

LIFESTYLE INFLUENCES ON AIRWAY HEALTH  
IN CHILDREN AND YOUNG ADULTS

by

SARA K. ROSENKRANZ

B.A., University of Kansas, 1993  
M.S., Kansas State University, 2001

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Department of Human Nutrition  
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## Abstract

The overall aim of this dissertation was to ascertain the influences of lifestyle factors on airway health in children and young adults. In Study 1 (Chapter 2) the effect of a high-fat meal on airway inflammation and hyperresponsiveness was examined. Results revealed a post-prandial increase ( $p < 0.05$ ) in total cholesterol (~4%), triglycerides (~93%), and exhaled nitric oxide (a marker of airway inflammation, ~19%) two-hours following a high-fat meal ( $74.2 \pm 4.1$  g fat). These novel findings suggest that a high-fat meal may contribute to impaired airway function. In study 2 (Chapter 3) we assessed the role of body fat and physical activity (PA) on airway health in prepubescent children. This study revealed that children with higher-body-fat levels (>21%), who were not meeting current PA recommendations, experienced greater ( $p < 0.05$ ) amounts of post-exercise airway narrowing ( $FEV_1$ , forced expiratory volume in 1-second, ~11%), as compared to children with lower-body-fat (<21%), who were meeting PA guidelines. These findings suggest that elevated adiposity and low PA levels may place children at risk for development of asthma and asthma-like symptoms. In study 3 (Chapter 4), based on study 2 results, we assessed the impact of 8 weeks of high-intensity interval training on airway health in children who were not meeting PA guidelines. We determined that high-intensity training significantly increased  $VO_{2max}$  (~24%), and decreased total cholesterol (~11%) and LDL cholesterol (~35%). Additionally, we found improvements ( $p < 0.05$ ) in  $\Delta FEV_1$  both post-exercise (pre:  $-7.6 \pm 2.2\%$ , post:  $-1.3 \pm 1.8\%$ ) and post-eucapnic voluntary hyperventilation (pre:  $-6.7 \pm 2.2\%$ , post:  $-1.4 \pm 1.5\%$ ) with training. Further, Lower-body-fat and higher  $VO_{2max}$  subjects experienced significantly greater improvement in  $\Delta FEV_1$  following training than higher-body-fat and lower  $VO_{2max}$  subjects ( $r = -0.80$ ,  $r = 0.73$ , respectively). These results suggest that in children, high-intensity training can ameliorate the negative health consequences of inactivity. However, increased body fat, and low  $VO_{2max}$  levels may constrain these improvements. This series of studies underscores the importance of dietary habits, body composition, and PA for airway health in children and young adults. These findings may be useful in determining policies and practices impacting children's health, and could facilitate protocol development for prevention of asthma-like symptoms.

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Approved by:

Major Professor  
Craig A. Harms, PhD

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## **Dedication**

*I dedicate this Dissertation to my father, James Joseph Coleman Jr. It was during my Doctoral work that I learned of my father's diagnosis of frontal temporal dementia.*

*To my father, who once possessed a gift for words that left me in awe, but has been stricken with an illness that has left him without his voice.*

*These words are for you.*

## **CHAPTER 1 - Introduction**

The etiology of asthma and other pulmonary diseases has been studied primarily from the perspectives of family history, environmental pollutants, indoor and outdoor allergens, and atopy. Often, these factors may be more relevant to asthma exacerbation and not to the pathophysiology of asthma or asthma-like symptoms (Beasley et al. 2000; Subbarao et al. 2009; Sykes et al. 2008). In addition to the aforementioned triggers, there is ample epidemiological evidence to suggest that in part, asthma and other pulmonary disease is associated with lifestyle influences such as dietary habits, physical activity, and adiposity.

Health outcomes of interest in the physical activity, obesity, and dietary literature, especially in the younger population, have primarily been related to cardiovascular and metabolic disease such as diabetes, heart disease, and cancer. Pulmonary function, however, has rarely been studied as a health outcome of interest in the physical activity and dietary literature. Asthma prevalence has been increasing over the past few decades and is the number one chronic childhood disease, making this topic relevant for this population (Guerra et al. 2002). Alongside this increase in asthma prevalence, there has also been a dramatic increase in the prevalence of childhood obesity (Ogden et al. 2008). A large body of research has shown an association between obesity and pulmonary function, but most has not controlled for lifestyle factors which play a part in the development of obesity (Shore & Johnston 2006; Stenius-Aarniala et al. 2000). Additionally, the bulk of this research has been either epidemiological or cross-sectional in nature, not allowing for mechanistic or causal inferences to be made. The relationship between lifestyle factors and airway health is essential in determining specific preventive

practices and potential treatment protocols for those with confirmed pulmonary disease as well as those at risk for developing such disease. With these issues in mind, the following set of studies was designed to elucidate our understanding of the importance of lifestyle influences on airway health in children and young adults.

An association between obesity and asthma and asthma-like symptoms has been pervasive in the research literature. Potential mechanisms for this relationship include the mechanical effects of obesity on the airway (Li et al. 2003; Jones & Nzekwu 2006), and inflammation resulting from elevated circulating adipocytokines (Mehta & Farmer 2007; Zulet et al. 2007). A high-fat diet is known to contribute to the development of obesity (McAuley et al. 2006). The consumption of a single high-fat meal is also known to elevate levels of systemic inflammatory markers such as C-reactive protein and other cytokines in addition to triglycerides (Austin 1997; Miller et al. 2005; Shome et al. 2006). Therefore, study 1 (Chapter 2) was designed to determine the post-prandial response to a high-fat meal, systemically, and also in the airways of healthy college-age subjects. We hypothesized that: 1) a high-fat meal would increase post-prandial C-reactive protein (systemic inflammation) and exhaled nitric oxide (eNO; marker of airway inflammation), 2) those subjects with the largest increases in post-prandial C-reactive protein would also have the largest increases in eNO, 3) those subjects with highest body fat levels would see the largest increases in total cholesterol, triglycerides, C-reactive protein, and eNO, and 4) subjects who experienced increases in eNO post-prandially, would also show decreases in airway function.

Physical activity is another important lifestyle factor influencing both the development of overweight and obesity, and also poor airway health in young people.

Accumulation of inadequate amounts of physical activity has been linked to development of airway hyperresponsiveness and asthma in previous research (Lucas & Platts-Mills 2005; Lucas & Platts-Mills 2006; Rasmussen et al. 2000). One methodological limitation with this literature however has been the lack of accounting for chronic levels of physical activity. In study 2 (Chapter 3), we classified subjects according to body fat percentage utilizing dual energy x-ray absorptiometry, and also classified them according to whether or not they met the current United States Department of Health and Human Services (USDHHS) physical activity guidelines (PAGAC 2008). Therefore, the purpose of this study was to determine whether body fat and physical activity levels would have an impact on airway health in healthy prepubescent children. We hypothesized that prepubescent children: 1) who were physically inactive would show a greater decrease in FEV<sub>1</sub> post-exercise than children who were physically active; 2) who had increased body fat would show a greater decrease in FEV<sub>1</sub> than children who had lower body fat, and 3) who had higher levels of body fat and were physically inactive, would show greater decreases in FEV<sub>1</sub> post-exercise than children with lower body fat levels and were physically active.

The cross-sectional nature of the study we performed in prepubescent non-asthmatic children revealed many unanswered questions regarding potential mechanisms and potential reversibility of post-exercise airway narrowing. Therefore, in study 3 (chapter 4), we utilized a controlled randomized trial including a high-intensity running protocol that has been shown to be effective in improving V<sub>O<sub>2</sub>max</sub> in preadolescent children (Backer and Ulrik 1992; Nourry 2005). We hypothesized that in healthy prepubescent children not meeting current physical activity guidelines, 8-weeks of high-



intensity running training would reduce post-exercise airway narrowing and airway resistance. We also hypothesized that children with the highest levels of body fat would experience greater amounts of airway narrowing and airway resistance at baseline and following the training protocol, they would experience greater improvements as compared to their leaner counterparts

The series of studies completed for this dissertation was designed to improve our understanding of the influences of lifestyle factors such as physical activity, adiposity levels, and dietary habits, on airway health in children and young adults. Each of the following chapters is a separate study (Abstract, Introduction, Methods, Results, Discussion and Conclusions and References).

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## **CHAPTER 2 - Effects of a High-Fat Meal on Pulmonary Function in Healthy Subjects**

Now in publication as Rosenkranz SK, Townsend DK, Steffens SE, Harms CA. Effects of a high-fat meal on pulmonary function in healthy subjects. *Eur J Appl Physiol*. 2010.

## Abstract

Obesity has important health consequences, including elevating risk for heart disease, diabetes, and cancer. A high-fat diet is known to contribute to obesity. Little is known regarding the effect of a high-fat diet on pulmonary function, despite the dramatic increase in the prevalence of respiratory ailments (e.g., asthma). **PURPOSE:** The purpose of our study was to determine whether a high-fat meal (HFM) would increase airway inflammation and decrease pulmonary function in healthy subjects. **METHODS:** Pulmonary function tests (PFT)(forced expiratory volume in 1-sec, forced vital capacity, forced expiratory flow at 25-75% of vital capacity) and exhaled nitric oxide (eNO; airway inflammation) were performed in 20 healthy (10 men, 10 women), inactive subjects (age  $21.9 \pm 0.4$  yrs) pre and 2 hours post HFM (1 gm fat/1 kg body weight;  $74.2 \pm 4.1$  gms fat). Total cholesterol, triglycerides, and C-reactive protein (CRP; systemic inflammation) were determined via a venous blood sample pre and post HFM. Body composition was measured via dual energy X-ray absorptiometry (DXA). **RESULTS:** The HFM significantly increased total cholesterol by  $4 \pm 1\%$ , and triglycerides by  $93 \pm 3\%$ . ENO also increased ( $p < 0.05$ ) due to the HFM by  $19 \pm 1\%$  (pre:  $17.2 \pm 1.6$ ; post:  $20.6 \pm 1.7$  ppb). ENO and triglycerides were significantly related at baseline and post-HFM ( $r = 0.82, 0.72$  respectively). Despite the increased eNO, PFT and CRP did not change ( $p > 0.05$ ) with the HFM. **CONCLUSION:** These results demonstrate that a HFM, which leads to significant increases in total cholesterol, and especially triglycerides, increases exhaled NO. This suggests that a high-fat diet may contribute to chronic inflammatory diseases of the airway and lung.

## Introduction

Obesity is a known risk factor for many diseases including coronary heart disease, hypertension, type 2 diabetes, stroke, gallbladder disease, osteoarthritis, some cancers, sleep apnea and respiratory problems (Fan 2006). Concurrent with the rise in obesity prevalence, an escalation in asthma and other respiratory ailments has occurred. There is a large body of research that has linked obesity to asthma and asthma-like symptoms (Shore and Johnston 2006; Stenius-Aarniala et al. 2000). One possible explanation for this relationship is thought to be that of mechanical effects on the airway. Specifically, obese individuals tend to have reduced functional residual capacity and they assume a higher frequency breathing pattern with a lower tidal volume than lean individuals (Li et al. 2003; Jones and Nzekwu 2006). This reduction in tidal volume creates a downward spiral where less airway stretch is occurring, creating airway stiffness in the smooth muscle. This stiffness leads to greater difficulty in creating airway stretch and increased airway muscle shortening and hyper-responsiveness.

Another potential mechanism for the link between obesity and the development of asthma is an immune modification. There is increasing evidence that obesity is an inflammatory condition (Mehta and Farmer 2007; Zulet et al. 2007). Studies to date have shown associations between markers of inflammation, namely TNF- $\alpha$ , interleukin 6 (IL-6), C-reactive protein (CRP), and the excess adiposity (Tantisira and Weiss 2001). IL-6 and TNF- $\alpha$  have both been found to be expressed by adipocytes and to be correlated with total fat mass. In addition to airway inflammation, there is also evidence that systemic inflammation is problematic for asthmatics as compared to people without asthma

(Rasmussen et al. 2009). Since asthma and obesity are so closely linked (Habib 2009), we questioned whether obesity creates an inflammatory state that leads to the development of asthma.

The consumption of a single high-fat meal is known to elevate levels of triglycerides and systemic inflammatory markers such as C-reactive protein and other cytokines (Austin 1997; Miller et al. 2005; Shome et al. 2006). Inflammation in the airways, however, has not been previously studied in relation to the impact of a high-fat meal. It is well established that airway inflammation is associated with airway hyper-responsiveness (a hallmark symptom of asthma) in asthmatics. Whether or not a single high-fat meal would lead to decreased airway function in healthy subjects is not known.

Therefore, the purpose of this study was to determine the relationship between a high-fat meal and the post-prandial response in systemic and airway inflammatory markers in healthy, college-aged males and females. We hypothesized that: 1) a high-fat meal would increase post-prandial C-reactive protein (systemic inflammation) and exhaled nitric oxide (eNO; marker of airway inflammation) significantly, 2) those subjects with the largest increases in post-prandial C-reactive protein would also have the largest increases in eNO, 3) those subjects with highest body fat levels would see the largest increases in total cholesterol, triglycerides, C-reactive protein, and eNO, and 4) subjects who experienced increases in eNO post-prandially, would also show decreases in airway function.



## Methods

### *Subjects*

Twenty healthy adults with no diagnoses or history of acute or chronic diseases were recruited via advertisement and word of mouth from the student population at Kansas State University. All subjects had low fruit and vegetable intake (< 3 servings fruits and vegetables per day) as verified by a screening questionnaire. Females were studied during the early follicular phase of their menstrual cycle, verified by progesterone (P<sub>4</sub>) levels  $\leq 1.5$ ng/ml. Each participant served as his/her own control. Informed consent was obtained following both a written and verbal explanation of the possible risks and discomforts of the experimental protocol. All research components were reviewed and approved by the Institutional Review Board of Human Subjects at Kansas State University, Manhattan, KS.

### *Exclusion Criteria*

Subjects were excluded if they had a history of smoking or chronic illness, were currently on a weight loss or dietary modification program, or on a diet that included a combination of >4 servings fruit and vegetables/day. Subjects were also excluded if they were on medications including: bronchial dilators, hormone therapy or hormonal contraception (except low dose combination ethinyl estradiol/progestin pills), anti-hypertensives, lipid lowering medications, drugs to control blood sugar, or psychoactive medications. Subjects were also excluded if their 12 hour fasting glucose levels were  $\geq 100$  mg/dl on the day of the screening, as measured by a hand held glucometer (FreeStyle Flash, TheraSense, Alameda, CA) or if blood pressure was  $\geq 140/95$  mmHg.

Data were rejected if biochemical analysis indicated triglycerides levels  $\geq 300$  mg/dl, cholesterol levels  $\geq 210$  mg/dl, or 2 hr. post OGTT  $\geq 140$  mg/dl.

### ***Experimental Design***

Subjects reported to the lab on two separate occasions, 2 hours apart, during the same day. Subjects refrained from alcohol and exercise for 24 hours, caffeine for 12 hours, and were fasted or had consumed a very low-fat snack prior to coming to the lab. During session one, subjects were familiarized with the equipment and procedures. Baseline blood samples were taken and total cholesterol, C-reactive protein and triglyceride levels were determined. Progesterone ( $P_4$ ) levels were also determined at baseline (Progesterone RIA DSL-3900, Diagnostics Systems Laboratory, Inc., Webster, TX). Each subject underwent standard pulmonary function tests (PFT);(forced expiratory flow in 1-sec, forced vital capacity, forced expiratory flow at 25-75% of vital capacity) maximal inspiratory pressure ( $P_{I_{max}}$ ) maximal expiratory pressure ( $P_{E_{max}}$ ) tests (SensorMedics 229 Metabolic Cart, SensorMedics Corp, Yorba Linda, CA), and exhaled nitric oxide tests (eNO; a marker of airway inflammation; Kharitonov et al. 1994; Rodway et al. 2009) via chemiluminescence (Sievers Nitric Oxide Analyzer 280, Sievers Instruments Inc, Boulder, CO) prior to and 2-hours following the ingestion of a high-fat meal (HFM). All tests were performed in triplicate, with the average value used in analysis. Exhaled nitric oxide (eNO) has been used as a non-invasive marker of airway inflammation (Ashutosh 1999; Becklake and Kauffman 1999; Gabbay et al. 1998; Kharitonov et al. 1994; Rodway et al. 2009; Tsang et al. 2001), and there are dramatic differences between asthmatics and non-asthmatics in terms of the amount of eNO

exhaled. Asthmatics can have 3-4 times the normal levels of eNO and the numbers tend to worsen as asthma symptoms worsen.

### ***Blood Lipids and Cytokines***

Total cholesterol, triglycerides, and C-reactive protein (CRP; systemic inflammation) were determined via a venous blood sample pre and post HFM. All analyses were performed by Lafene Health Center on Kansas State University's campus. Triglycerides, total cholesterol, and C-reactive protein levels were determined via an automated lipid analyzer (Cobas Integra 800 Clinical Chemistry Analyzer, Roche Professional Diagnostics, Basel, Switzerland).

### ***High-Fat Meal***

The high-fat meal consisted of ice cream (Edy's Grand Vanilla) and whipping cream (Reddi wip original). Serving size was determined by body weight)(1 gm fat/1 kg body weight). Calculations for ice cream were bodyweight in kg x 4.0625= g of ice cream. Whipping cream was measured as bodyweight in kg x 1.5=ml of whipped cream. The high-fat meal was consumed within a 20-minute time frame. The nutritional makeup of the high-fat meal was 4.5 gm saturated fat per serving (27.1gm-72.3gm), 30 mg cholesterol per serving (180.8mg-482.3mg), and 16g of carbohydrate (13g sugar)(96.4gm-257.2gm) per serving.

### ***Body Composition***

Total body composition was measured by use of a whole body DXA system (v5.6, GE Lunar Corp., Milwaukee, WI) and regions of interest were specified between the 7<sup>th</sup> rib and the iliac crest for measurement of visceral adiposity (abdominal fat %).

Subjects lay in a supine position with arms separated from trunk and legs slightly spaced apart. Shoes and metal objects were removed prior to scanning. Instructions were to lie as still as possible during the scanning procedure. DXA scanning has been validated and uses two x-ray beams with differing energy levels to find differences in absorption and therefore lean body mass (LBM), body fat percentage, and body fat distribution (Haarbo et al. 1991).

### ***Statistics***

SigmaStat statistical software (Jandel Scientific Software) was used for data analysis. Data is expressed as mean  $\pm$  standard deviation. Differences between genders were determined using ANOVA. Relationships were determined by Pearson Product Moment Correlation. Significance was set at  $p < 0.05$  for all analyses.

## Results

Subject characteristics are presented in Table 1. The ratio of males to females was equal (10 males, 10 females). Subjects were college-aged with a wide range of body fat percentages. The mean body fat percentage for women was  $31.7 \pm 8.0\%$ , and for men was  $23.2 \pm 7.9\%$ .

### *Pulmonary Function Tests*

Pulmonary function test values pre and post-HFM are shown in Table 2. Baseline values for all pulmonary function tests were not significantly different from predicted values for subjects of the same age, gender, and height (Crapo et al. 1982). Exhaled Nitric Oxide (eNO) pre and post HFM are shown in Fig. 1. The HFM increased eNO in 16 of 20 subjects (80%) by an average of approximately 19%. Despite the significant increase in eNO, other standard pulmonary function measures did not change ( $p > 0.05$ ) after the ingestion of the HFM.

### *Total Cholesterol and Triglycerides and C-reactive protein*

Total cholesterol and triglycerides pre and post HFM are shown in Fig. 2. Total cholesterol increased in 16 subjects by approximately 4% ( $p < 0.05$ ) and triglycerides increased in all subjects by approximately 92% ( $p < 0.05$ ) after the HFM. The HFM increased total cholesterol in 16 of 20 (80%) participants. The HFM increased triglycerides in all 20 participants. C-reactive protein levels did not change after the HFM ( $p > 0.05$ ) (Table 3).

### *Sex Differences*

Because previous studies have indicated that men have higher baseline levels of eNO, we divided subjects by sex for further analysis of eNO at baseline as well as

changes from pre to post-HFM. Exhaled Nitric Oxide pre- and post-HFM by sex are shown in Fig. 3. While the levels of eNO were significantly higher for men (M) versus women (W) at baseline (M:  $20.8 \pm 2.2$ , W:  $14.0 \pm 1.3$  ppb) and post-prandially (M:  $24.3 \pm 8.2$ , W:  $16.5 \pm 4.8$  ppb), the increase from pre to post HFM was similar ( $p > 0.05$ ) between sexes (M:  $115.1 \pm 22.3\%$ , W:  $120.1 \pm 21.2\%$ ).

Triglycerides by sex are reported in Table 4. There were no differences by sex ( $p > 0.05$ ) at either pre or post-HFM. Changes in TG from pre to post-HFM were significantly higher in men (M) than in women (W) (M:  $124.44 \pm 39.34$  mg/dL, W:  $60.50 \pm 30.23$  mg/dL).

## **Discussion**

### ***Major Findings***

The primary purpose of our study was to determine the relationship between a high-fat meal and the post-prandial response in systemic and airway inflammatory markers in healthy, college-aged males and females. In support of our first hypothesis, the results of our study demonstrate for the first time that a high-fat meal, which led to significant increases in total cholesterol and especially triglycerides, significantly increased exhaled nitric oxide in healthy subjects. Contrary to our hypothesis that the HFM would also lead to increases in C-reactive protein, we saw no changes in C-reactive protein post- HFM. Because there was no change in CRP post-prandially, our second hypothesis, that those subjects with the largest increases in post-prandial C-reactive protein would also have the largest increases in eNO, was not supported. Our third hypothesis, that those subjects with highest body fat levels would see the largest increases in total cholesterol, triglycerides, C-reactive protein, and eNO, was not supported. Results did not vary by body fat percentage. Finally, subjects who experienced increases in eNO post-prandially, did not show decreased lung function.

### ***Potential Mechanisms***

Similar to previous findings, the HFM in our study led to an increase in total cholesterol and triglycerides post-prandially (Maffeis et al. 2001; Knuth et al. 2008; Mattes 2009). While the mechanisms linking the increases in triglycerides, total cholesterol, and eNO are unknown, several potential mechanisms could be possible explanations for the increase seen in eNO values post-prandially. One explanation for the rise in airway inflammation post HFM is similar to that of the explanations arising from a

chronically high-fat diet. A chronically high-fat diet has been linked with and implicated in the development in obesity (McAuley et al. 2006). While there are currently no widely accepted norms for body fat percentage, according to the American College of Sports Medicine (ACSM), DXA results for our study population indicated that 7 out of 10 of the females ( $31.7 \pm 8.0\%$ ) and 8 out of 10 of the males ( $23.2 \pm 7.9\%$ ) had body fat values that were higher than the range associated with optimal health risk (ACSM 2010). A recent study by Patel et al. (2007) found that a high-fat, high-carbohydrate meal (62% CHO, 30% fat) caused a prolonged reactive oxygen species (ROS) generation and nuclear factor KappaB activation post-prandially, indicating increased systemic inflammation. While non-obese subjects also showed increases in ROS and inflammation, obese subjects' responses remained elevated significantly longer than in non-obese subjects. Since the mean body fat percentage of our subjects was 27% (for men and women), and 15 out of 20 were obese according to DXA, the post-prandial increases in eNO may have also been accompanied by ROS and inflammatory marker increases.

### ***High-Fat Diet and Inflammation (TNF- $\alpha$ and C-RP)***

A recent study has also shown that subsequent to a high-fat diet in rodents, the muscle tissue over expressed tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) (Borst and Conover 2005). According to the researchers, TNF- $\alpha$  is an inflammatory cytokine that has been implicated as a possible mediator of insulin resistance. The researchers further speculate that a high-fat diet can increase levels of TNF- $\alpha$ , which is over-expressed in adipose tissue, and subsequently create insulin resistance. Even during weight loss, when participants were losing weight via a high-fat, low carbohydrate diet, increases of 25% in C-reactive protein were observed (Rankin and Turpyn 2007; Peairs and Rankin 2008). In



contrast, when healthy post-menopausal women were placed on an ad-libitum low-fat, high-carbohydrate diet, inflammatory markers, including C-reactive protein were favorably altered (Rankin and Turpyn 2007). A more recent study indicated that in ApoE-deficient mice, a 12-week high-fat (HF) diet induced production of TNF- $\alpha$  as well as other inflammatory cytokines systemically (Naura et al, 2009). The proposed mechanism for this increase in lung and systemic inflammation was that of hypercholesterolemia, or more specifically, oxidized-LDL exposure on the smooth muscle cells. Because the subjects recruited into the current study were sedentary and likely had low antioxidant levels, they may have had chronically elevated levels of TNF- $\alpha$ , which are over-expressed in adipose tissue (see Introduction), leading to increased levels of inflammation. One further possible explanation is that the high saturated-fat levels of the HFM hindered the anti-inflammatory qualities of the HDL cholesterol, causing eNO levels to rise (Ansell 2007).

### ***Sex Differences in eNO***

Peak eNO levels differ between men and women. Men tend to have eNO levels that are about 50% higher than women (Tsang et al. 2001). This gender difference indicates that there is a hormonal influence determining eNO levels, perhaps due to the effect of estradiol on NO production in the airway. Previous research has shown the inducible isoform iNOs is regulated by estrogens (Darblade et al. 2002; Chen et al. 1999). The endothelial isoform of eNO synthase is also affected by 17 $\beta$ -estradiol which can lead to increases in eNO synthase within minutes, indicating that at midcycle the protective hormonal effect found in women may no longer exist. Studies have shown that eNO fluctuates with a woman's menstrual cycle (Becklake and Kauffman 1999). At midcycle,

eNO levels are about twice that of pre- and peri-menstrual levels (Kharitonov et al. 1994). In the current study, eNO levels pre-HFM were significantly higher for men than women (M:  $20.8 \pm 2.2$ , W:  $14.0 \pm 1.3$  ppb). Though previous work has indicated men have increased post-prandial lipemia following a high-fat, high-carbohydrate meal, and in our study, males had significantly higher increases in triglycerides post-prandially (M:  $124.44 \pm 39.34$  mg/dL, W:  $60.50 \pm 30.23$  mg/dL), the post-HFM increases in eNO were proportional between the two sexes.

### ***Limitations***

In the asthmatic airway, it is well established that airway inflammation and airway hyper-responsiveness are associated (Hancox et al. 2007; Porsbjerg et al. 2009; Rasmussen et al. 2009). In the current study, pulmonary function tests didn't change from pre- to post-HFM, yet airway inflammation increased. However, it is important to note the airway hyper-responsiveness was not assessed in the present study. It is possible that the introduction of a spasmogen, such as Methacholine or saline, would have increased airway hyper-responsiveness after a HFM also.

Because this was an assessment of post-prandial response to a single high-fat meal, our results were limited in terms of the ability to find differences in airway hyper-responsiveness post-HFM. Had we utilized a fat-loading protocol over a number of weeks, we may have actually seen a decrement in pulmonary function. In a recent study by Naura et al (2009) the authors showed that following a 12-week HF diet, ApoE-deficient mice showed elevated levels of TNF- $\alpha$  as well as lung inflammation and an increase in MMP-9 indicating lung remodeling was occurring. Other authors have shown that similar remodeling occurs in various chronic lung diseases (Maddox & Schwartz,

2002; Broide, 2007). Such findings indicate that airway remodeling can occur in response to, and perhaps in parallel with airway inflammation.

Increased exhaled nitric oxide levels were not associated with changes in systemic inflammation (CRP). The timing of the blood sample, which occurred only 2-hours post-prandially, may not have allowed adequate time for C-reactive protein levels to increase. Evidence from other studies indicates that C-reactive protein may take as long as 24 – hours to increase following a HFM. Additionally, other markers of systemic inflammation may have been more appropriate (IL-6, TNF- $\alpha$ ) than the use of CRP, since recent studies such as (Blackburn, 2006; Carrol & Schade, 2003) have shown changes in IL-6 and TNF- $\alpha$  post-prandially. Additionally, studies of chronically HF diets have also shown increased levels of TNF- $\alpha$  primarily, accompanied by hypercholesterolemia and airway remodeling (Naura et al, 2009).

Increases in post-prandial triglyceride levels may have been caused by the high-carbohydrate content of the ice cream rather than the fat or saturated fat content. Abbasi et al. (2000) for example found that on a low-fat, high-carbohydrate diet, after two weeks, plasma triglycerides were significantly elevated. Others, however, have found that when low-fat or high-carbohydrate diets are ad libitum, and not prescribed in a laboratory, significant weight loss occurs, and triglyceride and HDL levels are not significantly impacted (Kasim-Karakes et al. 2000; Turley et al. 1998). The authors have suggested that the differences in findings may be due to the eucaloric versus ad libitum nature of the diets. Also, perhaps the quality of the carbohydrate content is different, where ad libitum diets contain more fiber, and more complex carbohydrates versus simple carbohydrates in the laboratory prepared meals. Recently, in a study by Knuth et al. (2008) adding

carbohydrate to a HFM was shown to actually blunt post-prandial lipemia in women. While it is entirely possible that the increases in total cholesterol and triglycerides found in our study may have been due in part to the carbohydrate content of the ice-cream, the response in exhaled nitric oxide occurred, whether due to the high-fat content of the meal, the high-carbohydrate content, or a combination thereof. Often, high-fat diets also contain high-carbohydrate levels, and therefore this type of meal is a true-to-life example of what occurs post-prandially when such meals are consumed. Future studies, however, should examine the role of high-fat without high-carbohydrate meals on post-prandial airway inflammation and also airway hyper-responsiveness.

### ***Conclusions***

Asthma and other respiratory ailments have been on the rise alongside the increase in obesity levels over the past few decades. A high-fat meal, particularly when coupled with a high-carbohydrate content increases airway inflammation as evidenced by elevated exhaled nitric oxide levels post-prandially. This increase in exhaled nitric oxide parallels the post-prandial increases seen in plasma triglyceride and total cholesterol levels. These results suggest that a diet that is consistently high in fat content and perhaps also in carbohydrate content may contribute to chronic levels of airway inflammation placing individuals at risk for developing chronic inflammatory diseases of the airway and lung.

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## Tables

**Table 2.1 Subject Characteristics**

	N = 20 (M =10, F=10)	
	mean $\pm$ SD	Range
Age (yr)	21.9 $\pm$ 1.9	19.0 – 26.0
Weight (kg)	75.2 $\pm$ 19.1	49.1 – 129.5
Height (cm)	147.9 $\pm$ 8.9	160.0 – 193.0
Body Fat (%)	27.7 $\pm$ 9.0	11.3 – 42.7
Abdominal Fat (%)	34.9 $\pm$ 10.4	14.5 – 52.0
Total Fat (g)	74.7 $\pm$ 18.4	48.2 $\pm$ 128.6
BMI (kg/m <sup>2</sup> )	24.4 $\pm$ 4.5	18.5 – 34.8

**Table 2.2 Pulmonary Function Tests**

	Pre-HFM		Post-HFM	
	mean $\pm$ SD	range	mean $\pm$ SD	range
FVC (L/sec)	4.93 $\pm$ 1.03	3.70 – 7.97	4.89 $\pm$ 0.99	3.66 – 7.82
PEF (L/sec)	7.92 $\pm$ 0.37	5.66 – 11.44	7.77 $\pm$ 1.74	11.22 - 4.61
FEV <sub>1</sub> (L/sec)	3.89 $\pm$ 0.78	2.34 – 6.27	3.86 $\pm$ 0.81	2.27 – 6.33
FEV <sub>1</sub> /FVC (%)	79.4 $\pm$ 8.9	50.0 -91.0	79.3 $\pm$ 8.4	50.0 – 88.0
FEF <sub>25-75%</sub> (L/sec)	3.67 $\pm$ 0.88	1.26 – 5.71	3.64 $\pm$ 0.96	1.25 – 6.34
P <sub>I</sub> max (cm H <sub>2</sub> O)	102.4 $\pm$ 38.6	57.7 – 185.0	101.07 $\pm$ 39.71	38.5 – 179.5
P <sub>E</sub> max (cmH <sub>2</sub> O)	105.2 $\pm$ 37.9	26.5 – 205.5	106.6 $\pm$ 36.6	35.7 – 204.3
eNO (ppb)	17.3 $\pm$ 7.0	9.3 – 33.8	*20.6 $\pm$ 7.6	10.9 – 36.0

FVC, forced vital capacity; PEF, peak expiratory flow; FEV<sub>1</sub>, forced expiratory volume in 1 sec; FEV<sub>1</sub>/FVC (%), ratio of forced expiratory volume in 1 sec to forced vital capacity; FEF<sub>25-75%</sub>, forced expiratory flow between 25-75%, P<sub>I</sub>max, maximal inspiratory pressure; P<sub>E</sub>max, maximal expiratory pressure; eNO, exhaled nitric oxide \* Significantly higher than pre high-fat meal (p<0.05)

**Table 2.3 Blood Lipids and CRP**

	Pre-HFM		Post-HFM	
	mean $\pm$ SD	range	mean $\pm$ SD	range
Total Cholesterol (mg/dL)	166.2 $\pm$ 24.8	128.0 – 213.0	*173.2 $\pm$ 26.5	134.0 – 220.0
Triglycerides (mg/dL)	95.7 $\pm$ 51.4	38.0 – 225.0	*184.5 $\pm$ 81.7	67.0 – 344.0
C-reactive protein	1.73 $\pm$ 1.67	0.25 – 5.77	1.61 $\pm$ 1.55	52.00 – 159.26

Total cholesterol; Triglycerides, C-reactive protein pre to post high-fat meal

\*Significantly higher than pre high-fat meal (p<0.05)

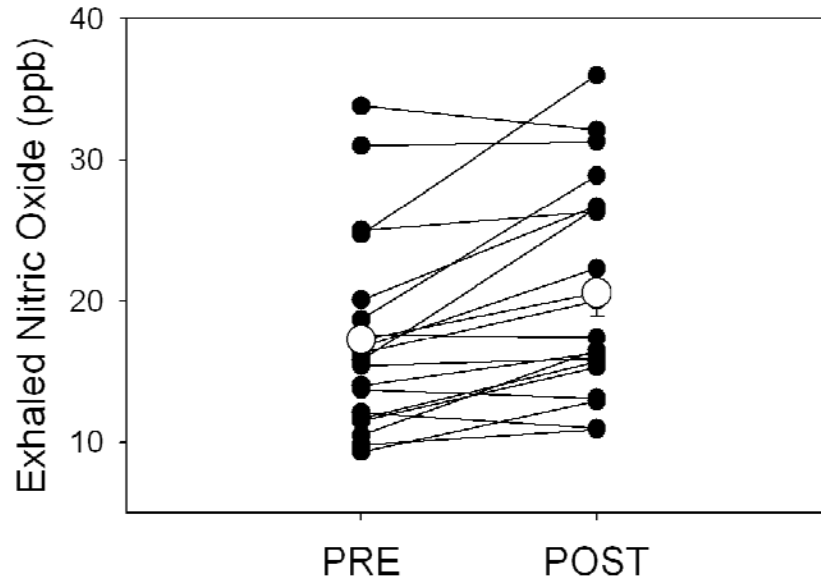
**Table 2.4 Triglycerides and Gender**

Triglycerides (mg/dL)			
Men (n=9) (1 subject missing data)		Women (n=10)	
Pre-HFM	Post-HFM	Pre-HFM	Post-HFM
96.0 $\pm$ 33.8	220.4 $\pm$ 55.7	91.5 $\pm$ 64.2	152.00 $\pm$ 90.2
(128.0-161.0)	(143.0-298.0)	(38.0-225.0)	(67.0-344.0)

Triglycerides Pre and Post-HFM for Men versus Women. No differences pre or post-HFM by gender  $p > 0.05$ . Change in TG from pre to post-HFM was significantly greater in men than in women.  $F = 15.982$ ,  $p < 0.001$ . Values are mean  $\pm$  SD.

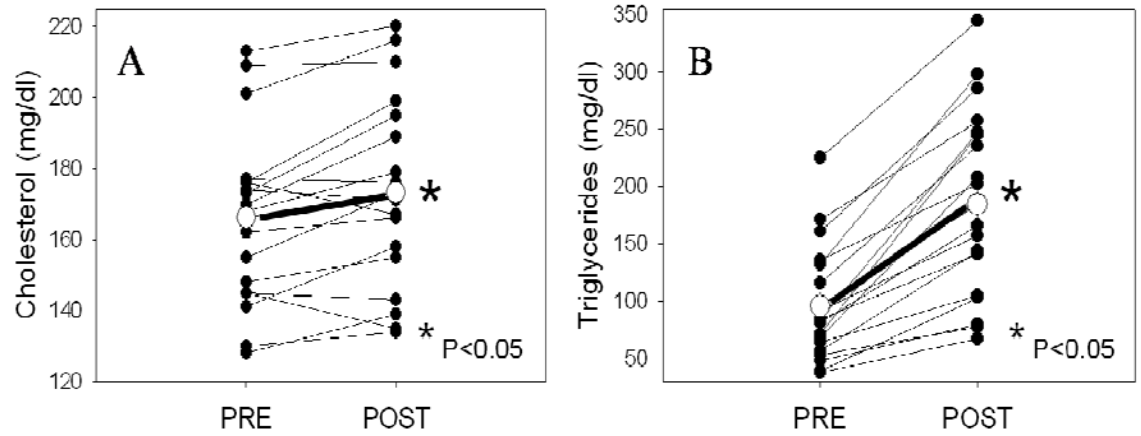
## Figures

Figure 2.1 Exhaled Nitric Oxide



Exhaled Nitric Oxide pre- and post- high fat meal. Filled circles are individual data and open circles are mean  $\pm$  SD. Exhaled Nitric Oxide (airway inflammation) increased  $\sim 19\%$  ( $P < 0.05$ ) after a high fat meal. Baseline and also post- HFM triglyceride values were significantly related to eNO ( $r = 0.82, 0.72$  respectively).

**Figure 2.2 Total Cholesterol and Triglycerides**



Total Cholesterol (a) and Triglycerides (b) pre- and post- high fat meal. Filled circles are individual data and open circles are mean  $\pm$  SD. Cholesterol increased  $\sim 4\%$  ( $P < 0.05$ ) and triglycerides increased  $\sim 93$  ( $P < 0.05$ ) after a high fat meal. The HFM increased total cholesterol in 16 of 20 (80%) participants by an average of  $4 \pm 1\%$  (pre:  $166.2 \pm 5.8$  mg/dL; post:  $173.2 \pm 6.1$  mg/dL). The HFM increased triglycerides in all 20 participants by an average of  $93 \pm 3\%$  (pre:  $95.7 \pm 12.1$  mg/dL; post:  $184.5 \pm 18.7$  mg/dL).



### Figure 2.3 Exhaled Nitric Oxide by Gender

Exhaled Nitric Oxide pre- and post-high fat meal. Black bars indicate mean eNO values for men (ppb) and grey bars indicate mean eNO values for women (ppb). Baseline eNO values were significantly higher for men than women. Post-test eNO values were also significantly higher for men than women. The difference from pre to post-HFM was similar between men and women. Asterisks indicate a significant gender difference, ( $p < 0.05$ ) and the plus sign indicates a significant difference from pre to post-HFM ( $p < 0.05$ ).

**CHAPTER 3 - Body Composition and Physical Activity Impact  
Airway Health in Non-Asthmatic Prepubescent Children**

## Abstract

The prevalence of both childhood obesity and childhood asthma have increased dramatically over the past few decades. Little is known concerning the role of body composition and lifestyle influences on airway health in children. **PURPOSE:** To determine whether body composition and physical activity (PA) would impact airway health in healthy prepubescent children.

**METHODS:** Pulmonary function tests (forced expiratory flow in 1-sec, forced vital capacity, forced expiratory flow at 25-75% of vital capacity) and exhaled nitric oxide (eNO) were measured pre- and post-exercise in 40 healthy (20 boys, 20 girls), non-asthmatic prepubescent children (age  $9.7 \pm 0.8$  yrs). Sedentary behavior, PA, and fruit and vegetable (FV) intake were assessed via questionnaire. Each subject completed an incremental cycle-ergometer exercise test to exhaustion ( $V_{O_{2max}}$ ). Body composition was measured via Dual Energy X-ray Absorptiometry. Subjects were divided into groups by attainment of Physical Activity Guidelines Advisory Committee (PAGAC) activity guidelines or not (active, inactive) and by body fat (high-fat,  $\geq 21\%$ , low-fat,  $< 21\%$ ; 50<sup>th</sup> percentile for our subjects). **RESULTS:** The change in FEV<sub>1</sub> (pre-post exercise) was inversely related ( $r=0.47$ ,  $P<0.05$ ) to % body fat; subjects with the highest body fat demonstrated the greatest decrease in FEV<sub>1</sub> (i.e., airway narrowing). The changes in FEV<sub>1</sub> and eNO were not different ( $P>0.05$ ) between active and inactive groups. Change in FEV<sub>1</sub> (pre-post exercise) was related ( $r=0.41$ ,  $P<0.05$ ) to  $V_{O_{2max}}$ ; subjects with the lowest  $V_{O_{2max}}$  had the greatest decrease in FEV<sub>1</sub>. Servings of FV were significantly related to PA status ( $r=0.33$ ,  $P<0.05$ ). Subjects meeting PA guidelines reported consuming significantly greater servings of FV per week ( $33.0 \pm 4.0$  servings) than subjects not meeting guidelines ( $23.3 \pm 1.9$  servings) ( $P<0.05$ ). The high-fat inactive group had a significantly greater decrease in FEV<sub>1</sub> post exercise ( $-11.0 \pm 3\%$ ), compared to the high-fat active ( $-7.1 \pm 2.3\%$ ), low fat inactive ( $-4.0 \pm 2.6\%$ ) and low-fat active group ( $-1.3 \pm 1.5\%$ ). **CONCLUSION:** These results suggest that physical inactivity and increased body fat negatively impact airway health in prepubescent children.

## **Introduction**

Despite widespread acceptance that lifestyle factors are key to combating the obesity epidemic (Jebb & Moore 1999), little is known regarding the relationship between physical activity, body composition, and health outcomes in children. One particular area where there is even less research is the impact of elevated body fat and inadequate moderate to vigorous physical activity (MVPA) on airway health in children. This topic lends itself well to research in children since, concurrent with the rise in obesity, an increase in asthma and other respiratory ailments has occurred (Lucas & Platts-Mills 2006).

There is evidence to suggest that physical activity can improve pulmonary health outcomes in children. For example, Nourry et al (2005) found that high-intensity interval training over an eight week period improved pulmonary function in non-athletic, prepubescent children. Physical activity can also play a protective role against the development of asthma and asthma-like symptoms (Rasmussen et al 2000; Lucas & Platts-Mills 2005; Lucas & Platts-Mills 2006). In a prospective study by Rasmussen et al (2000) involving 757 children who were nine years old at the beginning of the study, low physical fitness in childhood was significantly associated with development of asthma in their adolescent years. The same study also showed a moderate but significant correlation between low physical fitness in childhood and bronchial hyper-reactivity to methacholine at follow-up, even without an asthma diagnosis.

Healthy children who have asthma-like symptoms (specifically airway hyper-responsiveness), but no diagnosis of asthma, have been shown to be more likely than their non-reactive peers to develop asthma in the future (Rasmussen et al, 2002). The

impact of controllable lifestyle factors such as increasing physical activity, improving body composition, and increasing fruit and vegetable consumption, could provide valuable knowledge for the prevention and treatment of asthma and asthma-like symptoms. To date, there is little evidence regarding the impact of an inactive lifestyle and/or elevated body fat on airway health in children.

Therefore, the purpose of this study was to determine whether body fat and physical activity levels would have an impact on airway health in healthy prepubescent children. We hypothesized that prepubescent children: 1) who were physically inactive would show a greater decrease in FEV<sub>1</sub> post-exercise than children who were physically active; 2) who had increased body fat would show a greater decrease in FEV<sub>1</sub> post-exercise than children who had lower body fat, and 3) who had higher levels of body fat and were physically inactive, would show greater decreases in FEV<sub>1</sub> post-exercise than children with lower body fat levels or those who were physically active.

## **Materials and Methods**

### ***Subjects***

Forty healthy prepubescent children (20 girls, 20 boys) ages 7-11 years, with no diagnoses or history of acute or chronic diseases (determined via medical history questionnaire) volunteered to participate. All subjects were prepubescent, as defined by Tanner stage one (Tanner 1962) according to parental or guardian questionnaire responses. Subjects were not taking medications, including inhalers. We purposely attempted to recruit subjects with a wide range of physical activity levels and body fat percentages. None of the subjects were participating in swim training, which has been suggested to lead to increases in pulmonary function in children (Mickleborough et al 2008). An age appropriate physical activity questionnaire was completed (BS-BAQ, Treuth et al 2004) and subjects were classified as active or inactive based on whether or not they met physical activity guidelines for children on their responses for the previous day. The current physical activity guidelines from the United States Department of Health and Human Services (Physical Activity Guidelines Advisory Committee (PAGAC) 2008) were utilized. Each subject had a parent or guardian present to provide medical history information and informed consent, as well as assistance on the questionnaires. All research components were reviewed and approved by the Institutional Review Board of Human Subjects at Kansas State University, Manhattan, KS.

### ***Experimental Design***

Subjects reported to the lab on two separate occasions. A parent or guardian was present at both sessions. During session one, height and weight were first recorded by a

trained research assistant using a calibrated eye-level physical scale with height rod (Detecto, Webb City, MO). Subjects were then familiarized with the equipment and procedures. Standard pulmonary function tests (PFTs) via maximal flow volume loops were completed following several practice trials. These tests consisted of forced expiratory flow in 1-sec, forced vital capacity, forced expiratory flow at 25-75% of vital capacity (SensorMedics 229 Metabolic Cart, SensorMedicsCorp, Yorba Linda, CA), and exhaled nitric oxide (eNO; a marker of airway inflammation; Becklake & Kaufman 1999, Kharotinov et al 1994, Rodway et al 2009, Tsang et al 2001). All tests were performed in triplicate, with the average value of acceptable trials used in analysis. Subjects then completed an incremental cycle ergometer test until exhaustion to determine maximal oxygen uptake ( $VO_2max$ ). PFTs were performed at approximately 2 minutes and 10 minutes post-exercise. A second exercise test at constant work load (105%  $VO_2max$ ) was performed 15 minutes after the conclusion of the initial incremental test to verify  $VO_2max$  (Poole et al, 2008).

During the second session, total body composition was measured by use of a whole body DXA system (v5.6, GE Lunar Corp., Milwaukee, WI). Physical activity and nutrition questionnaires were also administered during the second session.

### ***Maximal Oxygen Uptake ( $VO_2max$ )***

An incremental exercise test to exhaustion (12-15 minutes) was performed using an electronically braked cycle ergometer to exhaustion (Ergometer 800S, Sensor Medics Corp., Yorba Linda, CA) to determine  $VO_2max$ . Subjects were given detailed instructions explaining the protocol of the test to ensure maximal volitional effort. Prior to testing, known gas concentrations spanning the range of expected measurements were

used to calibrate gas analyzers. Flow sensor calibration was also performed utilizing a 3-L calibration syringe. Resting metabolic measurements were taken for three minutes. Subjects then began with a warm-up for approximately two minutes at a work rate of 20 watts, pedaling between 50-60 revolutions per minute (rev/min). Subjects were instructed to remain seated and maintain this pedaling speed while the work rate was increased by 10 watts each minute. Metabolic and ventilatory data were assessed continuously through breath-by-breath analysis (SensorMedics 229 Metabolic Cart, SensorMedicsCorp., Yorba Linda, CA). Heart rate (HR) was monitored throughout the test via a four lead ECG interfaced to the metabolic software. The sensor from a pulse oximeter (Datex-Ohmeda, 3900P, Madison, WI) was secured to the left earlobe to estimate arterial oxygen saturation (SpO<sub>2</sub>). The pulse oximeter was calibrated before each test. Subjects continued to exercise until reaching volitional exhaustion (< 16 minutes). Verbal encouragement was provided throughout the test. The VO<sub>2</sub>max test concluded when subjects could not maintain a pedal frequency of >50 rev/min for five consecutive revolutions. Work rate for the confirmatory exercise test (constant work rate of 105% VO<sub>2</sub>max) was determined from the final work rate (watts) during the incremental test. Subjects were given a warm-up period of 90 seconds pedaling 50 rev/min at 20 watts. Work rate was increased during a period of approximately 30 seconds, until reaching the calculated work rate. Subjects were instructed to maintain 50 rev/min until volitional fatigue.

### ***Body Composition***

Total body composition was measured by use of a whole body DXA system (v5.6, GE Lunar Corp., Milwaukee, WI). Subjects lay in a supine position with arms



separated from trunk and legs slightly spaced apart. Shoes and metal objects were removed prior to scanning. Instructions were to lie as still as possible during the scanning procedure. DXA scanning has been utilized frequently in the pediatric population and is safe and valid for this population (Jensky-Squires et al 2008; Aasen et al 2006). DXA scanning uses two x-ray beams with differing energy levels to determine differences in absorption and therefore lean body mass (LBM), body fat percentage, and body fat distribution (Haarbo et al 1991).

### *Questionnaires*

In order to assess physical activity status we administered the BS-BAQ from Baranowski's Boy Scout project (Treuth et al 2004). This is an age appropriate, validated questionnaire with 37 activities. The parent and child completed the form together. To determine whether or not the subject was meeting current PAGAC physical activity guidelines for the previous day, the number of categories of physical activities were totaled for both the less than 15 minutes and the 15 minutes or more categories. If a subject had 4 or more physical activities checked for the "15 minutes or more", or 8 or more checked for the "less than 15 minutes" in the yesterday category, they were considered to have met guidelines for the previous day. If the parent or guardian indicated that yesterday was not a typical day for the child, the "usually" category was utilized. If the child had 4 or more physical activity categories marked in the "a lot" column, they were considered to be meeting guidelines. In order to determine current nutrition intake for fruits and vegetables and fruit juices, we administered the BS-FJV-FFQ (Cullen et al 1999; Thompson et al 2009). This is an age appropriate 7-day recall of fruit and vegetable intake which has been previously validated (Cullen et al, 1999). The

questionnaire consisted of four-100% fruit juice options, 15-fruit categories, 32-vegetable categories, and 34-drink categories including primarily sugar sweetened beverages.

Since mean estimates were shown to be high previously in the age group utilized in the current study, we did not utilize mean estimates to approximate actual consumption. The parent and child completed the questionnaire together.

Subjects who were above the mean body fat percentage ( $21 \pm 10\%$ ) for the entire group of 40 subjects were placed into the high body fat group. Subjects with body fat percentages below 21% were placed in the low body fat group. Subjects were divided into four groups based on whether or not they were above or below the group mean value for body fat percentage (High Fat vs. Low Fat) and whether or not they were meeting PAGAC guidelines for physical activity (Inactive vs. Active). The four groups were high-fat inactive (HFI), high-fat active (HFA), low-fat inactive (LFI), and low-fat active (LFA).

### ***Statistics***

SigmaStat statistical software (Jandel Scientific Software) was used for data analysis. Data is expressed as mean  $\pm$  standard deviation. Differences between groups were determined using ANOVA. Relationships were determined by Pearson Product Moment Correlation. Significance was set at  $P < 0.05$  for all analyses.

## Results

### *Subject Characteristics*

Subject characteristics are presented in Table 1. Subjects grouped together according to activity status were not significantly different for any anthropometric characteristics other than age. The active group was significantly older than the inactive group ( $P=0.02$ ). Boys and girls had similar ( $P>0.05$ ) height, weight, and body composition and were therefore grouped together. Medical history information provided by a parent or guardian confirmed that all subjects were in Tanner maturation stage 1 (Tanner 1962).

### *Pulmonary Function*

Table 2 displays baseline resting pulmonary function. Baseline PFT's were not significantly different between groups or from predicted values (Knudson et al 1983) for prepubescent children for comparable height, sex, and ethnicity. Percentage body fat versus the change in  $FEV_1$  from pre to post exercise is shown in Figure 1. Subjects with higher percent body fat had larger decrements in post-exercise  $FEV_1$  ( $r=0.47$ ,  $P<0.05$ ). Values for change in eNO and change in  $FEV_1$  are presented in Table 3. The change in exhaled nitric oxide (eNO) did not differ between groups ( $P>0.05$ ). Additionally, the change in  $FEV_1$  from pre to post exercise did not differ between groups ( $P>0.05$ ). The relationship between  $V_{O_{2max}}$  and change in  $FEV_1$  from pre to post exercise is shown in Figure 2. Those subjects with the lowest  $V_{O_{2max}}$  values showed the largest decrease in post-exercise  $FEV_1$  ( $r=0.41$ ,  $P<0.05$ ).

### ***Fruit and Vegetable Consumption***

We found that children who met guidelines for physical activity also had higher levels of fruit and vegetable consumption as compared to their less physically active peers (Active:  $33.0 \pm 4.0$ , Inactive:  $23.3 \pm 1.9$  servings per week). This finding confirms previous research showing that children who are more physically active are also more likely to consume more fruits and vegetables (Sallis et al 2000). There were no differences between those in the higher body fat group and lower body fat group in terms of self-reported fruit and vegetable intake. The variability of responses for number of servings per week was high (High FV 27-75 servings per week, Low FV 6-24 servings per week), and no significant relationships between fruit and vegetable consumption and post-exercise airway narrowing (i.e.  $\Delta FEV_1$ ) were found ( $F=0.1$ ,  $P=0.1$ ).

### ***Body Fat and Physical Activity***

The total body fat percentage, relative  $VO_{2max}$  and change in  $FEV_1$  are shown in Table 4 and Figure 3. The HFI group had a significantly greater decrease in  $FEV_1$  after exercise, compared to the HFA, the LFI, and the LFA groups. The HFI group also had a significantly lower  $VO_{2max}$  than the LFA group.

## **Discussion**

### ***Major Findings***

The results of our study did not directly support our first hypothesis, that children who were physically inactive would show a greater decrease in FEV<sub>1</sub> post-exercise than children who were physically active. While we did find that children with lower V<sub>O</sub><sub>2</sub> max levels had greater decrements in FEV<sub>1</sub> post-exercise, the division of groups by questionnaire data for physical activity did not show significant differences in post-exercise changes in FEV<sub>1</sub>. Our second hypothesis, that children who had higher body fat would show a greater decrease in FEV<sub>1</sub> than children who had lower body fat, was supported. Children who were in the high body fat group showed larger decrements in FEV<sub>1</sub> post-exercise than children in the low body fat group. Our third hypothesis, that children who had higher levels of body fat and were inactive would show the highest amount of post-exercise airway narrowing compared to children with lower body fat levels who were more physically active, was also supported. In fact, children with higher levels of body fat and lower levels of physical activity showed decrements in FEV<sub>1</sub> post-exercise that were large enough to be considered meeting diagnostic criterion for exercise induced bronchoconstriction (i.e. >10%, Rundell 2000).

### ***Potential Mechanisms***

Obesity may be a risk factor for asthma and asthma-like symptoms (Li et al 2003; Lucas & Platts-Mills 2006). This association, however, may not be due simply to excess adiposity, but in large part to lifestyle influences that tend to encourage development of obesity. Aerobic fitness, for example, has been shown to act protectively for several cardiometabolic health outcomes, and also for respiratory disease (Nickerson 1983;

Rasmussen et al 2000). Several studies have shown that asthmatic children are often less aerobically fit than their non-asthmatic counterparts, but that even the most severely asthmatic subjects can train to achieve normal levels of cardiorespiratory fitness (Ram et al 2000; Williams et al 2008). Although the subjects in our study were not asthmatic, the children with a lower  $\dot{V}O_{2\max}$  showed greater airway narrowing following exercise than the children with higher  $\dot{V}O_{2\max}$  values. Our study indicates that maximal aerobic capacity seems to confer some protection against asthma-like symptoms even in non-asthmatic subjects. One hypothesis that could help to explain the hyper-responsiveness seen in the children with lower  $\dot{V}O_{2\max}$  levels in the current study, is that hyper-responsiveness may result from a lack of chronic airway smooth muscle stretch, leading to stiffening of the airway wall and airway remodeling over time (Naghshin et al 2003). The effect of increased levels of ventilation on airway smooth muscle remodeling may act to decrease airway hyper-responsiveness and according to some in vitro studies, may also potentially activate endothelial derived nitric oxide synthase and further enhance mucociliary clearance (Button et al 2004).

Airway hyper-responsiveness and airway inflammation are hallmark symptoms of asthma. Hyper-responsiveness and inflammation have repeatedly been shown to be highly related, but do not always occur together (Baroffio et al 2009; Rasmussen et al. 2009; Porsbjerg et al. 2009). It is possible that it is less likely for hyper-responsiveness and inflammation to occur together in non-asthmatic children than asthmatic children. This possibility is also supported by previous research that indicates that healthy children who have airway hyper-responsiveness, but no diagnosis of asthma, are more likely than

their non-reactive peers to develop asthma in the future (Siersted et al 1996; Rasmussen et al, 2002).

Subjects in our study who were inactive, and also in the high-fat group (HFI), had the highest levels of airway narrowing following heavy exercise. The group with the second largest decrease in FEV<sub>1</sub> post-exercise was the HFA group. It appears that elevated body fat alone is placing children at risk for negative airway health outcomes, even in the face of higher activity levels. It is widely accepted that excess adipocytes place humans at risk for chronically high levels of pro-inflammatory adipocytokines circulating throughout their bodies, including the airways (Mehta and Farmer 2007; Zulet et al. 2007). Studies to date have shown associations between markers of inflammation, namely TNF- $\alpha$ , interleukin 6 (IL-6), C-reactive protein (CRP), and the excess adiposity (Tantisira and Weiss 2001). In addition to airway inflammation, there is also evidence that systemic inflammation is problematic for asthmatics as compared to people without asthma (Rasmussen et al. 2009). It is possible that this existed in our higher-fat subjects, and that our measure of airway inflammation (eNO) was not sensitive enough to detect differences post-exercise. It is interesting to note that when simply comparing the active and inactive groups, the eNO changes from pre to post exercise showed a tendency towards the active group having smaller decreases than the inactive group (-0.56 $\pm$ 1.91%, -0.90 $\pm$ 1.42%,  $p>0.86$ ). Such a tendency would indicate that with adequate power, subjects in the active group would show less of a drop in eNO from pre to post exercise, indicating that less airway narrowing was occurring and therefore, more eNO was able to be exhaled from the larger airways post-exercise as compared to the Inactive group where

more airway narrowing was occurring, leading to smaller amounts of eNO being exhaled post-exercise as compared to pre-exercise.

Dietary differences could also help to explain the post-exercise airway narrowing in the HFI group. Dietary fat was not assessed, but potentially could have played a role in airway inflammation via a TNF- $\alpha$  mediated process (Naura et al 2009). Additionally, since the subjects in the low active group consumed fewer servings of FV per week than the high active group, potentially low antioxidant status could have had a negative impact on airway health (Denny et al 2003). The high antioxidant content of fruits and vegetables, specifically vitamins C and E, and carotenoids are thought to act protectively for many different health outcomes. Anti-oxidants act to oxidize free radicals, thought to be a contributor in mediating asthma and COPD (Romieu and Trenga 2001). A relative deficiency of dietary antioxidants could potentially increase risk for development of asthma and COPD (Smit 2001).

### ***Limitations***

In the current study there was no relationship between airway inflammation (eNO) and airway narrowing ( $\Delta$ FEV<sub>1</sub>). This may be due to the use of a single marker of airway inflammation (eNO) for which there is limited data, particularly in healthy children with no diagnosis of asthma. Other methods of ascertaining inflammation such as cytokine panels, or induced sputum might provide evidence for extant inflammation in the children who also showed airway narrowing post-exercise. Additionally, other systemic markers of inflammation such as TNF- $\alpha$  or IL-6 could provide more information about potential mechanisms leading to the asthma-like symptoms observed.



Activity status alone, did not predict change in FEV<sub>1</sub>(%). It may be that the questionnaire assessment of physical activity was simply not sensitive enough to detect true differences in physical activity. Future studies should validate these findings of this study through the use of more objective physical activity measures such as pedometry, accelerometry, heartrate monitoring, or a combination of these methods.

Because of the intensive nature of the study, we utilized a one-time questionnaire assessment to serve as a 7-day recall for fruit and vegetable and sugar-sweetened beverage consumption. We attempted to increase the accuracy of these questionnaires by having the parent and child complete them together and had a trained researcher present to clarify any part of the questionnaire that they needed assistance with. Multiple 24-hour food recalls or a food-diary may have improved validity of these measures and would have provided additional dietary information beyond fruits and vegetables that could have provided valuable insight to antioxidant as well as other dietary differences between groups. Based on these limitations, the lack of relationship between fruit and vegetable consumption and post-exercise airway narrowing may simply be due to assessment methodology.

### ***Implications***

It is often argued that asthmatic children may be physically inactive for fear of an asthma attack. Since our study population was not diagnosed with asthma, we were able to show relationships between asthma-like symptoms, obesity and inactivity suggesting that lifestyle influences, at least in some cases, do come first and may lead to asthma-like symptoms and potentially asthma. Armed with more knowledge in these areas, more effective prevention policies and treatment protocols can be implemented to help to

reverse the rise in childhood obesity and childhood asthma. Finally, we addressed a health outcome that is often not studied. In terms of children, airway health is a key issue, particularly because asthma is the most common childhood chronic disease (Lucas and Platts-Mills 2006).

### ***Conclusions***

Thus far, minimal work has evaluated the influences of body composition and physical activity on health outcomes in children, specifically airway health. The results of our study demonstrate that increased body fat and low physical activity are associated with larger post-exercise decreases in FEV<sub>1</sub> as compared to lower fat, higher activity peers. In other words, when a child was not meeting physical activity guidelines, children with higher body fat levels were more likely to have a larger decrease in FEV<sub>1</sub> following heavy exercise. This finding suggests that children who fall into this HFI category are experiencing clinical levels of airway narrowing (EIB) at the very least, and are potentially at risk for developing asthma in the future if their course is not altered. Intervention studies as well as longitudinal types of research should be undertaken to help us better understand the relationships of physical activity, body composition, and measurable health outcomes in children.

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## Tables

**Table 3.1 Subject Characteristics**

	Inactive (n=20)		Active (n=20)	
	mean $\pm$ SD	range	mean $\pm$ SD	range
Age (yrs)	9.5 $\pm$ 0.9	7.6 – 10.9	9.8 $\pm$ 0.6*	8.7 – 11.3
Weight (kg)	33.2 $\pm$ 8.4	24.8 – 55.6	25.4 $\pm$ 11.8	25.4 – 78.2
Height (cm)	138.4 $\pm$ 6.2	130.8 – 149.9	125.7 $\pm$ 7.2	125.7 – 151.1
Body Fat (%)	22.4 $\pm$ 10.3	9.3 – 42.8	19.8 $\pm$ 10.7	7.9 – 50.5
BMI (kg/m <sup>2</sup> )	17.3 $\pm$ 4.0	12.1 – 27.0	17.6 $\pm$ 4.4	14.3 – 34.2

\*Significantly higher for active group than inactive group (p < 0.05)



**Table 3.2 Resting Pulmonary Function**

	Active (n=20)		Predicted	Inactive (n=20)		Predicted(%)
	mean $\pm$ SD	range		mean $\pm$ SD	range	
FVC (L/sec)	2.1 $\pm$ 0.4	1.6 - 2.8	100 $\pm$ 1	2.1 $\pm$ 0.3	1.4 - 2.7	100 $\pm$ 1
PEF (L/sec)	3.2 $\pm$ 0.8	1.8 - 4.9	_____	3.4 $\pm$ 0.7	2.0 - 4.8	_____
FEV <sub>1</sub> (L/sec)	1.8 $\pm$ 0.3	1.3 - 2.4	95 $\pm$ 1	1.7 $\pm$ 0.3	1.3 - 2.5	95 $\pm$ 1
FEV <sub>1</sub> /FVC (%)	83.0 $\pm$ 8.1	64 - 96	98 $\pm$ 1	84.6 $\pm$ 5.3	72 - 96	98 $\pm$ 1
FEF <sub>50%</sub> (L/sec)	2.1 $\pm$ 0.5	1.0 - 3.1	_____	2.2 $\pm$ 0.4	1.5 - 2.9	_____
FEF <sub>25-75%</sub> (L/sec)	1.9 $\pm$ 0.5	1.8 - 2.7	_____	1.9 $\pm$ 0.5	1.0 - 2.8	_____
eNO (ppb)	19.9 $\pm$ 35.1	4.3 - 166.8	_____	25.8 $\pm$ 32.8	3.8 - 137.9	_____

Predicted equations from Knudson et al (24)

FVC, forced vital capacity; PEF, peak expiratory flow; FEV<sub>1</sub>, forced expiratory volume in 1 sec; FEF<sub>50%</sub>, forced expiratory flow at 50%; FEF<sub>25-75%</sub>, forced expiratory flow between 25-75%; eNO, exhaled Nitric Oxide.

No differences between groups (p > 0.05)

**Table 3.3 Change in FEV<sub>1</sub> Following Exercise and Exhaled Nitric Oxide in Active and Inactive Subjects**

	ACTIVE (n = 20)	INACTIVE (n = 20)	
Change in FEV <sub>1</sub> (%)	-3.33 ± 1.38	-7.15 ± 2.11	P=0.137
Change in eNO (%)	-0.56 ± 1.91	-0.90 ± 1.42	P=0.855

Values are mean ± SD

**Table 3.4 Physical Activity Status and Body Fat**

	Low Fat Active (LFA) n = 11	Low Fat Inactive (LFI) n = 13	High Fat Active (HFA) n = 7	High Fat Inactive (HFI) n = 9
Total Body Fat (%)	13.5 ± 1.0	14.8 ± 1.0	31.6 ± 3.5	31.7 ± 2.5
VO <sub>2</sub> max (ml/kg/min)	38.2 ± 1.4	34.5 ± 1.8	27.0 ± 2.6	26.2 ± 1.6 **
Δ FeV1 (%)	-1.27 ± 1.51	-3.97 ± 2.61	-7.14 ± 2.33	-11.00 ± 3.10 *

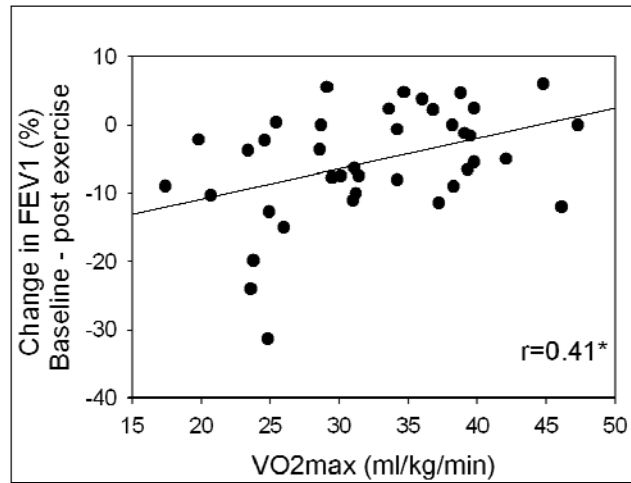
Values are mean ± SD

\*Significantly different than low fat active and low fat inactive groups (P<0.05).

\*\*Significantly lower than low fat active, low fat inactive, and high fat active groups (P<0.05).

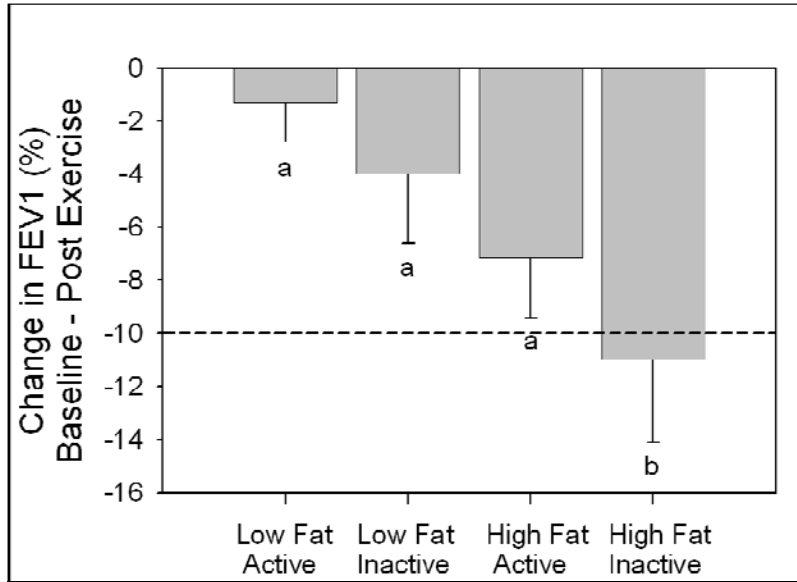


**Figure 3.2 Role of Physical Activity on Airway Health**



Change in FEV<sub>1</sub> vs. VO<sub>2max</sub> from pre to post-exercise is shown in Figure 3. The change in FEV<sub>1</sub> (pre-post exercise) was related ( $r=0.41$ ,  $P<0.05$ ) to VO<sub>2max</sub>; subjects with the lowest VO<sub>2</sub> max had the greatest decrease in FEV<sub>1</sub> (i.e., airway constriction).

**Figure 3.3 Combined Effects of Body Fat and Activity on Airway Health**



The total body fat (%), relative  $\dot{V}O_{2\max}$  and change in  $FEV_1$  are shown in Fig. 1 and Table 2. The high fat inactive group had a greater ( $p < 0.05$ ) decrease in  $FEV_1$  after exercise ( $-11.0 \pm 3\%$ ), compared to the high fat active ( $-7.1 \pm 2.3\%$ ), the low fat inactive ( $-4.0 \pm 2.6\%$ ) and the low fat active groups ( $-1.3 \pm 1.5\%$ ). The high fat inactive group had a significantly ( $p < 0.05$ ) lower  $\dot{V}O_{2\max}$  than the low fat active group.

**CHAPTER 4 - High-intensity interval training improves  
airway health in inactive non-asthmatic children**

## Abstract

**PURPOSE:** The relationship between physical activity (PA) and airway health in children is not well understood. The purpose of this study was to determine whether 8-weeks of high-intensity exercise training would improve airway health in inactive, non-asthmatic prepubescent children.

**METHODS:** Sixteen healthy, prepubescent children were tested (training group (TrG) n=8, control group (ConG) n=8). Subjects wore accelerometers for seven days prior to the 8-week training period to determine activity level. Prior to and following 8-weeks of training (or no training), subjects completed pulmonary function tests (PFTs) including forced expiratory flow in 1-sec (FEV<sub>1</sub>), forced vital capacity, forced expiratory flow at 25-75% of vital capacity (FEF<sub>25-75</sub>), exhaled nitric oxide (eNO), and impulse oscillometry (IOS). Subjects also completed an incremental cycle VO<sub>2max</sub> test, eucapnic voluntary hyperventilation (EVH), anthropometric tests, and blood tests to determine fasting blood glucose, total cholesterol, HDL, LDL, and triglycerides. Body composition was determined using dual-energy x-ray absorptiometry (DXA) pre-training and bioelectrical impedance analysis (BIA) pre- and post-training.

**RESULTS:** There were no differences (p>0.05) in anthropometric measures or PFTs between TrG and ConG at baseline. VO<sub>2max</sub> was significantly lower at pre-test for TrG (23.6±7.7 ml/kg/min) than ConG (33.1±6.2 ml/kg/min). In the TrG there was a significant increase in VO<sub>2max</sub> (~24%), and a decrease in total cholesterol (~13%) and LDL cholesterol (~35%). Additionally, we found improvements (p<0.05) in ΔFEV<sub>1</sub> both post-exercise (pre: -7.60±2.10%, post: -1.10±1.80%) and post-eucapnic voluntary hyperventilation (pre: -6.71±2.21%, post: -1.41±1.58%) with training. The change in FEF<sub>25-75</sub> pre-post exercise also improved with training (pre: -16.10±2.10%, post: -6.80±1.80%; p<0.05). Lower-body-fat and higher VO<sub>2max</sub> subjects experienced significantly greater improvement in ΔFEV<sub>1</sub> following training than higher-body-fat and lower VO<sub>2max</sub> subjects (r=-0.80, r=0.73, respectively).

**CONCLUSION:** These results suggest that physical inactivity negatively impacts airway health in non-asthmatic prepubescent children, which can be improved with high-intensity training. However, increased body fat, and low VO<sub>2max</sub> levels may constrain these improvements.

Keywords: high-intensity training, airway hyper-responsiveness, airway resistance, prepubescent children



## **Introduction**

Childhood asthma prevalence has increased significantly over the past few decades and is the most common chronic disease in childhood (Lucas & Platts-Mills 2006; Williams et al. 2008). While many factors contribute to the development of asthma, the role of physical activity on airway health in children is not well understood and attainment of inadequate levels of moderate to vigorous physical activity (MVPA) may be contributing to the increase in asthma prevalence rates.

Lack of physical activity, particularly at higher intensity levels (MVPA) may increase the risk of asthma development in two ways. First, it is well established that lack of physical activity is a potent risk factor for development of obesity which has been shown to be associated with asthma prevalence in children as well as adults (Dunstan et al. 2007; Foster et al. 2006; Hu et al. 2003; Jakes et al. 2003; Jebb & Moore 1999; Stamatakis 2008). Second, low levels of MVPA has been shown to be an independent risk factor for asthma development (Huovinen et al. 2001; Ludwick et al. 1986; Shore & Johnston 2006; Shaaban et al. 2007; Stenius-Aarniala et al. 2000). Additionally, lung function has been observed to be the highest in physically active children as compared to their less active peers (Berntsen et al. 2008). A few studies in children with asthma have also demonstrated improved lung function in addition to decreased use of medications following completion of a physical activity program (Araki et al. 1991; Bonsignore et al. 2008; Lucas & Platts-Mills 2006;). Similarly, low physical fitness in childhood has been found to be significantly associated with development of asthma in adolescence (Rasmussen et al. 2000).

In contrast to the beneficial effects of training, healthy children undergoing a short length high-intensity training protocol have shown significant decreases in pre- to post-training pulmonary function values (Nourry et al. 2005). The authors speculate that these results could be explained by heat and or water loss from the airways triggering bronchoconstriction resulting from higher ventilation levels and increased exercise intensity post-training. These studies, however, have not controlled for previous levels of chronic physical activity.

In adults, when physical activity levels were controlled for, a negative association between physical activity and bronchial hyperresponsiveness to methacholine was found (Shaaban et al. 2007). In children with stable, mild asthma, a decreased bronchial hyperresponsiveness was seen following a 12-week training protocol, which the authors speculated could be due to repeated airway stretch associated with increased ventilation during regular training that modified the contractile mechanism of the airway smooth muscle (ASM)(Bonsignore et al. 2008). A lack of smooth muscle stretch in the airway obtained through moderate to vigorous physical activity may be a risk factor for asthma-like symptoms in childhood. It is well known that ASM stretch relaxes the tone of muscle in the airway which reduces airway reactivity (Bonsignore et al. 2007). It is possible that physical training (and therefore, chronic airway stretch) will make the airway less susceptible to developing increased reactivity and asthma-like symptoms.

Therefore, we were interested in determining whether a high-intensity running training program would improve airway health in non-asthmatic prepubescent children who were not meeting current physical activity guidelines. We hypothesized that in this population, 8-weeks of high-intensity running training would: reduce post-exercise

airway narrowing and airway resistance. Additionally, we hypothesized that children with the highest body fat percentages would experience greater amounts of airway narrowing and airway resistance at baseline, and further, in these subjects, training would impart superior improvements in airway health relative to leaner subjects.

## **Methods**

### ***Subjects***

Eighteen healthy prepubescent children (16 girls, 2 boys) ages 7-12 years, with no diagnoses or history of acute or chronic diseases (determined via medical history questionnaire) volunteered as subjects. Two subjects were excluded from data analyses due to interruption in training for greater than one week. Sixteen subjects (14 girls, 2 boys) were included in the data analysis. All subjects were free of asthma or disease and demonstrated normal lung function as measured by standard pulmonary function tests (Knudson et al. 1983). According to parent or guardian completed questionnaires, children were prepubescent and were in the first stage of maturation, as defined by Tanner stage 1 (Tanner 1962). All subjects, along with their parent or guardian, reported their current level of physical activity (within the past 6 months) and were categorized as meeting USDHHS physical activity guidelines or not (Physical Activity Guidelines Advisory Committee 2008) ( $\leq 4$  days per week of 60 minutes of more moderate to vigorous physical activity). Each subject had a parent or guardian present to provide medical history information and informed consent. All research components were reviewed and approved by the Institutional Review Board of Human Subjects at Kansas State University, Manhattan, KS.

### ***Experimental Design***

All subjects reported to the lab on four separate occasions. A parent or guardian was present at each session. Sessions one and two were completed prior to group randomization. Subjects either completed eight weeks of training or were asked to continue their usual physical activity and dietary habits for the eight-week duration.

Sessions three and four were completed at the conclusion of the eight-week period of training or no training.

### *Sessions 1 and 3*

Procedures in sessions 1 and 3 were identical. Height and weight were recorded using a calibrated eye-level physical scale with height rod (Detecto, Webb City, MO). Subjects were then familiarized with the equipment and procedures. After demonstration and explanation, impulse oscillometry (IOS; Jaeger MS-IOS, Höchberg, Germany; LAB Manager Software version 8.0) was performed for approximately 30 seconds in triplicate, with the average of the three measurements being utilized for analyses. After several practice trials and demonstrations, standard pulmonary function measures were performed using an automated pulmonary function testing system (SensorMedics 229 Metabolic Cart, SensorMedicsCorp., Yorba Linda, CA) following recommended protocols (discussed below). Total lung capacity (TLC) was determined via nitrogen washout technique. Subjects completed exhaled nitric oxide measurements (eNO) via chemiluminescence (Sievers Nitric Oxide Analyzer 280, Sievers Instruments Inc, Boulder, CO). Subjects then completed an incremental cycle exercise test to exhaustion to determine maximal oxygen uptake  $VO_{2max}$  (discussed below). Two minutes following the maximal exercise test, IOS was again performed, followed immediately by maximal flow volume loops and eNO measurement. Then at 10 minutes post-exercise test, the IOS was repeated. A second exercise test at constant work load (105%  $VO_{2max}$ ) was performed following 15 minutes of rest to verify  $VO_{2max}$ .

Assisted by the parent or guardian, subjects completed the Day in the LIFE Questionnaire (DILQ, Edmunds & Ziebland 2002). Additionally, the parent or guardian

and the child completed a two-item questionnaire to determine previous 7-day physical activity and physical activity in a typical week (Prochaska et al. 2001). Subjects also had an Actigraph GT1M (The Actigraph, Shalimar, FL) accelerometer placed on their right hip as well as an Actical (Mini-Mitter, Bend, OR) accelerometer placed on their right ankle utilizing a hospital type wristband to wear for the next seven days. Subjects returned approximately one week later for their second visit. Subjects were asked to complete a log for the Actigraph GT1M accelerometers indicating time on and time off each day as well as any periods of non-wear time.

#### ***Sessions 2 and 4***

Procedures in sessions 2 and 4 were identical with the exception of no repeat DXA scan in session 4. Subjects reported for their second visit following a 10-12 hour overnight fast. A fingerstick was used to determine fasting blood glucose (BG), total cholesterol (TC), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), and triglycerides (TG). Following five minutes of quiet sitting, each subject had waist circumference (WC), tetrapolar bioelectrical impedance (BIA) (discussed below) and blood pressure (BP) via stethoscope auscultation assessed. Following the resting measurements, subjects repeated the IOS, followed by maximal flow-volume loop (MFVL) tests. Subjects then performed a six-minute eucapnic voluntary hyperventilation (EVH) test at 30 times their best pre-test FEV<sub>1</sub> from that day's testing. Two minutes following the EVH test, subjects performed IOS and then immediately performed MFVLs. Then at ten minutes post-EVH test, IOS was again performed. Following the breathing testing, each subject underwent a DXA scan to determine body composition.

### ***Impulse Oscillometry***

The IOS procedure is independent of participant effort where random 5-35Hz pulses are generated by a small loudspeaker mounted in series with a pneumotach during quiet tidal breathing (Evans et al. 2006). IOS measurements were performed while the subject was seated with his or her head in a neutral position and feet flat on the ground. Each subject supported his or her cheeks with both hands and nose-clip was worn. The subject was instructed to breathe normally and quietly. Each measurement was approximately 30 seconds in duration. The R5 (KPa/l/sec, resistance at 5 Hz) values are indicative of total airway resistance, and have been shown to be sensitive in detecting airway obstruction as well as bronchodilation response in children (Bar-Yishay et al. 2009; Ducharme & Davis 1998). Additionally, the R5 value has been shown to be highly correlated with the FEV<sub>1</sub> measurements, and more sensitive to airflow obstruction than traditional spirometry (Evans et al. 2006; Goldman et al. 2002).

### ***Pulmonary-function measurement***

Each subject underwent standard pulmonary function tests (PFT);(forced expiratory flow in 1-sec (FEV<sub>1</sub>), forced vital capacity (FVC), forced expiratory flow at 25-75% of vital capacity(FEF<sub>25-75</sub>)) (SensorMedics 229 Metabolic Cart, SensorMedicsCorp, Yorba Linda, CA), and exhaled nitric oxide tests (eNO; a marker of airway inflammation) (Sievers Nitric Oxide Analyzer 280, Sievers Instruments Inc, Boulder, CO) prior to as well as post exercise test. All tests were performed in triplicate, with the average value used in analysis.

Total lung capacity (TLC) and MFVLs were assessed prior to exercise testing (SensorMedics 229 Metabolic Cart, SensorMedics Corp, Yorba Linda, CA). TLC was

determined using the nitrogen wash-out technique. MFVLs were performed in triplicate, with the average value used in analysis.

### ***Maximal Oxygen Uptake (VO<sub>2max</sub>)***

VO<sub>2max</sub>, heart rate, and an estimate of arterial oxygen saturation (SpO<sub>2</sub>) were determined via a maximal exercise test on an electronically braked cycle-ergometer (Ergometer 800S, Sensor Medics Corp., Yorba Linda, CA). Subjects were given consistent instructions explaining the protocol of the test to ensure maximal volitional effort. Prior to testing, known gas concentrations spanning the range of expected measurements were used to calibrate gas analyzers. Flow sensor calibration was also performed utilizing a 3-L calibration syringe. Resting metabolic measurements were taken for three minutes. Subjects then began with a warm-up for approximately two minutes at a work rate of 20 watts, pedaling between 50-60 revolutions per minute (rev/min). Subjects were instructed to remain seated and maintain this pedaling speed while the work rate was increased by 10 watts each minute. Metabolic and ventilatory data were assessed continuously through breath-by-breath analysis (SensorMedics 229 Metabolic Cart, SensorMedicsCorp., Yorba Linda, CA). Heart rate (HR) was monitored throughout the test via a four-lead electrocardiograph (ECG) interfaced to the metabolic software. The sensor from a pulse oximeter (Datex-Ohmeda, 3900P, Madison, WI), calibrated before each test, was secured to the left earlobe to estimate arterial oxygen saturation (SpO<sub>2</sub>). Subjects continued to exercise until reaching volitional exhaustion (< 16 minutes). Verbal encouragement was provided throughout the test. The VO<sub>2max</sub> test concluded when subjects could not maintain a pedal frequency of >50 rev/min for five consecutive revolutions.



Following the  $VO_{2max}$  test, subjects performed IOS, followed immediately by pulmonary function tests, repeated the IOS at 10-minutes post-exercise, and then rested for the remainder of a 15 minute period. At the end of the 15 minute period, a constant load (105%  $VO_{2max}$ ) exercise test was performed until exhaustion to verify  $VO_{2max}$  (Poole et al. 2008). Work rate for the test was determined from the final work rate (watts) during the incremental test. Subjects were given a warm-up period of 90 seconds pedaling 50 rev/min at 20 watts. Work rate was increased until reaching calculated work rate (~30 seconds) and subjects were instructed to maintain 50 rev/min until volitional fatigue.

### ***Eucapnic Voluntary Hyperventilation (EVH)***

In addition to the post-exercise airway narrowing assessment, EVH was utilized to determine the changes in airway function following voluntary hyperventilation. The target ventilation for EVH was 30 x FEV1 which is intended to be approximately 85% of predicted maximal voluntary ventilation (MVV)(Spiering et al. 2004). The subject then hyperventilated while breathing on a bag containing 5% carbon dioxide dry gas mixture for six minutes. Subjects wore a nosepiece and looked at a computer monitor indicating their ventilation level in order to maintain target ventilation throughout the six-minute test.

### ***Body Composition***

#### ***Dual Energy X-ray Absorptiometry***

During the pre-test week, body composition was measured by use of a whole body DXA system (v5.6, GE Lunar Corp., Milwaukee, WI), as the criterion measure of body composition. Subjects removed shoes and any metal objects prior to scanning, and

lay in a supine position with arms separated from trunk and legs slightly spaced apart. Subjects were asked to lie as still as possible during the scanning procedure. DXA has previously been validated, and uses two x-ray beams with differing energy levels to find differences in absorption and therefore lean body mass (LBM), body fat percentage (BF%), and fat mass (FM) (Haarbo et al. 1991; Margulies et al. 2005). Additionally, DXA is considered to be safe and valid for use in the pediatric population (Aasen et al. 2006; Jensky-Squires et al. 2008).

### ***Bioelectrical Impedance***

Whole-body resistance and reactance were measured using a tetrapolar bioelectrical impedance analyzer (RJL systems, Quantum II, Detroit, MI). Calibration was performed using the 500-ohm resistor provided by the manufacturer. Electrodes were placed at the ankle and wrist as indicated by the manufacturer. Body fat percentage, fat-free mass (FFM), and body water were determined via the NHANES III algorithm included in the manufacturer provided body composition software.

### ***Questionnaires***

#### ***Physical Activity Status***

To determine whether or not the subjects were meeting current physical activity guidelines, subjects were asked to report on the previous week as well as a normal or usual week how many days per week they accumulated at least 60 minutes of moderate to vigorous physical activity, not including physical education or gym class (Prochaska et al. 2001). This two-item physical activity screening tool has been previously validated for meeting physical activity guidelines or not, in a similar age group (Prochaska et al. 2001).

### ***Nutrition Questionnaire***

To determine current nutrition intake for fruits and vegetables and fruit juices, we administered the Day in the Life Questionnaire (DILQ). The DILQ has been previously validated as a measure of fruit and vegetable consumption in 7-9-year-old children (Edmunds & Ziebland 2002).

### ***Accelerometer Data Reduction***

Raw Actigraph GT1M accelerometer count data for the 7 days of wear were uploaded to a customized data processing software program. The MET prediction equation from Freedson et al. (2005) was used to estimate time spent in moderate-to-vigorous (MVPA;  $\geq 4$  METS) physical activity. The age-specific counts per minute thresholds were divided by two to accommodate our 30 second epoch length. Non-wear time for each 24-hour period was determined by summing the consecutive zero counts that were 10 minutes or longer. MVPA per day was determined only if total wear time reached a 10 hour minimum. Participants met physical activity guidelines if their total daily accumulated MVPA time was equal to or exceeding 60 minutes per day on five or more days.

### ***Training protocol***

Training group subjects participated in two weekly training sessions spaced at least 48 hours apart for eight weeks in addition to their usual activities. To determine appropriate distances for each interval, prior to the first training session, subjects performed a PACER test which is a multistage 20m run test to determine their maximal aerobic speed (MAS) (Baquet et al. 2002; Nourry et al. 2005). Each training session lasted about 30 minutes and included approximately eight to nine minutes of vigorous

activity. Each training session began with a standardized warm-up performed at 100% MAS. The warm-up set was followed by four more sets of 10 x 10s, or later in the eight-week progression, 5 x 20s intervals at 100-130% of MAS. Rest intervals were performed at a 1 to 1 work to rest ratio and were passive recovery rest periods.

### ***Statistics***

SigmaStat statistical software (Jandel Scientific Software) was used for data analysis. Data are expressed as mean  $\pm$  standard deviation. Differences between groups and conditions were determined using mixed factorial ANOVA. Relationships were determined by Pearson Product-Moment Correlation. Significance was set at  $p < 0.05$  for all analyses.

## Results

### *Subject Characteristics*

Subject characteristics are presented in Table 1. There were no significant differences between ConG and TrG for anthropometric data or body composition. Additionally, anthropometric data and body composition did not change ( $p>0.05$ ) over the course of the study.

### *Physical Activity Status and Fruit and Vegetable Consumption*

Utilizing accelerometer data for analysis, the control group achieved MVPA guidelines on  $1.4\pm 1.3$  days and the TrG met guidelines on  $1.8\pm 1.8$  days per week ( $p>0.05$ ). All subjects met inclusion criterion by meeting guidelines on four or fewer days per week. Based on the two-item questionnaire, the ConG indicated meeting PA guidelines  $2.7\pm 0.5$  days per week, while the TrG reported meeting guidelines  $1.4\pm 0.8$  days per week ( $p>0.05$ ). Based on the DILQ questionnaire, there were no differences for fruit and vegetable intake between groups. The ConG mean fruit and vegetable intake was  $1.2\pm 0.7$  servings per day and the TrG mean intake was  $2.3\pm 1.7$  servings per day ( $p>0.05$ ).

### *Resting Pulmonary Function*

Table 2 displays baseline lung volumes and resting pulmonary function including exhaled nitric oxide and impulse oscillometry. Baseline PFTs were not significantly different from predicted values for prepubescent children (Knudson et al. 1983). Baseline PFTs were also not significantly different between the ConG and TrG. There were no significant differences in total lung capacity between groups from pre to post training.

### ***Change in Pulmonary Function***

Pre and post training pulmonary function baseline values as well as percent change in pulmonary function data from pre to post-training are shown in Table 3. Figure 1 shows individual and mean data for the percent change in FEV<sub>1</sub> for the ConG versus TrG from pre to post-exercise, indicating that the TrG showed a decrease in the percentage of  $\Delta$ FEV<sub>1</sub> following training. Six out of eight subjects in the TrG improved post-exercise  $\Delta$ FEV<sub>1</sub> from pre-post training ( $6.50 \pm 2.10\%$ ). The two subjects who did not improve were the subjects with the least decrease in FEV<sub>1</sub> post-exercise prior to training (0.5% increase and 0.7% decrease). Similar results ( $p < 0.05$ ) were observed with the EVH test. The pre to post-EVH  $\Delta$ FEV<sub>1</sub> was also significantly improved in the TrG (Pre:  $-6.71 \pm 2.21\%$ , Post:  $-1.41 \pm 1.58\%$ ;  $5.30 \pm 1.90\%$  improvement). Seven out of eight of the TrG subjects improved  $\Delta$ FEV<sub>1</sub> following training and the subject who did not improve showed a positive pre-post FEV<sub>1</sub> change with EVH. Figure 2 shows individual and mean data for the percent change in FEF<sub>25-75</sub> from pre to post-exercise. FEF<sub>25-75</sub>, a measure of small airway function, also improved ( $-16.10 \pm 2.10\%$  to  $-6.80 \pm 1.80\%$ ,  $p < 0.05$ ) in the TrG following the training program, but did not change in ConG. Significant improvement in post-exercise PEF was also seen following the training protocol for the TrG only ( $-14.26 \pm 2.00\%$  to  $-2.94 \pm 1.40\%$ ).

### ***Exercise Test Data Pre-Post eight-week Training Program***

Data collected during the incremental cycle ergometer test at VO<sub>2max</sub> are shown in Table 4. There was no difference ( $p > 0.05$ ) in VO<sub>2max</sub> between the incremental test and the constant load test at 105% VO<sub>2max</sub>. VO<sub>2max</sub> was significantly lower in the TrG versus the ConG at baseline. The training protocol was effective in increasing VO<sub>2max</sub> in all

subjects in the TrG by an average of approximately 24.5%. Ventilation (VE) also increased significantly (20.6%) in the training group. Figure 3 shows a significant relationship between  $V_{O_{2max}}$  and the percent change in pre to post-exercise  $\Delta FEV_1$  from pre to post-training. Subjects with the highest  $V_{O_{2max}}$  values at baseline showed greater improvements in post-exercise  $\Delta FEV_1$  from pre to post- training.

### ***Body Fat Percentage and Airway Health***

Figure 4 shows there was a significant relationship between body fat percentage at baseline and the percent change in pre to post-exercise  $\Delta FEV_1$  from pre to post-training. Subjects in the TrG with higher body fat percentages improved less in post-exercise  $\Delta FEV_1$  from pre to post-training than subjects with lower body fat percentages.

## Discussion

The results of the current study suggest that insufficient moderate-to-vigorous physical activity negatively impacts airway health in non-asthmatic prepubescent children, and high-intensity training can ameliorate these negative airway health outcomes. Our first hypothesis was supported by the finding that airway narrowing was reduced following training. However, we did not observe any change in airway resistance following training. Our data did not support our second hypothesis; i.e., at baseline, body fat percentage was not associated with airway narrowing. In fact, children with the highest levels of body fat experienced less of an improvement in airway narrowing following training compared to leaner subjects. Also, subjects in the training group who did not improve pulmonary function were the subjects with the least amount of airway narrowing prior to training. Finally, subjects with the highest  $V_{O_{2max}}$  at baseline experienced greater improvements in airway function than subjects with lower  $V_{O_{2max}}$  values, suggesting that a low  $V_{O_{2max}}$  puts children at risk for negative airway health outcomes.

### *Pulmonary Adaptations and Physical Activity*

Excessive airway narrowing that occurs due to contraction of airways smooth muscle (ASM) is known to be a risk factor for future development of asthma and asthma-like symptoms (Kopriva et al. 2007). A low level of physical activity is also associated with the future development of asthma in otherwise healthy children (Huovinen et al. 2001; Ludwick et al. 1986; Rasmussen et al. 2000; Shaaban et al. 2007; Shore & Johnston 2006; Stenius-Aarniala et al. 2000). One previous study in non-asthmatic children undergoing short length high-intensity training protocols showed significant



decrements in pre- to post-training pulmonary function (Nourry et al. 2005). Our data, however, indicate that in children not meeting current physical activity guidelines, a short-term high-intensity training protocol can improve airway responsiveness. There are important distinctions between our subject population and the previously studied subject population that may account for this discrepancy. For example, the average  $\dot{V}O_{2\max}$  from the training group from Nourry et al. (2005) was nearly 60% higher than our group's baseline average. By design, our subject population was inactive (none were meeting guidelines for physical activity). While Nourry and colleagues did not account for physical activity levels in their study, it is likely that their population would have been more physically active than ours. Nourry and colleagues (2005) also showed a 16% increase in ventilation following training in their subjects, reaching levels that were much higher than the average ventilation of our subject population following training. The authors attributed changes in post-exercise airway function to heat or water loss in the airway due to increases in ventilation (Nourry et al. 2005). Greater ventilation may also lead to increased airway smooth muscle stretch which may affect airway function (see below). The mean body fat percentage of Nourry's training group was approximately seven percent less than the mean of our subjects. This is potentially another important difference that could help to explain mechanisms responsible for disparate results between studies. Differences in habitual activity, ventilation,  $\dot{V}O_{2\max}$ , and body fat percentages between the two study populations could be keys in the mechanistic explanation of the differences between studies.

### *Airway smooth muscle stretch*

We believe that the reason for improved airway function following training in our study was increased airway smooth muscle (ASM) stretch and lung inflation associated with high-intensity training. Under normal conditions, ASM has reduced tone, allowing it to maintain the stiffness of the airways when there are large swings in transmural pressure, such as in exercise (Stephens & Hoppin 1986). Many pathology studies have documented an increased ASM mass in asthmatic airways along with faster proliferation, producing more chemokines and cytokines, and extracellular matrix proteins that are different from non-asthmatic ASM (Ebina et al. 1993). With increased ASM mass comes an increase in force development and airway narrowing (Lambert et al. 1993). Further, Skloot and colleagues (2003) reported that the differential effect of deep inhalation on induced bronchoconstriction in asthmatic versus normal subjects might be caused by different velocities of smooth muscle shortening in asthmatic airways. Although our subject population was not asthmatic, it is possible that similar mechanisms could account for the observed changes in airway hyperresponsiveness.

Lung inflation is known to have a beneficial effect on the airways of healthy subjects, but the mechanisms through which it exerts its beneficial role in healthy subjects, and the factors impairing such an effect in those with airway hyperresponsiveness, remain unclear (Scichilone et al. 2001). Lung inflation, or deep inspiration, acts as both as a bronchoprotector, and as a bronchodilator, in that it reverses bronchial obstruction. Asthmatics show an impairment in bronchoprotection, which is also lost in individuals with AHR, but no diagnosis of asthma. Stretch that is imposed on the airway by the parenchyma, not only opposes bronchoconstriction, but can remodel the

ASM by breaking actin-myosin cross-bridges and/or by changing their configuration (Fredberg et al. 1997; Gunst & Wu 2001). Additionally, stretch is stronger with deeper inspirations and with higher ventilation levels (Scichilone & Togias 2004). The effect of increased levels of ventilation on airway smooth muscle remodeling may act to decrease airway hyperresponsiveness and according to some *in vitro* studies, may also potentially activate endothelial derived nitric oxide synthase and further enhance mucociliary clearance (Button et al. 2004). Similarly, in an animal model, it has been shown that repeated bouts of aerobic exercise at a moderate intensity decrease total lung resistance, airway smooth muscle thickness, and attenuate airway hyper-responsiveness via a beta2-AR mechanism (Hewitt et al. 2009). One hypothesis that could help to explain the hyperresponsiveness seen in the children with lower  $VO_{2max}$  levels in the current study, is that hyperresponsiveness may have resulted from a lack of chronic airway smooth muscle stretch from less ventilation (due to lack of intense PA), leading to stiffening of the airway wall and airway remodeling over time (Naghshin et al. 2003). Further research is required to determine this mechanistic postulate.

### ***Impact of body fat on airway health***

It is well established that obesity is associated with asthma (Shore 2010; Shore & Johnston 2006; Stenius-Aarniala et al. 2000) and it has also been associated with asthma-like symptoms in otherwise healthy adults (Torchio et al. 2009), but the mechanisms for this relationship are unclear. Importantly, in a review by Scichilone et al. (2009), 16 of 17 potential prospective studies involving more than 200,000 adults and children indicated that obesity precedes asthma, and obesity also worsens asthma control (Scichilone et al. 2009). A few studies have shown that obesity is also a risk factor for

AHR (Celedon et al. 2001; Chinn et al. 2002; Litonjua et al. 2002). Although studies examining the relationship between obesity and AHR in children have shown inconsistent results, when exercise was the method of provocation, results have consistently indicated that obese children have more exercise induced bronchoconstriction (EIB) than non-obese children, even in non-asthmatics (Shore 2010). Although our subject population was not obese, the fact that body fat was associated with airway responsiveness suggests that similar mechanisms may be involved. Our trained subjects with higher-body-fat percentages had less of an improvement in  $\Delta FEV_1$  than leaner subjects suggesting that excess adiposity places children at risk for asthma-like symptoms and acts to diminish potential benefits derived from engaging in a vigorous physical training program. The mechanistic basis for increased body fat contributing to diminished airway health benefits may be greater systemic inflammation.

Adipose tissue acts as an endocrine organ, generating pro-inflammatory cytokines and creating inflammation systemically as well as in the airways (Mehta & Farmer 2007; Tantisira & Weiss 2001; Zulet et al. 2007). Changes in many adipose-derived inflammatory markers, including TNF- $\alpha$ , leptin, and adiponectin, have the capacity to promote AHR and may thus contribute to asthma in the obese (Rasmussen 2009). In addition to adipocytes, ASM may produce multiple inflammatory mediators (prostanoids, cytokines and chemokines) (Johnson & Knox 1997), contributing to the exacerbation of the inflammatory process in the airway wall. Related to the previous discussion of AHR, in response to stretch, the contractile myocytes of the airway smooth muscle (ASM) have a mechanical plasticity that is thought to be directly influenced by inflammation (Halayko & Amrani 2003; Seow et al. 2000). Chronic inflammation is

likely to be one possible mechanism by which airway remodeling occurs, eventually leading to airway hyperresponsiveness. In agreement with this hypothesis, a recent study from Lowder and Colleagues (2010) indicated that moderate intensity aerobic exercise training may attenuate airway inflammation within the asthmatic airway via regulatory T cells. It is possible that in our study, training led to increased ventilation and therefore, also to stretch in the airway smooth muscle. However, in our higher-body-fat subjects, systemic inflammation persisted, not allowing improvement in airway narrowing to the extent of the leaner subjects. Physical inactivity as well as adiposity, may have contributed to the hyperresponsiveness seen in the higher-body-fat subjects. Therefore, through training, improvement was seen in one contributing factor, but not the other, diminishing the overall improvement in airway function.

### ***Training Adaptations***

In addition to changes in pulmonary function, other adaptations occurred with training. The improvement in  $\dot{V}O_{2\max}$  seen in our group of children is consistent with previous reports with similar training protocols (Bacquet 2002; Nourry et al. 2005). Other positive improvements included improved total cholesterol and LDL cholesterol. These results indicate that children who are not meeting current physical activity guidelines, in addition to improved pulmonary function, may improve their aerobic capacity and blood lipid profile through increased high-intensity physical activity.

### ***Limitations***

Several limitations exist which may have influenced our results. By simply participating in the study, it is possible that alterations in lifestyle were made in order to augment the physical training, or in the case of the subjects in the control group, to take

place of participation in the training protocol. However, group differences at baseline were minimal, and subjects were encouraged to maintain physical activity and dietary habits throughout the study.

Because asthma is characterized by airway inflammation as well as airway hyperresponsiveness, and since acute as well as chronic inflammation can affect airway hyperresponsiveness (Motomura et al. 2009), we chose to assess airway inflammation via exhaled nitric oxide. Exhaled nitric oxide has been utilized as a marker of airway inflammation previously, but its use has been primarily in asthmatics (Kharitonov et al. 1994; Rodway et al. 2009). In our study, due to the fact that our subjects were non-asthmatic, no subjects had significant levels of airway inflammation evidenced as exhaled nitric oxide. Additionally, no differences were found in exhaled nitric oxide following training. These discordant inflammation and airway narrowing values lend support to previous findings that in non-asthmatic individuals, airway inflammation and airway hyperresponsiveness are only loosely related to one another (Brusasco et al. 1998; Gavreau et al. 2000; Jarjour & Calhoun 1992). It would be useful in future studies to include other markers of inflammation in order to determine whether changes in airway responsiveness were occurring in concert with systemic changes in inflammation.

In our study, we utilized two methods of airway perturbation (exercise and EVH) in order to make certain that subjects who may have responded to only one method and not to the other would not be misclassified. Previous literature has indicated that it is common for subjects to have inconsistent results for airway hyperresponsiveness depending on the method of perturbation (Holzer & Douglass 2006). The results of the EVH perturbation were much less consistent than the exercise condition for pulmonary

function measures other than FEV<sub>1</sub>, perhaps indicating inadequate airway irritation. However, our subjects experienced airway narrowing under both perturbation conditions, and also post-training improvements under both conditions, indicating that the response to airway stretch is positive for multiple methods of perturbation, and not just exercise.

Interestingly, our study primarily attracted parents and guardians of girls. We are unsure why girls were such a large majority of our sample, but girls are less likely to meet PA guidelines as compared to boys (Sallis et al. 2000). It is uncertain whether a subject population with a larger sample of boys would indicate sex differences in post-training improvement in pulmonary function.

### ***Public Health Implications***

The negative impact of insufficient activity at higher intensities may be of particular importance to young girls, as physical activity literature indicates that girls tend to be less active than boys and perhaps more importantly, less active at moderate and vigorous intensities (Ridgers et al. 2006; Sallis et al. 2000; Trost et al. 2008). Although asthma prevalence rates are higher in pre-adolescent boys than girls, in adulthood, females have a higher prevalence of asthma (National Center for Health Statistics 2001, 2003). This trend may be due in part to females becoming less active and engaging in inadequate MVPA. Our data suggests that in pre-adolescent girls, the negative airway health outcomes of physical inactivity can be reversed, potentially reducing risk for future development of asthma and asthma-like symptoms.

### ***Future Directions***

There are several compelling questions that should be answered based on the current study. Further investigations should track children through adolescence to

determine whether physical activity levels, specifically at higher intensities provide the same benefit as in pre-adolescence. In light of adult research indicating that moderate-level physical training provides beneficial anti-inflammatory effects, but strenuous training may worsen inflammation (Pastva et al. 2004), less intense exercise protocols should be used in future studies to determine the most effective treatment and prevention protocols for children. As mentioned previously, similar studies should investigate potential sex differences. Further studies should also examine such training protocols for treatment use in children with mild and stable asthma.

### ***Conclusions***

In conclusion, these research findings provide information regarding the importance of lifestyle factors including physical activity and body composition for children at risk for development of asthma. These results suggest that physical inactivity negatively impacts airway health in non-asthmatic prepubescent children, which can be improved with high-intensity training. However, increased body fat and low aerobic capacity may diminish these improvements. There are many factors contributing to the rapid increase in asthma and asthma-like symptoms in children over the past few decades, but early intervention in harmful lifestyle factors is one potential way to reverse the trend of increasing asthma prevalence rates in children.



## Tables

**Table 4.1 Subject Characteristics**

	Pre		Post	
	Control Group (n=8)	Training Group (n=8)	Control Group (n=8)	Training Group (n=8)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Age (yr)	9.6 ± 1.4	8.6 ± 0.6	-----	-----
Weight (kg)	35.8 ± 5.5	32.7 ± 11.6	37.0±6.2	32.0±10.4
Height (cm)	139.9 ± 7.0	131.3 ± 12.3	141.4±7.0	131.9±12.6
Body Fat (%)	22.6 ± 5.5	22.2 ± 12.0	21.6±6.3	20.0±11.5
Waist Circumference (cm)	61.0 ± 5.4	61.6 ± 12.6	62.3±5.7	60.6±11.3
BMI (kg/m <sup>2</sup> )	18.3 ± 2.2	19.5 ± 5.3	18.3±2.3	18.1±4.3
Systolic Blood Pressure (mmHG)	108±3	106±5	105.5±3.7	103.8±2.5
Diastolic Blood Pressure (mmHG)	73±4	70±5	71.3±4.9	67.5±4.2
Total Cholesterol (mg/dL)	166.4 ± 19.9	171.9 ± 26.0	168.8±20.2	149.9±20.5*†
HDL (mg/dL)	45.3 ± 14.2	46.0 ± 10.6	48.9±14.9	55.9±18.6
LDL (mg/dL)	103.5 ± 18.8	97.5 ± 18.8	97.9±22.0	62.7±20.2*†
Triglycerides (mg/dL)	120.5 ± 40.7	137.5 ± 13.28	117.0±32.5	114.0±54.5
Fasting blood glucose (mg/dL)	94.0 ± 8.2	89.1 ± 8.0	96.4±8.5	94.3±16.9

\*Significantly different between pre and post-test within each group (p<0.05)

†Significantly different from control group (p<0.05)

**Table 4.2 Resting Pulmonary Function**

	Pre				Post			
	Control (n=8)		Training (n=8)		Control (n=8)		Training (n=8)	
	mean $\pm$ SD	Predicted (%)	mean $\pm$ SD	Predicted (%)	mean $\pm$ SD	Predicted	mean $\pm$ SD	Predicted
TLC (L)	2.3 $\pm$ 0.5	99 $\pm$ 1	1.9 $\pm$ 0.6	97 $\pm$ 2	2.3 $\pm$ 0.5	99 $\pm$ 1	1.9 $\pm$ 0.6	97 $\pm$ 2
FVC (L/sec)	2.2 $\pm$ 0.4	100 $\pm$ 1	1.9 $\pm$ 0.4	98 $\pm$ 2	2.3 $\pm$ 0.3	100 $\pm$ 1	1.9 $\pm$ 0.5	98 $\pm$ 1
RV (L)	0.3 $\pm$ 0.2	100 $\pm$ 1	0.2 $\pm$ 0.2	100 $\pm$ 1	0.3 $\pm$ 0.2	100 $\pm$ 1	0.2 $\pm$ 0.1	100 $\pm$ 1
PEF (L/sec)	3.2 $\pm$ 0.7	-----	3.4 $\pm$ 0.8	-----	3.7 $\pm$ 1.5	-----	2.7 $\pm$ 0.8	-----
FEV <sub>1</sub> (L/sec)	1.8 $\pm$ 0.3	100 $\pm$ 1	1.5 $\pm$ 0.3	97 $\pm$ 2	1.8 $\pm$ 0.3	100 $\pm$ 1	1.4 $\pm$ 0.3	97 $\pm$ 2
FEV <sub>1</sub> /FVC (%)	81.0 $\pm$ 10.3	-----	84.1 $\pm$ 4.9	-----	79.5 $\pm$ 10.0	-----	76.9 $\pm$ 9.2	-----
FEF <sub>25-75%</sub> (L/sec)	1.8 $\pm$ 0.6	-----	1.7 $\pm$ 0.3	-----	2.0 $\pm$ 1.0	-----	1.3 $\pm$ 0.4	-----
eNO (ppb)	17.1 $\pm$ 9.1	-----	15.3 $\pm$ 12.6	-----	15.8 $\pm$ 7.1	-----	14.7 $\pm$ 11.8	-----
R <sub>5</sub> (KPa/l/sec)	0.8 $\pm$ 0.2	87 $\pm$ 23	0.8 $\pm$ 0.3	84 $\pm$ 30	0.7 $\pm$ 0.2	75 $\pm$ 23	0.8 $\pm$ 0.3	84 $\pm$ 34

Predicted values from Knudson et al. (1983)

TLC, total lung capacity; FVC, forced vital capacity; RV, residual volume; PEF, peak expiratory flow; FEV<sub>1</sub>, forced expiratory volume in 1 sec; FEF<sub>25-75%</sub>, forced expiratory flow between 25-75%; eNO, exhaled nitric oxide; R<sub>5</sub>, airway resistance at 5 Hz. No differences between groups (p > 0.05)

**Table 4.3 % Change Pulmonary Function Pre to Post-Training**

	Pre				Post			
	Control (n=8)		Training (n=8)		Control (n=8)		Training (n=8)	
	Mean ± SD	% change exercise	Mean ± SD	% change exercise	Mean ± SD	% change exercise	Mean ± SD	% change exercise
PEF (L/sec)	3.2 ± 0.7	5.61 ± 21.68	3.4 ± 0.8	-14.26 ± 2.00†	3.7 ± 1.5	-5.13 ± 11.53	2.7 ± 0.8	-2.94 ± 1.40*†
FVC (L/sec)	2.2 ± 0.4	2.39 ± 6.30	1.9 ± 0.4	-1.02 ± 5.56	2.3 ± 0.3	0.79 ± 5.03	1.9 ± 0.5	-4.41 ± 1.35
FEV <sub>1</sub> (L/sec)	1.8 ± 0.3	-3.01 ± 1.72	1.5 ± 0.3	-7.60 ± 2.10†	1.8 ± 0.3	-4.22 ± 1.86	1.4 ± 0.3	-1.13 ± 1.81*†
FEV <sub>1</sub> /FVC (%)	81.0 ± 10.3	-3.37 ± 8.60	84.1 ± 4.9	-5.90 ± 3.68	79.5 ± 10.0	-6.63 ± 2.33	76.9 ± 9.2	-4.17 ± 8.85
FEF <sub>25-75%</sub> (L/sec)	1.8 ± 0.6	-6.60 ± 5.90	1.7 ± 0.3	-16.10 ± 2.10†	2.0 ± 1.0	-4.50 ± 4.70	1.3 ± 0.4	-6.80 ± 1.80*†

\*Significantly different between pre and post-test within each group (p<0.05)

†Significantly different from control group (p<0.05)

**Table 4.4  $\dot{V}O_{2\max}$  Pre-test and Post-test**

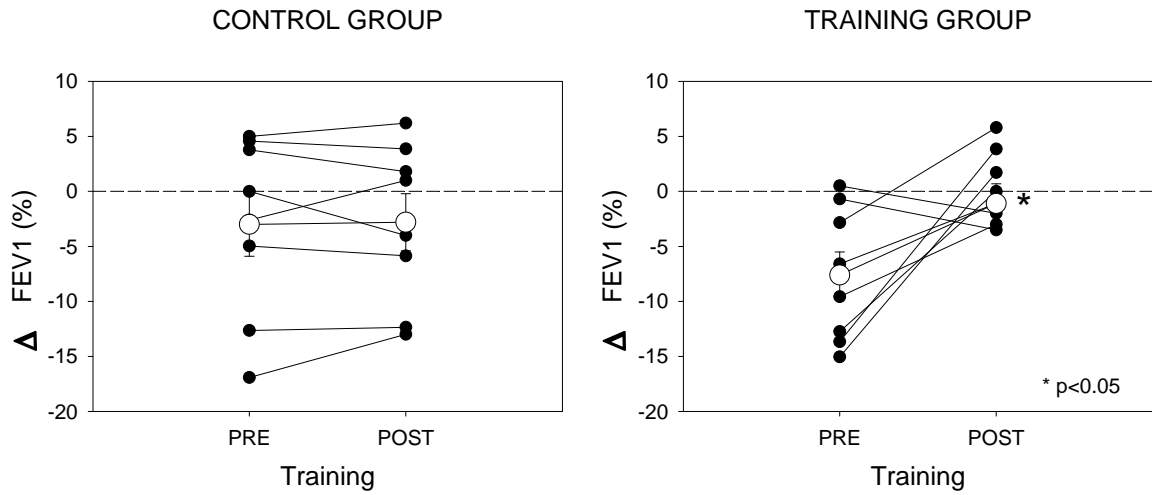
	Pre				Post			
	Control Group (n=8)		Training Group (n=8)		Control Group (n=8)		Training Group (n=8)	
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
<b><math>\dot{V}O_2</math> (L/min)</b>	1.20 $\pm$ 0.34	0.64 – 1.83	0.80 $\pm$ 0.27†	0.31 – 1.25	1.12 $\pm$ 0.32	0.66 – 1.65	0.92 $\pm$ 0.31†*	0.47 – 1.38
<b><math>\dot{V}O_2</math> (ml/kg/min)</b>	33.1 $\pm$ 6.2	20.9 – 40.9	23.6 $\pm$ 7.7†	14.2 – 34.4	30.4 $\pm$ 7.5	21.5 – 46.8	29.4 $\pm$ 8.2†*	15.6 – 36.7
<b><math>\dot{V}CO_2</math> (L/min)</b>	1.29 $\pm$ 0.36	0.68 – 1.91	0.84 $\pm$ 0.29†	0.32 – 1.33	0.99 $\pm$ 0.34	0.70 – 1.78	1.10 $\pm$ 0.32†	0.52 – 1.52
<b>VE (L/min)</b>	55.1 $\pm$ 13.6	36.0 – 78.6	40.0 $\pm$ 10.2†	19.3 – 52.3	47.2 $\pm$ 11.0	35.9 – 65.7	48.2 $\pm$ 13.3 †	30.6 – 66.9
<b>VE/<math>\dot{V}O_2</math></b>	47.1 $\pm$ 6.8	39 - 60	52.8 $\pm$ 10.8	42 – 73	43.8 $\pm$ 6.6	36 – 55	54.1 $\pm$ 10.8*	42 – 70
<b>VE/<math>\dot{V}CO_2</math></b>	44.0 $\pm$ 6.0	37 – 55	50.4 $\pm$ 9.6	40 - 66	44.5 $\pm$ 10.0	35 - 64	51.3 $\pm$ 8.5	42 – 66
<b>RER</b>	1.08 $\pm$ 0.02	1.05 – 1.12	1.06 $\pm$ 0.06	0.97 – 1.13	1.00 $\pm$ 0.13	0.98 – 1.08	1.09 $\pm$ 0.06	1.03 – 1.21
<b>HR (bpm)</b>	190.4 $\pm$ 13.7	165 - 205	176.1 $\pm$ 14.9	147-192	188.8 $\pm$ 10.3	172 - 199	184.5 $\pm$ 10.6	169 – 196
<b>SpO<sub>2</sub> (%)</b>	98 $\pm$ 1	97 – 99	98 $\pm$ 1	96 - 100	-----	-----	-----	-----
<b>Workload (watts)</b>	102.5 $\pm$ 26.6	60 – 150	66.7 $\pm$ 20.0†	40 - 110	95.0 $\pm$ 22.0	70 - 130	76.3 $\pm$ 24.5†	40 - 120

\*Significantly different between pre and post-test within each group (p<0.05)

†Significantly different from control group (p<0.05)

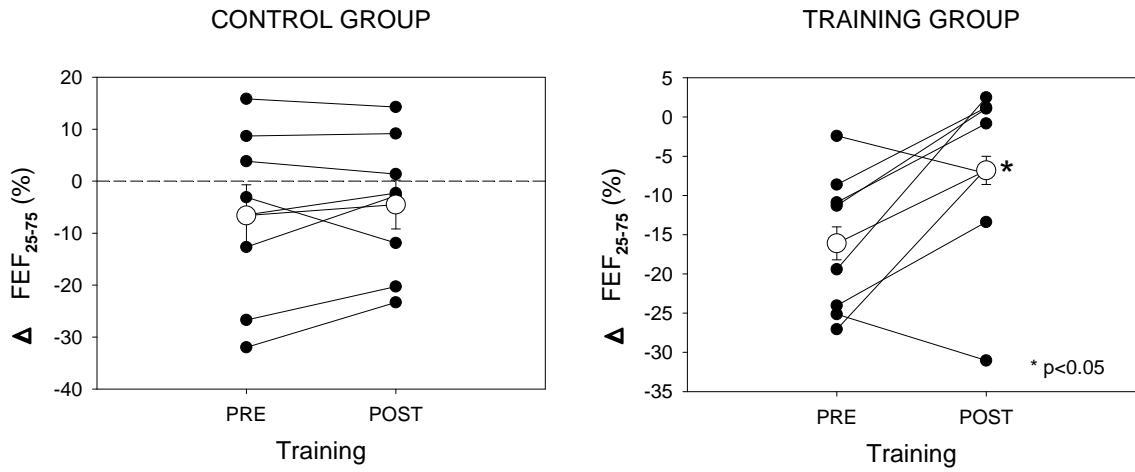
## Figures

**Figure 4.1  $\Delta$  FEV<sub>1</sub>% Pre-Post Training**



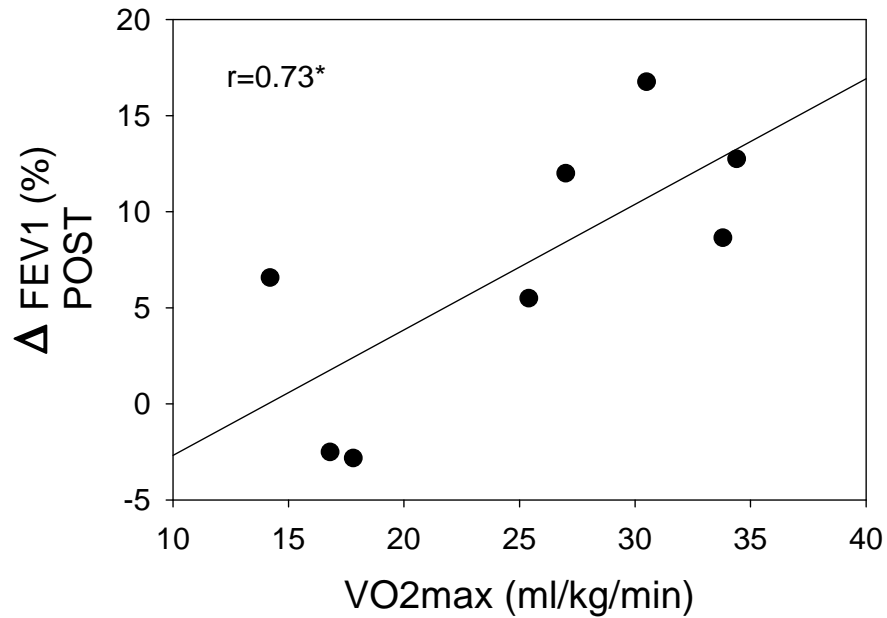
*Effect of training on post-exercise airway narrowing.* Filled circles are individual data and open circles are mean  $\pm$  SD. Training led to significant improvement in  $\Delta$ FEV<sub>1</sub>, with six of eight subjects in the TrG showing improvement in  $\Delta$ FEV<sub>1</sub> following training.

**Figure 4.2  $\Delta$  FEF<sub>25-75</sub>% Pre-Post Training**



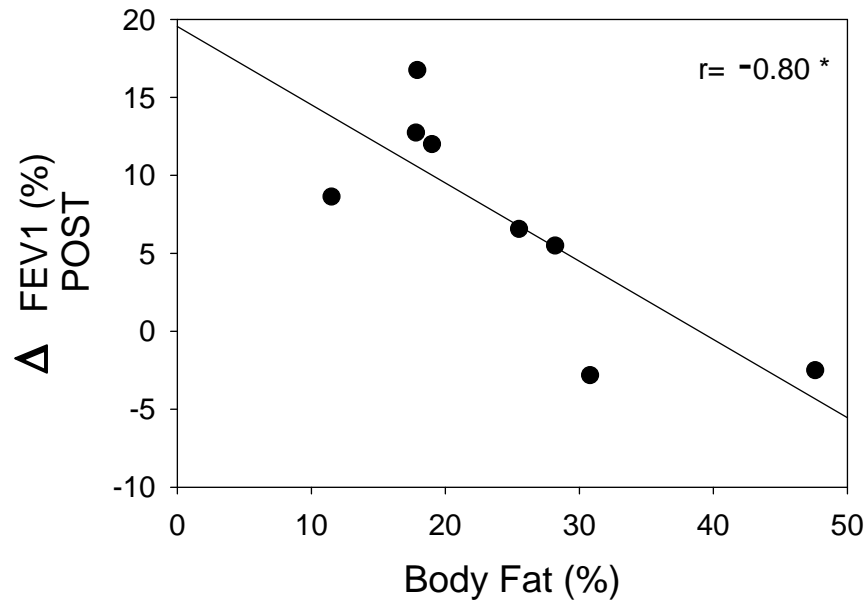
*Effect of training on FEF<sub>25-75</sub>.* Filled circles are individual data and open circles are mean  $\pm$  SD. Training led to significant improvement in  $\Delta$ FEF<sub>25-75</sub>, with six of eight subjects in the TrG showing improvement in  $\Delta$ FEF<sub>25-75</sub> following training.

**Figure 4.3  $VO_{2max}$  vs.  $\Delta FEV_1$ % Change Pre to Post-Training**



*Relationship between  $\Delta FEV_1$  following training and  $VO_{2max}$  at baseline.* There was a significant relationship ( $r=0.73$ ,  $p<0.05$ ) between  $VO_{2max}$  at baseline and post-exercise  $\Delta FEV_1$  following training. Subjects with the lowest  $VO_{2max}$  levels at baseline showed the least improvement in post-exercise airway narrowing ( $\Delta FEV_1$ ).

**Figure 4.4 Body Fat % vs.  $\Delta$  FEV<sub>1</sub> % Change Pre to Post-Training**



*Relationship between body fat % at baseline and  $\Delta$ FEV<sub>1</sub> following training.* There was a significant inverse relationship ( $r=-0.80$ ,  $p<0.05$ ) between body fat % at baseline and post-exercise  $\Delta$ FEV<sub>1</sub> following training. Subjects with the highest body fat % at baseline showed the least improvement in post-exercise airway narrowing ( $\Delta$ FEV<sub>1</sub>).



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## CHAPTER 5 - Conclusions

When considering the overall implications of this dissertation, it can be concluded that lifestyle factors such as diet, physical activity, and adiposity, have a significant impact on airway health in children and young adults. Specifically, we found that a high-fat meal was associated with increased airway inflammation (eNO), and inactivity was associated with increased airway hyperresponsiveness. Since airway inflammation and hyperresponsiveness are the key components of asthma, these associations suggest that left unaltered, unhealthy lifestyles can contribute to negative airway health consequences.

In the first study (Chapter 2) we observed a significant increase in airway inflammation (eNO) following a high-fat meal in healthy college aged subjects. The magnitude of the increase was related to the baseline levels of triglycerides in these subjects, indicating that people who have elevated triglycerides are more likely to experience increases in airway inflammation following the consumption of a high-fat meal. These findings suggest that a high-fat meal, and potentially a high-fat diet may be detrimental to airway health even in apparently healthy young adults.

In our second study (Chapter 3) we changed our focus from diet to physical activity and from young adults to children aged 8-10 years. Utilizing an incremental cycle-ergometer test to exhaustion, we found that increased body fat and low physical activity were associated with larger post-exercise decreases in FEV<sub>1</sub> as compared to leaner, more active children. Additionally, children who had higher-body-fat and were physically inactive, experienced clinically significant levels of post-exercise airway narrowing. This finding indicates that higher-body-fat children, who are not meeting

physical activity guidelines, are potentially at risk for asthma-like symptoms and for developing asthma in the future if their lifestyles are not altered.

Finally, in Chapter 4, building from our previous cross-sectional observations, we utilized a randomized controlled study design to observe the impact of 8-weeks of high-intensity physical training on airway health in children. Since the previous cross-sectional study indicated that children who were higher-body-fat and had lower  $\dot{V}O_{2\max}$  values had significantly more airway narrowing post-exercise as compared to their lower-body-fat, higher  $\dot{V}O_{2\max}$  peers, we wondered whether this airway narrowing could be alleviated through physical training. In contrast to previous research in the same age range, our population included only children who were not meeting current physical activity guidelines. These subjects had low  $\dot{V}O_{2\max}$  values, and we speculated that they were not obtaining adequate airway smooth muscle stretch due to low levels of high-intensity physical activity. Results indicated that airway narrowing, both post-exercise and post-eucapnic voluntary hyperventilation, could be ameliorated with a high-intensity training program. This is an important finding because airway hyperresponsiveness, increases the risk for future development of asthma. Additionally, other health outcomes including:  $\dot{V}O_{2\max}$ , post-exercise PEF and FEF<sub>25-75</sub>, total cholesterol, and LDL cholesterol improved with training. These findings suggest that inadequate physical activity negatively impacts airway health in non-asthmatic prepubescent children, which can be improved with high-intensity training. Elevated body fat, and low  $\dot{V}O_{2\max}$  levels, however, may diminish these improvements.

In conclusion, this series of studies furthers our knowledge regarding the importance of diet, physical activity, and adiposity for airway health outcomes. These

research findings may provide important information for development of policies, practices, for children at risk for development of asthma. Early intervention for children engaging in harmful lifestyle could help to stem the tide of the increasing prevalence of childhood asthma and asthma-like symptoms.