

**Title of Study:** Respiratory Effects of Obesity in Children  
**Document:** Study Protocol and Statistical Analysis Plan  
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## Overall Strategy

We will measure respiratory function at rest, exercise tolerance during graded cycle ergometry, and ratings of perceived breathlessness during constant load submaximal cycling in all subjects before and after 1 yr. We will study 72 prepubescent obese boys (n=36) and girls (n=36) including 12 boys and 12 girls misdiagnosed with asthma (BMI > 95<sup>th</sup> percentile, but less than 150% of the 95<sup>th</sup> percentile), and 24 prepubescent normal weight boys (n=12) and girls (n=12) (BMI between 16<sup>th</sup> and 84<sup>th</sup> percentile). Recently, there has been a shift towards classifying child BMI over the 95<sup>th</sup> percentile as a percent of the 95% percentile because the statistical procedures used to generate percentile curves are not accurate at the extreme tails of BMI distribution<sup>40</sup>. We have chosen 150% of the 95<sup>th</sup> percentile as a reasonable cut-off or upper-limit because it will exclude children with an average age and sex specific BMI of approximately > 35 kg/m<sup>2</sup> (BMI Range 33.2–36.3 in boys and 34.5–37.9 in girls), which roughly corresponds to Class II obesity in adults. Our approach will enable us to study and compare boys and girls while pubertal effects on lung function and exercise responses are minimized<sup>76</sup>. Furthermore, we are **not** studying the effectiveness of the intervention stimulus, the dose response of diet and exercise, or the rate of weight loss but only the response to 1) weight loss and regular exercise or 2) continued weight gain.

### Specific Aim 1: Respiratory Function at Rest in Obese Children

**Aim 1a. Introduction: at Rest Seated Upright.** The *working hypothesis* is that childhood obesity will alter pulmonary function and breathing mechanics while seated upright but to a greater extent in obese children with respiratory symptoms misdiagnosed as asthma. The *rationale* behind the proposed research centers on the fact that nonobese children already breathe at very low lung volumes<sup>21</sup> and have compliant chest walls<sup>2,21,89</sup> and excess fat on the chest will push FRC to lower levels<sup>28,81</sup> where obese children may experience breathing limitations, all because of a simple reduction in lung volume. Furthermore, we propose that obese children with respiratory symptoms misdiagnosed as asthma will have greater changes in lung volumes and/or differences in peripheral airways function (e.g., resistance or reactance or both) making them more susceptible to low lung volume breathing.

**Methodology, experimental protocol, and measurements.** The strategy will be to measure body composition and respiratory function while seated upright. Height, weight, circumference measurements (chest, waist, hip, and neck), BMI, and body composition by DEXA (e.g., %body fat, fat mass, lean body mass, & distribution of chest wall fat) will be used to characterize differences in overall body size and general fat distribution (e.g., weight-to-height, waist-to-hip ratios, android/gynoid measures, & chest wall fat distribution by segmental analysis of the DEXA scan) among groups. Baseline respiratory function tests will include spirometry before and after bronchodilators (i.e., to assess airway hyperresponsiveness), airway resistance (including impulse oscillometry system to examine central & peripheral airway function), lung volumes by plethysmography, diffusing capacity, MVV, MIP, and MEP. Participants who show clinically significant airway hyperresponsiveness (i.e., to bronchodilator or bronchial challenge) will be referred for treatment and excluded from further study (~8-9% prevalence in children, CDC). The absence of asthma will be confirmed by a negative response to spirometry before and after bronchodilator and by a negative bronchial challenge test (i.e., methacholine) in obese children with a prior diagnosis of asthma without confirmation by lung function testing<sup>1,45</sup>.

**Expected results.** We *expect* that altered respiratory function and breathing mechanics will be both prevalent and significant in obese children, primarily a reduced FRC, which may be correlated with other obesity-related changes in body composition. Furthermore, we expect that obese children with respiratory symptoms misdiagnosed as asthma will have greater changes in obesity-related respiratory function and/or differences in peripheral airways function making them more susceptible to low lung volume breathing. We also *expect* to have a better understanding of the magnitude, mechanism, and importance of the respiratory effects in obese children and the differences (if any) between obese boys and girls.

Interpretation of results. We will interpret these results to suggest that obese children have significant obesity-related alterations in respiratory function, which predispose them to potentially clinical breathing limitations especially in obese children misdiagnosed with asthma, all through a common mechanism of low lung volume breathing. This is important knowledge for the healthcare provider who may assume obese children have mostly normal lung function and that any respiratory symptom in obese children is primarily related to asthma.

**Aim 1b.** Introduction: at Rest Lying Supine. The *working hypothesis* is that childhood obesity will alter respiratory function and breathing mechanics when lying supine. Also, we propose that obese children with respiratory symptoms misdiagnosed as asthma will have greater changes in lung volumes when supine and/or differences in peripheral airways function making them more susceptible to low lung volume breathing. The *rationale* behind the proposed research centers on the fact that excess fat on the chest wall will push FRC to extremely low levels in the obese children especially when lying supine, thus creating the potential for significant breathing limitations especially in obese children misdiagnosed with asthma. To further test the hypothesis, we will add an external weight (2.5kg) between the xiphoid process and umbilicus of supine nonobese children and measure the change in FRC. This weight is lower than the 5kg that we used in preliminary testing, and is representative of the difference in chest wall fat observed between our obese and nonobese children.

Methodology, experimental protocol and measurements. The strategy will be to measure respiratory function and breathing mechanics when lying supine at the cardiopulmonary exercise station. Baseline data will consist of 15 min of sitting upright in a chair. After obtaining a steady-state, we will measure HR, SpO<sub>2</sub>% (by pulse oximetry), P<sub>ET</sub>CO<sub>2</sub>, ratings of perceived breathlessness (RPB; Borg 0-10 scale), respiratory function (FVC&IC by spirometry, FRC by helium dilution, & calculate TLC=IC+FRC) including breathing pattern and tidal flow-volume loops<sup>8,9,15,47</sup>. Measurements will be made in duplicate, and averaged for analysis, which has worked well in preliminary studies in children. During 15 min of lying supine, we will measure the same variables in the same way as above. In nonobese children only, we will add a 2.5kg external weight between the xiphoid process and umbilicus to simulate the effects of abdominal fat on FRC and make the same measurements.

Expected results. We expect that in comparison with nonobese children many obese children will have a marked reduction in FRC when lying supine, which may be correlated with other obesity-related measures of body composition. Furthermore, we expect that obese children with respiratory symptoms misdiagnosed as asthma will have greater changes in obesity-related respiratory function when lying supine. We anticipate a decrease in FRC with the external abdominal weight in the nonobese children. We expect this change will be *qualitatively* similar to that observed in obese children. We also *expect* to have a better understanding of the importance of changes in supine respiratory function in obese children, and the differences (if any) between obese boys and girls.

Interpretation of results. We will interpret these results to suggest that obese children have significant obesity-related alterations in respiratory function when lying supine, which predispose them to potentially clinical breathing limitations, especially in obese children misdiagnosed with asthma, all through a common mechanism of low lung volume breathing. This is important knowledge for the healthcare provider who sees obese children with symptoms of orthopnea or sleep related complaints. These findings would stress the need for further clinical/physiological testing in obese children with symptoms when lying supine (i.e., more testing than simple seated spirometry).

### **Specific Aim 2: Exercise Tolerance in Obese Children**

Introduction: Exercise Tolerance. The *working hypothesis* is that respiratory effects of obesity in children will decrease exercise tolerance (i.e., decreased peak  $\dot{V}O_2$  in ml/min/kg) but **not**

cardiorespiratory fitness (i.e., normal peak  $\dot{V}O_2$  in percent predicted based on ideal body weight), except in those misdiagnosed with asthma. The *rationale* behind the proposed research centers on the fact that conventional thinking suggests that obese children are 'out of shape' or deconditioned, but when displayed appropriately, cardiorespiratory fitness will be within normal limits during graded cycle ergometry. However, in obese children misdiagnosed with asthma, cardiorespiratory fitness may be decreased also due to respiratory symptoms and/or avoidance of exercise.

Methodology, experimental protocol and measurements. The strategy will be to measure HR, BP, SpO<sub>2</sub>%, P<sub>ET</sub>CO<sub>2</sub>, gas exchange ( $\dot{V}_E, \dot{V}O_2, \dot{V}CO_2$ , RER), RPB (Borg 0-10 scale), RPE (6-20 scale), and breathing mechanics (IC, EELV, EILV) including breathing pattern and tidal flow-volume loops continuously during maximal graded cycle ergometry (step increments of 10W/min) using standard methods as required for participant safety. A maximal flow-volume loop will be measured before and immediately after the exercise to assess the possibility of bronchodilation or bronchoconstriction (i.e., exercise induced airway reactivity) during exercise.

Peak exercise capacity will be evidenced by peak HR, peak work rate, peak  $\dot{V}O_2$ , and peak RER obtained during graded cycle ergometry as well as by verification at 105% of maximal work rate to exhaustion<sup>67</sup>.

Expected results. We expect that in comparison with nonobese children, obese children will have decreased exercise tolerance due to increased body weight but normal cardiorespiratory fitness when peak  $\dot{V}O_2$  is properly evaluated, despite altered breathing mechanics (e.g., EELV, expiratory flow limitation) during peak exercise. However, we also expect cardiorespiratory fitness may be decreased in obese children misdiagnosed with asthma. We also expect to have a better understanding of obesity-related alterations in respiratory function during peak exercise in obese children and the differences (if any) between obese boys and girls.

Interpretation of results. We will interpret these results to suggest that obese children are not deconditioned despite significant obesity-related alterations in breathing mechanics during peak exercise, except in obese children with respiratory symptoms misdiagnosed as asthma where cardiorespiratory fitness may be reduced also. This could be due to greater breathing alterations or an avoidance of exercise. These findings are extremely important for healthcare providers who examine fitness and associations with chronic disease in obese children, and prescribe weight loss and exercise programs.

### **Specific Aim 3: Dyspnea on Exertion in Obese Children**

Introduction: DOE During Constant Load Submaximal Exercise. The *working hypothesis* is that childhood obesity will increase DOE and alter respiratory function during constant load submaximal cycling, but to a greater extent in obese children with respiratory symptoms misdiagnosed as asthma. The *rationale* behind the proposed research centers on the fact that exercising will force EELV to even lower levels in obese children creating the potential for significant breathing limitations. If low enough, obese children could experience tidal expiratory flow limitation, constrained expiratory tidal volume expansion, and decreased ventilatory capacity. It is unclear whether these respiratory effects or changes in body composition will be linked with DOE in obese children (i.e., increased RPB as it is in obese adults). Furthermore, we propose that obese children with respiratory symptoms misdiagnosed as asthma will have greater changes in EELV or differences in peripheral airways function making them more susceptible to the limitations of low lung volume breathing and/or increased ratings of dyspnea on exertion.

Methodology, experimental protocol, and measurements. The strategy will be to determine respiratory function, breathing mechanics, and DOE during 6 minutes of constant load submaximal exercise cycling at 30W (8-9 yr olds) or 40W (10-12 yr olds) and during 6 minutes of constant load cycling at 50% of peak  $\dot{V}O_2$ . We will measure HR, BP, SpO<sub>2</sub>%, P<sub>ET</sub>CO<sub>2</sub>, gas

exchange ( $\dot{V}_E$ ,  $\dot{V}O_2$ ,  $\dot{V}CO_2$ , RER), RPB (Borg 0-10 scale), RPE (Borg 6-20 scale), and breathing mechanics (IC, EELV, EILV) including breathing pattern and tidal flow-volume loops continuously by standard methods used in prior studies<sup>8,9,15,47</sup>. RPB and RPE will be measured every 2 min during the tests and the last values recorded will be used for analysis. We chose 30W and 40W to represent roughly ventilatory threshold where the exercise is not too easy or too difficult, and the participants can exercise continuously for 6–10 min as needed to get adequate assessment of RPB. We chose 50% to examine RPB and EELV at the same relative intensity. We selected cycling exercise because stationary cycling is affected less by body weight (and in a predictable manner) than treadmill exercise, we can impose smaller work rates more precisely, and we can compare the results in obese children to our previous findings in obese adults.

Expected results. We expect to have shown that in comparison with nonobese children otherwise healthy obese children have an increase in RPB and altered respiratory function (i.e., reduced EELV and increased expiratory flow limitation) during submaximal cycling, which may be correlated with other obesity-related changes in body composition and resting respiratory function. However, we expect that obese children with respiratory symptoms misdiagnosed as asthma will have greater changes in EELV or airway function making them more susceptible to low lung volume breathing and/or greater DOE. We also *expect* to have a better understanding of the importance of obesity-related alterations in respiratory function and breathing mechanics in obese children during exercise, and the differences (if any) between obese boys and girls.

Interpretation of results. We will interpret these results to suggest that obese children have significant obesity-related alterations in DOE and respiratory function during submaximal exercise, which predispose them to potentially clinical breathing limitations, especially in obese children misdiagnosed with asthma, all through a common mechanism of low lung volume breathing. This is important knowledge for the healthcare provider who sees obese children with symptoms on exertion. On the other hand, if obese children have RPB values similar to that in nonobese children during exercise, without association with other obesity-related changes, this would imply that increased chest wall fat and a lower EELV do not impair breathing during exercise as in obese adults, which would be important to document.

#### **Specific Aim 4: Effects of Weight Loss and Regular Exercise and Weight Gain in Obese Children**

Introduction. The *working hypothesis* is that weight loss (or an equivalent reduction in BMI percentile) and regular exercise will improve respiratory function, exercise tolerance, and DOE in obese children, including obese children misdiagnosed with asthma, while continued weight gain (or increase in BMI percentile) will worsen respiratory function, exercise tolerance, and DOE in obese children, including obese children misdiagnosed with asthma, as compared with normal weight children after 1 yr. The *rationale* behind the proposed research centers on the fact that FRC at rest, EELV during exercise, and peak  $\dot{V}O_2$  in ml/min/kg will be increased in obese children after weight loss and regular exercise but decreased by continued weight gain. The cumulative effect of the weight loss program would thereby reduce the potential for breathing limitations at rest and/or during exercise, increase exercise tolerance, and potentially reduce DOE in obese boys and girls, including those misdiagnosed with asthma, but weight gain would have the reverse effects.

Methodology, experimental protocol and measurements. The strategy will be to repeat the measurements detailed in Aims 1-3 after 1 yr: 1) in 12 obese boys and 12 obese girls after weight loss (or an equivalent reduction in BMI percentile) and regular exercise, 2) in 12 obese boys and 12 obese girls after weight gain (or an increase in BMI percentile), 3) in 12 obese boys and 12 obese girls misdiagnosed with asthma regardless of weight changes or exercise participation, and 4) in 12 normal weight boys and 12 normal weight girls. We will test at 1 yr because parents **prefer** to have testing done during summer vacation months only.

*Weight Loss and Regular Exercise* will be prescribed by attending physicians at the **COACH**

Program at Children's Medical Center (Director Dr. Olga Gupta is co-investigator, see Facilities & Letter of Support). However, all obese children will receive standard of care information regarding weight loss and physical activity if they elect not to enter **COACH**. At **COACH**, the participant and parents will meet with a registered dietician who will perform a diet assessment and offer a meal plan for an initial weight reduction of  $\approx 10\%$  of body weight or an equivalent reduction in BMI percentile (e.g., growth of 2.5 cm would lower BMI percentile by  $\approx 1$  based on CDC growth charts). This change in weight or in BMI should yield significant changes in respiratory function based upon our prior adult data<sup>9</sup> and our preliminary data. Participants will then meet with nurses, and physical therapists in the **COACH** behavior modification class where they will be encouraged to participate in 60-min of moderate intensity physical activity per day<sup>3</sup>. Finally, they will meet with the physician who will discuss clinical test results (i.e., blood work to eliminate diabetes, impaired glucose tolerance, impaired fasting glucose, dyslipidemia, liver enzyme elevation, vitamin D deficiency or insufficiency), and review the overall diet and physical activity plan. We will schedule retesting after 1 yr.

To increase the likelihood of success of the weight loss and regular exercise, we will establish a biweekly phone-contact schedule with **COACH** participants. We will not interfere with the dietary and medical recommendations set forth by **COACH** faculty, but we will act as liaisons and supporters. Specifically, we will be available to answer any questions that might arise regarding diet and physical activity recommendations. The results of the constant load and incremental exercise tests will be used to provide a guide of appropriate physical activity intensities (e.g., based on VTh, peak HR, peak  $\dot{V}O_2$ , & ACSM guidelines). We will invite the participants and parents to meet with us and we can demonstrate these physical activity intensities on multiple exercise devices depending on the preferences of the patient (e.g., stationary cycle, treadmill, etc.). Our goal will be to encourage regular exercise (i.e., increase energy expenditure), not to design an exercise training program for improvement of cardiorespiratory fitness. Consequently, activity levels will not be quantified (i.e., controlled & supervised exercise intensity, duration, & frequency). We will note biweekly whether the children have been active as recommended and we will administer the Physical Activity Questionnaire-Children (PAQ-C) before and after 1 yr.

All children who completed Aims 1-3 will be tested at 1 yr whether they lose weight or gain weight. The retesting in nonobese children will be used as control data to document the effect of age and growth on respiratory function, exercise tolerance, and DOE.

Expected results. We expect that a program of weight loss (or an equivalent reduction in BMI percentile) and regular exercise will increase FRC at rest and EELV during exercise while weight gain will decrease these. The change in FRC and EELV may be correlated with changes in body composition (if any). These improvements in respiratory function and breathing mechanics may also be associated with decreased DOE as well, while weight gain would have the reverse effects. Exercise tolerance will be increased in ml/min/kg but we expect no change in cardiorespiratory fitness (i.e., Peak  $\dot{V}O_2$  as percent predicted based on ideal body weight), which may actually be slightly decreased, including those misdiagnosed with asthma. With weight gain, exercise tolerance will be decreased but cardiorespiratory fitness would be unchanged as well. We also *expect* to have a better understanding of the importance of weight loss and regular exercise and weight gain on respiratory function and breathing mechanics in obese children, and the differences (if any) between obese boys and girls.

Interpretation of results. We will interpret these results to suggest that weight loss (or an equivalent reduction in BMI percentile) and regular exercise will have a significant improvement on respiratory function, exercise tolerance, and DOE, while weight gain will worsen respiratory function, exercise tolerance, and DOE in obese children all through a common mechanism of increasing or decreasing EELV. Weight loss may also reduce potential clinical breathing limitations at rest and during exercise (e.g., expiratory flow limitation) but weight gain would increase limitations. This would be important knowledge for healthcare providers who may not appreciate the obesity-related respiratory effects in obese children, and who may assume that

symptoms such as breathlessness are just due to cardiovascular deconditioning and/or asthma. In contrast, if obese children have a simple increase in EELV with weight loss without association with other changes in respiratory function, exercise tolerance, and DOE, this would be clinically important to document as well. The same would be true if there were no negative effects of weight gain. These findings, while very unlikely, would suggest that chest wall fat and changes in respiratory function are not the lone sources of obesity-related breathing limitations and bring to question what effects weight loss and regular exercise, and weight gain might have on respiratory function, exercise tolerance, and DOE.

### **Subject Selection**

Prepubescence. We have elected to study the effects of obesity in otherwise healthy prepubescent children for three reasons. One, this level of obesity is very prevalent in prepubescent children. Two, they may benefit the most from early intervention to prevent the development of further obesity, increased cardiovascular risk factors, and/or comorbidities like type-2 diabetes, which usually manifest after puberty. Three, boys and girls should have very similar predicted respiratory function values<sup>64</sup> and pubertal effects on exercise responses should be minimal<sup>76</sup>.

Matching of groups. Nonobese children will have age and sex specific BMI between 16<sup>th</sup> and 84<sup>th</sup> percentile in order to exclude children who are overweight and/or possibly underweight. Both the groups will be otherwise healthy since most obesity-related metabolic issues occur after puberty.

Recruitment of subjects. We will recruit participants by BMI percentile, age, and gender only. We will recruit participants from large pediatric practices, Children’s clinics (My Children’s Clinics), emails throughout Texas Health Hospital System, paid sites such as Center Watch and Sunshine Day, community programs (e.g., 7 Day Fitness Academy, Parkland Hospital Health Camps, Get UP and Go programs), advertisements in local newspapers, and ads in other media sources). Also, approximately 1250 patients enter the **COACH** program per year with an average age of 11.6±2.9 yr. Males make up 42% of the population and race/ethnic background is approximately 55/25/4/16 (W/AA/H/O). We are very confident in our ability to recruit and retain participants. Our preliminary retention numbers are quite good (14 of 16). Also, based upon our preliminary data, 50% of the obese children we tested last summer lost weight and 50% gained weight, and we had no contact with these children since last summer. We will recruit heavily before and during summer. We will recruit, and search COACH and medical practice records for children diagnosed with asthma who have not had pulmonary function tests to confirm the presence of asthma.

Subjects. A total of 96 prepubescent children, 48 boys (36 obese) and 48 girls (36 obese), who are otherwise healthy, will complete all four aims. 24 of these obese children (12 girls & 12 boys) may have respiratory symptoms, which were misdiagnosed as asthma (i.e., not confirmed by lung function tests). We will confirm the absence of asthma by spirometry before and after bronchodilator, and by bronchial challenge test with methacholine<sup>1,45</sup>. We will include children between **8-12** years old as long as they have a **Tanner stage ≤ 3** and can successfully perform pulmonary function tests, exercise tests, and ratings of breathlessness. We anticipate that children less than 8 yr old could have difficulty performing accurate pulmonary function and exercise tests and will be excluded from participation<sup>45</sup>. Including children within 8-12 years old should increase the likelihood of meeting our recruitment goals. In future studies we anticipate studying older children and adolescents (also see Subject criteria Human Subjects Section).

### **Statistical Considerations**

Co-investigator Beverley Huet (UT Southwestern Division of Biostatistics) is the statistical co-investigator on this project and has assisted in the development of the statistical plan; she will also

Total n=96	Obese	Obese misdiagnosed with asthma	Non- obese
Boys	n=24	n=12	n=12
Girls	n=24	n=12	n=12

direct the data analysis on an on-going basis.

Statistical Plan. Our plan is to complete testing in nonobese and two obese groups, boys and girls separately but concurrently. Thus, sample size calculations are based upon within-gender comparisons per allocation in table at right. In Aims 1-3, comparisons will be made between nonobese, obese, and obese children misdiagnosed with asthma within the same gender with analysis of variance models (ANOVA). Pairwise group comparisons will be made using least squares means contrasts derived from the linear models. Data transformations or nonparametric tests will be utilized as needed to meet analysis assumptions. At baseline, ISSAC and ECRHS asthma screening questionnaire scores will be compared among groups with the Kruskal-Wallis test. Comparisons for Aim 4 will be made similarly, but with the addition of repeated measurements to assess the two obese groups and the nonobese children of the same gender at baseline and at 1 year of follow-up. Additional assessments of gender effects will be performed as part of a secondary analysis, but we are not powering the study to detect interaction by gender. Alpha is set at 0.05 for ANOVA and planned contrast power (PASS 11, NCSS, LLC, Kaysville, UT), described below <sup>75</sup>. Data will be analyzed using SAS version 9.4 (SAS Institute, Inc., Cary, NC).

Aim 1. Our primary variable is FRC measured while seated upright (Aim 1a) and when lying supine (Aim 1b). Based upon our preliminary data for seated FRC (%TLC) in nonobese boys (mean± SD, 42.4±2.5) and obese boys (36.5±6.2), it is calculated that a mean difference in FRC (%TLC) of 5.1 can be detected with statistical power of 81%, alpha=0.05, pooled SD of 5.0, comparing 24 obese to 12 nonobese children or to 12 obese children misdiagnosed with asthma. Our data also show that the differences may be larger for girls than boys, so power for a test between obese girls and obese girls misdiagnosed with asthma will be at least 80%. Similarly, for children lying supine, nonobese boys have an FRC (%TLC) of 34.4±8.8 vs. 23.1±3.4 obese, a more conservative difference of 6.0 %TLC is detectable with 80% power. In our formal protocol, we anticipate being able to make precise and reproducible measurements, and have confidence that we can meet our goals with the sample size proposed. Preliminary data demonstrate that these measures and effect sizes are feasible.

Aim 2. Our primary variable is peak  $\dot{V}O_2$ . From our preliminary peak  $\dot{V}O_2$  (ml/min/kg) for nonobese (41.9±4.9) and obese boys (26.3±3.9), it is calculated that our study will yield power of 80% to detect a clinically relevant mean difference of 4.7 ml/min/kg for obese versus nonobese children or obese children misdiagnosed with asthma, anticipating smaller differences than the 16 ml/min/kg mean difference observed from our data in obese versus nonobese. Effect size estimates for girls are anticipated to be similar.

Aim 3. Our primary variable for Aim 3 is RPB during constant load exercise. Preliminary data from children in our lab show that RPB at constant load is very similar between nonobese and obese children of both genders (e.g., girls, 3.4±0.6 vs. 3.3±1.5), which would not be detectable with a reasonable sample size. This is in contrast with our previous studies of RPB in nonobese (2.1±1.3) <sup>46</sup> and obese women with DOE (4.7±1.2) <sup>8</sup>, where only 6 subjects would have been necessary per group to detect significant differences. At this time our preliminary sample of children is small, and we anticipate that future experience could be more like that in the adults, but we need to carry out the designed studies at a feasible sample size (96 total including nonobese controls) to find out. These effects contrast with another important variable for Aim 3; the EELV during constant load exercise. Based upon EELV (%TLC) data from obese girls (28±3) during exercise, we would be able to detect differences of 3.2%TLC between the 3 groups at greater than 80% power (also note how low the EELV is in the obese children during exercise). We expect similar differences and power in studies with boys.



**Aim 4.** Primary variables for Aim 4 are change in FRC at rest, peak  $\dot{V}O_2$ , RPB, and EELV during constant load exercise from before to after 1 year of follow-up. We are gaining experience with studies in children; current preliminary data from n=7 obese children yield similar mean differences and standard deviations as in adults. Therefore, based upon the change in EELV during constant load exercise in obese men before ( $35 \pm 5$ ) and after ( $39 \pm 3$ ) a loss of 13% of body fat<sup>9</sup>, and the change in RPB during constant load exercise in obese women before ( $4.7 \pm 1.1$ ) and after ( $3.2 \pm 1.6$ ) weight loss, our sample will have sufficient power (.80,  $\alpha=0.05$ ) to detect meaningful paired differences (e.g., 1.5 in RPB) with weight loss and regular exercise. We will retest all children after ~1 yr. We expect less than 10% attrition and repeated data on nearly all children will be available for this Aim. Combining the two obese groups (with and with asthma misdiagnosis), the children will be divided into obese who lost weight, obese who gained weight, and control normal weight children and respiratory changes will be compared between groups with ANOVA as described for Aims 1-3. While these categorized weight changes have clinical appeal, weight change analyzed as a continuous variable may be more powerful and informative. Hence, change at 1 year minus baseline will also be evaluated with Spearman or Pearson correlations to quantify the association between 1-year weight change and the change for respiratory function, exercise tolerance, and DOE variables and, with the 36 obese participants analyzed (by gender), a correlation of 0.45 can be detected with 80% power.

To evaluate gender effects, baseline and follow-up measurements for FRC, peak  $\dot{V}O_2$ , RPB, and EELV will be analyzed in mixed-effects repeated measures models with the study participant included as a random effect. Interactions between gender, study group (obese, obese misdiagnosed with asthma, nonobese), and time can be assessed as fixed effects; weight change effect will be modeled as a covariate. PAQ-C score changes will also be assessed with repeated measures models.

#### **Anticipated problems and potential solutions**

The anticipated changes in respiratory function (particularly FRC) should be apparent at this level of obesity. A 10.5 yr old boy at 4' 8 1/2" or 143.3 cm at the 95<sup>th</sup> percentile will have a body mass of 103-105 lb. The upper limit for that same child at 150% of the 95<sup>th</sup> BMI percentile is 155 lbs. Thus, the range for this example child is ~50 lbs (22.7 kg). This increase in weight is significant and should produce the type of effects we expect as confirmed in our preliminary data. However, if this does not hold true, we do not believe increasing the range of obesity to include those with BMI scores > 150% of the 95<sup>th</sup> percentile would yield a subject population that is representative of the majority of obese children. We intend to perform testing for Aims 1a and 1b in two sessions ( $\approx 2.5$  hr each). This approach has been very attractive to the children and parents without complaint and the children have performed upright and supine respiratory function tests without difficulty. Also, if the weight placed on the abdomen is not tolerable, we will investigate other methods like a weighted vest or broader distribution of the weight over the chest wall. We have not found our standard graded cycle ergometry protocol to be a problem when testing peak performance in obese or nonobese children, especially when confirmed at supra-maximal work rates. However, if we find the protocol difficult for them, we can try different stage increments, or try a non-continuous exercise test to determine peak exercise capacity. Also, we believe that the level of obesity planned for this study will not preclude the obese children from completing exercise testing at 30 or 40W as in the nonobese children or with our ability to examine respiratory function, breathing mechanics, and DOE during exercise at 30 or 40W. Nevertheless, we will complete a second exercise session at 50% of peak  $\dot{V}O_2$  just to be sure. So far all procedures and testing has been very well-tolerated and without complaint. Weight loss success is always a concern that involves many personal and family-related issues.

However, all of the investigators are aware of these difficulties and have the insight and flexibility to try different techniques to overcome obstacles that may arise. However, by testing the participants who are not successful with weight loss but actually gain weight, we believe we have dramatically increased the value of Aim 4. However, some subjects may have no change in body composition or %body fat. We anticipate this will not occur very often since all children will gain weight, fat weight, and/or fat free weight over the course of 1 yr.

#### **Tentative Timetable**

It will take  $\approx$ 5 yr to complete the proposed studies. Roughly 30 to 60 children will be studied each year. Starting in year two, half will be repeat studies and half will be new participants. The last year will be devoted mostly to repeat studies. All projects will run concurrently but most of the data collection will take place in the summers with the exception of the obese children with respiratory symptoms misdiagnosed with asthma. These children may be able to complete testing throughout the year. We are highly confident that we can successfully complete this extremely important but ambitious project in 5 yr as planned.