was not correlated with the decrease in eNO in this population.

Interestingly we found eNO was not reduced by exercise in those who were regularly physically active, which may be explained by exercise adaptation producing an attenuated inflammatory response to acute moderate intensity exercise. Indeed, the increases in IL-6, IL-1ra and IL-10 were only statistically significant in the physically inactive participants. In line with our observations, Fischer et al[17] studied seven healthy men who underwent knee extensor training for 10 weeks. Prior to the training program, an acute bout of eccentric exercise resulted in a 76-fold increase in skeletal muscle IL-6 gene expression; however, following the training program this response was blunted, with only an 8-fold increase in IL-6 gene expression[17]. Similarly, Croft et al[32] found that six weeks of high intensity interval training reduced the acute exercise-induced increase in plasma IL-6 and IL-8 compared with pre-training increases. Exercise training increases muscle glycogen content, with Fischer et al[17] demonstrating a 74% increase in muscle glycogen following their 10 week training program. Pre-exercise muscle glycogen content is inversely correlated with the post-exercise skeletal muscle IL-6 mRNA expression, suggesting that muscular IL-6 mRNA expression is enhanced once muscle glycogen is depleted below a certain level[17]. Therefore, exercise training appears to assist with maintaining metabolic homeostatis within skeletal muscle during an acute bout of exercise, which dampens

the inflammatory response[32]. Our study suggests that this dampened response extends to both the blood and airways of those with asthma.

NRF2 gene expression was increased following acute moderate-intensity exercise in this cohort of adults with asthma, with this increase only significant in those who were regularly active. *NRF2* is a transcription factor that is involved in the transcriptional regulation of various antioxidant genes including *SOD2*, *HO-1* and *GSTP1*[33]. Acute exercise has been shown to increase *NRF2* expression in the hearts of mice resulting in enhanced antioxidant defences[34], while exercise training leads to activation of *NRF2* in human skeletal muscle[35]. Further investigation of exercise-induced modifications to gene expression in relation to asthmatic airway inflammation should be investigated in a larger asthmatic population.

A limitation of this study is the small sample size, which limits the statistical power of the analysis. Despite this, important and novel observations were made, providing direction for future trials. Airway inflammation was measured indirectly by eNO, however eNO has been shown to be an acceptable surrogate marker of eosinophilic airway inflammation[36], with Mendes et al[3] demonstrating reductions in both eNO and sputum eosinophils following exercise training. In addition, this trial only examined exercise of a moderate intensity. Future trials should examine whether vigorous-intensity exercise has different effects upon airway inflammation. Furthermore, this study is unable to elucidate the long term effects of exercise training on asthma outcomes. However, the purpose of this study was to better understand the anti-inflammatory effects of exercise on asthma in general because in order to fully

understand how exercise impacts asthma, it is important to understand how acute exercise modifies inflammation and any factors that may impact upon this.

In conclusion, this study has demonstrated that a single bout of moderate-intensity exercise reduces eNO in physically inactive adults with asthma and that this reduction is strongly correlated with a rise in plasma levels of the anti-inflammatory myokine IL-1ra. Interestingly, the inflammatory response to moderate intensity exercise appears to be attenuated in those who are regularly physically active, which is likely due to sustained anti-inflammatory effects of training. Future studies are needed to establish which intensity and type of exercise produces the most favourable effects upon airway inflammation in those with asthma. In addition, future studies should examine the long term effects of exercise training on chronic inflammation in asthma.

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Table 1. Baseline Characteristics.

	All Subjects	Physically Inactive	Physically Active	P-value
N	14	6	8	0.005
Sex (%Female)	78.6	100.0	62.5	0.091
Age (years)	43.3 (37.0, 57.8)	40.2 (37.0, 44.5)	52.8 (38.2, 59.7)	0.366
BMI (kg/m^2)	29.1 (26.7, 29.7)	28.4 (26.7, 29.7)	29.1 (24.0, 31.4)	0.949
Waist Circumference (cm)	92.3 (86.1, 104.4)	94.7 (86.1, 101.2)	92.3 (84.4, 105.7)	0.897
Total Body Fat (%)	44.4 (39.4, 47.7)	44.6 (40.4, 48.4)	42.0 (31.1, 47.5)	0.366
Pre-BD FEV_1 (%)	74.7 (63.2, 96.8)	74.7 (69.2, 76.8)	73.4 (56.0, 97.5)	0.699
Pre-BD FVC (%)	86.6±14.1	88.4±12.1	85.2±16.0	0.908
Pre-BD FEV_1/FVC (%)	69.9±10.3	71.2±10.5	69.0±10.7	0.704
Atopy (%)	85.7	83.3	87.5	0.825
ICS Dose (µg/day) †	300 (0, 1000)	500 (0, 1000)	300 (0, 750)	0.892
Ever Smokers (%)	35.7	33.3	37.5	0.872
Pack Years	0 (0, 1.35)	0 (0, 0.07)	0 (0, 3.95)	0.547
Age at Asthma Diagnosis (years)	7.5 (4.0, 16)	9 (2, 32)	8 (6, 13)	0.948
ACQ Score	0.71 (0.57, 1.00)	0.71 (0.71, 1.00)	0.72 (0.22, 1.07)	0.558
AQLQ Score	6.5 (6.4, 6.7)	6.5 (6.2, 6.7)	6.6 (6.5, 7.0)	0.395
GINA Classification (%)				
(Intermittent/Mild/Moderate/Severe)	36/0/50/14	17/0/83/0	50/0/25/25	0.086
Exhaled Nitric Oxide (ppb)	25.9±15.9	19.5±15.2	30.7±15.7	0.205
Total Physical Activity (METS)	4278 (2374, 10245)	2074 (1719, 3275)	8950 (5205, 11 157)	**0.010**
Vigorous Physical Activity (METS)	40 (0, 160)	61 (0, 160)	40 (0, 200)	0.890
Moderate Physical Activity (METS)	2550 (1560, 6780)	1410 (970, 1920)	5475 (2550, 10 448)	**0.014**
Walking (METS)	619 (454, 2525)	516 (215, 644)	1056 (545, 2995)	0.197
Physical Activity Classification (%)				
(Low/Moderate/High)	43/43/14	100/0/0	0/75/25	**0.001**

Data presented as median (IQR), mean±SD or % the percentage of subjects with the specified variable. BMI – body mass index; BD – bronchodilator; FEV_1 – forced expiratory volume in 1 second; FVC – forced vital capacity; ICS – inhaled corticosteroid; ACQ – asthma control questionnaire; AQLQ – asthma-related quality of life questionnaire; GINA – Global Initiative for Asthma; METS - Metabolic Equivalent Tasks (minutes/week).

Table 2. Baseline levels of inflammatory markers, antioxidants and relative gene expression

	All Subjects	Physically Inactive	Physically Active	P-value
Blood Biomarkers				
IL-1ra (pg/mL)	230 (211, 374)	227 (211, 259)	256 (200, 389)	0.699
IL-6 (pg/mL)	0.92 (0.70, 1.33)	0.79 (0.74, 1.52)	1.06 (0.69, 1.31)	0.796
IL-10 (pg/mL)	0.24±0.10	0.25±0.08	0.22±0.13	0.616
SOD (U/10mg Hb)	10.8 (6.6, 12.2)	11.9 (10.4, 12.2)	6.9 (5.6, 12.8)	0.317
GSH-Px (nmol/min/10mg Hb)	28.3 (26.1, 37.6)	36.2 (27.8, 37.6)	27.4 (23.3, 36.2)	0.661
Eosinophils ($\times 10^9$/L)	0.2 (0.1, 0.3)	0.2 (0.1, 0.3)	0.3 (0.2, 0.3)	0.640
Neutrophils ($\times 10^9$/L)	3.5±0.9	4.0±0.8	3.2±0.9	0.098
Gene Expression*				
IL1RA mRNA	16.08±0.83	15.91±0.63	16.22±0.99	0.515
IL10 mRNA	23.44±0.76	23.64±0.52	23.27±0.92	0.400
SOD2 mRNA	10.88±0.74	10.52±0.66	11.19±0.69	0.103
HO-1 mRNA	17.51±0.66	17.43±0.64	17.58±0.72	0.703
NRF2 mRNA	17.18±1.12	16.84±1.16	17.46±1.09	0.345
GSTP1 mRNA	16.81±0.44	16.85±0.48	16.77±0.45	0.783

Data presented as mean±SD, median (IQR) or % the percentage of subjects with the specified variable. *Data expressed as the change in cycle threshold (Ct) compared to the housekeeping gene 18s rRNA (ΔCt). A lower ΔCt corresponds to a stronger expression of the target gene. IL1RA – interleukin 1 receptor antagonist; IL10 – interleukin 10; SOD2 – superoxide dismutase 2, mitochondrial; HO-1 – Heme oxygenase 1; NRF2 – nuclear factor, erythroid 2-like 2; GSTP1 – glutathione S-transferase pi 1.

Table 3. Effect of acute exercise on exhaled nitric oxide and systemic inflammation

Outcome	Change After Rest (n=14)	P-value	Change After Exercise (n=14)	P-value	ΔR vs ΔE (P-value)
Exhaled Nitric Oxide					
Δ Immediately Post	-0.3±4.6	0.793	-0.9±5.2	0.542	0.696
Δ 30 Minutes Post	0.5±5.8	0.731	-1.0±8.3	0.645	0.275
Δ 1 Hour Post	0.3±3.2	0.722	0.2±5.3	0.875	0.946
Δ 2 Hours Post	0.2 (-0.2, 2.9)	0.346	-0.05 (-2.1, 1.8)	0.975	0.826
Δ 4 Hours Post	-1.2 (-4.1, 2.0)	0.177	-0.5 (-5.2, 1.6)	0.346	0.433
Δ 24 Hours Post	2.8±4.1	**0.029**	2.5±8.1	0.286	0.894
IL-6 (pg/mL)					
Δ Immediately Post	-0.09 (-0.20, 0.06)	**0.033**	0.27 (0.06, 0.41)	**0.013**	**0.007**
Δ 2 Hours Post	0.06 (-0.05, 0.19)	0.109	0.21 (0.12, 0.64)	0.056	0.470
Δ 24 Hours Post	-0.02 (-0.38, 0.03)	0.382	-0.07 (-0.30, 0)	0.072	0.917
IL-1ra (pg/mL)					
Δ Immediately Post	-11.1 (-16.9, 9.0)	0.074	-2.1 (-9.0, 18.5)	0.807	0.116
Δ 2 Hours Post	3.0 (-6.7, 20.7)	0.272	14.8 (4.9, 29.7)	**0.009**	0.683
Δ 24 Hours Post	1.1 (-16.4, 12.7)	0.753	-6.8 (-23.5, 7.1)	0.249	0.249
IL-10 (pg/mL)					
Δ Immediately Post	0.09±0.23	0.241	0.13±0.29	0.200	0.990
Δ 2 Hours Post	0.17 (-0.09, 0.41)	0.230	0.24 (0.01, 0.56)	**0.009**	0.328
Δ 24 Hours Post	0±0.32	0.964	0.13±0.30	0.211	0.455
SOD (u/10mg Hb)					
Δ Immediately Post	-1.5 (-6.3, 1.9)	0.158	-2.3 (-3.3, 1.6)	0.600	0.534
Δ 2 Hours Post	-2.5±3.6	**0.045**	-1.1±3.9	0.352	0.487
Δ 24 Hours Post	-3.4 (-5.9, -0.4)	**0.033**	-2.3 (-3.7, 1.1)	0.100	0.398
GSH-Px (nmol/min/10mg Hb)					
Δ Immediately Post	4.7 (-6.8, 9.6)	0.683	1.8 (-7.2, 5.3)	0.938	0.693
Δ 2 Hours Post	6.9 (-1.7, 14.6)	0.116	4.0 (1.7, 9.5)	0.087	0.814
Δ 24 Hours Post	7.1 (-0.9, 8.6)	0.213	-1.3 (-9.2, 7.2)	0.959	0.441
Neutrophils ($\times 10^9$/L)					
Δ Immediately Post	0 (0, 0.3)	0.130	0.3 (0.1, 0.6)	**0.002**	**0.039**
Δ 2 Hours Post	0.5±0.4	**<0.001**	1.0±0.6	**<0.001**	**0.009**
Δ 24 Hours Post	0±0.5	0.812	-0.1±0.7	0.541	0.659
Eosinophils ($\times 10^9$/L)					
Δ Immediately Post	0 (-0.1, 0)	0.379	0 (0, 0)	0.387	0.853
Δ 2 Hours Post	0 (-0.1, 0)	0.078	0 (-0.1, 0)	0.149	0.294
Δ 24 Hours Post	0 (0, 0)	0.863	0 (0, 0)	0.532	0.600
IL1RA mRNA Fold Change					
Δ Immediately Post	1.00 (0.73, 1.49)	0.780	1.08 (0.68, 1.58)	0.308	0.530
Δ 2 Hours Post	1.08 (0.84, 1.70)	0.328	1.07 (0.77, 1.34)	0.401	0.754
IL10 mRNA Fold Change					
Δ Immediately Post	0.73 (0.60, 1.08)	0.133	0.84 (0.57, 1.44)	0.938	0.530
Δ 2 Hours Post	0.71 (0.66, 1.10)	0.311	0.87 (0.64, 1.47)	0.753	0.754
SOD2 mRNA Fold Change					
Δ Immediately Post	0.85 (0.77, 1.05)	0.442	1.21 (0.75, 1.43)	0.239	0.136
Δ 2 Hours Post	1.12 (0.89, 1.32)	0.263	1.04 (0.79, 1.32)	0.364	0.875
HO-1 mRNA Fold Change					
Δ Immediately Post	0.99±0.29	0.904	1.13±0.46	0.355	0.309
Δ 2 Hours Post	0.93 (0.74, 1.29)	0.807	0.72 (0.47, 1.07)	0.221	0.480
NRF2 mRNA Fold Change					
Δ Immediately Post	1.19 (1.08, 1.36)	**0.046**	1.15 (0.88, 1.39)	0.170	0.814
Δ 2 Hours Post	0.89 (0.80, 1.22)	0.861	1.37 (1.00, 1.57)	**0.014**	0.272
GSTP1 mRNA Fold Change					
Δ Immediately Post	0.89 (0.67, 1.02)	0.345	0.95 (0.64, 1.57)	0.937	0.875
Δ 2 Hours Post	1.05 (0.61, 1.16)	0.753	0.72 (0.63, 0.89)	0.421	0.754

Data presented as mean±SD or median (IQR) for the change from baseline at each time point specified. SOD – superoxide dismutase; GSH-Px – glutathione peroxidase, IL1RA – interleukin 1 receptor antagonist, IL10 – interleukin 10; SOD2 – superoxide dismutase 2, mitochondrial; HO-1 – Heme oxygenase 1; NRF2 – nuclear factor, erythroid 2-like 2; GSTP1 – glutathione S-transferase pi 1. *P*-value respresents the difference in change from baseline to the specified time point. ΔR vs ΔE *p*-value compares the difference in change between the rest condition versus the exercise condition.

Table 4. Post-exercise change in airway and systemic inflammation, in physically inactive and physically active adults with asthma.

Outcome	Physically Inactive (n=6)	P-value	Physically Active (n=8)	P-value	ΔPI vs ΔPA (P-value)
eNO (ppb)					
Δ Immediately Post	-2.0±3.6	0.227	0±6.2	0.996	0.498
Δ 30 Min Post	-1.5±3.2	0.314	-0.7±10.9	0.857	0.876
Δ 1 Hour Post	-1.5±3.0	0.280	1.5±6.4	0.528	0.315
Δ 2 Hours Post	-1.8 (-5.4, -0.6)	0.116	1.4 (-0.4, 2.8)	0.161	**0.028**
Δ 4 Hours Post	-4.8 (-6.4, -0.5)	**0.028**	0.8 (-1.0, 5.0)	0.362	**0.024**
Δ 24 Hours Post	-5.1 (-7.6, 2.5)	0.225	3.8 (1.6, 10.6)	**0.042**	**0.040**
IL-1ra (pg/mL)					
Δ Immediately Post	17.5 (-4.4, 29.9)	0.463	-2.8 (-13.2, 7.5)	0.398	0.199
Δ 2 Hours Post	25.4 (9.6, 32.5)	**0.028**	6.7 (-0.4, 22.5)	0.161	0.093
Δ 24 Hours Post	-6.8 (-24.3, 7.2)	0.500	-3.2 (-21.3, 5.6)	0.401	0.884
IL-6 (pg/mL)					
Δ Immediately Post	0.25 (0.09, 0.45)	**0.046**	0.27 (-0.09, 0.41)	0.128	0.568
Δ 2 Hours Post	0.20 (0.14, 0.73)	**0.028**	0.21 (-0.21, 0.48)	0.484	0.651
Δ 24 Hours Post	-0.07 (-0.13, 0.19)	0.893	-0.06 (-0.35, 0)	**0.030**	0.377
IL-10 (pg/mL)					
Δ Immediately Post	0.24±0.27	0.081	-0.05±0.25	0.747	0.136
Δ 2 Hours Post	0.40 (0.23, 0.65)	**0.028**	0.19 (0, 0.36)	0.174	0.144
Δ 24 Hours Post	0.22±0.30	0.179	0.17±0.23	0.797	0.347
SOD (u/10mg Hb)					
Immediately Post	-2.9 (-3.3, 1.4)	0.463	0.9 (-2.9, 3.2)	0.866	0.475
2 Hours Post	-2.3±3.9	0.249	0.1±3.8	0.753	0.262
24 Hours Post	-3.9 (-4.3, -1.7)	0.080	-0.3 (-3.0, 2.3)	0.612	0.088
GSH-Px (nmol/min/10mg Hb)					
Δ Immediately Post	1.4 (-7.6, 4.4)	0.893	2.1 (-6.7, 6.2)	0.735	0.808
Δ 2 Hours Post	9.5 (4.5, 13.0)	0.225	2.6 (-0.4, 5.3)	0.263	0.188
Δ 24 Hours Post	12.3 (2.6, 31.8)	0.144	-7.3 (-13.1, -0.9)	0.116	0.055
Neutrophils ($\times 10^9$/L)					
Δ Immediately Post	0.3 (0, 0.6)	0.052	0.3 (0.2, 0.6)	**0.018**	0.519
Δ 2 Hours Post	1.3±0.6	**0.004**	0.9±0.6	**0.004**	0.366
Δ 24 Hours Post	-0.1±1.0	0.827	-0.1±0.5	0.483	0.951
Eosinophils ($\times 10^9$/L)					
Δ Immediately Post	0 (-0.1, 0)	0.159	0 (0, 0)	0.914	0.256
Δ 2 Hours Post	-0.1 (-0.1, 0)	0.086	0 (-0.1, 0)	0.682	0.346
Δ 24 Hours Post	0 (0, 0)	>0.999	0 (0, 0)	0.317	0.621
IL1RA mRNA Fold Change					
Δ Immediately Post	1.33 (0.82, 1.55)	0.463	1.03 (0.54, 1.61)	0.463	0.749
Δ 2 Hours Post	1.00 (0.72, 1.00)	0.753	1.34 (0.77, 2.73)	0.149	0.199
IL10 mRNA Fold Change					
Δ Immediately Post	1.42 (0.74, 2.12)	0.249	0.57 (0.41, 0.93)	0.075	**0.037**
Δ 2 Hours Post	1.01 (0.64, 1.47)	0.753	0.87 (0.42, 1.92)	0.866	0.886
SOD2 mRNA Fold Change					
Δ Immediately Post	1.18±0.52	0.435	1.22±0.48	0.313	0.893
Δ 2 Hours Post	0.91 (0.72, 1.04)	0.753	1.28 (0.92, 2.74)	0.128	0.153
HO-1 mRNA Fold Change					
Δ Immediately Post	1.18±0.45	0.386	1.08±0.51	0.709	0.743
Δ 2 Hours Post	0.60 (0.44, 1.07)	0.116	0.78 (0.51, 1.65)	0.866	0.391
NRF2 mRNA Fold Change					
Δ Immediately Post	1.15 (1.08, 1.26)	0.075	1.10 (0.74, 1.43)	0.463	0.749
Δ 2 Hours Post	1.12 (0.98, 1.57)	0.292	1.39 (1.29, 1.67)	**0.028**	0.391
GSTP1 mRNA Fold Change					
Δ Immediately Post	0.89 (0.69, 1.69)	0.917	0.97 (0.59, 1.57)	0.753	0.873
Δ 2 Hours Post	0.69 (0.46, 0.77)	**0.028**	0.89 (0.63, 2.19)	0.612	0.224

Data presented as mean±SD or median (IQR) for the change from baseline at each time point specified. SOD – superoxide dismutase; GSH-Px – glutathione peroxidase, IL1RA – interleukin 1 receptor antagonist, IL10 – interleukin 10; SOD2 – superoxide dismutase 2, mitochondrial; HO-1 – Heme oxygenase 1; NRF2 – nuclear factor, erythroid 2-like 2; GSTP1 – glutathione S-transferase pi 1. *P*-value respresents the difference in change from baseline to the specified time point. ΔPI vs ΔPA *P*-value compares the difference in change for physically inactive versus physically active adults with asthma.

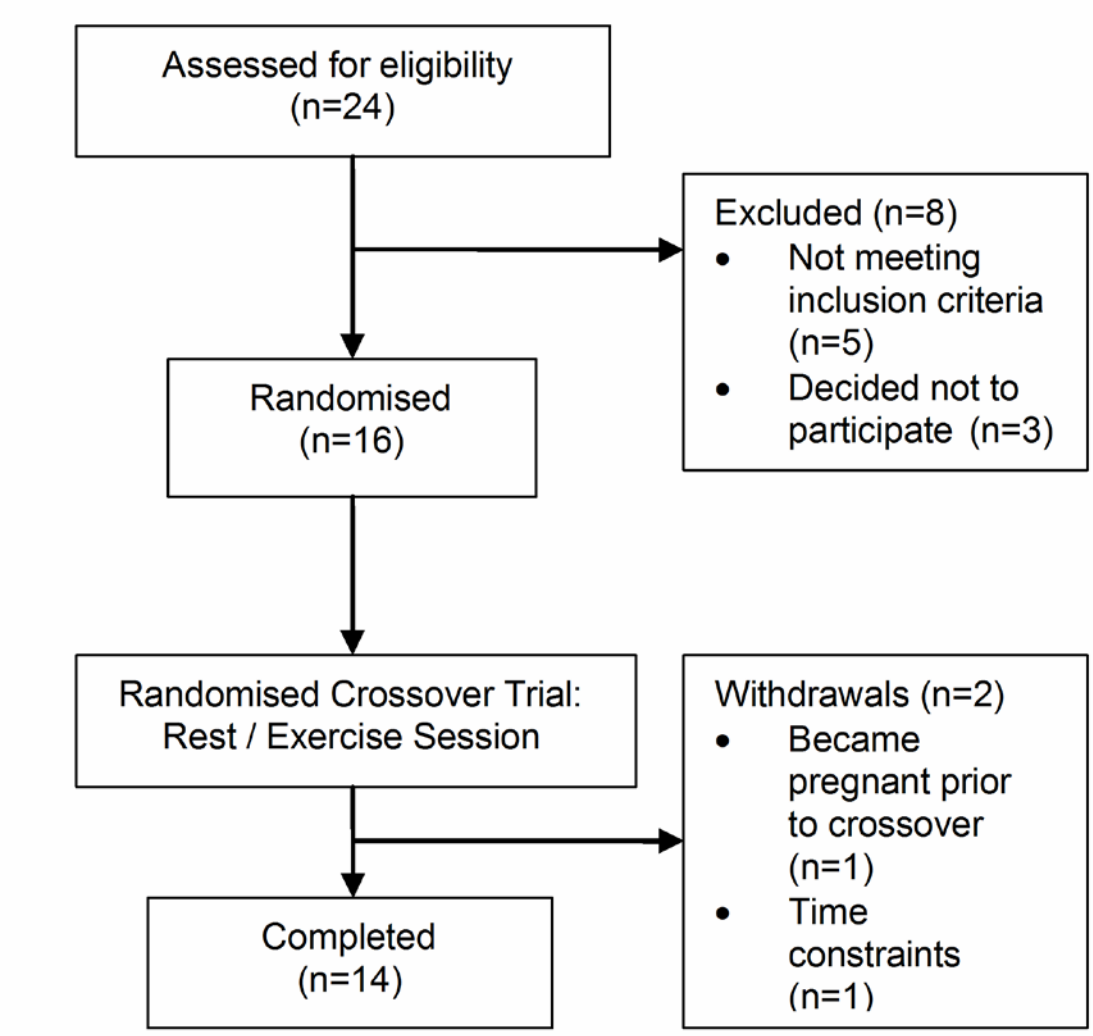

Figure 1. Flowchart of study participants.

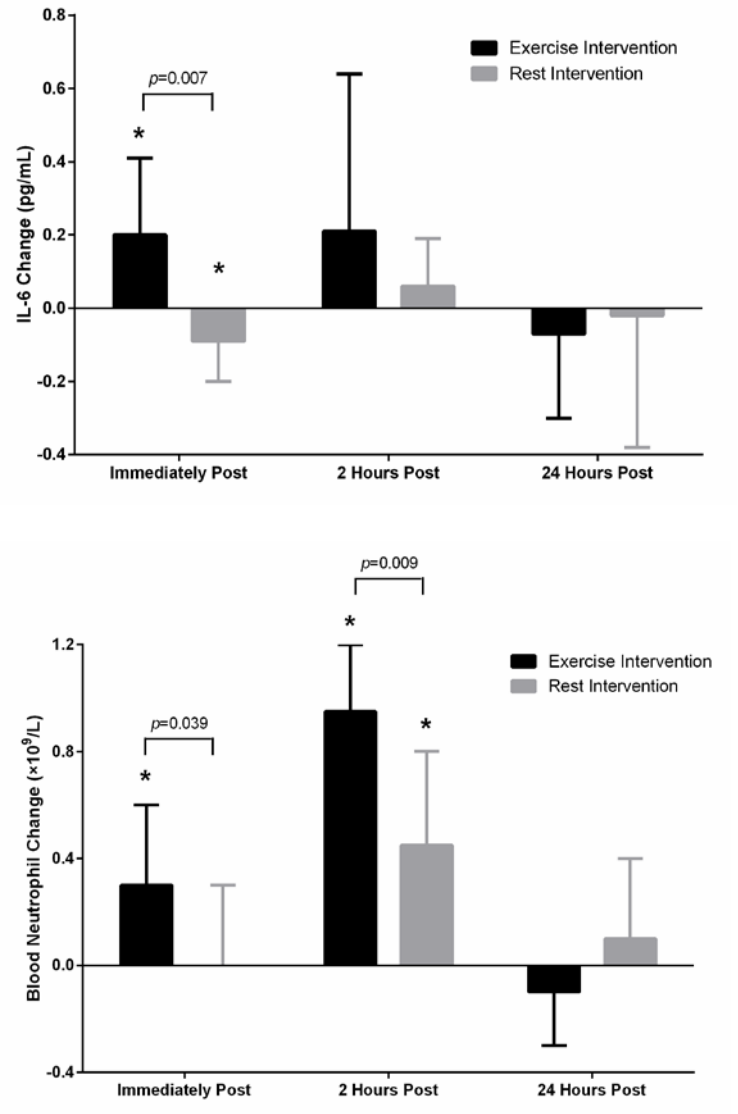

Figure 2. Median (interquartile range) for (A) interleukin-6 (IL-6) concentration and (B) change in peripheral blood neutrophil number after exercise and after rest. *P <0.05 compared with baseline concentration.

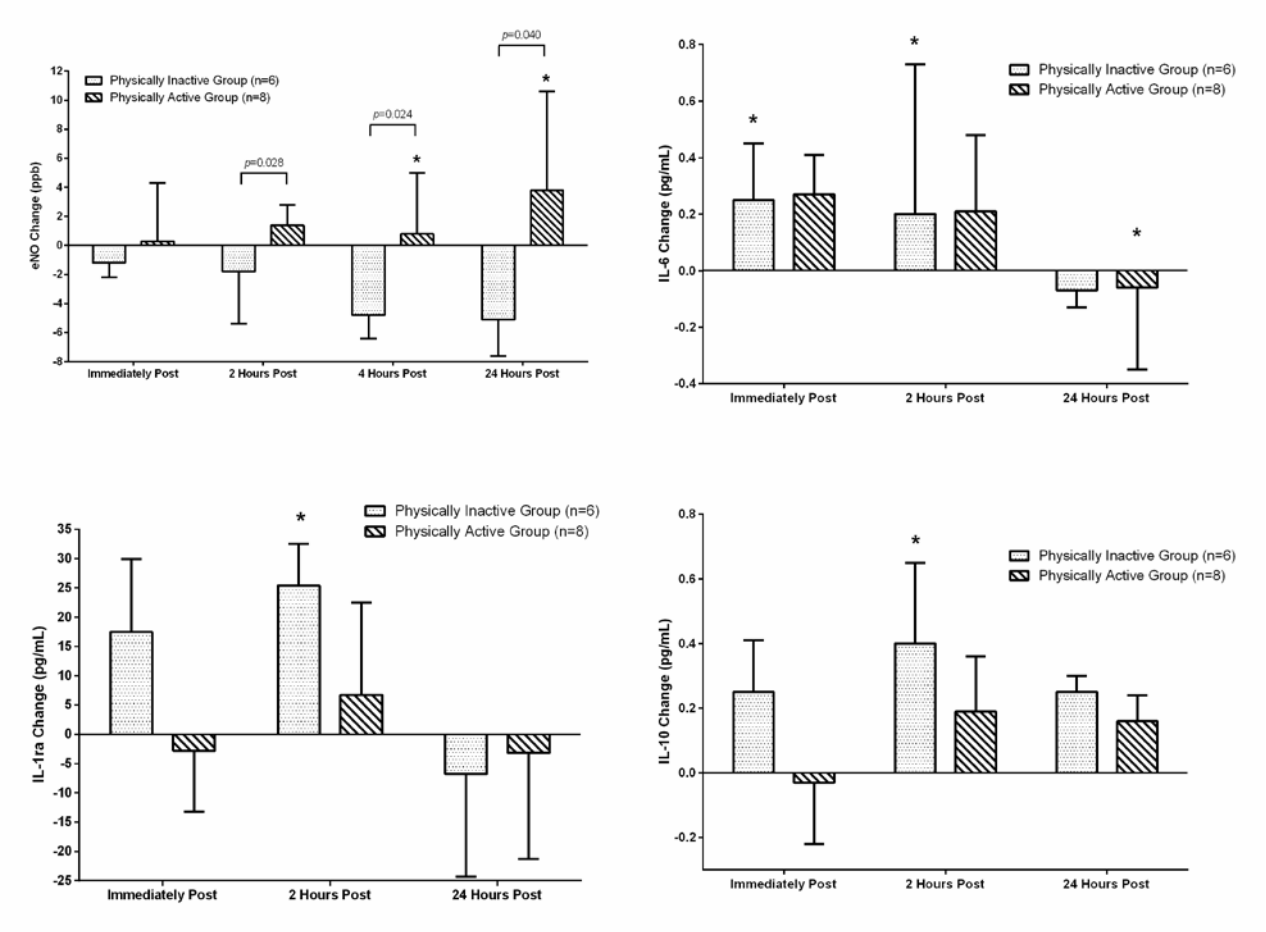

Figure 3. Median (interquartile range) for (A) exhaled nitric oxide (eNO), (B) interleukin-6 (IL-6), (C) interleukin-1 receptor antagonist (IL-1ra), and (D) interleukin-10 (IL-10) after exercise in physically inactive and physically active adults with asthma. *P < .05 compared with baseline concentration.

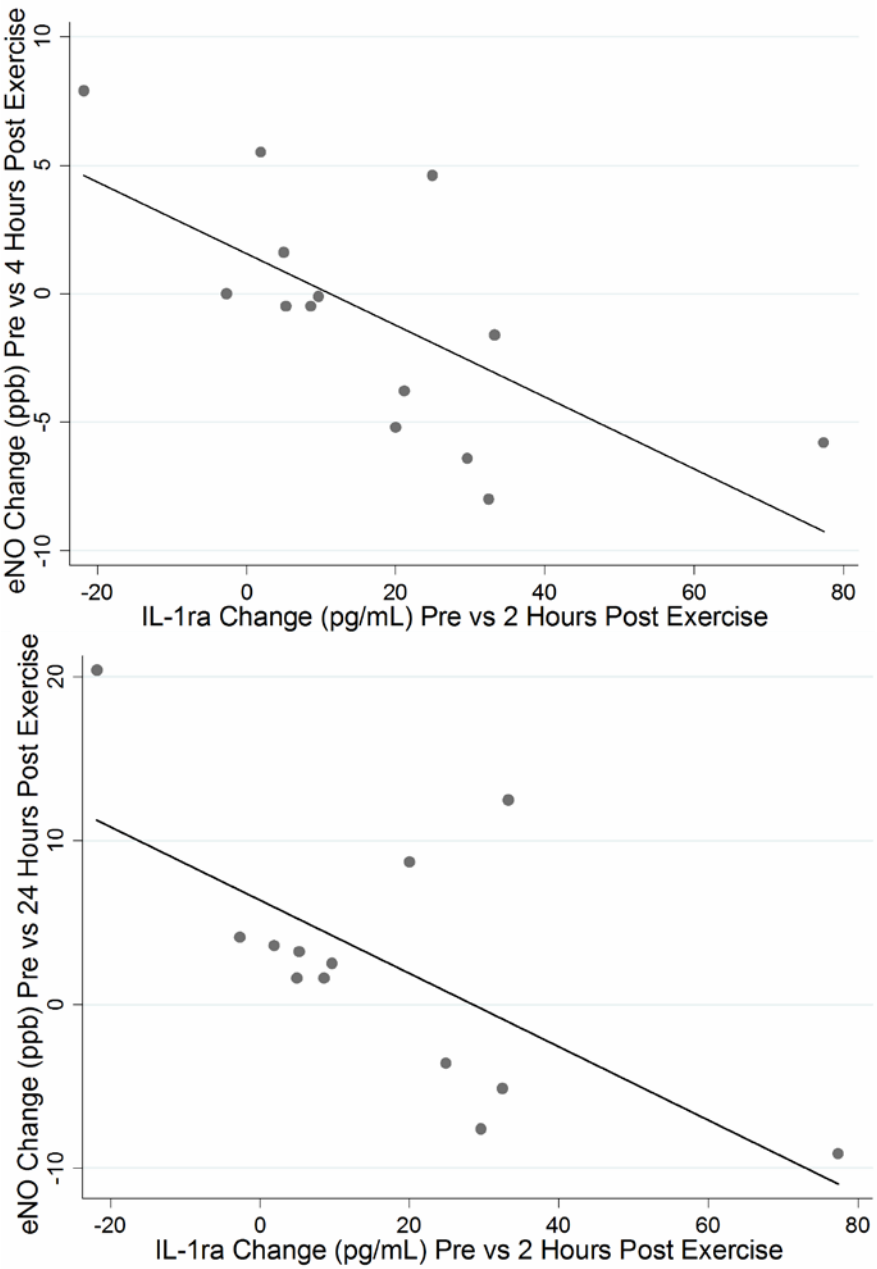

Figure 4. Correlation between interleukin-1 receptor antagonist (IL-1ra) change at 2 hours with exhaled nitric oxide (eNO) change at (A) 4 hours (R=0.685, P= 0.007) and (B) 24 hours (R=0.659, P=0.014) after exercise.