THE INFLUENCE OF MATURATION ON THE OXYGEN UPTAKE EFFICIENCY SLOPE

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Background

Exercise testing with spirometry is an invaluable method of measuring physiological function. By measuring the amount of air inspired and expired, and the amount of oxygen consumed and carbon dioxide produced, spirometric measurements are a non-invasive means of assessing a number of cardiorespiratory and metabolic responses during exercise. Typical measures include oxygen uptake ($VO_2$), pulmonary ventilation, and the respiratory exchange ratio (RER). $VO_2$ is a measure of the rate at which an individual utilizes oxygen. The measure of $VO_2$ at a given movement speed is a measure of the economy or efficiency of exercise (67). A common $VO_2$ measurement during graded exercise testing is the maximal oxygen consumption of an individual ($VO_{2\text{max}}$). $VO_{2\text{max}}$ is an indicator of cardiorespiratory fitness and the maximum aerobic capacity of the body (78). The kinetic response of $VO_2$ to a step change in work rate reflects the oxygen and metabolic demands of skeletal muscle (39). Pulmonary ventilation is a measure of the rate of air flow in or out of the lungs per unit of time. When pulmonary ventilation is plotted as a function of
VO₂ during the course of a graded exercise test, the ventilatory threshold can be determined as the point where pulmonary ventilation begins to increase disproportionally to the increase in VO₂ (27). The RER provides insight into the fuel utilization pattern during exercise, whether carbohydrate or fat, as the stoichiometric ratios of CO₂ produced and O₂ used for oxidation of these energy sources differ (38).

The oxygen uptake efficiency slope (OUES) is a relatively new spirometric measurement in the assessment of the cardiorespiratory response to graded exercise. The OUES is established by calculating the slope of the line of the absolute oxygen consumption volume over the log transformed volume of ventilation: $VO_2 = a \log V_E + b$ where $a$ is the OUES (17). The log transformation of the Vₕ response allows for a linearization of the VO₂-Vₕ relationship and thus the calculation of the OUES. The OUES utilizes data from the entire duration of a graded exercise test; however, it is not necessarily dependent on achieving a maximal exercise response as is required when VO₂max is assessed (15, 17).

The physiological factors thought to influence OUES in addition to the influence of VO₂ and Vₕ are the arterial CO₂ set point (PaCO₂), VCO₂, and the ratio of pulmonary dead space to tidal volume (17). However, recent studies also have shown that age and size influence OUES, which prevents meaningful comparisons of OUES between age groups and people of different sizes (5, 32, 53). In addition, it is possible maturation may also influence OUES as measures
of VO$_2$, VCO$_2$, and V$_E$ during exercise are influenced by metabolism and metabolism is in turn affected by maturation (52).

**Statement of Purpose**

This proposed study sought to evaluate the OUES across different maturation stages (early puberty through young adult) in males of similar fitness levels (VO$_{2\text{max}}$) by examining two different means to size adjusting the OUES response among these groups. If normalizing across body size fails to eliminate differences between groups, the differences are likely a function of maturation. By following this approach, the independent effect of size versus maturation on OUES can be determined. Specifically, it was hypothesized that the normalized OUES will be higher in less mature versus more mature subjects. Because of the maturational influence on exercise metabolism, it was hypothesized that normalizing the OUES for differences in body size will not entirely eliminate differences across maturation groups in subjects with similar VO$_{2\text{max}}$ values.

**Significance of the Problem**

Assessment of cardiorespiratory fitness utilizing data that does not require a maximal effort could provide a useful measurement for researchers and health professionals alike given the demanding nature of a maximum graded exercise test and the effort needed to achieve a true measurement. This is especially
important with chronic disease populations whose condition may prevent them from achieving the standard criteria of a maximal exertion.

However to have meaningful comparisons between people of different sizes, there is a need to establish how different measures of body size influence the OUES. As an example of this, VO$_2$ (submaximal) is often expressed relative to body mass, as it is more relevant to ambulatory activity and VO$_2$ (maximal) is expressed relative to body mass order to normalize the variable to measure fitness with respect to varying physical sizes, metabolic needs, and maximum work rates. This allows for fitness and functional comparisons across different subject populations. Recent studies have shown that OUES is influenced by age, body size, and even the musculature of the secondary respiratory muscles (5, 32, 53, 72). Additionally, the two variables comprising the OUES are known to be affected by metabolism; so it is possible that the OUES might vary by maturation as a function of muscle metabolism (14). However, no such normalizing variable has been evaluated to provide comparisons between subjects of different size with a similar level of fitness, while the influence of maturation on the pulmonary and metabolic factors mediating the OUES has yet to be examined.
**Assumptions**

The following assumption was made in this investigation: Prior to each testing session, the subjects complied with the guidelines regarding food and fluid intake, restriction of vigorous physical activity, and avoidance of caffeine, alcohol, and tobacco.

**Delimitations**

The subject population was restricted to males 8-29 years of age. All subjects were healthy, physically active, not presently taking medications that would alter their cardiorespiratory responses to exercise, and none were trained athletes.
CHAPTER II
REVIEW OF LITERATURE

Introduction

This chapter will provide an overview of the scientific literature relevant to this study. The chapter will begin by discussing principles of gas exchange measurements and the physiologic insights gained from the use of these measurements. Growth and maturation patterns of children will then be presented followed by a discussion of appropriate scaling measures of data in the context of studying pediatric exercise. The chapter will continue with an examination of differing metabolic responses to exercise in children compared to adults. Finally, the chapter will expound upon the OUES, going through its origins and interpretation, as well as its metabolic mediation and specific applications in the pediatric population. A brief summary will conclude the chapter.
Principles of Gas Exchange

Gas exchange measurements are a fundamental means of assessing physiological function during exercise. By measuring the rate at which $O_2$ is consumed, $CO_2$ is produced, and the pulmonary ventilation over the course of a bout of exercise a number of physiological insights can be quantified in a safe, noninvasive manner that is valid and reproducible. Measurement of gas exchange is based on following equations derived from the research conducted by Haldane (42):

\[
V_I = V_E \cdot \left(100 - (F_{EO2} + F_{ECO2})\right) \div F_{IN2}
\]

\[
VO_2 = (V_I \cdot F_{iO2}) - (V_E \cdot F_{EO2})
\]

\[
VCO_2 = (V_E \cdot F_{ECO2}) - (V_I \cdot F_{iCO2})
\]

Where $VO_2$ is the volume of oxygen consumed, $VCO_2$ is the volume of carbon dioxide produced, $V_I$ is the volume of inspired air per minute, $V_E$ is the volume of expired air per minute, and $F$ designates the fraction of gas as a percent of total air composition from a particular air sample, expired ($E$) or inspired ($I$). By measuring $V_I$ over a period of time multiplied by the percent concentration of oxygen at room air (accepted as 20.93%) and subtracting from it by the measured $V_E$ multiplied by the measured percent oxygen, the rate of oxygen consumption can be calculated. Conversely, by measuring $V_E$ over a period of time multiplied by the measured percent concentration of carbon dioxide and subtracting from it by the measured $V_I$ ventilation multiplied by the carbon
dioxide concentration at room air (accepted as 0.04%) the rate of carbon dioxide production can be calculated (86). In modern applications, these calculations are performed rapidly with computers using input values derived from electronic gas analyzers as the subject is exercising. By assessing pulmonary ventilation and gas exchange response during exercise, investigators are able to gain insight into a variety of metabolic processes in the body including VO$_{2\text{max}}$, locomotion economy, oxygen uptake kinetics, selective fuel utilization with RER, the ventilatory threshold, and ventilatory efficiency. These gas exchange measurements and their significance will be explained in the following paragraphs.

Maximal Oxygen Uptake

Perhaps the most common measurement assessed in the exercise physiology laboratory is VO$_{2\text{max}}$. VO$_{2\text{max}}$ is the accepted standard measurement of cardiorespiratory fitness (87) and is a strong prognostic indicator of patient mortality (26, 71). VO$_{2\text{max}}$ is also used to assess athletic performance in endurance and aerobically demanding sports (31), as it measures the aerobic capacity of an individual and subsequently the level of sustainable work that one can perform without rapid fatigue (30). As such, it is commonly assessed before and after an exercise training program in order to evaluate the effectiveness of the training regimen as well as to determine target training intensities (78). VO$_{2\text{max}}$ is commonly expressed in absolute terms as L·min$^{-1}$ and relative to body size as mL·kg$^{-1}$·min$^{-1}$. 
A graded exercise test, in conjunction with the measurement of pulmonary ventilation and gas exchange, is used to determine VO$_{2\text{max}}$, where the initial power output on a cycle ergometer (or speed and percent incline on a treadmill) is set low and increases at a set rate over predetermined time intervals until the subject can no longer continue to maintain the required power output (87). Numerous graded exercise testing protocols exist, running a spectrum of different modes of exercise, stage durations and workload rate increases. The Bruce (28) and Balke (19) protocols are two examples of tests used in clinical exercise evaluations. Graded exercise tests are generally targeted to last 8-12 minutes in duration with stage durations, workloads achieved, and workload changes customized to be feasible for the subject population and relevant to the measurements collected. The graded exercise test is able to reveal the subject's VO$_{2\text{max}}$ due to the direct relationship between power output (watts) and oxygen consumption presented in Figure 1 (41).

In order to ensure that the subject has performed to a maximal level of exertion, a number of criteria are often employed. The classically accepted
criteria is a plateau in the rise of VO$_2$, where VO$_2$ fails to increase despite a further increase in power output by the subject, indicating the achievement of the maximum rate of aerobic metabolism (77). This plateau is a challenging criteria to meet, and as such is seldom definitively observed at the end of a graded exercise test (49, 59, 71). Often investigators will report VO$_{2\text{peak}}$ values, indicating the highest VO$_2$ measured during a graded exercise test in the absence of a plateau in the power output / VO$_2$ relationship (9). Due to the rigid demands of the VO$_2$ plateau, other criteria are often utilized to help identify a maximal level of exertion that include, but are not limited to, an RER over a specified threshold, achieving a percentage of predicted maximal heart rate, or a high rate of perceived exertion (46).

Locomotion Economy

The ability to simultaneously monitor VO$_2$ and external power output during exercise also allows for measurements of locomotion economy. Economy refers to the rate of energy expenditure or VO$_2$, for a given speed of movement (if swimming or running) or a given power output (if using a bicycle or other type of ergometer) (46). Economy is an important endurance sport performance measurement, particularly in high level athletics as a better economy will allow an athlete to maintain a faster pace with less energy expended along with decreased heat production and thermal stress, both leading to a longer time to fatigue (58). Though not always observed to improve over the course of a training program (58), a preponderance of data reveals that athletes of higher
training status are more economical than their less trained or untrained counterparts (58, 69). Economy should not be confused and interchanged with efficiency, as efficiency deals with the effective driving force over the total force exerted on an object and does not involve measures of energy expenditure (87).

**Oxygen Uptake Kinetics**

In recent years, researchers have been able to use the time course of changes in VO$_2$ across stepwise changes in exercise intensity, referred to as VO$_2$ kinetics, in order to gain a quantifiable insight into the VO$_2$ of the working skeletal muscle. Through averaging repeated measures on a breath-by-breath basis, researchers can plot the rise in VO$_2$ in order to separate the curve of the VO$_2$ transition into distinct phases. The first initial rise in VO$_2$ is contributed directly to increases in blood flow through the action of the heart and lungs, followed immediately by phase II, whereby the VO$_2$ increases rapidly to the expected VO$_2$ levels for the given power output, reflecting the time course for the uptake and oxygen demand of the working muscle. Mapping the time course of these VO$_2$ kinetics through gas analysis can provide physiological insight to the metabolic response to changing exercise intensities, without invasive blood flow or muscle measurements (6).

**Ventilatory Threshold**

Ventilatory threshold (T$_{vent}$) is a common submaximal exercise measurement assessed during the course of a graded exercise test. One
common definition of $T_{vent}$ is the point during graded exercise when $V_E$ begins to increase out of proportion to $VO_2$ (29). This excess ventilation is thought to arise due physiologic stimulation of the central and peripheral chemoreceptors in response to the ensuing metabolic acidosis from $H^+$ ion accumulation brought on by intense exercise and the resultant in increase in $CO_2$ production. As $H^+$ ions are released from lactic acid, a product of anaerobic glycolysis, they are buffered in the blood by $HCO_3^-$ ions to become carbonic acid ($H_2CO_3$), which is transformed by enzyme carbonic anhydrase in the lungs into $H_2O$ and $CO_2$ to be expelled by the lungs (24). $V_E$ is chemostatically regulated primarily by blood $CO_2$, and hence is driven by $CO_2$ production, rather than $O_2$ consumption. Other physiological mechanisms that feedback to increase the respiratory response during exercise that can influence the $T_{vent}$ include, but are not limited to increased core temperature, increased potassium levels, increased circulating catecholamines, and efferent nerve responses from the working muscle (27, 61). $T_{vent}$ is often used as a performance measurement and functional assessment because it marks an intensity range beyond which point sustaining exercise for extended periods of time becomes difficult (63, 83).

$T_{vent}$ can be evaluated through multiple methods by examining a number of respiratory parameters in relation to one another. A common method of assessment is done by examining the breakpoint in the initial linearity of the relationship between $V_E$ over $VO_2$ and has been demonstrated to correlate well with the anaerobic threshold obtained from the more invasive blood lactate
measurement (29). Another method of evaluating the ventilatory threshold is the ventilatory equivalents method, where $T_{vent}$ is defined as the $VO_2$ value where the ventilatory equivalent for oxygen ($V_E/VO_2$ ratio) increases without a coinciding increase in the ventilatory equivalent for carbon dioxide ($V_E/VCO_2$ ratio) (37). More recently a method assessing the change in the relationship between $VCO_2$ over $VO_2$ has developed. This methods is termed the V-slope method, as it was based on analyzing the volume curves of the two gasses and was developed due to the dependence of other methods on the accumulation of metabolic acidosis, which occurs after the subject is above the anaerobic threshold (23). However, $T_{vent}$ as a cardiorespiratory measurement is not without shortcomings as the selection of particular breakpoints of various graphical displays of the ventilatory response to exercise can vary between mode of exercise, exercise protocol, and examining investigators (88) and in some individuals or under certain conditions may not even be detectable (62).

**Respiratory Exchange Ratio**

The RER is a non-invasive gas analysis measurement that provides insight into substrate utilization (9). This is due to the stoichemetric differences in the amount of CO$_2$ produced per amount of O$_2$ used to oxidize fat versus carbohydrates (24):

Glucose: $C_6H_{12}O_6 + 6 O_2 = 6CO_2 + 6H_2O$

Palmitic Acid: $HOOC(CH_2)_{14}CH_3 + 23O_2 = 16CO_2 + 16H_2O$
RER is calculated by dividing the volume of CO$_2$ produced, and the O$_2$ consumed. Using the molar ratio for the oxidation of glucose above, the RER equivalent equals 1.00 because 6 CO$_2$ molecules are produced relative to 6 O$_2$ molecules consumed. With the oxidation of a fatty acid, the RER equals .70 because 16 molecules of CO$_2$ are produced relative to 23 O$_2$ molecules consumed. Thus, an RER of 1.00 indicates that purely carbohydrate is being utilized for fuel in the body, whereas an RER of .70 indicates that purely fat is being utilized for fuel in the body, with RER values that lie in between representing a continuum of relative contribution of either source of fuel (27).

RER values are affected by exercise intensity and duration. The body will increasingly depend on carbohydrates as a fuel source as the rate of energy expenditure increases, thereby increasing RER with increasing exercise intensity (9). However, at a constant intensity, fat utilization increases over time as fatty acids are mobilized in the blood and given time to be taken up by the cells and transported into the mitochondria for oxidation. As a result, RER usually declines over time (27). The relationship of RER to intensity and duration is most readily observable in the fasted state. Fuel utilization patterns are also dictated by substrate availability. With the ingestion of carbohydrate, a subject will display greater RER values for a given submaximal intensity in the fed state as compared to the fasted state due to the increased availability of glucose (25). Other factors that influence the RER response to exercise include aerobic fitness (25, 27), prior exercise (4), and maturation (14, 73).
It should be noted that when using RER measurements to ascertain fuel utilization while exercising that the protein contribution to energy expenditure is assumed to be negligible, that the subject is operating under steady state conditions during exercise and that CO₂ production is from oxidative metabolism (27). RER values can increase above 1.00, but this does not represent an increase in the fractional use of carbohydrates. An RER value above 1.00 is a product of additional CO₂ production associated with metabolic acidosis and hyperventilation that typically occur during high intensity exercise. However, the degree to which peak RER rises past 1.00 can still be a useful indicator of effort, related to how hard the lungs are working to offload excess CO₂, and thus is often used as a maximum exercise test criteria around the threshold of an RER \geq 1.00 (46).

**Ventilatory Efficiency**

Ventilatory efficiency, often referred to as the $V_E$-$VCO_2$ slope, is a submaximal respiratory gas exchange measurement that quantifies the amount of ventilation needed to expel a given volume of CO₂ produced from the body’s metabolic processes. The assessment of ventilatory efficiency is made by analyzing gas exchange measurements and obtained by plotting $V_E$ over the volume of CO₂ produced (45, 74, 80). The $V_E$-$VCO_2$ slope is driven by arterial CO₂ partial pressure (PaCO₂) and the physiologic dead space to tidal volume ratio, whereby an increased pulmonary dead space and a reduced PaCO₂ set point will increase the $V_E$-$VCO_2$ slope; these factors are primarily determined by
the body’s ability to adequately match pulmonary perfusion in proportion to total ventilation (80). The slope value is the indicator of ventilatory efficiency and can be divided into subsections, such as below or above the $T_{\text{vent}}$ or the respiratory compensation point, for more specific analysis (74). It is necessary to make clear, that unlike most other respiratory measurements and exercise testing parameters, a higher $V_E$-$VCO_2$ slope is undesirable, indicating a higher ventilation cost for the removal of expired CO$_2$, and that it is inversely correlated with VO$_{2\text{max}}$ (45). Ventilatory efficiency is considered clinically impaired if the $V_E$-$VCO_2$ slope exceeds 35. As such, this measurement is often used as a prognostic indicator in heart failure patients; when examining the prognostic value of ventilatory efficiency, one study found that subjects who had a measured slope $V_E$-$VCO_2$ above 130% of their predicted value based on their age and sex suffered a >40% 1 year mortality rate (45).

**Growth and Maturation**

Few things are as characteristic of healthy child physiology as growth (84). Growth specifically refers to an increase in body size, whether as a whole or in reference to specific body parts (36). This can encompass increases in body mass and stature as well as changes in physique/somatotype and body composition (49). Closely paired with growth, but not necessarily similar, is maturation. Maturation concerns the rate and specific timing of growth and developmental processes that advance towards a final adult physiology (36, 49). Though the cascade of physiologic processes that drive growth and maturation
are cellular, mediated through hyperplasia, hypertrophy, and the actions of hormones, quantifying measurements of growth and maturation are most often based off of physical changes and clinical observation (49). When examining the physiologic response to exercise in children, the physical dimensions and degree of maturation are important considerations in the interpretation of the findings. Muscular strength, metabolism, hormonal response, and cardiovascular responses are all affected by the degree of physical growth and maturation (21). Although loosely paralleled with maturation, chronological age alone cannot be used to precisely quantify maturation (36); therefore the following paragraphs will examine some common, noninvasive means of assessing growth and biological maturity in children that are relevant to the discussion.

Growth

Growth is defined as a physical change in a body dimension and is often quantified with measurements of mass and height. These measurements are commonly collected through the usage of standard, non invasive instrumentation such as scales (mass), stadiometers (height), tape measures (segment length or circumference) or calipers. In addition to or in combination with standard height and mass measurements, other measurements can give insights into developmental changes in body proportion such as leg length to sitting height ratio, waist to hip ratio, BMI (mass/height^2), or ponderal index (height/mass^3) (50).
Children undergo a remarkable increase in size throughout their lifetime as they reach adulthood. On average, a 2-year-old male child will grow from 13.7 kg at 91.2 cm to 78.2 kg at 176.6 cm by the time he is 19 (60). Total lung volume increases on average from 1400 to 4500 ml between the ages of 5 and 14 (66). Due to increases in heart size and blood volume, maximal cardiac output increases on average from 12.5 to 21.1 and 10.5 to 15.5 L/min from the ages of 9-20 years in males and females respectively (10). Though certainly only one of many aspects, physical growth is a major contributor towards children developing a mature adult physiology.

Maturation

Perhaps the most common means of evaluating maturation in children and adolescence is in reference to peak height velocity (PHV). PHV is defined as a specific time period within adolescence where a maximal rate of increase in stature occurs (36). PHV is convenient to use as it is usually time related to other maturation events, such as the development of secondary sex characteristics, and provides an anchoring event along the continuation of physical maturity whereby maturation is often expressed in years ± PHV. PHV is determined retrospectively or prospectively through a series of height measurements every few months over the course of a few years (42). PHV can also be estimated through the timing and changes of various anthropometric measurements including seated height, limb length, and stature (55). There is wide variation in both the PHV obtained as well as the age of PHV among children. PHV values
range from 6-11 cm/year for girls and 7-12 cm/year for boys; PHV typically occurs between 9 and 15 years for girls and 12 and 16 years for boys with an average of 12 and 14 years respectively (36).

Alternatively, maturation also is often assessed through examining skeletal age. Though a number methods and criteria used to evaluate skeletal age all of them involve comparing an X-ray image of the subject (usually the left hand) to either reference figures or a set of maturation characteristics present in the features of the bone structure (36). This maturation assessment is based on the increasing degree of ossification in the bone tissue and the progressive closure of the epiphyseal plates as the child approaches adulthood where bone growth ceases (40). Skeletal age is regarded as a strong indicator of biological age, and has the benefit of being usable throughout the entire developmental span, in contrast to sexual maturity, which is only measureable surrounding the developmental stages of puberty (36).

Despite the more limited range of assessment, measurements of sexual maturation show a strong relation to overall physical maturation and can be assessed rather easily through mere observation, and without the need for X-ray imaging or repeated height measurements. Sexual maturity is commonly assess through observational development of breast, genital, or pubic hair with the usage of a 1-5 rating system developed by Tanner (76), where stage I indicates no pubertal development of the sex characteristics to stage V, which indicates a fully mature adult development of the sex characteristics. Individual subject's
characteristics of breasts, genitals, or pubic hair are compared to reference figures that represent each stage and are selected accordingly. This method of determination is often carried out through self assessment, parent assessment or clinical observation (33).

**Biometric Scaling**

Physiologic measurements are often expressed relative to specific body parameters to provide comparisons across subjects of different sizes. This is warranted as one would expect an 80 kg adult of similar body composition to have a higher VO$_{2\text{max}}$ than a 30 kg child by virtue of the former’s greater mass of metabolically active tissue and absolute work capacity. Indeed without a methodology for normalizing, many physiological measurements would be incomparable across different populations; specific physiological differences between men and women as well as adults and children would often be impossible to delineate. Common parameters used to normalize physiologic data across subjects are body mass, fat free mass, body surface area, height, or other more specific anthropometric measurements.

Perhaps the most common form of normalizing physiologic measurements is by expression relative to body mass. Most exemplary, relative VO$_{2}$ expressed as ml·kg$^{-1}$·min$^{-1}$ is found throughout the literature to measure aerobic capacity and subsequent sports performance (68), and clinical health classification (26). Other times investigators may want to express a measurement relative to fat free mass, particularly when measuring metabolic parameters in order to quantify a
result relative to metabolically active tissue. Body surface area is also commonly employed as a scaling measurement, which can be used to scale physiological measurements such as those relating to the circulatory system and thermoregulation where the diffusion rate and transport of heat proportion to the surface area of the body (34). Height is sometimes used with reference to delineate specific body proportions differences across people of various somatotypes expressed relative to measurements such as wingspan or seated height (55). However, while these methods of adjustment for body size are common and widespread, they are not without limitations. In the following section, alternative modeling concepts that may enhance the interpretation of physiologic data will be described.

Scaling is accomplished through the use of two general methods: ratio and allometric. Typical ratio scaling usually consists of a simple ratio: \( Y/X \), where \( Y \) is the size influenced variable and \( X \) is the adjusting measurement. This is case for \( \text{VO}_2 \) where \( Y \) is \( \text{L} \cdot \text{min}^{-1} \) and \( X \) is \( \text{kg} \), where \( Y/X \) is expressed as \( \text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \). However it should be pointed out that this simple ratio scaling is only appropriate when the data reflects a simple linear model: \( Y = a + b \cdot X \) (3, 85). One such means to evaluate if a selected ratio is indeed removing the influence of size, is through graphical analysis where adjusted variable \( Y/X \) is plotted over \( X \); if the size adjusting variable has removed the influence of size, the least-squares regression line of the plot should have a slope of zero (3). The standard
Body mass adjusted VO₂ tends to over-scale in its adjustment, inflating the values of those with lower body masses (85).

An alternative to ratio scaling suggested in the literature is allometric scaling, whereby the general model is expressed as, \( Y = a + b \cdot X^k \) (3) where the theoretical exponent \( k \) is solved for to reveal the proper relationship. These optimized exponent values are usually determined by plotting a least squares regression to the entire data set of the subject population studied using the log transformed derivative in order to solve for \( k \): \( \log Y = \log b + k \cdot \log X + a \) (7). Theoretical exponents common in the literature are .67 and .75 both of which try to scale for metabolic rate increases proportional to mass based on respective theories; however, specific exponents determined in the literature range from .37 to 1.07 (85). This wide variance of potential scaling exponents is thought to be a reflection of the heterogeneity of the respective subject populations studied, but may also demonstrate the limits of allometric scaling to improve understanding how VO₂ measurements are influenced by size.

Proper modeling is key to a proper interpretation of findings. When comparing modeling methods, Batterham et al. (22) found that when adjusting VO₂peak values by body mass, the exponent for mass varied from .65 to 1.00 depending upon whether a simple or full allometric model respectively was employed. Conversely it was reported that when scaling VO₂peak to FFM that the exponent for FFM only varied by .97 to 1.10 with the simple and full allometric models; this indicates an essentially linear relationship between FFM and
VO_{2\text{peak}}, where the allometric exponent does not significantly differ from 1 as with simple ratio scaling. Additionally, the FFM scaled equation model accounted for a greater percentage of variance in the study population. This lends credence to the superiority of FFM as a scaling variable for VO_{2\text{peak}}. In light of the goal to eliminate the influence of size with respect to physiological measurements (an important aspect of evaluating pediatric exercise performance), and given the data that has been presented on biometric scaling, due consideration should be made with respect to the utilization of appropriate scaling variables and scaling models when interpreting relationships between size and exercise responses.

**Unique Exercise Responses in Children**

When studying child physiology and attempting to measure the physiologic response to exercise, it must be recognized that children are not merely small adults. As children grow and mature their stature, somatotype, and metabolism progress along a continuum towards an adult profile. Examining the child’s unique physiological responses to exercise allows investigators opportunities to chronicle and quantify this progressive development of physiological maturation. Numerous parameters of exercise function have been studied in the pediatric exercise literature and provide information on similarities and discrepancies between children and adults. These include: substrate metabolism, muscular strength, fatigue, anaerobic power, and aerobic power, to name a select few (8). However, in the interest of space, this review will only elaborate upon specific pulmonary and respiratory gas exchange responses to
exercise in children as they are relevant to the measurements examined in this study. Although most child-adult differences to be discussed are irrespective of gender, there are some unique differences specific to boys and men versus girls and women, and where applicable, this will be noted.

**Ventilation**

One of the most basic respiratory parameters measured in exercise testing is $V_E$. In children, absolute ventilation increases with age, but when expressed relative to body mass, the value will decrease with age at both submaximal and maximal levels of exercise (21). This is due to the strong relationship vital capacity has with stature and body mass during growth (7, 21). With smaller vital capacities, and subsequent small tidal volumes, breath frequency is higher in children for a given level of submaximal exercise. However, even compensating for the smaller lung volume with increased breathing frequency, children achieve much lower maximum exercise ventilations. Children also have poorer ventilatory efficiency (high $V_E/VO_2$ ratio), requiring more ventilation for a given amount of oxygen uptake by the lungs. These differences are possibly due to a lower $PaCO_2$ threshold required for ventilatory drive, as evidence by lower measured $PaCO_2$ values in children and a greater ventilatory dead space to tidal volume ratio from rapid, shallow breathing (21, 66).
**Oxygen Uptake**

$\text{VO}_2$, consists of two physiological components, oxygen delivery (comprised of $O_2$ extraction from the atmosphere in the lungs and subsequent transport via the heart and circulatory vessels along with blood hemoglobin) and oxygen extraction (the ability of the muscles to uptake and utilize oxygen to synthesize ATP), both of which are altered as a child matures to adulthood (10, 11). The factors that influence $\text{VO}_2$ can be illustrated by the Fick Equation: $\text{VO}_2$ is the product of cardiac output and the difference between arterial and mixed venous oxygen content (11, 65). When comparing children to adults exercising at the same absolute $\text{VO}_2$, children exhibit a higher heart rate and a greater arterial-venous oxygen difference (10) in order to compensate for their diminished stroke volume and cardiac output (65).

Although comparing children and adults at the same $\text{VO}_2$ provides a basis to understand differences in cardiovascular function, often times, comparisons are made at the same submaximal level of exercise. On the treadmill $\text{VO}_2$ values at a given walking or running speed differ between adults and children, as children display lower absolute and higher relative $\text{VO}_2$ values. The lower absolute $\text{VO}_2$ can largely be attributed to a small body. The higher mass relative $\text{VO}_2$ has been attributed to increased fat use, higher heart rate and respiratory rate, increased basal metabolic rate and increase stride frequency (57). On a cycle ergometer, absolute $\text{VO}_2$ is the same or slightly lower (for small children) when compared to adults. Factors that may account for the difference in mass-
relative VO$_2$ include some of the factors already noted above as well as a decrease in muscular efficiency (58).

At maximal exercise, VO$_2$ responses between adults and children also vary. Absolute VO$_{2\text{max}}$ values are strongly related to body size and steadily increase with age up to young adulthood (11). These changes can largely be attributed to an increase in the size and dimensions of the oxygen transporting and utilizing organs as the child grows and matures, assuming an absence of physical training or disease conditions which can increase and decrease respectively, changes in function during this period of time. In contrast, mass-relative VO$_{2\text{max}}$ is not significantly altered as a result of growth and maturation between the ages 8 to 18 in boys (13).

**Respiratory Exchange Ratio**

Though child-adult differences are often discussed in terms of body size, a striking feature of child metabolism is the apparent lack of development of the glycolytic metabolic pathway and increased reliance on oxidative metabolism during submaximal exercise in comparison to adults (14). Due to ethical concerns with more invasive methods of examining substrate metabolism, such as muscle biopsies to assess glycogen usage, and the expense of labeled tracers, substrate utilization is often assessed in children by measuring RER (14). As exercise intensity increases, so does the relative contribution of carbohydrate to energy expenditure, which is reflected in increasing RER values. This relationship is consistent in both adults and children; however, lower RER values
for a given submaximal relative exercise intensity are reported for children (14) and relative fat and carbohydrate usage has been demonstrated to be affected by maturation through early puberty to adolescence (73).

Lower relative carbohydrate utilization and subsequent lower RER values in children may be indicative of a glycogen sparing mechanism, reflecting the lower glycogen stores in children’s muscle (14). This may explain the relative increased ability for boys to utilized exogenous sources of glucose compared with men during prolonged exercise with glucose ingestion (79). Lower carbohydrate utilization in children could also be explained by children’s decreased capacity to utilize carbohydrate sources, as lower concentrations of an important glycolytic enzyme, phosphofructokinase, were observed in boys compared to men (35), but this finding is not universally supported in the literature (43). Consistent with the lower submaximal exercise RER values, blood lactate concentrations for a given exercise intensity are lower in children as well (54); Lower RER values are also observed in children compared to adults (73), and as such should be considered when evaluating maximal exercise response criteria between the two populations.

**OUES**

The oxygen uptake efficiency slope (OUES) is determined by the slope of the line taken from the oxygen consumption (VO₂ L·min⁻¹ STPD) and the log₁₀ transformed Vₑ (L·min⁻¹ BTPS) relationship: VO₂ = a log₁₀Vₑ +b where a = OUES (17). Semilog transformation of the x-axis results in a linear relationship between
VO₂ and Vₑ with a steeper slope indicating a greater uptake of oxygen per unit of Vₑ. An example of the OUES is shown in Figure 2, where the graph on the left is not log transformed and is modeled by a logarithmic relationship and the graph on the right is log transformed to reveal a simplified modeled linear relationship, where the slope of the line given is the OUES.

**Figure 2** – A representative plot of the OUES.

The OUES was originally developed in response to a need for a submaximal measurement of cardiorespiratory function that was accurate, reliable, and easily interpretable in individuals who might not be able to produce a maximal exercise effort. Due to unique challenges and physiological limitations, it can prove difficult to reliably gather maximal exercise data on various clinical populations. To exemplify this point, one OUES study utilizing severely overweight adolescents and nonoverweight adolescents in the subject population
found that 24% of severely overweight and 10% of nonoverweight subjects failed to achieve the VO$_{2peak}$ criteria set forth in the study, representing a lack of potential data if one were to employ maximal exercise measurements only (32). The OUES however sidesteps this issue by utilizing data from the entire duration of the graded exercise test, making it independent from the level of subject effort. Other submaximal measurements do exist in the literature as noted previously. For example, T$_{vent}$, a common submaximal measurement, can be valuable for assessing the level of dysfunction in heart disease patients or as a measure of athletic performance, but has poor reproducibility between measurements conducted with different exercise protocols, methods of assessment, and individual investigators (16, 18, 88). Another slope variable, the V$_{E}$-VCO$_{2}$ slope, has also been utilized to assess ventilatory efficiency in heart failure patients, but has been observed to have a poor relationship to VO$_{2max}$ (17). The OUES is an attractive alternative to present submaximal cardiorespiratory fitness measurements due to their reported limitations, with its strong relationship to VO$_{2max}$, and objectively interpretable linear slope output (17, 44).

Due to the linear nature of the OUES, it was theorized that the OUES would be uniform regardless of the test interval measurement or the termination point (17). To verify this hypothesis, OUES values were assessed at 100%, 90%, and 75% of exercise duration with 100% and 90% not being significantly different from each other but 75% being slightly but significantly less. Values for VO$_{2max}$ and OUES at 100%, 90%, and 75% were significantly correlated ($r > .94$)
providing a higher correlation coefficient to VO\textsubscript{2max} than the other established submaximal measures of cardiorespiratory function including: ventilatory anaerobic threshold, V\textsubscript{E}-CO\textsubscript{2} slope, and the extrapolated VO\textsubscript{2max}. From this initial study, it was concluded that OUES did in fact offer an objective, effort-independent method of assessing cardiorespiratory function.

From a physiological perspective, three factors are known to influence the V\textsubscript{E}-VO\textsubscript{2} relationship: partial pressure of arterial CO\textsubscript{2} (PaCO\textsubscript{2}), the volume of expelled CO\textsubscript{2} from the lungs (VCO\textsubscript{2}), and pulmonary dead space to tidal volume ratio (V\textsubscript{d}/V\textsubscript{t}) (17). Elevated CO\textsubscript{2} production and metabolic acidosis stimulates hyperventilation; this in turn will decrease the OUES value, as commonly seen in heart failure patients (81). Hyperventilation during exercise in the presence of heart failure is associated with a greater pulmonary dead space. The physiologic basis for the stronger correlation to VO\textsubscript{2max} of OUES than the VE-VCO\textsubscript{2} slope was that it is influenced by the volume of pulmonary dead space and metabolic acidosis, which are indicative of both pulmonary and systemic perfusion, while the VE-VCO\textsubscript{2}, which is not influenced by systemic acidosis, but mainly mediated through the degree of lung perfusion (17).

Reliability and Reproducibility

Investigations have revealed the OUES to be a consistent and reliable measurement, while being able to obtain similar slope values for maximal and submaximal tests. Interprotocol variability of same mode of exercise (treadmill) was found to be low (-18% to 17%) and better than VO\textsubscript{2max} (-20% to 24%), when
comparing the standard Bruce protocol and a rapidly increasing speed protocol (18). Reproducibility was found to be high as well, with an intraprotocol coefficient of repeatability (COR, a measure of 2 standard deviations of the difference between two measurements, represented as % of the mean) between two maximal exercise tests was and comparable to VO$_{2\text{max}}$ (OUES COR = 20% and VO$_{2\text{max}}$ COR = 16%) (16). A later study of a sizable subject cohort did not find significant differences in OUES values taken from 75%, 90% and 100% exercise duration (44), an improved result compared to an earlier study (17) where a slight, but significant difference was seen at 75% compared to the other durations. In a study involving obese children and healthy controls, pooled group average OUES derived up to the anaerobic threshold differed only by 1.1% with OUES derived up to 100% exercise duration (51). A recent study also found high intratest reliability and reproducibility for the OUES (18.7% COR), but noted that test-retest reproducibility declined with shortening percentages of exercise duration being measured (82). Likely the most important factor when evaluating the OUES's value as an index of cardiorespiratory function, is the consistent finding across numerous studies of its high correlation value to peak VO$_2$ (15-17, 44, 51, 53, 56, 62, 81, 82).

**OUES in Children and Adolescents**

Perhaps due to some of the challenges applying in exercise testing procedures in children and youth (64), several studies have examined OUES in this population (1, 17, 18, 32, 51, 53). The first study to introduce the concept of
the OUES utilized a pediatric population (11.7 ± 4.4 years) and compared healthy controls to children with congenital heart disease with the idea of developing an objective, maximal effort independent measurement of cardiorespiratory function in a population that would have difficulty achieving maximal test criteria (17). However, the study pooled means of both subject groups and did not report differences in OUES values between groups. A subsequent study by the same research group also used child subjects (12.7 ± 2.8 years) to evaluate interprotocol agreement for measuring OUES and concluded that OUES’s protocol independence provided evidence in favor of its use in evaluating exercise tolerance in children (18). Marinov et al. (53) conducted a large pediatric population based study evaluating OUES in boys and girls 7-18 years old in order to delineate factors influencing the OUES across ages. The study revealed a number of interesting findings concerning OUES in the pediatric population, noting that sex differences in OUES values emerged after age 14, and adjusting by fat free mass (FFM) eliminated sex differences, but not between age and height groups. It was noted that in addition to VO$_{2\text{peak}}$, very high correlation values with the OUES were found for anthropometric variables, including body surface area, weight, FFM, height, in addition to age. A recent study involving boys and girls 7-17 found no differences between boys and girls in absolute values of VO$_{2\text{peak}}$, V$_{E\text{peak}}$, and OUES, but when adjusted for body mass, FFM, and BSA that VO$_{2\text{peak}}$ and V$_{E\text{peak}}$ were higher in boys with still no difference in OUES (1). In light of these findings, it was recommend that given the influence of size parameters on OUES, especially in the pediatric
population, it is appropriate to express OUES relative to anthropometric measurements, with FFM as the best means to correct for the influence of size.

**Summary**

This chapter has provided a brief overview of the relevant measurements, issues, and unique responses to exercise in children as compared to adults. The principles of gas exchange and their application as child appropriate, non-invasive measurements of numerous physiological processes were presented. These measurements were then placed in context of the outlined growth and maturation patterns of children with special attention to the potential limits associated with simple ratio scaling. From this, select respiratory measurements in exercise physiology were compared between children and adults, highlighting both similar and divergent responses to exercise. Finally, the end of the chapter introduced the OUES and expounded upon its relationship to accepted measurements of aerobic fitness, robustness of its measurements, and physiological mediation to offer evidence for its potential as a viable measurement of aerobic capacity requiring only submaximal exercise.
CHAPTER III
METHODOLOGY

Subjects

Forty three male subjects from a previous study at the Ball State Human Performance Laboratory formed half of the subject pool (73). Additional subjects (n = 32) 8 to 29 years of age, were recruited. Subject recruitment commenced upon approval of the study by the Institutional Review Board at Ball State University. All subjects recruited were healthy, recreationally active, but not well trained, as indicated by responses to a health history questionnaire and based on VO$_{2\text{max}}$ (see below for criterion). Subjects were excluded from participation if they currently had a disease or were presently taking any medications known to alter the cardiorespiratory and metabolic responses to exercise. Due to comparisons being drawn across subjects of maturational stages and the need to determine normalized values of OUES, subjects required similar aerobic fitness; therefore, the study was limited to subjects who exhibit VO$_{2\text{max}}$ values between 35 and 55 ml·kg$^{-1}$·min$^{-1}$, a range typical for children of this cohort and recreationally active adults (12, 21). The study was also limited to male
subjects as to isolate the influence of maturation on the metabolic and pulmonary response measured in the OUES apart from the influence of gender. The subjects were categorized into four different maturational groups according to their pubertal stage (early-pubertal (EP), mid-pubertal (MP), late-pubertal (LP) and young adult (YA)). Assessment of pubertal status, based on pubic hair development, was determined during a complete physical examination conducted by a medical doctor prior to exercise testing in accordance with the criteria developed by Tanner (76). For this study, EP was defined as pubertal stage I; MP was defined as pubertal stages II and III; LP was defined as pubertal stages IV and V; and YA was defined as ages 18 - 29 years. YA subjects did not undergo the physical examination.

**Procedures**

Child subjects came into the laboratory on three separate occasions; adult subjects reported to the laboratory on two separate occasions. During the first visit for children, parental permission and child assent were obtained followed by a physical examination administered by the physician in order to assess pubertal status. Height and weight were also assessed at this time.

During the second visit for children, skinfold measurements were taken at six different sites (subscapular, triceps, suprailliac, abdomen, thigh, and calf) and measured to the nearest millimeter (mm) using a Harpenden 228 skinfold caliper. Each site measured was duplicated and averaged whereby if duplicate measured differed by more than 1.0 mm a third measure was obtained and the two closest
measurements were averaged. Percent body fat was estimated from skinfold thickness using equations developed by Slaughter et al. (70). Percent body fat was then used to calculate fat mass, which was then subtracted from total mass to obtain FFM values of each subject. This was followed by an orientation session to familiarize the participants with the exercise testing procedures. Depending upon the subject’s age and size, children exercised at either 25, 50, and 75 watts (W), 30, 60, and 90 W, or 40, 80, and 120 W for four minutes at each stage. Heart rate (HR) and rating of perceived exertion (RPE) were recorded. Prior to the exercise test the child read a standardized set of instructions regarding the use of the RPE scale.

During the first visit for adult subjects, informed consent was obtained. Following informed consent, skinfold measurements and estimation of percent body fat and FFM were obtained in the same manner as the child subjects outlined in the previous paragraph. Additionally, subjects underwent an orientation session to familiarize the participants with the testing procedures. The adult protocol commenced at 75 or 100 W and increase 25 W every four minutes for three stages. HR and RPE were recorded. Prior to the exercise test the subject read a standardized set of instructions regarding the use of the RPE scale.

During the final visit, both children and adult subjects performed a graded exercise test to maximal voluntary effort in order to determine $\text{VO}_{2\text{max}}$. $\text{VO}_{2\text{max}}$ constituted the highest $\text{VO}_2 \text{ L-min}^{-1}$ value (one minute rolling average) obtained
during the course of the graded exercise test. The McMaster All-Out Progressive Continuous Cycling Test (20) was performed on an electronically-braked cycle ergometer. Criteria used to verify the achievement of a maximal effort were as follows: 1) failure to maintain the pedal rate of 60-70 revolutions per minute, 2) RER ≥ 1.00 for EP and MP, 1.05 ≥ for LP, ≥ 1.10 for YA and 3) peak heart rate at the end of the test that was ≥ 95% of age-predicted maximum (220-age). If at least two of these criteria were not achieved, a second maximal test was performed on another day. This test protocol and criteria mimic the procedures of Stephens et al. (73). The subjects wore shorts, a t-shirt, and gym shoes during the exercise tests, and were told to abstain from vigorous physical activity, caffeine intake, alcohol consumption, and tobacco use the day of prior to exercise testing. Also, it was requested that the subjects refrain from food and fluid intake approximately two hours prior to the time of testing, which subjects affirmed with verbal confirmation.

**Instrumentation**

All exercise testing took place on a Lode cycle ergometer (Groningen, Holland). Gas exchange measurements were obtained continuously throughout the test using standard open-circuit spirometry techniques. Subjects breathed through a mouthpiece connected to a two-way breathing valve (Hans Rudolph, Kansas City, MO) while wearing a nose clip. Two sizes of mouthpieces and breathing valves were used (model 2600 for the smallest children and model 2700 for the larger children and adults). The $V_I$ was assessed using a Parkinson-
Cowan dry gas meter (Rayfield Equipment, Waitsfield, VT). Aliquots of expired air were sampled from a plexiglass mixing chamber, dehydrated with a drying line (Perma Pure, Inc., Toms River, NJ), and analyzed for oxygen and carbon dioxide concentrations using a S-3A/I oxygen and CD 3A carbon dioxide analyzers (Applied Electrochemistry, Inc., Pittsburgh, PA). Prior to testing, the analyzers were calibrated with room air and a gas mixture of a known concentration. The analyzers and dry gas meter are interfaced with an IBM personal computer. A metabolic software program provided computer average recordings of oxygen uptake (VO$_2$), carbon dioxide output (VCO$_2$), RER, and pulmonary ventilation (V$_E$) at 30-second intervals using a 60 second rolling average. HR was measured using a Polar monitor (Polar Electro Oy, Kempele, Finland) set to record at 15-second intervals attached to the subject’s chest. RPE was assessed at the end of each exercise test stage using the Borg 6-20 scale.

**Calculations**

The OUES was calculated as the slope of the line of the absolute oxygen consumption (VO$_2$ expressed in L/min) over the log transformed V$_E$ (log$_{10}$ V$_E$): $VO_2 = a \log V_E + b$ where $a$ is the OUES (17). VO$_2$ and V$_E$ values over the course of a maximal effort graded exercise test were used to calculate the OUES, minus the gas exchange response from the first minute of the exercise, which was excluded from the calculation of the OUES in order to help eliminate confounding influence of hyperventilation common at the onset of exercise. Absolute OUES
was determined, but in order to account for the influence of size, OUES was expressed relative to body mass and FFM.

**Statistics**

The physical characteristics of the subjects, cardiorespiratory responses at maximal exercise, as well as absolute and size adjusted OUES values were analyzed using a one-way ANOVA using PASW Statistics 18 software. A Bonferroni post-hoc test was used to determine specific differences between groups in the event a significant F-test from the ANOVA was observed. In the event of a violation of homogeneity of variances, main effects were assessed using Welch statistic, where significant interactions were then evaluated using a Dunnett T3 post hoc test for between group comparisons. The independent variable for all analysis was group (EP, MP, LP, and YA). Statistical significance was established if \( P \leq .05 \).
CHAPTER IV
RESULTS

Descriptive Statistics

With the inclusion of the previous subject cohort (73), 55 boys and 20 young adult males completed the study. All subjects who completed the study achieved the required maximal exercise criteria set forth in the methods. From the 75 subjects, two boys and one adult did not meet the minimum fitness criteria for inclusion in the study (≤ 35 ml·kg⁻¹·min⁻¹) and their data was not used in the statistical analysis. Additionally, one boy displayed a prolonged and exaggerated hyperventilatory response throughout his exercise test that confounded his OUES measurement and was also excluded from statistical analysis. Therefore, the final sample size used in the data analysis is as follows: EP n = 15, MP n = 20, LP n = 17, and YA n = 19.

Descriptive statistics are displayed in Table 1. As expected, significant differences were observed between groups for age, height, and weight. Percent body fat however, was not significantly different between groups. Age and height
variables across groups did not meet the Levene’s test for homogeneity of variances; therefore the main effects were assessed using the Welch statistic and specific differences were assessed using a Dunnett T3 pos hoc test.

**Table 1 - Descriptive Statistics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>EP</th>
<th>MP</th>
<th>LP</th>
<th>YA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>10.1 ± 1.1(^{abc})</td>
<td>11.8 ± 1.6(^{bc})</td>
<td>15.3 ± 1.2(^{c})</td>
<td>23.0 ± 2.6</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>138.1 ± 6.6(^{abc})</td>
<td>149.3 ± 10.9(^{bc})</td>
<td>175.4 ± 8.4</td>
<td>179.4 ± 8.6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>34.0 ± 6.3(^{bc})</td>
<td>41.2 ± 11.0(^{bc})</td>
<td>68.3 ± 12.0(^{c})</td>
<td>81.5 ± 11.8</td>
</tr>
<tr>
<td>Body Fat (%)</td>
<td>19.2 ± 5.2</td>
<td>16.2 ± 7.5</td>
<td>20.2 ± 8.6</td>
<td>20.7 ± 6.8</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD. \(^a\)Significant difference from MP, \(^b\)significant difference from LP, and \(^c\)significant difference from YA (p<.05).

**Maximal Exercise Response**

Maximal exercise responses are listed on Table 2. No significant differences were observed between groups for both maximum heart rate and relative \(\text{VO}_{2\text{max}}\) (ml·kg\(^{-1}\)·min\(^{-1}\)). RER values at maximal exercise were significantly different between groups with EP being similar to MP, but both had a lower RER than LP and YA; the difference between LP and YA was not significant. Absolute \(\text{VO}_{2\text{max}}\) (L·min\(^{-1}\)) values across groups did not meet the Levene’s test for homogeneity of variances; therefore the main effect was assessed using the Welch statistic and specific differences were assessed using Dunnett T3 pos hoc test. Absolute \(\text{VO}_{2\text{max}}\) (L·min\(^{-1}\)) values were significantly different between
groups, with EP being similar to MP, but both had a lower $\text{VO}_{2\text{max}}$ (L·min$^{-1}$) than LP and YA; the difference between LP and YA was not significant. The only difference between groups for RPE was a lower value in EP versus LP.

### Table 2 - Maximal Exercise Response

<table>
<thead>
<tr>
<th>Variable</th>
<th>EP</th>
<th>MP</th>
<th>LP</th>
<th>YA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{VO}_{2\text{max}}$ (L·min$^{-1}$)</td>
<td>1.55 ± .23$^{bc}$</td>
<td>1.83 ± .39$^{bc}$</td>
<td>3.02 ± .61</td>
<td>3.54 ± .57</td>
</tr>
<tr>
<td>$\text{VO}_{2\text{max}}$ (ml·kg$^{-1}$·min$^{-1}$)</td>
<td>45.8 ± 4.2</td>
<td>45.2 ± 5.1</td>
<td>44.5 ± 5.4</td>
<td>43.6 ± 4.2</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>195.7 ± 9.0</td>
<td>193.2 ± 8.7</td>
<td>194.2 ± 10.1</td>
<td>188.9 ± 11.9</td>
</tr>
<tr>
<td>RER</td>
<td>1.12 ± .06$^{bc}$</td>
<td>1.13 ± .06$^{bc}$</td>
<td>1.19 ± .05</td>
<td>1.21 ± .07</td>
</tr>
<tr>
<td>RPE</td>
<td>17.5 ± 1.9$^{b}$</td>
<td>18.5 ± 1.7</td>
<td>19.2 ± 1.0</td>
<td>18.7 ± 1.3</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SD. $^a$Significant difference from MP, $^b$significant difference from LP, and $^c$significant difference from YA (p<.05).

### OUES

Absolute OUES ($\text{VO}_2 \text{L·min}^{-1}/\log_{10} V_E \text{ L·min}^{-1}$) values across groups did not meet the Levene’s test for homogeneity of variances; therefore main effect was assessed by the Welch statistic and specific differences were assessed using a Dunnett T3 post hoc test. There were significant differences observed for OUES between groups, with EP (1.74 ± .24) being similar to MP (2.03 ± .39), but both had a lower OUES than LP (3.03 ± .72) and YA (3.37 ± .58); the difference between LP and YA was not significant. These group differences were not eliminated when values were scaled relative to mass. The EP and MP groups
displayed higher ($p < .05$) relative OUES ($\text{VO}_2 \cdot \text{kg}^{-1} \cdot \text{min}^{-1} / \log_{10} \text{VE} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) values compared to the LP and YA groups (Figure 3). This relationship was slightly altered when scaling OUES to FFM where EP was significantly higher than LP and YA, and MP only significantly higher than YA (Figure 4).

**Figure 3** – OUES Scaled to Body Mass

OUES/kg units presented as $\text{VO}_2 \cdot \text{kg}^{-1} \cdot \text{min}^{-1} / \log_{10} \text{VE} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. Values are mean + SD. \(^a\)Significant difference from MP, \(^b\)significant difference from LP, and \(^c\)significant difference from YA ($p < .05$).
**Figure 4 – OUES Scaled to Fat Free Mass**

OUES/FFM units presented as $\text{VO}_2 \text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}/\log_{10} \text{VE} \text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. Values are mean + SD. aSignificant difference from MP, bsignificant difference from LP, and csignificant difference from YA ($p<.05$).
Purpose

The purpose of this study was to assess the influence of biological maturation on the OUES in healthy male subjects. The OUES was originally developed as a submaximal measurement of cardiorespiratory function (17) and has been shown to be highly correlated with VO$_{2\text{max}}$ (15, 17). Due to the difficulties associated with achieving the standard criteria used in the determination of a VO$_{2\text{max}}$ (a plateau in VO$_2$ despite an increasing workload) (59), investigation of submaximal indicators of cardiorespiratory function that are accurate and reliable is warranted. By gaining a better understanding the OUES and the factors that influence it, a strong foundation can be built for its use in evaluating cardiorespiratory function in graded exercise testing. This study sought to evaluate qualitative differences in OUES between subjects of different stages of maturation by controlling for cardiorespiratory fitness and adjusting for the influence of body size.
Findings

It was hypothesized that older maturation groups would display higher OUES values than younger maturation groups. Absolute OUES values were higher in LP and YA groups in comparison to EP and MP. This is consistent with previous findings and the known influence of body mass on OUES (5, 32, 53). When scaled to body mass, differences observed with absolute OUES essentially reversed with two least mature groups maintaining higher values compared to the two more mature groups. This suggests a maturation effect on the OUES that is independent of body mass whereby qualitative differences between groups remain after accounting for the influence of body size without discrepancies in cardiovascular fitness (similar VO$_{2\text{max}}$ mL·min$^{-1}$·kg$^{-1}$) between groups.

With consideration to the initial hypothesis that OUES would vary by maturation and that the influence of maturation would be metabolic in nature, OUES values were also scaled relative to FFM, which may be a better indicator of the primary tissue that is metabolically active during exercise; FFM was also recommended previously in the literature as a more appropriate scaling variable for OUES (1). In this comparison, OUES in EP and MP remained higher compared to YA, but there was no difference between MP and LP. This further supports a maturation influence on the OUES but suggest the influence of maturation may be more gradual, whereby MP is similar to LP and LP is similar to YA, but EP and MP are both different from adults. This maturational division
observed in this study is in some agreement with observed developmental changes in fuel use profile, where a more prominent utilization of the glycolytic pathway and increased reliance on carbohydrate as a fuel source observed in adults compared to children begins to manifest itself between MP and LP groups (73). Due to the hypothesized influence of the metabolic profile with children being more dependent on oxidative and less dependent on glycolytic metabolism compared to adults these differences in OUES may be due to increased metabolic acidosis and subsequent increased ventilatory drive from the utilization of the glycolytic pathway in the more mature subject groups, particularly during intensities above the $T_{vent}$. These maturation changes likely mediate factors known to influence the $V_E$-$VO_2$ relationship which would influence OUES values: $VCO_2$, arterial $CO_2$ set point $PaCO_2$, and pulmonary dead space to tidal volume ratio (17). $VCO_2$ values are higher in the more mature groups based on the higher RER values observed at maximal exercise. Lower $PaCO_2$ values are observed in children compared to adults but the pulmonary dead space to tidal volume ratio is found to be similar between children and adults (21). It can be speculated that the influence of a more mature metabolic profile increases $VCO_2$ and $PaCO_2$ which in turn increases ventilatory drive and allows for the achievement of higher absolute OUES values.

The OUES values obtained in this study are in agreement with others reported in the literature for healthy, untrained children and adults. Akkerman et al. (1) reported average OUES values in a group of boys (average age 11.8
years) doing maximal cycle ergometry of 2.185 (absolute OUES VO$_2$ L·min$^{-1}$/$\log_{10}V_E$ L·min$^{-1}$), 52.9 (OUES/kg VO$_2$ mL·kg$^{-1}$·min$^{-1}$/$\log_{10}V_E$ mL·kg$^{-1}$·min$^{-1}$), and 62.7 (OUES/FFM VO$_2$ mL·kg$^{-1}$·min$^{-1}$/$\log_{10}V_E$ mL·kg$^{-1}$·min$^{-1}$), compared to the observed values in the MP group of this study with an average age of 11.8 and OUES values of 2.03, 50.2, and 60.0 respectively. Drinkard et al (32) reported peak OUES value scaled to lean body mass at 52.8 in their control group of non-overweight audiences (average age 14.8) compared to our LP group (average age 15.3) OUES/FFM value of 57.8. The adult OUES values in this study (YA = 3.37 VO$_2$L·min$^{-1}$/$\log_{10}V_E$ L·min$^{-1}$) are also similar to the untrained adult values reported by Mollard et al. (56) at 3.23 obtained from peak exercise. This agreement with previously reported OUES values in this study provides evidence that the values reported may be representative of the population studied with regards to their specific age ranges and maturation status.

**Scaling**

The accepted measure of cardio-respiratory function, VO$_{2\text{max}}$, is commonly scaled relative to body mass and fat free mass; as a submaximal indicator of cardio-respiratory function, it was deemed appropriate to also scale OUES relative to body mass and FFM. Scaling OUES to body surface was not conducted despite precedence in previous OUES studies (1, 44) due to the questionable relation of a two dimensional parameter to a metabolic/mass related process in light of work by Tanner (75), illustrating a coincidental relationship to BSA and
oxygen consumption, rather than an appropriate proportional scaling variable. After maintaining differences in OUES values between groups by accounting for the influence of body mass on the OUES, it can be reasonably maintained that this is the result of maturation mediated physiological factors. However it may potentially reflect a tendency of body mass to over scale OUES values, disproportionally favoring subjects with smaller masses. As OUES is intended to be a submaximal indicator of cardiorespiratory function it is appropriate to discuss the potential for over scaling with OUES considering there are known issues in the literature with scaling VO$_{2\text{max}}$ to body mass whereby expressing VO$_{2\text{max}}$ as (mL·kg$^{-1}$·min$^{-1}$) disproportionally favors lighter individuals and fails to remove the mass relationship to the dependent variable (85). This tendency for higher values in lighter subjects is maintained when scaling OUES to FFM. In spite of recommendations to scale OUES to FFM in the literature (1) it may be more appropriate for future analysis to consider allometric scaling methods with regards to mass and FFM.

**Maximal Exercise**

All subjects achieved maximal exercise capacity in accordance with the criteria utilized for the purposes of this study which included the achievement of at least two of the three following criteria: 1) failure to maintain the pedal rate of 60-70 revolutions per minute, 2) RER $\geq 1.00$ for EP and MP, $1.05 \geq$ for LP, $\geq 1.10$ for YA and 3) peak heart rate at the end of the test that is $\geq 95\%$ of age-predicted maximum (220-age). Absolute VO$_{2\text{max}}$ values were significantly different between
groups, as expected due to the size dependent nature of the measurement. These between group differences were eliminated when \( \text{VO}_{2\text{max}} \) was expressed relative to body mass, indicating similar cardiorespiratory fitness levels between groups. Although maximal heart rate is known to be affected by age (78), significant differences between groups were not detected. The wide variation in maximal heart rate observed in our subjects and the truncated age range of the subject population may have limited the age-related differences between the child and adult subjects (48). Maximal RER was significantly lower in EP and MP versus LP and YA. This is the expected response commonly observed between children and adults (47) and consistent with maturational changes in glycolytic dependency.

**Strengths and Limitations**

This study’s comparison of OUES across maturation groups boasts a number of strengths that bring confidence to the measurements. Upon review of the literature, no previous studies have evaluated OUES with respect to pubertal development (2). Maturation, the primary independent variable controlled for in the study, was obtained in the most reliable manner via assessment by a physician. This method provides stronger confidence in maturation classification than the alternative of self or parent assessment. Fitness levels across subject groups were also well controlled with no significant differences in relative \( \text{VO}_{2\text{max}} \) (\( \text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \)) due to the accepted range of inclusion for data analysis (\( \geq 35 \) -
≤55 mL·kg⁻¹·min⁻¹). The tight range of average relative VO₂max values across groups (ranging from 43.6 in YA to 45.8 mL·kg⁻¹·min⁻¹ in EP) are within average, healthy values for men (78) and boys (12). With the limits of a small sample size acknowledge, it can be said that in light of the subject population displaying normal, healthy values of the accepted measurement of cardiorespiratory function (VO₂max mL·kg⁻¹·min⁻¹) that the average OUES values presented in this study reflect typical values for normal, healthy males across their respective age and maturation levels. Finally it should be highlighted that body fat percentage was not significantly different between maturation groups allowing for greater homogeneity of mass relative and FFM relative measurements of the OUES, wherein previous studies have shown discrepant comparisons of OUES values between subjects with significantly different body fat percentages (32).

However, there are some limitations that need to be recognized. The first limitation of the study is the relatively small sample size, in addition to uneven group size, specifically ranging from EP n = 15 to MP n = 20. Unequal variances (significant Levene’s test) were found for some of the dependent variables, including absolute VO₂max and OUES; statistical analysis using the Welch statistic for main effect and Dunett T3 post-hoc test for group by group differences was employed to help account for this. The distribution of the respective maturation stages in groups MP and LP was not equal; the MP group consisted of 14 pubertal stage II subjects compared to 6 stage III subjects while the LP group consisted of 13 stage IV subjects versus 4 stage V subjects. This uneven
distribution of maturation groups may have affected the clarity of what stages the observed maturation dependent changes in OUES take place. Another concern is hyperventilation at the onset of exercise that was common in some of the subjects. As a precaution against hyperventilation confounding the OUES measurement, the first minute of exercise data was not utilized for the calculation of the OUES. One subject however, displayed such an exaggerated hyperventilatory response that it confounded his OUES measurement. It is possible that hyperventilatory responses in other subjects extended beyond the first minute and may have affected the OUES measurement to a small degree. Finally this study's subject population was limited to males and the observed maturational influence on the OUES cannot readily be applied to the female population.

**Summary**

The results of this study suggest that the OUES is influenced by maturation with early and mid pubertal boys displaying lower OUES values than late pubertal boys and men while these group differences remained, though in a reverse relationship, when attempting to account for the influence of body size by scaling to body mass and FFM. The maturation influence is strengthened by between group differences despite similar cardio-respiratory fitness levels across all groups. These maturation linked differences may be reflective of the differing fuel use patterns between groups as the glycolytic metabolic pathway does not begin to fully develop until more advanced maturation stages.
**Future Directions and Recommendations**

In light of the OUES being used as a submaximal indicator of cardiorespiratory fitness and the known differences in the changes with VO$_{2\text{max}}$ during maturation between boys and girls (12), the observed maturational changes in OUES between EP and MP versus LP and YA can only be applied to the male population. The maturational influence on the OUES can be elucidated further through similar studies controlling maturation but studying female subjects instead. Furthermore, gender differences can be explored by incorporating both male and female subjects in a single study and conducting maturation by gender comparisons in OUES. Given the uneven distribution of maturation stages within groups MP and LP, future studies may desire to achieve a more even sampling of the respective maturation stages, or reclassify maturation group assignments to delineate specific differences between maturation stages that are closer to each other. Finally, it should be noted that one of the subjects exhibited a prolonged, exaggerated hyperventilatory response that confounded his OUES measurement, artificially inflating the value beyond physiologic reasonability. With this observation it should be considered that hyperventilation can confound the OUES measurement and that future studies should take precautions to account for and minimize hyperventilatory responses in subjects through adequate familiarization trials and data exclusion criteria.
REFERENCES


