

CHANGES IN EXPIRATORY FLOW LIMITATION DURING EXERCISE FROM PRE- TO  
POST-PUBERTY

by

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## Abstract

Expiratory flow limitation (EFL) during exercise can limit exercise tolerance. We have recently reported a high prevalence of EFL independent of sex in prepubescent children (Swain et al. 2010) that greatly exceeds that reported in adults. It is unknown how maturation and growth from pre- to post-puberty affects pulmonary function, specifically EFL, during exercise. The purpose of this longitudinal study was to investigate the changes in cardiopulmonary function from pre- to post-puberty in boys and girls. We hypothesized that EFL prevalence would decrease from pre- to post-puberty (with boys exhibiting a greater decrease than girls) and that the decrement could be explained by an increase in pulmonary function and a decrease in  $V_E/V_{CO_2}$ . Twenty-one children (ages 12-16 yrs; 11 boys, 10 girls) were recruited from 40 prepubescent children who completed testing in our laboratory ~5 years ago. Subjects completed pulmonary function tests before and after an incremental exercise test to exhaustion ( $VO_{2max}$ ) on a cycle ergometer. EFL was determined using the percent tidal volume ( $V_T$ ) overlap method. Nineteen of the 21 subjects (10 boys, 9 girls; 90%) exhibited EFL pre-puberty, while only 7 of the 21 subjects (5 boys, 2 girls; 33%) exhibited EFL post-puberty. Of the subjects who experienced EFL post-puberty, all had experienced EFL pre-puberty. Boys had a significantly greater vital capacity (VC) than girls both pre- (~15%) and post-puberty (B:  $4.73 \pm 0.53$ ; G:  $3.80 \pm 0.29$  L). Maximal aerobic capacity ( $VO_{2max}$ ) significantly increased (~110% in girls and ~120% in boys) from pre- to post-puberty and was greater ( $p < 0.05$ ) in boys post-puberty (B:  $2.76 \pm 0.43$ ; G:  $1.94 \pm 0.35$  L/min).  $V_E/V_{CO_2}$  also significantly decreased (~13%) in both boys and girls. Post-puberty subjects regulated

tidal breathing at higher lung volumes (greater ERV/FVC and lower IRV/FVC) during exercise compared to pre-puberty. None of the subjects experienced significant arterial desaturation pre-puberty or post-puberty. Our findings suggest that the prevalence of EFL declines as children mature from pre- to post-puberty, likely due to increases in lung size, decreases in  $V_E/V_{CO_2}$ , and/or changes in breathing mechanics that are greater than increases in maximal ventilation that occur with increased pulmonary gas exchange.

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## **Dedication**

I would like to dedicate this paper to my parents, Bob and Keri Emerson. Thank you for making my education possible and always providing encouragement and support.

# Chapter 1 - Literature Review

## Introduction

Exercise tolerance is not typically limited by the pulmonary system in normal, healthy adult men (Dempsey, 1986). This is reflected by maintenance of arterial oxygen partial pressure ( $PO_2$ ) at near resting levels even during maximal exercise (Dempsey et al. 1984). However, pulmonary limitations to exercise tolerance can occur, typically manifested as gas exchange impairment, fatigue of the respiratory muscles, or ventilatory constraint. Expiratory flow limitation (EFL), a form of ventilatory constraint, occurs when tidal breathing ( $V_T$ ) approaches and intersects the maximal flow-volume loop (MFVL) boundary (Johnson et al. 1999). Through a concomitant dynamic hyperinflation of the lungs, EFL presents a noteworthy limitation to exercise tolerance (Babb et al. 1991).

With regard to healthy adults, there are structural and morphological differences in the pulmonary system of women that may result in increased pulmonary limitations during exercise compared to men (McClaran et al. 1998; Harms et al. 1998). Hallmark sex differences in pulmonary function include smaller lungs and airways and lower maximal flow rates in women compared to men (Thurlbeck, 1982; Schwartz et al. 1988; McClaran et al. 1998). It has been suggested that these differences result in greater ventilatory constraint, specifically expiratory flow limitation, during exercise in women compared to men (McClaran et al. 1998; Guenette et al. 2007).

It has recently been reported that the prevalence of ventilatory constraint, i.e. EFL, in prepubescent children is very high (>90%), independent of sex or aerobic

capacity (Swain et al. 2010). However, there are sex differences in children with regard to the pulmonary system. Boys have larger lungs and more alveoli compared to height-matched girls (Thurlbeck 1982). These factors contribute to significantly greater lung volumes and expiratory flow rates (e.g. FVC, FEV<sub>1</sub>, FEF<sub>25-75%</sub>) in boys compared to girls (Wang et al. 1993). However, boys exhibit greater maximal aerobic capacity (Krahenbuhl et al. 1985) and exercise ventilation (Rutenfranz et al. 1981) compared to girls, representing an enhanced ventilatory demand in boys during exercise. Thus, most boys and girls demonstrate EFL during heavy exercise, although from different mechanisms (Swain et al. 2010).

The prevalence of ventilatory constraint (i.e. EFL) in prepubescent children is much greater than that reported in normal, healthy adults (Johnson et al. 1999). This difference in prevalence of EFL between children and adults could be due to multiple structural and physiological changes that occur from pre- to post-puberty. Notably, significant changes in height, weight, body composition, lung size, expiratory flow rates, and aerobic capacity are all known to occur from pre- to post-puberty (Rogol et al. 2002; Wang et al. 1993; Sprynarova et al. 1997). However, the specific changes that occur during puberty that precipitate a decrease in the prevalence of EFL are currently unknown. This review will initially focus on ventilatory constraint during exercise and its manifestation in adults and children, followed by a discussion on pulmonary function, body composition, and gas exchange variables that change from pre- to post-puberty that may help explain alterations in ventilatory constraint.

## **Pulmonary Limitations During Exercise**

### ***Expiratory Flow Limitation***

The pulmonary system generally does not limit exercise tolerance in normal, healthy individuals at sea level (Dempsey et al. 1986). However, there are some conditions, such as expiratory flow limitation (EFL), in which the pulmonary system has been shown to limit exercise tolerance. Expiratory flow limitation is defined as the percent of a tidal flow-volume loop that intersects with the maximal flow-volume loop (MFVL). The regulation of lung volumes (i.e. end-inspiratory lung volume [EILV] and end-expiratory lung volume [EELV]) also affects the amount of ventilatory constraint experienced during exercise. Exercise leads to an increase in minute ventilation ( $V_E$ ) and tidal volume ( $V_T$ ), which is accomplished via an increase in EILV and a decrease in EELV (Henke et al. 1988). As EELV decreases, expiratory reserve volume (ERV) is reduced. Conversely, EELV typically begins to rise with the occurrence or impending occurrence of EFL (Babb et al. 1991) and, as a result, EILV approaches total lung capacity (TLC; McClaran et al. 1999). When EELV surpasses resting values or resting functional residual capacity (FRC), it is termed dynamic hyperinflation (Johnson et al. 1999). Dynamic hyperinflation can limit exercise tolerance in healthy individuals via decreased inspiratory muscle length, increased work of breathing and oxygen cost of breathing, and ultimately a decreased inspiratory muscle endurance (Tzelepis et al. 1988). Further, it has been shown that with increased exercise intensity and duration, ventilatory constraints worsen (Johnson et al. 1991; Johnson et al. 1995; Aaron et al. 1992).

While the prevalence of EFL has not been established, it commonly occurs in highly trained young men (Johnson et al. 1992), older endurance-trained individuals

(Johnson et al. 1991), women (McClaran et al. 1998), and children (Swain et al. 2010). Endurance-trained young men are likely to develop EFL due to the substantial ventilatory ( $V_E$ ) requirement necessary to sustain extraordinary rates of pulmonary gas exchange (high oxygen uptake [ $VO_2$ ] and  $CO_2$  production [ $VCO_2$ ]) during vigorous exercise (Johnson et al. 1992). In older endurance-trained individuals, EFL is precipitated by normal age-related declines in lung function (e.g. reduced maximal flow rates and vital capacity) met by a relatively high aerobic capacity ( $VO_{2max} > 40$  ml/kg/min; Johnson et al. 1991). The difference in prevalence of EFL in women versus men can be explained by the disadvantageous morphological differences that exist between the pulmonary systems of men and women. Specifically, women have smaller lungs and airways compared to men (Mead 1980), resulting in lower maximal flow rates and smaller maximal flow-volume loops; ultimately, these can lead to a larger prevalence of ventilatory constraint during exercise in women compared to men (McClaran et al. 1998; Dominelli et al. 2011).

Few studies have investigated EFL in healthy children (Nourry et al. 2005; Nourry et al. 2006; Swain et al. 2010). Recently, Swain et al. (2010) reported a high prevalence (~93%) of EFL in 40 prepubescent children (20 boys; 20 girls). There were no sex difference in EFL prevalence between boys (EFL: 19/20 subjects) and girls (EFL: 18/20 subjects). However, there were differences in the factors that led to EFL. Boys had a larger vital capacity (VC) and higher maximal mid-flow rates ( $FEF_{50\%}$ ) compared to girls. However, boys also had a greater maximal aerobic capacity ( $VO_{2max}$ ), maximal ventilation ( $V_{Emax}$ ), and maximal workload compared to girls. Therefore, while the capacity of the pulmonary system in boys was larger than the girls, boys nevertheless

experienced EFL due to a large ventilatory demand that exceeded their maximal expiratory flow rates. Conversely, girls had a lower  $VO_{2max}$  than boys, but also had a smaller pulmonary capacity, and consequently also experienced EFL. Therefore, despite the substantial sex-based variability present in children in regard to  $VO_{2max}$ ,  $V_{Emax}$ , VC, and  $FEF_{50\%}$ , a high prevalence of prepubescent children experience EFL during maximal exercise.

### ***Consequences of Expiratory Flow Limitation***

Expiratory flow limitation has several potential deleterious outcomes that can limit exercise tolerance, including dynamic hyperinflation, dyspnea, respiratory muscle fatigue, and gas exchange impairments (McClaran et al. 1999; Iandelli et al. 2002; Walls et al. 2002). Dynamic hyperinflation can limit exercise via placing a large elastic load on the inspiratory muscles. Dynamic hyperinflation, typically caused by EFL, can shorten the initial length of the inspiratory muscles, compromising their force-generation potential resulting in a mechanical disadvantage (McClaran et al. 1999). The shortening of inspiratory muscle length has a significant effect on the pressure generation requirement of the inspiratory muscles, requiring a larger pressure gradient for a given change in lung volumes during inspiration at high lung volumes compared to inspiration at resting or lower lung volumes. As a result, the elastic load placed on the inspiratory muscles is increased. On the other hand, the occurrence of EFL without a rise in EELV (dynamic hyperinflation) may lead to an increased breathing frequency, resulting in a large resistive load placed on the inspiratory muscles. Therefore, the occurrence of EFL during exercise increases the likelihood of adverse outcomes that can limit exercise



tolerance, including dyspnea, respiratory muscle fatigue, and exercise-induced arterial hypoxemia.

### ***Dyspnea***

Dyspnea and perceived exertion intensify with increasing whole body effort and duration of an activity, often limiting exercise tolerance in an individual via respiratory or locomotor discomfort (Killian, 2006). Babb et al. (2008) reported that dyspnea during cycling exercise is strongly associated with an increased oxygen cost of breathing in obese women. Additionally, Eltayara et al. (1996) found ventilatory constraint (i.e. EFL) to be the strongest predictor of dyspnea during exercise in patients with chronic obstructive pulmonary disease. With regards to healthy individuals, it has been reported that substantial dyspnea occurs as a consequence of externally imposed EFL in healthy adults during maximal exercise (Iandelli et al. 2002). The authors reported that the increased requirement for pressure generation accounted for the majority of the variance in dyspnea between control trials and trials with externally imposed EFL. While dyspnea at the end of the incremental exercise test to exhaustion was very high for both trials, exercise performance was limited at ~65%  $W_{max}$  in trials with externally imposed EFL. Therefore, the sense of discomfort associated with inspiratory muscle work during whole body exercise with EFL may limit exercise tolerance.

### ***Respiratory Muscle Fatigue***

During prolonged exercise at intensities greater than 80%  $VO_{2max}$ , the diaphragm frequently fatigues towards the end of exercise (Johnson et al. 1993). Diaphragmatic fatigue leads to a reduction in blood flow to the working skeletal muscle brought on by sympathetically mediated vasoconstriction (Sheel et al. 2002). Vasoconstriction of

skeletal muscle via sympathetic outflow allows a larger portion of the cardiac output to be redistributed to the respiratory muscles to accommodate the large oxygen cost of breathing (Harms et al. 1997). The attenuation of locomotor muscle blood flow and oxygen uptake via “stealing” of the blood by the respiratory muscles has been shown to compromise exercise tolerance (Harms et al. 1997; Harms et al. 1998). Expiratory flow limitation, commonly accompanied by dynamic hyperinflation, presents an increased demand for pressure generation from the respiratory muscles, and thus a higher oxygen cost of breathing (Johnson et al. 1999). Therefore, the presence of EFL during exercise at high intensities may exacerbate fatigue of the respiratory muscles and further compromise exercise tolerance.

### ***Exercise-Induced Arterial Hypoxemia and Gas Exchange***

Exercise-induced arterial hypoxemia (EIAH) is characterized by a substantial decrement in arterial oxygen saturation (Dempsey and Wagner, 1999) and, like EFL, is a scenario in which exercise tolerance can be limited by the pulmonary system (Harms et al. 2000). Walls et al. (2002) reported a strong correlation ( $r=0.71$ ) between the extent of flow limitation and EIAH severity in healthy young women. Specifically, McClaran et al. (1998) reported that EFL is strongly associated with the degree of widening of the alveolar-arterial oxygen difference in a group of highly-fit women presenting with a high prevalence of EFL. However, the authors point out that even if the ventilatory constraint were removed, this still may have not been sufficient to counteract the excessively widened alveolar-arterial oxygen difference and would not have prevented EIAH. As EIAH has multiple etiologies (Dempsey and Wagner 1999), it is likely in highly-fit

subjects that EFL contributes to EIAH. So, although the exact mechanisms remain unclear, there appears to be a link between EFL and EIAH.

## **Sex Differences During Exercise in Adults**

### ***Physiological Bases for Pulmonary Function Differences***

There are sex differences with regard to pulmonary function during exercise, which primarily stem from differences in hormones and morphology/structure between men and women. Changes in levels of progesterone and estrogen in women dependent on menstrual cycle phase can impact ventilation during exercise (Moore et al. 1987). Specifically, the presence of circulating progesterone stimulates ventilation (Moore et al. 1987), increases hypoxic ventilatory response (HVR), and increases hypercapnic ventilatory response (HCVR; Moore et al. 1987). During exercise, progesterone can increase central ventilatory drive, potentially resulting in an enhanced drive to breathe in women. Sex differences in ventilation during exercise due to hormonal influences could potentially contribute to the higher prevalence of EFL in women. However, the exact role of hormones in regard to sex differences in pulmonary function during exercise is not fully understood.

Differences in body composition between men and women may also affect pulmonary function during exercise. Adult men have less fat mass and more lean mass than adult women of the same height (Wells, 2007). There are also sex differences in location of tissue storage, as men store less fat in the limbs and exhibit higher central adiposity compared to women (Wells, 2007). However, there are adverse outcomes of

weight gain and obesity on lung function independent of sex. Thyagarajan et al. (2008) conducted a longitudinal study investigating age-related changes in lung function relative to body mass index (BMI) in a cohort of 5,115 black and white men and women. They reported that lean subjects at baseline exhibited a significant increase in FVC and FEV<sub>1</sub> over the duration of the study, while overweight (BMI > 26.4) subjects showed a decrease in FVC and FEV<sub>1</sub> throughout the 10-year study. Further, subjects that exhibited the greatest weight gain over the 10 years showed the largest decreases in FVC. Therefore, there appears to be an influence of body composition on pulmonary function, which could play a role in the manifestation of EFL.

In addition to differences in body composition and hormones, sexual dimorphism exists with regards to pulmonary structure and functional capacity in adults. It has been reported that there is a substantial ventilatory reserve in normal, healthy young men even during maximal exercise (Dempsey, 1986). However, morphological differences between adult men and women suggest that a similar reserve for increases in ventilation may not exist in women. Men have larger lungs than women, even when matched for height (Thurlbeck, 1982). Larger lung size results in a greater number of total alveoli and consequently larger diffusion surfaces in men compared to women (Thurlbeck, 1982). It has also been documented that adult men have larger airway diameter than height-matched adult women (Mead, 1980). Further, Green et al. (1974) suggested that variability in maximal flows between individuals of similar lung size can be partly explained by differences in airway size. This independent pairing between lung volume and airway size has been termed “dysanapsis” (Mead et al. 1980). Recently, dysanapsis has been suggested to be a strong predictor of EFL (Dominelli et al. 2011). Specifically,

individuals with smaller airways (measured via mid-maximal expiratory flows; i.e. FEF<sub>50%</sub>) relative to lung size may be more likely to experience EFL at maximal exercise, independent of aerobic capacity (Dominelli et al. 2011). Given these structural differences in pulmonary function, it would be expected that women would be more likely to experience pulmonary limitations during exercise compared to men.

### ***Pulmonary Response to Exercise***

Due to the previously described sexual dimorphism with regard to pulmonary structure, there are important sex differences in pulmonary response to exercise. First, due to smaller lung size and similar maximal tidal volume to vital capacity ratio ( $V_{Tmax}/VC$ ), women experience a plateau in  $V_{Tmax}$  at a relatively smaller VC compared to men (McClaran et al. 1998). Second, due to smaller airways and lung volumes, women typically exhibit smaller maximal flow-volume loops compared to men, which can lead to increased prevalence of ventilatory constraint in women (McClaran et al. 1998).

In healthy untrained men, pulmonary function does not typically limit exercise tolerance, as there is substantial reserve for increases in ventilation during maximal exercise. However, due to smaller lungs, airways, and flow rates exhibited in women, it has been hypothesized that women are more likely to experience ventilatory constraint during exercise by encroaching on their ventilatory reserve earlier and/or to a greater extent than men. This question was examined by McClaran et al. (1998), who tested 29 healthy young women, with varying levels of aerobic capacity ( $VO_{2max}$ ), on a progressive treadmill running test. They found that endurance-trained subjects ( $VO_{2max} > 57$  ml/kg/min) exhibited significantly greater maximal ventilation ( $V_E$ ), tidal volume ( $V_T$ ), and breathing frequency compared to less-fit subjects ( $VO_{2max} < 56$  ml/kg/min). It was

also reported that more highly-fit subjects experienced expiratory flow limitation (EFL) compared to less-fit subjects. Specifically, 12 of 14 highly-fit subjects experienced EFL, while only 4 of 15 less-fit subjects showed EFL during maximal exercise. The findings were later supported by Guenette et al. (2007) who measured EFL in endurance-trained men (n=8) and women (n=10) during maximal exercise by applying a negative expiratory pressure at the mouth. Nine of ten women exhibited EFL during maximal exercise, while only three men experienced EFL. Women also regulated tidal breathing at higher lung volumes during maximal exercise compared to men, most likely as a compensatory “strategy” to avoid EFL. Finally, the authors reported that women displayed a greater work of breathing at a given absolute ventilation. Therefore, these studies suggest that women, especially endurance-trained women, are more likely to encroach upon their ventilatory reserve and experience mechanical constraint during maximal exercise due to smaller lungs and flow rates compared to age- and height-matched men.

### **Pulmonary Response during Exercise in Prepubescent Children**

There are pulmonary structure differences that exist between children and adults. Kivastik and Kingisepp (1997) reported that increases in lung function measures coincide with growth patterns of thoracic dimensions. Therefore, differences in lung function can partly be explained by differences in thoracic dimensions in children normalized for height, independent of sex. However, it has been reported that children also have smaller airways relative to lung size compared to adults (Mead, 1980). Further, with regards to the pulmonary response to exercise, children breathe “out of proportion” to metabolic demand and regulate tidal breathing at a lower  $PCO_2$  compared to adults (Cooper et al.

1987). These differences may play a role in the development of ventilatory constraint in children.

### ***Oxygen Uptake during Exercise***

Differences in oxygen uptake during exercise exist between children and adults. Turley et al. (1997) investigated the cardiovascular response during exercise in children and adults on both the treadmill and the cycle ergometer. Cardiac output at a given  $\text{VO}_2$  (L/min) was lower in children compared to adults during exercise on both modalities. However, the lower cardiac output was counteracted by a greater arterial-venous oxygen difference in children, resulting in the similar oxygen consumption.

In addition to the components of the Fick equation, Freedson et al. (1981) reported faster  $\text{VO}_2$  kinetics in children compared to adults. Armon et al. (1991) further clarified that this is only true during high-intensity exercise (above lactate threshold); during low-intensity exercise (below lactate threshold)  $\text{O}_2$  kinetics in children are not significantly different than adults when normalized for age and body size. Faster  $\text{O}_2$  kinetics during high-intensity exercise in children implies a smaller  $\text{O}_2$  deficit. This was supported by Fawkner et al. (2002) who compared 25 adults (ages 19-26 years) and 23 children (ages 11-12 years) and found that children had significantly faster time constants compared to adults. Overall, the faster time constants and  $\text{O}_2$  kinetics in children compared to adults suggest an enhanced capacity for aerobic metabolism in children.

### ***Ventilation during Exercise***

Ventilation at rest and during submaximal exercise is greater in prepubescent children compared to adults when normalized to body size. Rowland et al. (1997)

performed a longitudinal study measuring ventilation during submaximal exercise in 20 children (11 girls, 9 boys) between the ages of 9 and 13 years. It was found that tidal volume per kilogram of body mass did not change while breathing frequency progressively fell, resulting in a decline in submaximal ventilation per kilogram of body mass ( $V_E/\text{kg}$ ) with age. The fall in submaximal  $V_E/\text{kg}$  denotes an increase in ventilatory reserve with age, suggesting that children use a larger percentage of ventilatory reserve during submaximal exercise compared to adults.

Maximal ventilation ( $V_{E\text{max}}$ ) during exercise is determined by tidal volume and breathing frequency. Breathing frequency is higher during submaximal and maximal exercise in children compared to adults; conversely absolute tidal volume is lower in children compared to adults (Gratas-Delamarche et al. 1993, Mercier et al. 1991, Rowland et al. 1997).  $V_{E\text{max}}$  increases with age in proportion to increases in lean body mass (Mercier et al. 1991). In 1998, Nagano et al. reported that children regulated their alveolar  $\text{PCO}_2$  at lower levels than adults during exercise. Further, the slope of the relationship between ventilation and  $\text{CO}_2$  production ( $V_E/V\text{CO}_2$ ) decreased with age. This may be related to growth-related changes in tissue  $\text{CO}_2$  storage (Cooper et al. 1987). Since ventilation in children has been suggested to be “out of proportion” to end-tidal  $\text{PCO}_2$  and metabolic  $\text{CO}_2$  production, the efficiency of breathing in children during exercise appears to be less than that of adults. By having a high  $V_E/V\text{CO}_2$  it is suggested that children have a lower breathing efficiency compared to adults. In addition, it has been shown that chemosensitivity, measured via hypercapnic ventilatory response (HCVR) and hypoxic ventilatory response (HVR), decreases from pre- to post-puberty (Marcus et al. 1994) and it has been speculated that the responsiveness of the peripheral



chemoreceptors is higher in children compared to adults (Gozal et al. 1994). This increased ventilation could potentially contribute to ventilatory constraint. Children also differ from adults with regard to depth of ventilation, which is the amount of vital capacity (VC) used during tidal breathing ( $V_T$ ) and can be quantified as  $V_T/VC$ . Rutenfranz et al. (1981) reported that children have a significantly lower  $V_T/VC$  during exercise than adults. Therefore, children appear to respond to exercise with more shallow breathing and tachypnea relative to adults, which could lead to a greater work of breathing, increased dead space ventilation, and limited exercise tolerance in children.

### ***Ventilatory Limitation during Exercise in Children***

Due to differences in breathing regulation between children and adults, it could be expected that children will experience ventilatory constraint and consequently increased work of breathing during exercise. In 2006, Nourry et al. investigated expiratory flow limitation (EFL) in 18 healthy prepubescent children during maximal exercise. Ten of the eighteen children exhibited EFL at maximal exercise. Further, they found that there was a difference in lung volume regulation during exercise between the children that experienced EFL and those that did not. Specifically, children that were flow limited presented a significantly lower expiratory reserve volume relative to forced vital capacity (ERV/FVC) compared to the non-flow limited group, suggesting that children that experienced EFL during maximal exercise regulated breathing at lower lung volumes compared to children that did not experience EFL. This suggests that children that did not exhibit EFL operated at higher lung volumes during maximal exercise, avoiding EFL. Also, children that experienced flow limitation had significantly lower maximal minute ventilation ( $V_E$ ) and maximal oxygen uptake ( $VO_{2max}$ ) compared to children who did not

exhibit EFL. These findings suggest that children can present EFL even if they have a high  $VO_{2max}$ . There were no differences between EFL and non-EFL children in regards to vital capacity, BMI, body composition, age, or height.

Ventilatory constraint during exercise in children was further examined several years later by Swain et al. (2010). Forty healthy prepubescent children (20 boys, 20 girls) completed maximal exercise and pulmonary function testing in order to determine the prevalence and implications of EFL in children. Nineteen boys and eighteen girls exhibited flow limitation at maximal exercise, suggesting a very large prevalence of ventilatory constraint in children independent of sex. There was a significant relationship between severity of EFL and increase in end-expiratory lung volume (EELV) from rest to maximal exercise, suggesting that EFL does lead to dynamic hyperinflation in children.

### ***Sex Differences in Prepubescent Children***

In adults, sex differences during exercise are associated with body composition, hormones, maximal oxygen uptake ( $VO_{2max}$ ), and pulmonary function (Wells, 2007; McClaran et al. 1998). In children, sexual dimorphism does exist but is not greatly attributed to differences in hormone levels, as puberty onset accounts for a large amount of hormonal changes. Thus, differences in prepubescent boys and girls during exercise can be explained by differences in morphology, aerobic capacity, and pulmonary function.

### ***Structural Differences***

Lung function (e.g. FVC and expiratory mid-flows) cannot be easily predicted from age and anthropometric measures alone, as there is a complex relationship between age, morphology, and sex (Schwartz, 1988). However, several longitudinal studies have

demonstrated that lung function and growth tends to lag behind anthropometric growth (Degroodt et al. 1986; Borsboom et al. 1993). Therefore standing height may not be a strong predictor of lung development. Sitting height and thoracic dimensions are thus a more acceptable and accurate predictor of lung function during growth (Degroodt et al, 1986; Kivastik and Kingisepp, 1997). Kivastik and Kingisepp (1997) measured FVC, peak expiratory flow (PEF), and forced expiratory flow at 50% and 75% of FVC in 1187 schoolchildren (ages 6-18 years) to determine the relationships between standing height, thorax dimensions (chest width and depth and biacromial diameter) and lung function. While growth patterns were found to be similar, thoracic dimensions were typically greater in boys compared to girls of the same height. This suggests that differences in lung function at rest between boys and girls normalized for height may be explained in part by differences in thoracic size. This finding is also supported by the classical work of Thurlbeck in 1982, who reported larger lungs in boys compared to girls of the same height. Larger lung size results in a greater number of total alveoli in boys compared to girls. However, there are no sex differences in alveolar dimensions and number of alveoli per unit area and volume (Thurlbeck, 1982).

### ***Aerobic Capacity***

There is a sex difference with regard to aerobic capacity in prepubescent children (Krahenbuhl et al. 1985). Boys have significantly greater absolute  $VO_{2max}$  compared to girls. Also,  $VO_{2max}$  relative to body mass, lean body mass, and allometric scaling is greater in boys than girls (Denckner et al. 2007). However, some have suggested that the enhanced  $VO_2$  in prepubescent boys cannot be explained only by body composition; specifically, it has been proposed that there are different cardiovascular responses to

exercise in boys and girls (Rowland et al. 2000; Vinet et al. 2003). Rowland et al. (2000) studied cardiac functional responses to maximal exercise in 25 boys and 24 girls. Boys had a significantly greater relative  $\text{VO}_{2\text{max}}$  compared to girls, which was partly explained by a larger maximal stroke index measured via Doppler echocardiography. However, when maximal stroke index and aerobic capacity were normalized for lean body mass, sex differences did not exist. This work was supported by Vinet et al. (2003) who similarly investigated sex differences in cardiac function and  $\text{VO}_{2\text{max}}$  in prepubescent children. They reported higher mass-relative  $\text{VO}_{2\text{max}}$  in boys compared to girls, but when  $\text{VO}_{2\text{max}}$  was normalized to lean body mass (LBM) the difference disappeared. It was also found that boys had larger cardiac size and stroke volume. However, when normalized to body weight the differences in stroke volume and cardiac size disappeared as well. There was no difference in arterial-venous oxygen difference between boys and girls. The findings of Rowland et al. (2000) and Vinet et al. (2003) suggest that differences in maximal aerobic capacity between prepubescent boys and girls can be explained by the larger stroke volume in boys, but that this difference is no longer significant when normalized to lean body mass.

### ***Pulmonary Responses to Exercise***

Several differences are apparent between prepubescent boys and girls with respect to the pulmonary response to exercise. Thurlbeck (1982) reported that boys and girls have similar alveolar density but that boys have larger lungs, resulting in a greater number of total alveoli. Furthermore, many studies have demonstrated that lung volumes are larger in prepubescent boys compared to girls of the same height (Wang et al. 1993; Armstrong et al. 1998; Gonzales-Barcala et al. 2007). However, differences in lung function exist

between prepubescent boys and girls that cannot be explained by differences in lung size alone, as several studies have reported similar values for forced expiratory volume in 1 second (FEV<sub>1</sub>) and mid-maximal expiratory flows (MEFs) at rest between height-matched boys and girls (Zapletal, 1982; Gonzales-Barcala et al. 2007). On the other hand, some literature does suggest greater FEV<sub>1</sub> and MEF values in boys than girls (Schwartz et al. 1988; Kivastik and Kingisepp et al. 1997).

There does not appear to be a sex difference in chemosensitivity (measured via HCVR and HVR) between prepubescent boys and girls (Marcus et al. 1994). However, it has been well documented that boys exhibit larger tidal volume and maximal ventilation during exercise compared to girls, which can be almost completely explained by the larger VO<sub>2max</sub> levels exhibited in boys (Rutenfranz et al. 1981). However, despite the lower maximal ventilation during exercise exhibited in girls, prevalence of ventilatory constraint during exercise has been shown by one study with 40 subjects to be very similar between sexes (Swain et al. 2010). Therefore, despite substantial differences in the response to exercise between prepubescent boys and girls, the pulmonary system represents a potential limitation to exercise tolerance in nearly all normal, healthy children (Swain et al. 2010).

## **Changes in Cardiopulmonary Function from Pre- to Post-Puberty**

### *Physiological Bases*

#### *Height, Weight and Body Composition*

The onset of puberty typically occurs at an age of 13 years in boys and 11 years in girls (Tanner et al. 1975). A hallmark of puberty is the “growth spurt”, in which there is a

large increase in height and weight in both sexes. Peak height velocity in girls occurs at approximately age 12 with a rate of 9 cm per year (Kelch and Beitins, 1994). The total height gain during puberty in girls on average is 25 cm (Kelch and Beitins, 1994). In contrast, boys do not typically reach peak height velocity until age 14, in which height increases at a rate of approximately 10.3 cm per year (Marshall and Tanner, 1970; Kelch and Beitins, 1994). Further, growth essentially ceases at approximately age 15 in girls and 17 in boys (Tanner, 1990). On average, boys exhibit a total increase in height that is 13 cm greater than that of girls (Rogol et al. 2000). In this context, boys exhibit both a greater maximal height velocity and a longer duration of pre-pubertal growth, resulting in substantially greater height increases in boys compared to girls from pre- to post-puberty. Pubertal growth is also characterized by period of marked changes in weight gain. Almost half of adult body mass is gained during puberty (Rogol et al. 2000). Boys experience a peak weight gain of approximately 9 kg/year, while girls exhibit a peak weight gain of 8.3 kg/year on average (Tanner, 1990).

Changes in body composition, including the regional distribution of body fat, are very substantial from pre- to post-puberty and differ greatly between sexes (Rogol et al. 2002). During childhood, boys and girls exhibit similar rates of fat-free mass (FFM) accrual, although boys normally have approximately 1-3 kg more FFM compared to girls (Rogol et al. 2002). With regard to body fat, prepubescent boys and girls display similar absolute amounts of fat mass (Rogol et al. 2002). However, due to smaller body mass, girls typically have a slightly higher percentage of body fat, exhibiting approximately 6% more body fat than boys at 10 years of age (Rogol et al. 2002). During pubertal growth, boys experience significantly enhanced increases in FFM, with both the rate and duration

of FFM accrual being greater in boys (Malina et al. 2004). Girls typically reach the young adult amount of FFM at age 15 to 16, while boys do not reach the young adult amount of FFM until approximately age 19 to 20 (Malina et al. 2004). As adults, men have approximately 150% FFM presented by adult women (Cheek, 1974). Concerning fat mass, girls accumulate fat in the limbs during puberty, while fat mass in the limbs in boys decreases (Tanner, 1990; Johnston, 1992). In addition, while fat content increases in both boys and girls during puberty, the amount of fat deposition relative to body weight is greater in girls, resulting in a greater increase in percent body fat in girls from pre- to post-puberty (Rogol et al. 2002). Since body composition appears to be tied to lung function (Thyagarajan et al. 2008), changes in FFM and percent body fat from pre- to post-puberty could have a substantial impact on the prevalence of ventilatory constraint.

### ***Hormonal Influences***

Changes in height, weight, and body composition from pre- to post-puberty are primarily driven by hormonal influences. The primary hormones regulating body composition during pubertal growth include cortisol, growth hormone (GH), insulin, and the sex steroids (Roemmich et al. 1999). Hormones differ in function, as GH and the sex steroids promote lipolysis while cortisol and insulin stimulate fat deposition (Roemmich et al. 1999). The substantial sexual dimorphism that is a hallmark of pubertal growth can be largely explained by the influence of gonadal steroid hormones, specifically estradiol in girls and testosterone in boys, and the adrenal androgens (Rogol et al. 2002). Gonadarche, which represents the onset of gonadal steroid hormone production, typically occurs between 11.5 and 12 years in boys and between 8 and 13 years in girls (Rogol et

al. 2002). Changes in secondary sex characteristics, height, weight, and body composition closely follow gonadarche.

### *Aerobic Capacity*

There is an increase in absolute  $\text{VO}_{2\text{max}}$  during puberty that parallels anthropometric changes (Sprynarova et al. 1987). In a meta-analysis of 5793 males and 3508 females, Krahenbuhl et al. (1985) reported that absolute  $\text{VO}_{2\text{max}}$  increased from ~1.0 L/min at 6 years of age to ~3.2 L/min at 16 years of age in boys, while in girls there was an increase from ~1.0 L/min until plateau of ~2.0 L/min at 14 years of age. When normalized to body weight,  $\text{VO}_{2\text{max}}$  remains relatively stable in boys from age 6 to 16 years (Beunen et al. 1988). Conversely, relative  $\text{VO}_{2\text{max}}$  exhibits a gradual decrease in girls from age 6 to 16 years, which is attributed to increased deposition of body fat during puberty in girls (Beunen et al. 1988).

Changes in  $\text{VO}_{2\text{max}}$  during puberty are determined by changes in anthropometrics and body composition (Sprynarova et al. 1987; Janz et al. 1997). During puberty, boys increase FFM and decrease percentage of body fat, while girls display a lesser increase in FFM accompanied by increased percent body fat (Rogol et al. 2002). In 1987, Sprynarova et al. reported that FFM mass accounts for most interindividual differences in  $\text{VO}_2$  at rest and during maximal exercise in subjects age 11 to 18 years, while body weight accounts for most interindividual differences in  $\text{VO}_2$  during submaximal exercise. Hence, changes in weight and body composition appear to be a primary determinant in changes in  $\text{VO}_{2\text{max}}$  from pre- to post-puberty.



## ***Pulmonary Function***

### ***Pulmonary Structure***

There are substantial changes in pulmonary structure from pre- to post-puberty in both boys and girls. Lung and airway size increase during the pubertal growth spurt (Wang et al. 1993). This increase in lung size is due exclusively to enhancement of alveolar dimensions, as alveolar multiplication ceases after approximately age two (Thurlbeck 1982). With regard to pulmonary function, both boys and girls exhibit increases in FVC, FEV<sub>1</sub>, forced expiratory flow at 50% FVC (FEF<sub>50%</sub>), and forced expiratory flow at 25-75% FVC (FEF<sub>25-75%</sub>) from pre- to post-puberty (Wang et al. 1993). While the increase in lung size closely follows increases in height in both boys and girls, there are sex differences in pulmonary structure changes during puberty. When adjusted for height, boys have greater lung function values than girls. Boys and girls also differ in regard to timing and rate of lung growth. Lung growth is similar in both prepubescent boys and girls until approximately age 12 in boys and age 10 in girls (Wang et al. 1993). However, girls reach maturity in pulmonary function at approximately age 14, while boys do not reach pulmonary maturity until approximately age 18 (Seely et al. 1974). Therefore, boys exhibit a greater rate of growth and a longer growth duration than girls, resulting in a greater increases in pulmonary function in boys compared to girls during puberty (Wang et al. 1993). The improved pulmonary function in boys compared to girls from pre- to post-puberty may contribute to a lower prevalence of ventilatory constraint in boys compared to girls during puberty.

### ***Pulmonary Response to Exercise***

The increase in resting pulmonary function due to structural enhancements during puberty contributes to a coincident increase in ventilatory response during exercise. Several studies have observed increases in  $V_{E_{max}}$  during exercise in the same cohort of subjects from pre- to post-puberty (Prioux et al. 1997; Mercier et al. 1993; Seely et al. 1974). However, similar to pulmonary structure and resting function, there are also sex differences in the pulmonary response to exercise during puberty. Rutenfranz et al. (1981) conducted a 5-year longitudinal study examining changes in  $V_{E_{max}}$  during exercise in two cohorts of European schoolchildren: 62 Norwegian boys and girls from age 8 to 15 years and 52 German boys and girls from age 12 to 17 years. It was found that  $V_{E_{max}}$  increased significantly in boys and girls from age 8 to 15 years. However, while a significant increase in  $V_{E_{max}}$  was exhibited in boys from age 12 to 17 years, there was not a significant increase in  $V_{E_{max}}$  in girls from age 12 to 17 years, possibly supporting other reports that girls reach pulmonary maturity earlier than boys (Seely et al. 1974).

There is also a change in the coupling of  $CO_2$  production with ventilation ( $V_E/VCO_2$ ) during exercise from pre- to post-puberty. In 1987, Cooper et al. investigated  $V_E/VCO_2$  during exercise in a cohort of 128 subjects (68 boys, 60 girls) ranging in age from 6 to 18 years. They found a significant decrease in  $V_E/VCO_2$  with increasing age, height, and weight. Specifically, the mean slope of  $V_E/VCO_2$  was 27 in the youngest subjects (i.e. children) and 21 in the oldest subjects (i.e. teenagers). Further, it was found that end-tidal  $PCO_2$  was regulated at significantly lower levels during exercise in children compared to teenagers. These results suggest that children breathe “out of proportion” to metabolic demand and regulate  $PCO_2$  at lower levels compared to teenagers and adults.

### ***Pulmonary Limitations to Exercise***

Several reports in adult men and women suggest a prevalence of EFL that is substantially lower than that of children (Mota et al. 1999; Dominelli et al. 2011). This could potentially be explained by increases in pulmonary function and a decrease in ventilatory drive from pre- to post-puberty (Wang et al. 1993; Gratas-Delamarche et al. 1993). However, to date, prevalence of ventilatory constraint, specifically EFL, has not been determined from pre- to post-puberty. Further, most studies investigating EFL prevalence have used relatively small sample sizes ( $n < 25$ ), making comparisons between children and adults difficult. In addition, pubertal growth exhibits substantial sexual dimorphism, including growth differences in height, weight, body composition, aerobic capacity, and pulmonary function between boys and girls, which may contribute to ventilatory differences. Finally, if changes in EFL do occur from pre- to post-puberty, how do they affect operational lung volumes? Changes in lung volume regulation have not been investigated pre- and post-puberty. Therefore, additional research is needed to understand changes in EFL from pre- to post-puberty and how cardiopulmonary responses differ from adults.

## **Conclusion**

Pulmonary limitations to exercise tolerance include fatigue of the respiratory musculature, gas exchange impairment, and ventilatory constraint. Of specific interest to this project, expiratory flow limitation is a form of ventilatory constraint during exercise that is thought to be highly prevalent in prepubescent children, but presents a much lower prevalence in normal, healthy adults. Many physiological changes occur from pre- to post-puberty that can play a role in the prevalence of expiratory flow limitation. It is

currently unknown how maturation from pre- to post-puberty affects the prevalence of expiratory flow limitation during exercise and whether a sex difference exists.

Therefore, the purpose of this study was to investigate changes in cardiopulmonary function, specifically expiratory flow limitation, from pre- to post-puberty in boys and girls and determine whether a sex difference existed. We hypothesized that: 1) the prevalence of expiratory flow limitation would be lower in post-puberty subjects compared to pre-puberty subjects; 2) post-puberty girls would show a higher prevalence and severity of expiratory flow limitation compared to post-puberty boys due to smaller pulmonary structure in girls compared to boys; 3) post-puberty subjects would exhibit increased lung volume and expiratory flow rates and decreased  $V_E/V_{CO_2}$  compared to pre-puberty subjects.

## **Chapter 2 - Methods**

We recruited 21 healthy subjects (11 boys, 10 girls) from 40 subjects who were previously tested in our laboratory ~5 years ago (Swain et al. 2010). We were unable to make contact with the remaining 19 subjects. All subjects were non-asthmatic and free of pulmonary disease both at baseline and follow-up, as measured by pulmonary function tests (PFTs). Approximately five years ago, all children were determined to be pre-pubescent, as defined by Tanner stage 1 (Tanner, 1962). Tanner stage was also assessed post-puberty. We did not determine menstrual cycle phase in female subjects. Each subject had a parent or guardian present for each appointment 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, Kansas.

### **Experimental Design**

Subjects reported to the lab on a total of four occasions: two times at baseline (pre-puberty) and twice five years later for follow-up (post-puberty). The experimental design for baseline and follow-up were identical. On the first visit, height and weight for each subject were measured using a calibrated eye-level physical scale with height rod (Detecto, Webb City, MO). Standard PFTs were then performed, after several practice trials to ensure subject competence. These measures included lung volumes, lung diffusing capacity with carbon monoxide (DLCO), maximal inspiratory and expiratory pressures ( $P_{I_{max}}$  and  $P_{E_{max}}$ ), and maximal flow-volume loops (MFVL).  $P_{I_{max}}$ ,  $P_{E_{max}}$ , and

MFVL tests were performed in triplicate and averaged. The subject then completed an incremental maximal exercise test to exhaustion to determine maximal oxygen uptake ( $VO_{2max}$ ). The subject completed a second exercise test at 105% of maximal workload to verify  $VO_{2max}$ . On the second visit, each subject underwent a Dual-Energy X-Ray Absorptiometry (DEXA) scan to determine body composition.

## **Tests and Measurements**

### ***Pre-Exercise Pulmonary Function Tests***

Total lung capacity (TLC), residual volume (RV), lung diffusion capacity (DLCO), maximal inspiratory pressure ( $P_{I_{max}}$ ), maximal expiratory pressure ( $P_{E_{max}}$ ), and maximal flow-volume loops were measured prior to exercise testing at pre-puberty and post-puberty (SensorMedics 229 Metabolic Cart, SensorMedics Corp, Yorba Linda, CA). The nitrogen washout technique was used to measure TLC and RV. Diffusion capacity of the lung was determined using normalized alveolar air ( $DLCO/V_A$ ) using a test gas mixture of 0.3% acetylene, 0.3% carbon monoxide, 0.3% methane, 21%  $O_2$  with  $N_2$  balance via the intra-breath inhalation technique.  $P_{E_{max}}$  was measured at TLC and  $P_{I_{max}}$  was measured at RV. Following practice trials,  $P_{I_{max}}$ ,  $P_{E_{max}}$ , and MFVL tests were performed in triplicate and averaged.

### ***Maximal Aerobic Capacity ( $VO_{2max}$ )***

Maximal oxygen uptake ( $VO_{2max}$ ) was determined using a cycle ergometer (Ergometer 800S, SensorMedics Corp, Yorba Linda, CA). Clear and comprehensive instructions were given to subjects prior to exercise to ensure full volitional effort. A 3-L

calibration syringe was used to calibrate the flow sensor prior to testing. Known gas concentrations were used in the calibration of gas analyzers. Prior to onset of exercise, resting metabolic measurements were recorded for three minutes. Subjects then began a 2-minute warm-up at 20 watts. During the pre-puberty assessment, subjects were instructed to maintain a pedaling frequency between 50-60 revolutions per minute (rev/min), while in the post-puberty assessment subjects pedaled at 60-80 rev/min. Subjects were directed to maintain the instructed pedaling frequency for the duration of the warm-up and exercise test. After approximately two minutes of warm-up, the workload was increased at a rate of 10 watts per minute at pre-puberty and 25 watts per minute at post-puberty. Subjects remained seated throughout the entire test. Towards the end of each minute of an exercise stage, subjects were asked to report a rate of perceived exertion (RPE) and dyspnea using the modified Borg scale. Arterial oxygen saturation ( $\text{SaO}_2$ ) was estimated using a pulse oximeter (Datex-Ohmeda, 3900P, Madison, WI) secured to the left ear lobe. Heart rate (HR) was assessed both pre- (4-lead ECG) and post-puberty (heart rate monitor). Verbal encouragement from researchers was provided throughout the exercise test. Subjects exercised continuously until reaching volitional exhaustion (<16 minutes). The exercise test was terminated when the subject could no longer maintain a pedaling frequency within the instructed range for five consecutive revolutions. Ventilatory and metabolic data were measured and monitored continuously throughout the  $\text{VO}_{2\text{max}}$  test via breath-by-breath analysis (SensorMedics 229 Metabolic Cart, SensorMedics Corp, Yorba Linda, CA). After a rest period of approximately 15 minutes, subjects completed a second exercise test to volitional exhaustion at 105% of maximal workload (~2 minutes) to verify  $\text{VO}_{2\text{max}}$  (Poole et al. 2008). Subjects completed

a warm-up of 90 seconds at 20 watts with a pedaling frequency of 50-60 rev/min (pre-puberty) or 60-80 rev/min (post-puberty). The workload was then increased to 105% of maximal workload from the incremental test. Subjects were instructed to maintain pedaling frequency and exercise to volitional fatigue. The constant-load exercise test was terminated when the subject could no longer maintain the desired pedaling frequency for five consecutive revolutions.

### ***Expiratory Flow Limitation and Tidal Volume Regulation***

A bidirectional flow sensor combined with a gas analyzer was used to measure and record tidal flow-volume loops during the  $\text{VO}_{2\text{max}}$  test. Tidal flow-volume loops were placed inside post-exercise maximal flow-volume loops (Johnson *et al*, 1999). Subjects performed inspiratory capacity (IC) maneuvers from functional residual capacity (FRC) throughout the incremental exercise test, with approximately 20 seconds to go in each stage. IC maneuvers were used in the accurate placement of tidal flow-volume loops within the post-exercise MFVL. Subjects were given clear and concise instruction regarding the correct performance of IC maneuvers and were allowed to practice in a resting state prior to testing. Ventilatory drift was automatically accounted and corrected for appropriately via the flow sensor. Expiratory flow limitation (EFL) was deemed to be present when the tidal flow-volume loop intersected the exercise flow-volume loop to a degree of 5% or greater (Chapman *et al*, 1998; Nourry *et al*, 2006). End-expiratory lung volume (EELV) and end-inspiratory lung volume (EILV), expressed as ratios of expiratory reserve volume and inspiratory reserve volume (ERV/FVC; IRV/FVC), were used to describe changes in tidal volume regulation during the incremental exercise test.



During testing, any changes to breathing regulation or flow sensor drift were monitored by allowing for approximately five tidal breaths prior to performance of an IC maneuver.

### ***Body Composition***

A whole body DEXA system (v5.6, GE Lunar Corp, Milwaukee, WI) was used to measure total body composition. Subjects removed metal objects and shoes before scanning and were instructed to lay in a supine position with legs slightly spaced apart and arms separated from the trunk. The subjects were directed to lay as still as possible during scanning. DEXA scans are a validated means of determining lean body mass (LBM), body fat percentage, and body fat distribution via differences in absorption of two different high-energy x-ray beams (Haarbo et al. 1991).

### ***Statistical Analysis***

SigmaStat statistical software (Janel Scientific Software) was used for data analysis. Table data are expressed as mean  $\pm$  standard deviation. Figure data are expressed as mean  $\pm$  standard error. A 2X2 mixed ANOVA (time versus group) was used to determine differences between sexes and pre- and post-puberty. A Chi-square two-tailed test of independence was used to determine an association between sex and prevalence of expiratory flow limitation. Significance was set at  $p < 0.05$  for all analyses.

## Chapter 3 - Results

Pre- and post-puberty subject characteristics are displayed in Table 1. The male to female ratio was nearly equal (n = 10 girls, n = 11 boys). Pre-puberty boys had significantly greater LBM (kg and %) and lower BF (%) compared to pre-puberty girls. Post-puberty boys had significantly greater age, height, LBM (kg and %) and lower BF (%) compared to post-puberty girls. All variables were significantly different from pre- to post-puberty in boys and girls, except LBM (%) and BF (%).

**Table 3-1 Subject characteristics**

	Pre-puberty		Post-puberty	
	Boys n=11	Girls n=10	Boys n=11	Girls n=10
	<i>mean ± SD</i>	<i>mean ± SD</i>	<i>mean ± SD</i>	<i>mean ± SD</i>
<b>Age (yr)</b>	10.1 ± 0.5	9.4 ± 1.0	15.3 ± 0.5*†	14.1 ± 1.0 <sup>#</sup>
<b>Tanner stage (1-5)</b>	1.0 ± 0.0	1.0 ± 0.0	4.2 ± 0.6*	3.7 ± 0.7 <sup>#</sup>
<b>Height (cm)</b>	142.0 ± 6.1	136.3 ± 7.5	177.8 ± 6.5*†	166.0 ± 6.3 <sup>#</sup>
<b>Weight (kg)</b>	33.0 ± 5.2	32.3 ± 5.8	65.5 ± 9.9*	58.9 ± 8.8 <sup>#</sup>
<b>LBM (kg)</b>	26.6 ± 3.1	22.6 ± 2.4 <sup>^</sup>	53.4 ± 5.7*†	37.8 ± 3.4 <sup>#</sup>
<b>LBM (%)</b>	79.6 ± 6.0	72.5 ± 8.0 <sup>^</sup>	83.1 ± 4.0 <sup>†</sup>	65.2 ± 8.3 <sup>#</sup>
<b>BF (%)</b>	17.2 ± 6.3	24.8 ± 8.5 <sup>^</sup>	12.9 ± 4.3 <sup>†</sup>	32.0 ± 8.6 <sup>#</sup>

**LBM, lean body mass; BF, body fat**

\* Pre-puberty Boys significantly different from Post-puberty Boys (p<0.05)

<sup>#</sup> Pre-puberty Girls significantly different from Post-puberty Girls (p<0.05)

<sup>^</sup> Pre-puberty Boys significantly different from Pre-puberty Girls (p<0.05)

<sup>†</sup> Post-puberty Boys significantly different from Post-puberty Girls (p<0.05)

## Metabolic and Ventilatory Data

### *Resting Pulmonary Function*

Table 2 presents lung volumes and resting pulmonary function. Pre-puberty boys showed greater ( $p < 0.05$ ) TLC, FRC, DLCO, FVC, FEV<sub>1</sub>, and PEF compared to pre-puberty girls. FEF<sub>25-75%</sub>, FEV<sub>1</sub>/FVC, FEF<sub>50%</sub>, and P<sub>I</sub>max were not significantly greater in post-puberty boys compared to post-puberty girls. All resting pulmonary function values were significantly greater in post-puberty boys compared to pre-puberty boys, except FEV<sub>1</sub>/FVC and P<sub>I</sub>max. Similarly, resting pulmonary function values were significantly greater in post-puberty girls than pre-puberty girls, with the exception of FEV<sub>1</sub>/FVC and P<sub>E</sub>max.

### *Exercise Data*

Data recorded during maximal exercise for pre- and post-puberty boys and girls are displayed in Table 3. Absolute VO<sub>2max</sub>, ventilation, tidal volume, VCO<sub>2</sub>, V<sub>E</sub>/VCO<sub>2</sub>, respiratory exchange ratio, and work rate were greater ( $p < 0.05$ ) in post-puberty boys and girls compared to pre-puberty. Post-puberty girls showed greater VO<sub>2</sub> relative to lean body mass than pre-puberty. Pre-puberty sex differences were present with regards to relative VO<sub>2</sub>, absolute VO<sub>2</sub>, ventilation, VCO<sub>2</sub>, and work rate. Post-puberty boys displayed significantly greater relative VO<sub>2</sub>, absolute VO<sub>2</sub>, ventilation, tidal volume, VCO<sub>2</sub>, and work rate. V<sub>D</sub>/V<sub>T</sub> decreased significantly from pre- to post-puberty in both boys and girls and there was no sex difference with regard to V<sub>D</sub>/V<sub>T</sub> at either time point. Due to experimental limitations, valid dyspnea measures were unattainable during the pre-puberty measurement. When measured post-puberty, boys reported greater levels of dyspnea compared to girls during maximal exercise. Further, subjects that experienced

EFL reported higher levels of dyspnea compared to subjects that did not experience EFL during maximal exercise (EFL:  $9.29 \pm 0.49$ , NEFL:  $7.21 \pm 2.05$ ;  $p < 0.05$ ).

**Table 3-2 Resting Pulmonary Function**

	Pre-puberty		Post-puberty	
	Boys n=11	Girls n=10	Boys n=11	Girls n=10
	<i>mean ± SD</i>	<i>mean ± SD</i>	<i>mean ± SD</i>	<i>mean ± SD</i>
<b>FRC (L)</b>	0.94 ± 0.26	0.67 ± 0.27 <sup>^</sup>	2.03 ± 0.70 <sup>*†</sup>	1.51 ± 0.29 <sup>#</sup>
<b>IC (L)</b>	1.61 ± 0.44	1.45 ± 0.23	2.82 ± 0.31 <sup>*†</sup>	2.22 ± 0.19 <sup>#</sup>
<b>FVC (L)</b>	2.32 ± 0.28	1.96 ± 0.30 <sup>^</sup>	4.73 ± 0.53 <sup>*†</sup>	3.80 ± 0.29 <sup>#</sup>
<b>FEV<sub>1</sub> (L)</b>	1.94 ± 0.19	1.64 ± 0.26 <sup>^</sup>	3.96 ± 0.56 <sup>*†</sup>	3.30 ± 0.24 <sup>#</sup>
<b>FEV<sub>1</sub>/FVC (%)</b>	83.7 ± 5.3	83.7 ± 5.0	83.9 ± 10.1	87.0 ± 5.2
<b>FEF<sub>25-75%</sub> (L/sec)</b>	2.10 ± 0.37	1.78 ± 0.41	4.13 ± 1.31 <sup>*</sup>	3.69 ± 0.57 <sup>#</sup>
<b>FEF<sub>50%</sub> (L/sec)</b>	2.33 ± 0.36	2.06 ± 0.33	4.44 ± 1.32 <sup>*</sup>	4.01 ± 0.58 <sup>#</sup>
<b>PEF (L/sec)</b>	3.64 ± 0.62	2.99 ± 0.47 <sup>^</sup>	7.89 ± 1.17 <sup>*†</sup>	5.83 ± 0.68 <sup>#</sup>
<b>P<sub>I</sub>max (cmH<sub>2</sub>O)</b>	80.4 ± 27.4	68.1 ± 15.0	95.5 ± 30.6	109.2 ± 34.2 <sup>#</sup>
<b>P<sub>E</sub>max (cmH<sub>2</sub>O)</b>	77.5 ± 20.5	65.8 ± 23.9	108.0 ± 21.5 <sup>*†</sup>	82.4 ± 23.6
<b>DLCO (mmHg/L/min)</b>	16.0 ± 3.1	11.8 ± 3.8 <sup>^</sup>	29.8 ± 8.8 <sup>*†</sup>	18.5 ± 6.9 <sup>#</sup>

**TLC, total lung capacity; FRC, functional residual capacity; IC, inspiratory capacity; DLCO, lung diffusion capacity; FVC, forced vital capacity; FEV<sub>1</sub>, forced expiratory volume in 1 second; FEF<sub>25-75%</sub>, forced expiratory flow between 25-75% FVC; PEF, peak expiratory flow; FEF<sub>50%</sub>, forced expiratory flow at 50% FVC; P<sub>I</sub>max, maximal inspiratory pressure; P<sub>E</sub>max, maximal expiratory pressure**

**\* Pre-puberty Boys significantly different from Post-puberty Boys (p<0.05)**

**# Pre-puberty Girls significantly different from Post-puberty Girls (p<0.05)**

**^ Pre-puberty Boys significantly different from Pre-puberty Girls (p<0.05)**

**† Post-puberty Boys significantly different from Post-puberty Girls (p<0.05)**

**Table 3-3 Metabolic and ventilatory data at maximal exercise**

	Pre-puberty		Post-puberty	
	Boys n=11	Girls n=10	Boys n=11	Girls n=10
	<i>mean ± SD</i>	<i>mean ± SD</i>	<i>mean ± SD</i>	<i>mean ± SD</i>
<b>VO<sub>2</sub> (ml/kg/min)</b>	37.5 ± 7.9	29.2 ± 5.7 <sup>^</sup>	42.7 ± 4.7 <sup>†</sup>	33.0 ± 6.7
<b>VO<sub>2</sub> (ml/kg LBM/min)</b>	47.5 ± 6.6	41.9 ± 6.1	51.5 ± 5.2	51.1 ± 6.9 <sup>#</sup>
<b>VO<sub>2</sub> (L/min)</b>	1.26 ± 0.18	0.95 ± 0.18 <sup>^</sup>	2.76 ± 0.43 <sup>*†</sup>	1.94 ± 0.35 <sup>#</sup>
<b>V<sub>E</sub> (L/min)</b>	52.4 ± 8.8	40.5 ± 7.9 <sup>^</sup>	110.1 ± 16.2 <sup>*†</sup>	84.1 ± 17.1 <sup>#</sup>
<b>V<sub>T</sub> (L)</b>	0.92 ± 0.17	0.80 ± 0.18	2.16 ± 0.32 <sup>*†</sup>	1.70 ± 0.22 <sup>#</sup>
<b>Fb (breaths/min)</b>	59.9 ± 13.9	52.6 ± 11.1	51.5 ± 6.8	50.0 ± 8.9
<b>VCO<sub>2</sub> (L/min)</b>	1.28 ± 0.20	0.96 ± 0.19 <sup>^</sup>	3.07 ± 0.50 <sup>*†</sup>	2.19 ± 0.4 <sup>#</sup>
<b>V<sub>E</sub>/VCO<sub>2</sub></b>	41.7 ± 4.5	43.3 ± 4.9	36.1 ± 3.2 <sup>*</sup>	38.6 ± 4.2 <sup>#</sup>
<b>V<sub>E</sub>/VO<sub>2</sub></b>	42.0 ± 4.8	43.0 ± 6.8	40.4 ± 4.2	43.8 ± 6.1
<b>P<sub>ET</sub>O<sub>2</sub> (mmHg)</b>	110.6 ± 3.3	110.3 ± 6.4	110.6 ± 2.9	112.6 ± 4.8
<b>P<sub>ET</sub>CO<sub>2</sub> (mmHg)</b>	34.9 ± 2.3	34.6 ± 4.1	35.8 ± 2.9	33.5 ± 3.5
<b>V<sub>D</sub>/V<sub>T</sub></b>	0.22 ± 0.03	0.25 ± 0.04	0.15 ± 0.03 <sup>*</sup>	0.17 ± 0.03 <sup>#</sup>
<b>HR (beats/min)</b>	178.3 ± 18.1	181.9 ± 17.3	178.6 ± 10.3	186.6 ± 8.5
<b>SpO<sub>2</sub> (%)</b>	97.1 ± 2.3	97.9 ± 1.5	98.3 ± 1.1	98.0 ± 1.8
<b>RER</b>	1.02 ± 0.04	1.03 ± 0.08	1.12 ± 0.04 <sup>*</sup>	1.13 ± 0.06 <sup>#</sup>
<b>Work (W)</b>	104.5 ± 12.9	81.0 ± 19.7 <sup>^</sup>	259.1 ± 34.0 <sup>*†</sup>	187.5 ± 29.5 <sup>#</sup>
<b>Dyspnea (0-10)</b>	N/A	N/A	8.91 ± 1.22 <sup>†</sup>	6.80 ± 2.04

**VO<sub>2</sub>, oxygen uptake; V<sub>E</sub>, minute ventilation; V<sub>T</sub>, tidal volume; Fb, breathing frequency; VCO<sub>2</sub>, carbon dioxide production; P<sub>ET</sub>, end-tidal partial pressure; HR, heart rate; Sp, saturation measured via pulse oximetry; RER, respiratory exchange ratio**

**\* Pre-puberty Boys significantly different from Post-puberty Boys (p<0.05)**

**# Pre-puberty Girls significantly different from Post-puberty Girls (p<0.05)**

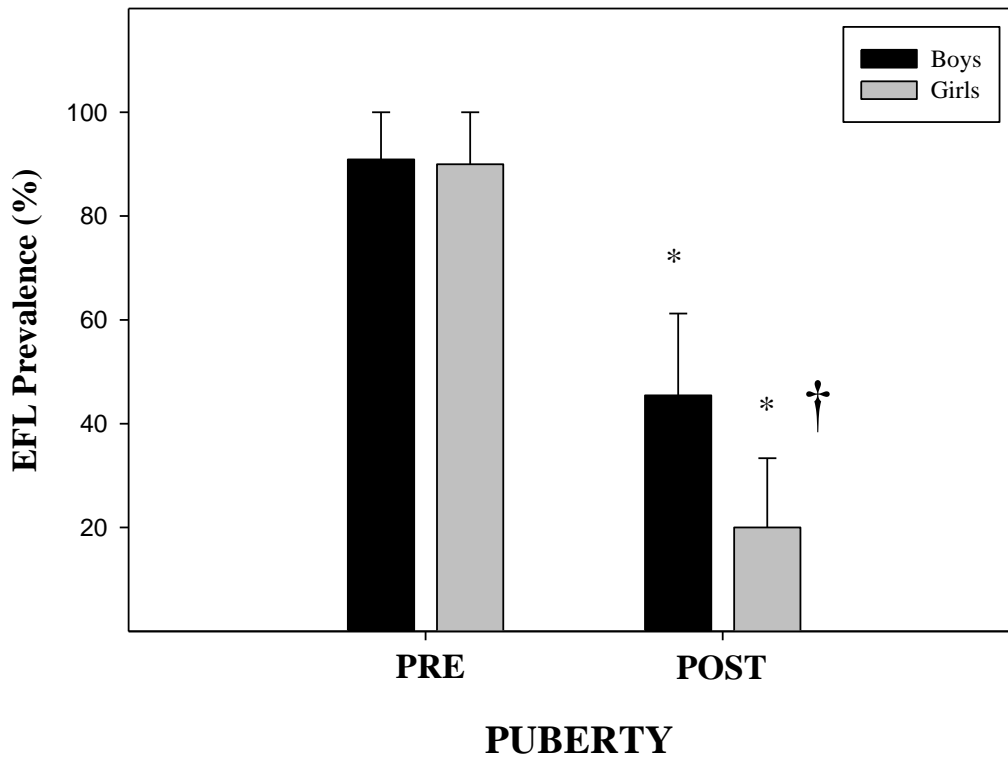
**^ Pre-puberty Boys significantly different from Pre-puberty Girls (p<0.05)**

**† Post-puberty Boys significantly different from Post-puberty Girls (p<0.05)**

## Expiratory Flow Limitation

### *Prevalence and Severity*

Figure 1 shows the change in EFL prevalence from pre- to post-puberty in boys and girls. Ten of eleven (~91%) pre-puberty boys and nine of ten (90%) pre-puberty girls exhibited EFL at  $VO_{2max}$ . Five of eleven (~45%) post-puberty boys and two of ten (20%) post-puberty girls experienced EFL at  $VO_{2max}$ . There was no sex difference in EFL prevalence pre-puberty ( $p>0.05$ ) but post-puberty boys showed a greater prevalence of EFL (5 of 11 subjects) than post-puberty girls (2 of 10 subjects;  $p<0.05$ ). Figure 2 demonstrates the severity (%  $V_T$ ) of EFL experienced during maximal exercise measured pre- and post-puberty in boys (A) and girls (B). There was a significant decrease in severity of EFL at  $VO_{2max}$  from pre- to post-puberty in both boys (10/11 subjects) and girls (9/10 subjects). Boys that displayed EFL both pre- and post-puberty displayed similar ( $p>0.05$ )  $VO_{2max}$  and  $V_E$  but lower expiratory flow rates compared to post-puberty boys that did not exhibit EFL. Girls that experienced EFL pre- and post-puberty showed similar ( $p>0.05$ ) expiratory flow rates but greater  $VO_{2max}$  compared to post-puberty girls that did not experience EFL. Figure 3 displays EFL severity (%  $V_T$ ) from 40% to 100%  $VO_{2max}$  in boys and girls measured pre- and post-puberty. Pre-puberty boys and girls showed significantly greater EFL severity at higher relative exercise intensities (80% and 100%  $VO_{2max}$ ) compared to post-puberty boys and girls.

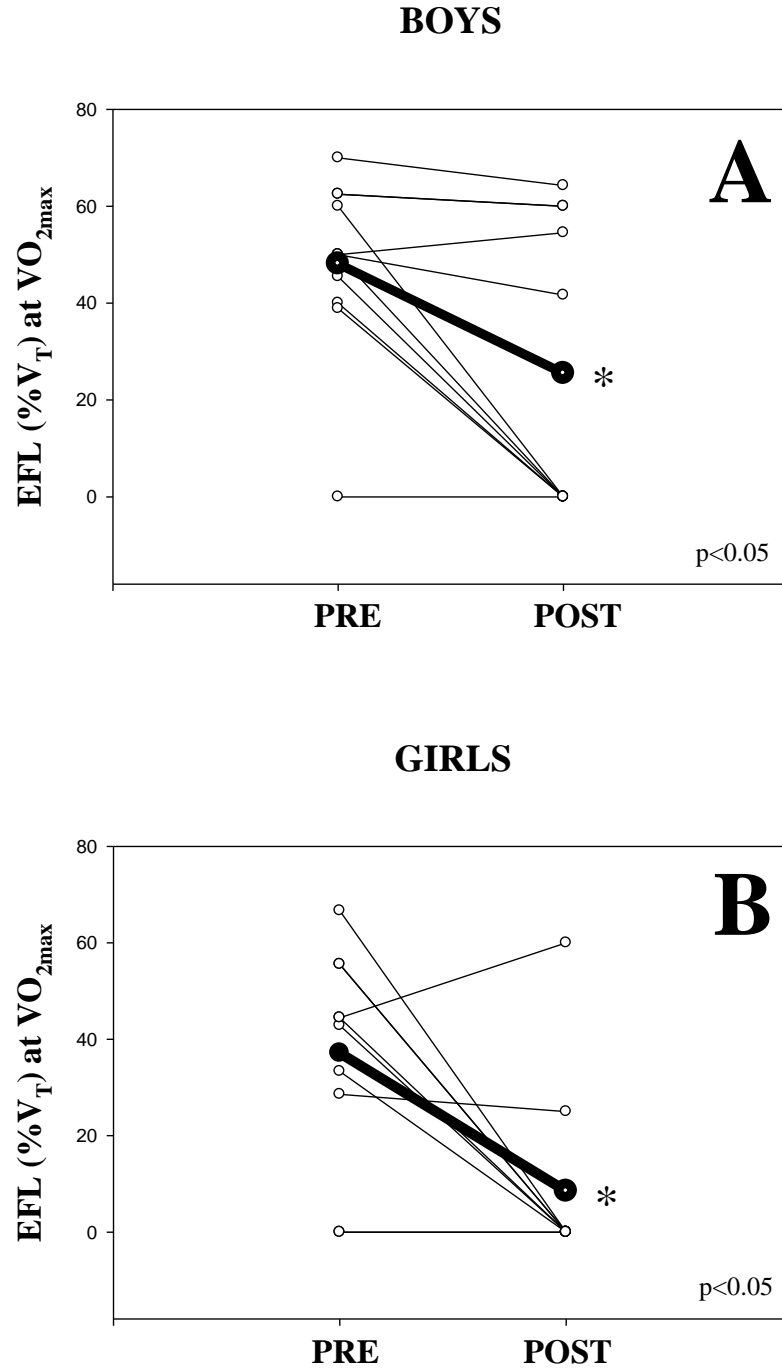


**Figure 3-1 Prevalence of EFL during maximal exercise.**

There was a significant decrease in EFL prevalence from pre- to post-puberty in both sexes ( $p < 0.05$ ). There were no sex differences with regard to prevalence of EFL pre-puberty. Post-puberty boys displayed a greater ( $p < 0.05$ ) prevalence of EFL than post-puberty girls.

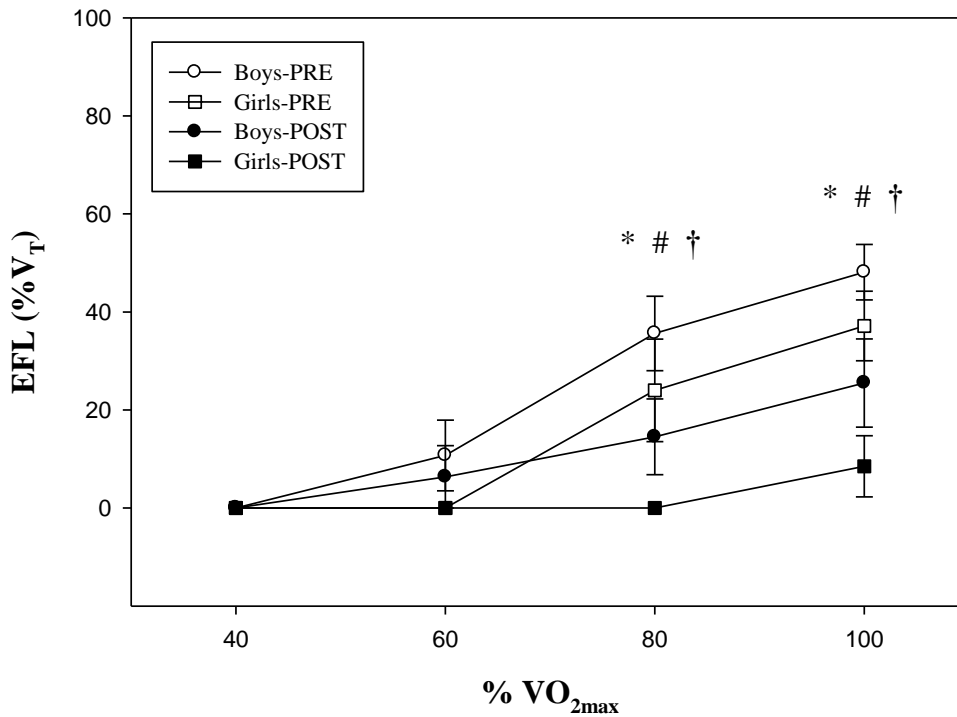
**\* Significantly different from Pre-puberty**

**† Post-puberty boys significantly different from Post-puberty girls**



**Figure 3-2 Change in EFL severity during maximal exercise from pre- to post-puberty**  
 Individual (open circles, thin lines) and mean (closed circles, thick lines) changes in EFL ( $\% V_T$ ) during maximal exercise in boys (A) and girls (B) from pre- to post-puberty. Significant decreases occurred in EFL severity from pre- to post-puberty in both sexes ( $p < 0.05$ ).





**Figure 3-3 Change in EFL severity from 40-100% VO<sub>2max</sub>**

Change in EFL (%V<sub>T</sub>) from 40% to 100% VO<sub>2max</sub> in boys and girls measured pre- and post-puberty. Pre-puberty boys and girls exhibited significantly greater EFL severity at 80% and 100% VO<sub>2max</sub> compared to post-puberty boys and girls (p<0.05). Post-puberty boys showed significantly greater EFL severity at 80% and 100% VO<sub>2max</sub> compared to post-puberty girls (p<0.05).

\* Pre-puberty Boys significantly different from Post-puberty Boys (p<0.05)

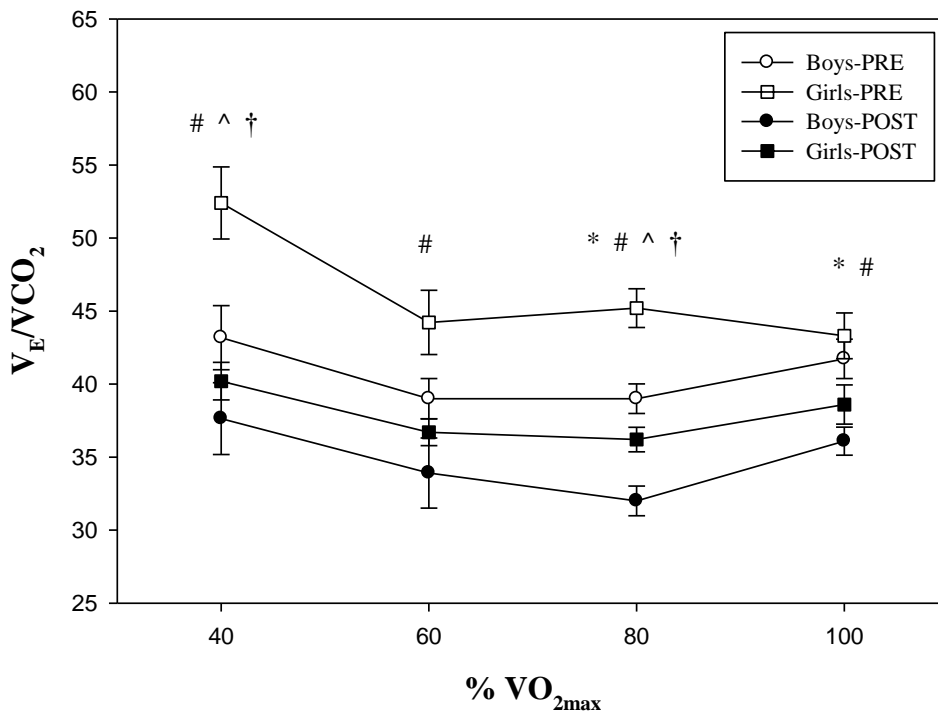
# Pre-puberty Girls significantly different from Post-puberty Girls (p<0.05)

† Post-puberty Boys significantly different from Post-puberty Girls (p<0.05)

### *Determinants of Expiratory Flow Limitation*

Figure 4 demonstrates minute ventilation relative to CO<sub>2</sub> production ( $V_E/V_{CO_2}$ ) in pre- and post-puberty boys and girls at different exercise intensities. Pre-puberty boys displayed greater ( $p < 0.05$ )  $V_E/V_{CO_2}$  than post-puberty boys at 80% and 100%  $VO_{2max}$ , while pre-puberty girls showed greater ( $p < 0.05$ )  $V_E/V_{CO_2}$  compared to post-puberty girls at all exercise intensities, reflecting a decreased relative ventilatory response during exercise from pre- to post-puberty in boys and girls.

Figure 5 displays changes in end-expiratory lung volume (EELV) and end-inspiratory lung volume (EILV) normalized to vital capacity (expressed as ERV/FVC (%) and IRV/FVC (%), respectively) at  $VO_{2max}$  from pre- to post-puberty in boys and girls. ERV/FVC (%) during maximal exercise significantly increased in boys from pre- to post-puberty, but did not in girls ( $p > 0.05$ ). However, there was a significant decrease from pre- to post-puberty in IRV/FVC (%) at  $VO_{2max}$  in both sexes. Figure 6 shows that post-puberty boys and girls breathed at higher ( $p < 0.05$ ) lung volumes across a range of exercise intensities. Specifically, post-puberty boys demonstrated a significantly higher ERV/FVC (%) and lower IRV/FVC (%) at every exercise intensity compared to pre-puberty boys. Post-puberty girls showed a higher ERV/FVC (%) at 40% and 60%  $VO_{2max}$  and a lower IRV/FVC (%) at 60%, 80%, and 100%  $VO_{2max}$ . Overall, the data suggest that our subjects predominately breathed at significantly higher relative lung volumes during exercise when measured post-puberty compared to pre-puberty.



**Figure 3-4 Changes in  $V_E/VCO_2$  from 40-100%  $VO_{2max}$**

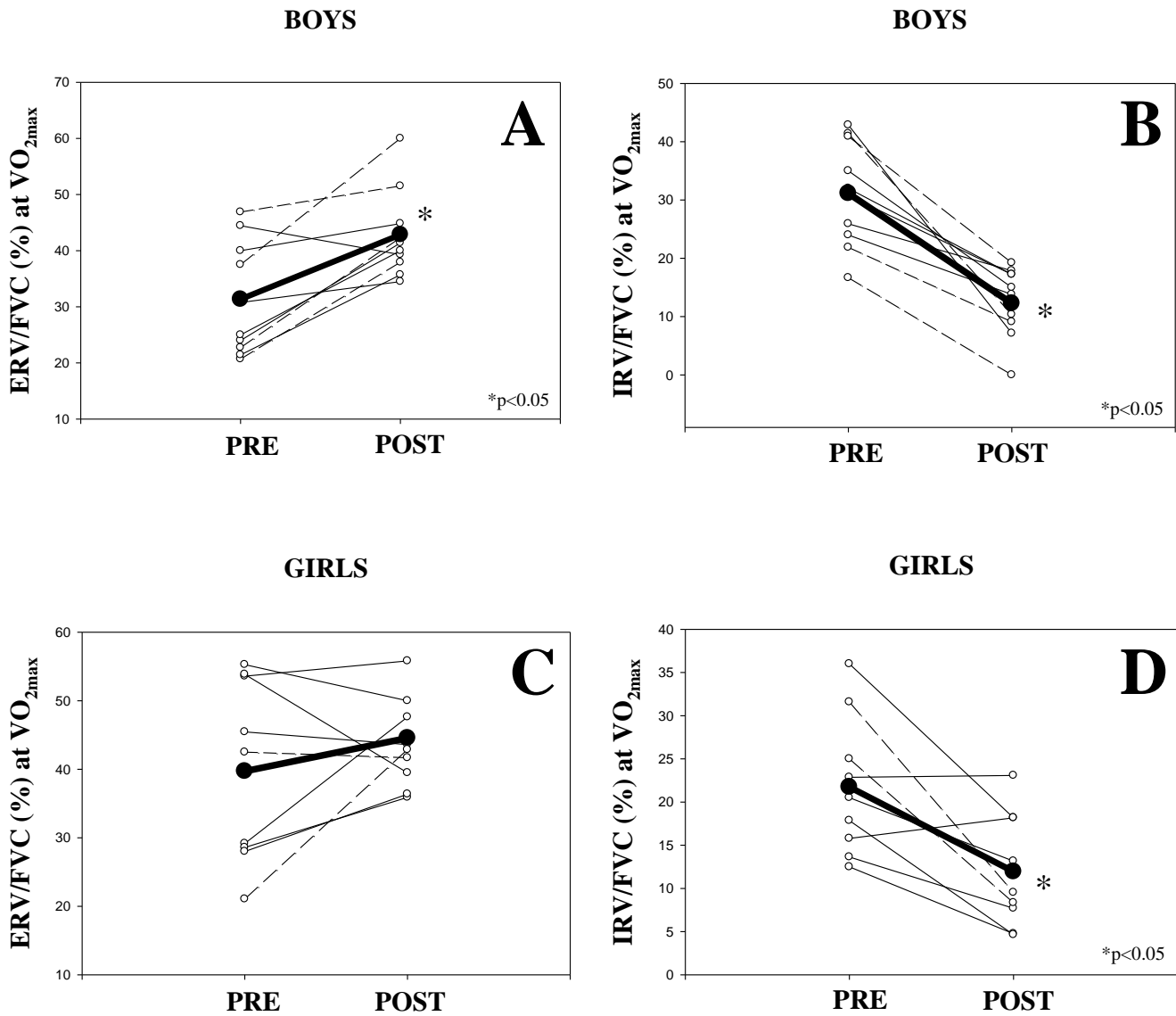
Changes in  $V_E/VCO_2$  at different intensities of exercise in pre- and post-puberty boys and girls.  $V_E/VCO_2$  was significantly greater ( $p < 0.05$ ) in pre-puberty boys compared to post-puberty boys at 80% and 100%  $VO_{2max}$ .  $V_E/VCO_2$  was significantly greater ( $p < 0.05$ ) in pre-puberty girls than post-puberty girls at all exercise intensities.

\* Pre-puberty Boys significantly different from Post-puberty Boys ( $p < 0.05$ )

# Pre-puberty Girls significantly different from Post-puberty Girls ( $p < 0.05$ )

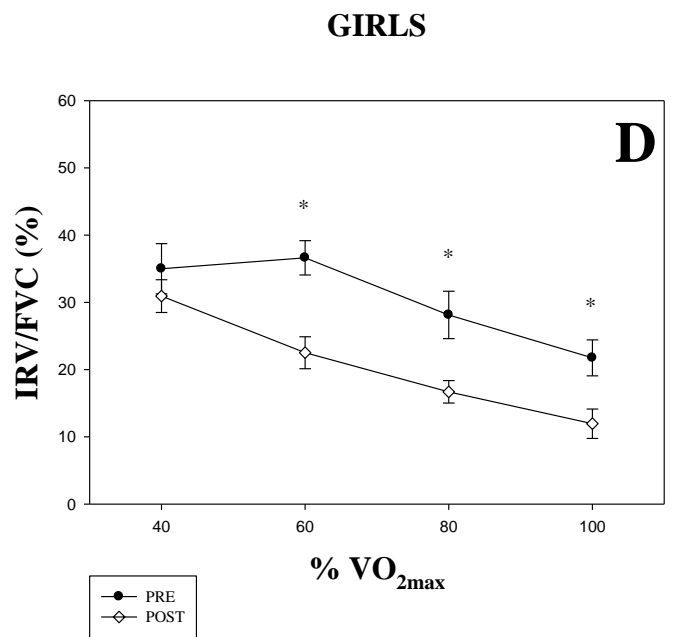
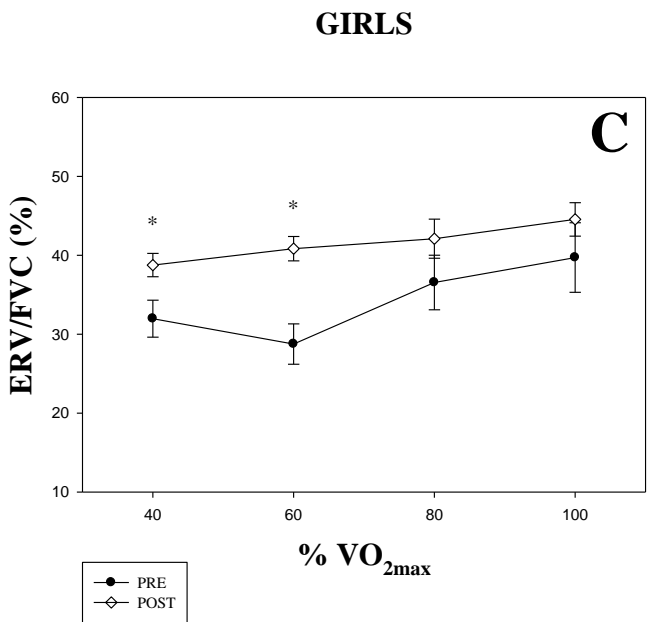
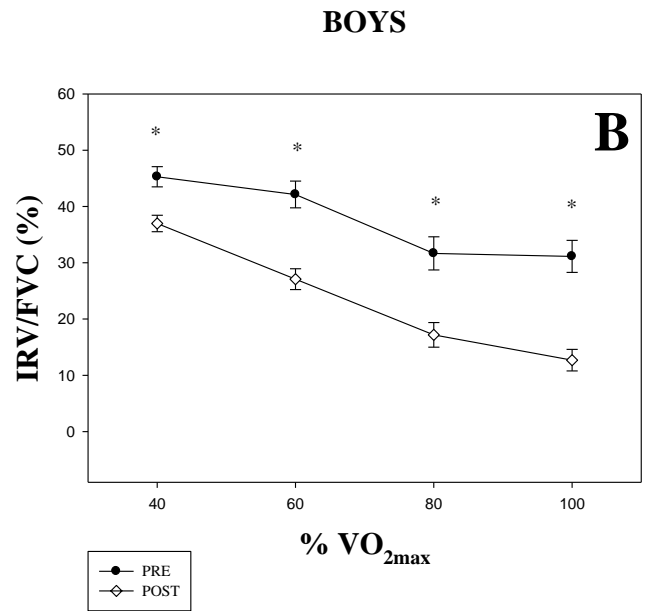
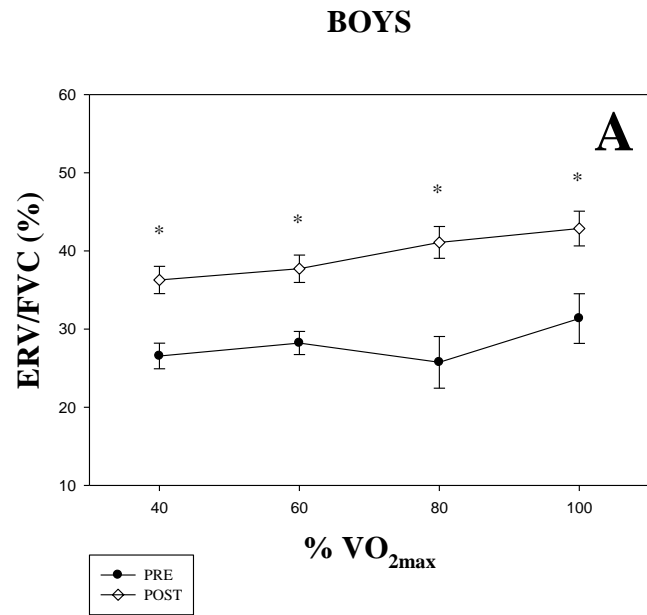
^ Pre-puberty Boys significantly different from Pre-puberty Girls ( $p < 0.05$ )

† Post-puberty Boys significantly different from Post-puberty Girls ( $p < 0.05$ )



**Figure 3-5 Lung volume regulation during maximal exercise**

Individual (open circles, thin lines) and mean (closed circles, thick lines) changes in operational lung volumes (ERV/FVC and IRV/FVC, %) during maximal exercise from pre- to post-puberty (PRE and POST, respectively) in boys (A,B) and girls (C,D). Significant changes occurred with regard to lung volume regulation at maximal exercise in both sexes from pre- to post-puberty ( $p < 0.05$ ), except for ERV/FVC (%) in girls (Panel C). Dashed lines are subjects that exhibited EFL.



**Figure 3-6 Lung volume regulation from 40-100% VO<sub>2max</sub>**

Change in operational lung volumes (ERV/FVC and IRV/FVC, %) from 40% to 100% VO<sub>2max</sub> measured pre- and post-puberty in boys (A,B) and girls (C,D).

**\* Post-puberty significantly different from pre-puberty (p<0.05)**

## **Chapter 4 - Discussion**

### **Major Findings**

The purpose of this study was to assess changes in cardiopulmonary function, specifically with regard to ventilatory constraint, during exercise from pre- to post-puberty in boys and girls. The primary finding was that the prevalence of expiratory flow limitation (EFL) decreased from pre- to post-puberty from >90% to ~33% in our population samples. Surprisingly, while there was no sex difference in the prevalence of EFL pre-puberty, more post-puberty boys exhibited EFL than post-puberty girls. The decreased prevalence of EFL post-puberty was likely due to substantial increases in lung size and expiratory flow rates relative to improvements in aerobic capacity and exercise ventilation. Finally, both post-puberty boys and girls regulated tidal breathing at significantly higher relative lung volumes during exercise, despite the significantly lower prevalence of EFL compared to pre-puberty.

### **Prevalence of Expiratory Flow Limitation**

Why did EFL prevalence significantly decrease from pre- to post-puberty? While it is known that both trained and untrained prepubescent children present a high prevalence of EFL (Nourry et al. 2005; Swain et al. 2010), there is currently no data to our knowledge on how EFL changes during maturation. The prevalence of EFL in normal untrained adults is thought to be lower than that of children (Johnson et al. 1999). Therefore, growth and changes in gas exchange that occur from pre- to post-puberty appears to promote a decrease in the prevalence of ventilatory constraint during exercise. The results of the present study support this hypothesis, as only 45% boys and 20% girls

exhibited EFL post-puberty. As expected, significant changes also occurred with regard to body composition, ventilation during exercise, and maximal aerobic capacity. Thus, the relatively greater structural and morphological changes that occur compared to changes in aerobic capacity and ventilation during the pubertal growth spurt likely decreases EFL prevalence during exercise.

There were no sex differences in the prevalence of EFL when measured pre-puberty. Previous data from our lab has described an equally high prevalence of EFL (>90%) in both prepubescent boys and girls (Swain et al. 2010). Interestingly, in the current study we found a higher prevalence of EFL in post-puberty boys compared to post-puberty girls. Studies investigating adult populations point to a greater prevalence of EFL in women, due to smaller lungs and airways and reduced maximal flow rates (McClaran et al. 1998). Consequently, we hypothesized that we would see a greater decrease in EFL prevalence in post-puberty boys compared to girls. However, this was not the case. It is likely (as discussed below) that this can be partly explained by differences between boys and girls in the timing of growth and maturation of the pulmonary system during puberty (Seely et al. 1974).

The prevalence of EFL in post-puberty boys differs from what has been reported in men. Lungs in adult men with aerobic capacity <165% predicted are typically considered to be “overbuilt” with regard to the ventilatory demand placed on them during exercise (Dempsey 1986). However, it has been described that the prevalence of EFL is high (>90%) in aerobically trained men (Johnson et al. 1992), although there is not universal consensus with regard to the prevalence of ventilatory constraint in this population (Mota et al. 1999; Guenette et al. 2007). Since the degree of ventilatory

constraint is modulated by the ventilatory response during exercise (Johnson et al. 1992), it is unlikely that the vast majority of healthy, untrained adult men experience EFL, even during maximal exercise. Hence, the finding that 45% of our boys experienced EFL during the post-puberty assessment deserves attention. This occurred despite an average  $\text{VO}_{2\text{max}}$  in this group. A likely explanation is that the boys in our study had not yet achieved full maturation of the pulmonary system, as maturation has been shown to occur at approximately age 18 years in males (Seely et al. 1974). In our sample, the five boys who demonstrated EFL exhibited significantly lower expiratory flow rates ( $\text{FEV}_1/\text{FVC}$ ,  $\text{FEF}_{25-75\%}$ , and  $\text{FEF}_{50\%}$ ) compared with boys who did not exhibit EFL, despite no differences in body composition,  $V_{\text{Emax}}$ , or  $\text{VO}_{2\text{max}}$ . Because growth of the pulmonary system has been shown to lag behind anthropometric growth during puberty (Seely et al. 1974; Wang et al. 1993), it is likely that the boys in the current study were still experiencing maturation of the lungs and airways. This may have contributed to a prevalence of EFL that is higher than what has been reported in men.

It is believed that girls reach full maturation of the pulmonary system at approximately age 14 years (Seely et al. 1974). In this context, it is likely that post-puberty girls presently studied had nearly mature lungs and airways. This postulation is supported by the finding that only 20% of our post-puberty girls exhibited EFL during exercise. Further, the two girls who demonstrated EFL displayed significantly greater  $\text{VO}_{2\text{max}}$  compared with non-EFL girls. In a study by McClaran et al. (1998), it was found that highly-fit women had a significantly higher prevalence of EFL compared to less-fit women, which agrees with our findings. So overall, possibly due to a nearly mature



pulmonary system, the prevalence of EFL that we found in post-puberty girls is similar to that of untrained women (McClaran et al. 1998).

### **Determinants of Expiratory Flow Limitation**

Several possibilities exist to explain the reduced prevalence of EFL from pre- to post-puberty, including increase in lung size, decrease in  $V_E/V_{CO_2}$ , and changes in aerobic capacity. Pertaining to increased lung size, it has been documented that the lungs and airways grow from birth until approximately age 14 in girls and age 18 in boys (Seely et al. 1974), demonstrated by improved functional measurements (e.g. FVC, FEV<sub>1</sub>, FEF<sub>25-75%</sub>) from childhood through the end of puberty (Wang et al. 1993). As expected, data from the current study demonstrate a substantial growth in lung and airway size from pre- to post-puberty, reflected by approximately twofold increases in TLC, FVC, FEV<sub>1</sub>, FEF<sub>25-75%</sub>, and DLCO in both boys and girls. It is likely that the improvement in resting pulmonary function had a considerable impact on reducing EFL prevalence from pre- to post-puberty.

Because structural differences in the pulmonary system have been shown to exist between men and women (Mead 1980), and have been implicated with an increased prevalence of ventilatory constraint in women (McClaran et al. 1998), we also wanted to examine sex differences with regard to resting pulmonary function. It has been well documented that prepubescent boys have larger lungs and airways than girls, even when matched for standing height (Thurlbeck 1982; Kivastik and Kingisepp, 1997). Our data is supportive of this previous work, as pre-puberty boys exhibited significantly greater lung volumes and maximal flows compared to girls at rest, despite no difference in height between sexes. Similarly, post-puberty boys in our study also showed significantly

greater lung volumes and maximal flows compared to post-puberty girls. In adults, it is thought that this sex difference in pulmonary function contributes to an increased prevalence of ventilatory constraint in women compared to men (McClaran et al. 1998; Guenette et al. 2007). However, in our subjects, despite greater resting pulmonary function in post-puberty boys compared to girls, more boys displayed EFL than girls. This was an unexpected finding, but can likely be explained by the incomplete maturation of the boys' lungs and airways, which does not typically occur until approximately age 18 (Seely et al. 1974).

Decreased ventilatory response during exercise could also reduce the prevalence of EFL. It has been described that children breathe “out of proportion” to ventilatory demand (Cooper et al. 1987) and that the slope of  $V_E$  and  $V_{CO_2}$  decreases with age (Nagano et al. 1998). Consequently, we also hypothesized that subjects would display a decreased  $V_E/V_{CO_2}$  from pre- to post-puberty. This hypothesis was supported by our data, because during high-intensity exercise (80% and 100%  $VO_{2max}$ ) both post-puberty boys and girls exhibited significantly lower  $V_E/V_{CO_2}$  levels compared to pre-puberty. Thus, there was a decrease in the relative ventilatory response to metabolic  $CO_2$  production. This aligns with previous research suggesting a decline in chemoresponsiveness from pre- to post-puberty in boys and girls (Marcus et al. 1994), which might explain in part a decrease in EFL prevalence during puberty. We also found a decrease in the dead space to tidal volume ratio ( $V_D/V_T$ ) from pre- to post-puberty in boys and girls. The decrease in relative dead space ventilation, alongside a decrease in chemoresponsiveness, possibly speaks to an overall increase in ventilatory efficiency from pre- to post-puberty. With respect to absolute minute ventilation during exercise,

there was a significant increase in  $V_{E_{max}}$  (~110%) from pre- to post-puberty, with both boys and girls displaying an approximate twofold increase. These findings agree with previous work demonstrating a substantial increase in  $V_{E_{max}}$  during the pubertal growth spurt (Prioux et al. 1997; Mercier et al. 1993). Although  $V_{E_{max}}$  was significantly greater in boys than girls at both time points, there was no difference in  $V_E/VCO_2$  between sexes when measured pre- or post-puberty. This is in agreement with previous literature that found no sex differences in chemosensitivity (determined by HVR and HCVR) in children, adolescents, or adults (Marcus et al. 1994). Therefore, although there is a sex difference in  $V_{E_{max}}$ , it does not appear to be determined by the relative ventilatory response to metabolic stimuli.

Finally, changes in aerobic capacity with maturation could explain the reduced prevalence of EFL. It has been well-documented that absolute  $VO_{2max}$  increases during pubertal growth in boys and girls (Sprynarova et al. 1987; Krahenbuhl et al. 1985). In our subjects, there was an increase in absolute (~110%) and relative (~15%)  $VO_{2max}$  in boys from pre- to post-puberty, while girls only showed a significant change with regard to absolute  $VO_{2max}$ . So overall both boys and girls showed an increased aerobic capacity from pre- to post-puberty, reflecting a greater ventilatory demand. It has been postulated that the prevalence of ventilatory constraint during exercise is determined in part by aerobic capacity, with both highly-fit men (Johnson et al. 1992) and women (McClaran et al. 1998; Guenette et al. 2007) demonstrating a high prevalence of EFL. This was true for post-puberty girls, as the two girls that demonstrated EFL presented a significantly greater  $VO_{2max}$  compared to girls that did not experience EFL. However, there was no difference in  $VO_{2max}$  between flow-limited boys and those that did not exhibit EFL.

Consequently, the relative importance of  $VO_{2max}$  in determining the prevalence of EFL, specifically with regards to changes from pre- to post-puberty, remains to be elucidated.

### **Regulation of Tidal Volume during Exercise**

There are several consequences of expiratory flow limitation. Studies in adults have suggested that EFL or impending EFL leads to an increase in end-expiratory lung volume (EELV), representative of dynamic hyperinflation (Babb et al. 1991; Pellegrino et al 1993; McClaran et al. 1999). Therefore, with decreased EFL prevalence from pre- to post-puberty in our subjects, we expected to see tidal volume regulated at a lower proportion of FVC when measured post-puberty compared to pre-puberty. However, our findings contradict this prediction. Overall, we found that post-puberty subjects breathed at higher lung volumes than pre-puberty subjects. Specifically, post-puberty boys and girls breathed at a higher ERV/FVC and lower IRV/FVC throughout the exercise protocol compared to when tested pre-puberty. These findings extend to both subjects that exhibited EFL and those that did not. Changes in lung volume regulation during exercise occurred despite a significant decrease in EFL prevalence from pre- to post-puberty in both sexes. A possible explanation could be that our post-puberty subjects breathed at higher lung volumes during exercise as a “strategy” to avoid EFL, utilizing the higher flow rates available with a leftward shift of the tidal flow-volume loop within the maximal flow volume loop (Babb et al. 1991). Conversely, our pre-puberty subjects increased ERV/FVC relatively less when they were prepubescent compared to when measured post-puberty and displayed a high prevalence of EFL. Thus, it is possible that the driving factor of the decreased EFL prevalence in our subjects from pre- to post-puberty was the change in lung volume regulation, especially considering the

proportional increases in  $V_{E\max}$  and FVC (representative of ventilatory demand and capacity, respectively). To our knowledge, there are currently no previous data suggesting that tidal breathing is regulated at a lower lung volume pre-puberty compared to post-puberty, or that these breathing regulatory changes lead to a higher prevalence of ventilatory constraint in prepubescent children. Hence, mechanistic conclusions must be drawn with caution. In adults, it is thought that dynamic hyperinflation only occurs in the presence of impending EFL and excessive ventilatory demand and that dynamic hyperinflation results in a greater elastic work of breathing (Babb et al. 1991; McClaran et al. 1999; Guenette et al. 2007). However, Rowland et al. (1997) suggested that lung compliance and airway resistance (determinants of work of breathing) may not develop in unison during maturation, which could partly explain changes in the most “economical” balance of tidal volume and breathing frequency used to accomplish exercise ventilation. Thus, for our post-puberty subjects it may have been more mechanically sustainable and advantageous to breathe at higher lung volumes and avoid EFL during exercise as compared to pre-puberty. Clearly, the regulation of tidal breathing within the maximal flow-volume loop is very important in the determination and occurrence of EFL. However, without objective work of breathing measures it is difficult to definitively attribute changes in lung volume regulation from pre- to post-puberty, and thus EFL prevalence, to differences in the work of breathing.

### **Implications of Expiratory Flow Limitation**

Several notable physiological outcomes that can limit exercise tolerance have been implicated for EFL and dynamic hyperinflation. Dynamic hyperinflation limits exercise tolerance by shortening the initial length of the inspiratory muscles and

compromising their force generation potential due to operating on a flatter portion of the compliance curve, creating a mechanical disadvantage (McClaran et al. 1999). The decreased inspiratory muscle length can lead to decreased inspiratory muscle endurance time (Tzelepis et al. 1988) and respiratory muscle fatigue (RMF; Johnson et al. 1993). During prolonged strenuous exercise, the body must accommodate the rising oxygen cost of breathing by redistributing blood from the active skeletal muscle to the respiratory muscles, thereby adversely affecting exercise tolerance (Harms et al. 1997). It is likely that our post-puberty subjects experienced high work of breathing during maximal exercise due to the dynamic hyperinflation that was demonstrated in this group. However, it is doubtful that post-puberty boys and girls experienced RMF since all incremental exercise tests in the present study were <15 minutes and therefore insufficient in duration to elicit RMF, based on findings in adults (Johnson et al. 1993).

Other deleterious outcomes have also been previously linked with the occurrence of EFL and dynamic hyperinflation during exercise. It has been reported that a high oxygen cost of breathing as seen in EFL and dynamic hyperinflation is strongly correlated with dyspnea or “perception of breathlessness” (Babb et al. 2008). It has also been shown that externally imposing EFL on healthy individuals during exercise intensifies reported dyspnea (Iandelli et al. 2002). Unfortunately, due to experimental limitations in the perception and reporting of dyspnea and perceived exertion in our young subjects, dyspnea levels are unavailable for the pre-puberty assessment. However, considering the high prevalence of EFL in our pre-puberty subjects, it is likely that many of our subjects experienced dyspnea during the maximal exercise test. Subjects that experienced EFL post-puberty reported greater levels of dyspnea compared to those that

did not experience EFL during maximal exercise. Also, post-puberty boys experienced higher levels of dyspnea compared to post-puberty girls.

With regards to gas exchange impairment and arterial desaturation, it has been reported in young women that there is a strong correlation between amount of EFL and severity of arterial desaturation (Walls et al. 2002). However, despite the high prevalence of EFL in our pre-puberty subjects, none experienced substantial arterial desaturation (<94%) as determined by pulse oximetry. Arterial desaturation also did not occur in any subjects post-puberty. It appears that, notwithstanding ventilatory constraint, pulmonary oxygen diffusing capacity and pulmonary capillary red blood cell transit time were adequate to prevent a decrement in SaO<sub>2</sub> during exercise.

### **Experimental Considerations**

A major strength of this study is the longitudinal design. To our knowledge, we are the first to investigate ventilatory constraint during exercise in the same cohort of subjects from pre- to post-puberty. Therefore, our research is a valuable contribution to the existing literature describing cardiopulmonary function during maturation. However, several considerations need to be addressed in the interpretation of our data. First, EFL measurement depends heavily on the accurate placement of tidal flow-volume loops within the maximal flow-volume loop (MFVL). In order to reduce variability, we recorded at least five tidal breaths before the subject performed the inspiratory capacity maneuver. Only tidal breaths accurately placed from inspiratory capacity maneuvers performed at functional residual capacity were used in the assessment of EFL. Also, our metabolic cart automatically corrects for ventilatory drift when there are differences in expiratory and inspiratory volumes. Finally, subjects performed maximal flow-volume

maneuvers at rest until consistent values were demonstrated and the subject felt confident with the procedure. Placement of tidal breaths within the MFVL (“overlap” method) is the most traditional and widely-used experimental practice for determination of EFL during exercise (Johnson et al. 1992; Johnson et al. 1999; McClaran et al. 1998; Swain et al. 2010). However, recently it has been postulated that the overlap method may result in false detection of EFL via thoracic gas compression and differences in volume and time history preceding the inspiratory capacity maneuver (Koulouris et al. 2002). In this context, several studies have utilized a newer technique that creates a negative expiratory pressure at the mouth in order to determine if there is an increase in flow when a larger pressure gradient is established (Guenette et al. 2007; Koulouris et al. 1997). However, while this methodology has been validated in mechanically ventilated patients (Valta et al. 1994) and patients with chronic obstructive pulmonary disease (Koulouris et al. 1995), to our knowledge it has never been validated in healthy subjects, specifically children. Several studies using negative expiratory pressures in adult men (Mota et al. 1999) and women (Guenette et al. 2007) have reported results incongruent with previous classical studies (Johnson et al. 1992; McClaran et al. 1998). Whether or not the use of negative expiratory pressures is superior for EFL detection as compared to the overlap method in healthy subjects across a range of ages remains to be elucidated. With regard to the current study, we believe that the overlap method was the most appropriate methodology, as it has been used previously in children (Swain et al. 2010; Nourry et al. 2005; Nourry et al. 2006).

Unfortunately, dyspnea and rating of perceived exertion (RPE) were recorded only post-puberty during testing, as values were deemed undependable in the pre-puberty



measurement. Children appeared to have a challenging time comprehending and/or reporting their perceptions of difficulty during exercise.

Finally, pulse oximetry was used in the measurement of arterial oxygen saturation during exercise. Arterial catheterization was ethically unfeasible in our young subjects, therefore pulse oximetry was the only practical methodology, and is an acceptable form of measurement in this population (Nourry et al. 2004). To best ensure accuracy of our measures, the oximetry sensor was securely fastened to the left ear lobe and visual waveforms were constantly monitored during the exercise tests. Nevertheless, no subjects desaturated during exercise.

### **Future Directions**

The results of the present study point to additional questions for future research. First, while considerable pubertal growth had occurred in all of our subjects from pre- to post-puberty, our subjects had not yet reached full maturity. As expected, the results of our post-puberty subjects differ slightly from previous data reported in adults. It would be worthwhile to further track our subjects from this “snapshot” of puberty into adulthood, thus providing resolution to the story of ventilatory constraint from childhood to adulthood. Next, a deeper look into other factors that may contribute to the decreased prevalence of ventilatory constraint from pre- to post-puberty would be valuable. Specifically, dysanapsis (lung and airway size mismatch) has been shown to be a strong determinant of ventilatory constraint in adult women (Dominelli et al. 2011). An investigation into changes in the dysanapsis ratio from pre- to post-puberty may prove worthwhile. Also, it has been shown that modifiable lifestyle factors, such as physical activity and excess adiposity, can affect pulmonary function in children (Rosenkranz et

al. 2011; Rosenkranz et al. 2012). The extent to which lifestyle habits such as diet and physical activity during maturation affect lung function and ventilatory constraint during exercise is yet to be determined.

## Conclusions

The findings of the present study indicate that the prevalence of ventilatory constraint (i.e. EFL) decreases from pre- to post-puberty in both boys and girls. Contributory moderators of this change are twofold increases in lung size (i.e. TLC, FVC) and maximal mid-flows (i.e.  $FEF_{50\%}$ ,  $FEF_{25-75\%}$ ) and less of a ventilatory response to metabolic stimuli (represented by  $V_E/VCO_2$ ) compared to prepubescence. However, there also was a ~110% increase in absolute  $VO_{2max}$ . Interestingly, there appears to be a change in lung volume regulation, with pre-puberty subjects (demonstrating a high prevalence of EFL) regulating tidal breathing at lower lung volumes during exercise compared to post-puberty subjects. The difference in lung volume regulation could also help explain differences in prevalence of EFL from pre- to post-puberty, as breathing at a lower lung volume places an individual at an increased risk of their tidal breath intersecting with the effort-independent portion of the maximal flow-volume loop. However, it is likely that other variables contribute to the decrease in EFL prevalence, such as dysanapsis and lifestyle factors, and we recommended that these be explored in the future.

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