EXCESS POST-EXERCISE OXYGEN CONSUMPTION AND SUBSTRATE UTILIZATION IN CHILDREN AND ADULTS

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ABSTRACT

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INTRODUCTION: The majority of available literature on excess post-exercise oxygen consumption (EPOC) focuses on the adult population. No study has quantified EPOC in children after exercise lasting more just a few minutes. Previous research has shown fat oxidation, measured by respiratory exchange ratio (RER), to be greater in children than in adults at rest and during exercise. Few studies have investigated substrate utilization post-exercise in children. **PURPOSE:** To examine young adult-child differences in EPOC and substrate utilization following moderate and vigorous intensity exercise performed on the cycle ergometer. **METHODS:** 19 children (7 to 9 years old) and 22 young adults (20 to 23 years old) visited our laboratory on three separate occasions and completed an exercise trial each time: VO_2max test, moderate exercise (MOD), and vigorous exercise (VIG). Maximum power output (MaxPO) during the VO_2max test was used to determine workload for MOD (35% MaxPO) and VIG (70% MaxPO) exercise. MOD and VIG trials were randomized and counterbalanced. Participants rested for 30 minutes (min) prior to exercise; the last 20 min were used for baseline VO_2 measures. Tests were 2-min square-wave intervals lasting 1 hour for the MOD trial and 30 min for the VIG trial. Expired gases were captured 10 min prior to the cessation of exercise and for 20 minutes post-exercise. EPOC was examined using ANOVA and repeated measures ANCOVA to control for sex, fitness, body composition, and caloric intake. Substrate utilization was examined using multivariate analysis of variance (ANOVA) for RER at minutes 1, 5, 10, 15, and 20 post-exercise. Another ANOVA model statistically controlling for sex, fitness, body composition, and caloric intake was also performed. **RESULTS:** After MOD and VIG, children had significantly lower volume of EPOC when compared to young adults (0.30 ± 0.13 vs. 1.18 ± 0.38 L; $F = 19.609$, $p < 0.001$, $d = 0.31$ for MOD and 0.71 ± 0.26 vs. 2.46 ± 0.95 L; $F = 59.73$, $p < 0.001$, $d = 2.44$ for VIG exercise). Children returned to baseline significantly faster than the young adults after both MOD (240 ± 105 vs. 552 ± 325 seconds, respectively; $F = 16.413$, $p < 0.001$, $d = 1.30$) and VIG (424 ± 345 vs. 925 ± 340 seconds, respectively; $F = 21.912$, $p < 0.001$, $d = 1.50$). Children's RER was significantly lower than young adults at 1, 5, and 10 min (0.84 ± 0.05 vs. 0.90 ± 0.05; 0.93 ± 0.05 vs. 1.00 ± 0.08; and 0.86 ± 0.03 vs. 0.91 ± 0.05, respectively; all $p < 0.01$; effect sizes of 0.61, 0.26, and 0.29, post MOD respectively). After VIG, RER was similar in children and young adults at min 1 and 10; however, children's RER in min 5 was significantly lower than adults (1.02 ± 0.06 vs. 1.15 ± 0.11, $p = 0.001$; effect size of 0.60). Children's RER in min 15 and 20 was significantly higher than adults' (0.83 ± 0.05 vs. 0.77 ± 0.06; 0.83 ± 0.05 vs. 0.75 ± 0.06, respectively; $p \leq 0.001$; effect sizes of 1.66 and 2.12, respectively). **CONCLUSIONS:** Children had lower EPOC and recovered faster than young adults after both MOD and VIG. Children had lower RER than young adults after MOD, but higher RER 15 and 20 min after VIG. Children relied more on fat oxidation at rest and during exercise than young adults. This study observed children to utilize a greater proportion of carbohydrates than young adults after VIG.

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To my loving and unbelievably supportive wife.
“some day, you will get the best of me…”

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"The LORD is my rock, my fortress and my deliverer; my God is my rock, in whom I take refuge." – Psalm 18:2

"Fear not, for I have redeemed you; I have summoned you by name; you are mine. When you pass through the waters, I will be with you…For I am the LORD, your God, the Holy One of Israel, your Savior." – Isaiah 43:2a, 3a

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Chapter 1 – Introduction

Total daily energy expenditure (TDEE) results from a combination of factors: resting metabolic rate (RMR), thermic effect of food (TEF), and physical activity (PA). Each factor is further comprised of smaller elements. RMR is the energy expenditure due to basal metabolic rate (energy required for vital functions) and arousal metabolism (energy required for a general state of awareness). RMR is highly dependent on an individual's fat free mass (FFM), sex, age, and hormonal balance[1]. The TEF can contribute significantly to TDEE, accounting for 3 – 25 percent of energy consumed in a meal depending on nutrient composition. All other things being equal a high protein meal elicits the highest TEF, followed by a high CHO meal, with high fat meals being the lowest[2-4]. The high TEF from protein is due to the greater cost of metabolism and storage when compared to CHO and fat[5]. Lastly, PA can significantly increase an individual's metabolic rate above resting values, both during and after the activity. PA bouts provide a unique opportunity to study the metabolic adaptations of the human body; these adaptations provide insight into control of metabolism[6,7].

Pre-exercise metabolism

Pre-exercise metabolic rate refers to the energy expenditure immediately prior to the exercise bout and has long been assumed to be slightly higher than RMR. The premise behind this assumption is that the human body, in anticipation and preparation of the work that is to come, increases sympathetic output, and as a result metabolic rate increases. One author, reviewing the literature of EPOC, suggests that pre-exercise measurements should be recorded with the understanding that RMR is not accurately represented by pre-exercise metabolic rate and; therefore, if RMR is desired, measurements should be taken well in advance of the exercise bout[8]. Other authors disagree. Thomas et al reported no significant differences between pre-exercise metabolic measurements and RMR during a control visit[9]. Turley et al reported no significant difference between inpatient and outpatient RMR[10]. Against commonly held assumptions some research suggests that pre-exercise, inpatient metabolism is not significantly different from controls.

Exercise metabolism

During exercise, aerobic metabolism increases from commencement to completion of exercise, and there are subtle differences between the onset of exercise and the attainment of a steady state of exercise. As exercise commences, the fuel needed to produce power initially is supplied by stored ATP-PCr in the muscle and, moments later, the slightly slower metabolic process of glycolysis. Concurrently, oxygen consumption increases as the slower aerobic system is able to provide the majority of energy needed to perform sustained work. The beginning of exercise provides a unique insight into the kinetics of oxygen uptake and has been considered as an important window into the understanding of control of the aerobic energy system[6,11]. At the onset of exercise, the difference between the required energy to perform an activity and the produced energy by the aerobic system, as measured by oxygen consumption, has long been referred to as oxygen deficit[12]. The measure of the total volume of oxygen deficit, or accumulated oxygen deficit (AOD), has been used as a valid indication of anaerobic capacity[13].

Oxygen uptake kinetics studied at the onset of exercise are critical to the understanding of the control of aerobic metabolism[6,11].

As sustained exercise continues beyond a minute or two, oxygen consumption increases until the energy produced is able to meet the aerobic demand of the activity - this is considered attainment of "steady state." While at metabolic steady state, an aerobically healthy individual can usually maintain the activity for extended periods of time. Steady state exercise allows for closer study of many variables, including the use of different substrates (CHO, fats, and protein) to fuel activity. Analyzing expired gases allows for the comparison of carbon dioxide production (VCO_2) and the oxygen consumption (VO_2), the ratio of which is known as the respiratory exchange ratio (RER). RER is calculated by VCO_2/VO_2 and can be used to interchangeably as the non-protein respiratory quotient (RQ) to estimate the percentage of CHO and fats that the individual uses to fuel oxidative phosphorylation if the contribution of protein to metabolism in a healthy individual is negligible. It is generally accepted that protein metabolism is minimal during aerobic exercise, therefore RER can be an appropriate estimation of RQ[14]. As in all measurement techniques, there are limitations to assessing substrate utilization via RER[15]; however, RER, as measured by indirect calorimetry, is widely accepted and especially practical since it is non-invasive[16]. As the ratio approaches 1.0 during steady state conditions, the reliance on CHO as the preferred substrate increases toward 100%. An RER of 0.7 is indicative of the sole reliance of fat to fuel metabolism. During heavy exercise the body relies preferentially on CHO as the desired fuel; therefore, RER drifts close to, or during intense exercise, greater than 1.0. Furthermore, RER exceeding 1.05 (or 1.1 in some laboratories) is used as a criterion measure for maximal exertion in adults. Thus, observing steady state exercise has been important for the understanding of metabolism and in particular, fuel utilization.

Post-exercise metabolism

Recovery from exercise provides a view of the metabolic characteristics of an individual not seen during rest or exercise. Unlike rest, onset of exercise, or steady state exercise, the post-exercise period includes time of elevated metabolism with little skeletal muscle activity and was first published as a scientific observation in 1910 by A.V. Hill who later coined the phrase “oxygen debt” to describe the phenomenon[17,18]. The phrase “oxygen debt” was designed to imply excessive oxygen consumption after exercise was “making up” for the slower oxygen uptake kinetics at the onset of exercise. Thus, Hill believed that the oxygen debt was equal to the oxygen deficit at the onset of exercise. The implications of oxygen deficit and oxygen debt being equal have led some researchers to use oxygen debt as a measure of anaerobic capacity[19-21]. However, the physiological differences between the onset of exercise and recovery from exercise led some researchers to reconsider the equality of oxygen deficit and oxygen debt. Thus, In 1984, Gaesser and Brooks addressed several faulty implications of the term “oxygen debt” and proposed a new term: excess post-exercise oxygen consumption (EPOC) citing that when compared to pre-exercise, recovery is characterized by: elevated body temperature, increased circulating catecholamines, increased uptake of calcium ions by the previously active muscles, continued liberation of fatty acids to fuel metabolism, and a host of other known and unknown factors which contribute to the uniquely elevated metabolic rate post-exercise[22]. Much of the literature concerning EPOC centers on the observation that

EPOC volume is dependent upon the intensity, duration, and mode of exercise[8,23-33]. In a review of the literature, EPOC is linearly correlated with the duration of the exercise and exponentially related with exercise intensity when exercising above 60% VO_2max[8]. Controversy exists concerning the duration of EPOC. Some investigations have observed EPOC after aerobic exercise lasting 12 hours or more after cessation of the exercise bout[34,35]. Others have reported EPOC lasting between three and 10 hours[8,36-38]. Still others have observed the duration of EPOC to be less than an hour[33,39,40]. Furthermore, at least one group of investigators found the duration of EPOC was as short as 13 minutes when exercising for 30-49 minutes at intensities below 60% of VO_2max[41]. Although controversy surrounds the duration of EPOC, intensity and duration of the exercise appear to be major contributors to the total volume of EPOC. Investigations into EPOC will contribute to the understanding of the body's attempt to maintain homeostasis in the context of post-exercise. Furthermore, insights into the mechanisms of EPOC could provide information regarding metabolic control during recovery.

The existence of EPOC has led some researchers to explore its potential impact on weight control in the adult population; however, the magnitude of the caloric expenditure from EPOC is not clear[26,37,42]. Although the majority of the literature has concluded that EPOC contributes minimally to TDEE[8], others have argued that more energy can be expended during EPOC than during the exercise itself[43]. The accumulated effect of EPOC on energy expenditure with consistent exercise can contribute to weight balance, since even a seemingly negligible positive caloric balance, such as 25 kilocalories/day, can lead to obesity over time[44].

The exact mechanisms driving EPOC are still not fully understood[22,24,34,35]. Researchers have proposed that mitochondrial respiration is the keystone to the control of EPOC[22]. In turn, increased mitochondrial respiration can be affected by factors such as increased core body temperature, ionic disturbance, increased circulating catecholamines, and release of fatty acid[22]. Based on the available literature it is clear that more research is warranted in order to better understand the control of post-exercise oxygen consumption.

The majority of the existing EPOC literature addresses the adult population. It is widely understood that the maturing child responds to exercise differently than the mature adult[45]. For example, healthy children have been observed, based on heart rate (HR), to reach a steady state faster and recover from exercise more quickly than adults[46]. Unfortunately, little is known about EPOC in children. The published research concerning oxygen consumption post-exercise in children is limited, scattered throughout different topics, and is rarely addressed as EPOC per se. Studies investigating recovery in children address issues in adult-child comparisons, assessment of anaerobic metabolism, effects of chronic exposure to altitude, and evaluation of children with various diseases[19-21,47,48]. Much research in children focuses on quantifying anaerobic metabolism by measuring oxygen debt[19-21]; however, EPOC is not simply an indication of the anaerobic metabolism that takes place at the onset of exercise[22]. The information presented in these various studies constitutes the bulk of what is known concerning EPOC in children. However, these data support no definite conclusions regarding EPOC in the pediatric population.

Substrate utilization

The body preferentially uses different substrates (carbohydrate, fat, or protein) as fuel for metabolism depending on the needs of the individual. The body shifts from relying on carbohydrates (CHO) during moderate to vigorous exercise, to primarily fats oxidation during recovery in normal, healthy individuals[49]. Wong and associates observed that there were no differences in substrate utilization between the lean and obese men during a standard exercise bout; however, after exercise, obese adults showed a lower reliance on fat oxidation and a reduced EPOC when compared to their lean counterparts[49]. It is of note that the degree of ability to utilize fats as a source of energy has been shown to be a significant component in the development of obesity[50,51]. Thus, it is possible that the suppressed fat oxidation in the obese individuals may only be observed during the post-exercise period of time.

Substrate utilization, as estimated by RER, during rest and exercise is known to differ between children and adults[16]. It is generally accepted that children have lower RER values than adults during rest and exercise, indicating that they rely more on fat oxidation for energy production[16,52]. Wong and Harber observed that after a 30-minute bout of cycling at ventilatory threshold RER was suppressed significantly below baseline in normal weight adults indicating a greater reliance on fat as a substrate for oxidative phosphorylation[49]. Since skeletal muscle composition does not differ significantly between children and adults, it stands to reason that children would have a similar post-exercise response in RER. However, to our knowledge, no research studies have been published concerning the substrate utilization during recovery from submaximal exercise bouts in children when compared to adults. It is unknown if children utilize the same fuel to recover from exercise as do adults. Therefore, more research is needed to investigate substrate utilization, particularly post-exercise, in children.

Problem statements

Investigations into exercise recovery contribute a unique view of metabolism and metabolic control in youth. However, little research is available concerning metabolism post-exercise in healthy children. Research on EPOC or "oxygen debt" available in the pediatric literature address exercise of differing intensity levels, acclimatization to altitude, and chronic diseases, making comparison across investigations difficult. In particular, it is unknown if EPOC differs as a function of moderate and vigorous intensity activities in which children are most likely to engage.

Currently there is little research available to provide insight into a child's metabolic profile, including oxygen consumption and substrate utilization, during recovery from moderate and vigorous exercise intensities. In addition, it is unknown if children continue to preferentially oxidize fats post-exercise when compared to adults. It is also unknown if children rely more on fat as a fuel after exercise than before exercise. It is important to first describe a normal response to exercise before researchers can identify anything deviates from normality. Once a normal response has been described, future research can begin to investigate different populations, specifically overweight and obese children.

Research aims and hypotheses

The aims of this investigation were to:

1) Describe the metabolic profile (oxygen consumption pre-, during and post-exercise) of children and young adults.

2) Examine differences in EPOC between young adults and children after moderate and vigorous intensity exercise.

> *Hypothesis A: Young adults would experience greater volume of EPOC than children after moderate intensity exercise.*
>
> *Hypothesis B: Young adults would experience greater volume of EPOC than children after vigorous intensity exercise.*
>
> *Hypothesis C: Young adults would experience longer duration of EPOC than children after moderate intensity exercise.*
>
> *Hypothesis D: Young adults would experience longer duration of EPOC than children after vigorous intensity exercise.*

3) Examine differences in EPOC between moderate and vigorous intensity exercise in children and young adults.

> *Hypothesis A: Vigorous exercise would elicit a greater volume of EPOC than moderate exercise in children and young adults.*
>
> *Hypothesis B: Vigorous exercise would elicit a longer duration of EPOC than moderate exercise in children and young adults.*

4) Examine if differences exist between pre-exercise oxygen consumption and one-hour post-exercise (moderate and vigorous) in children and young adults.

Hypothesis A: Children's oxygen consumption would not be significantly different (<1.3ml/kg•min^{-1})[1] *between pre-exercise and one-hour post-exercise for moderate or vigorous exercise.*

Hypothesis B: Young adults' oxygen consumption would be significantly elevated (>1.3ml/kg•min^{-1})[53] *one-hour post exercise when compared to pre-exercise values.*

5) Examine if any differences exist in RER between young adults and children after moderate and vigorous intensity exercise at minutes 1, 5, 10, 15, and 20 post-exercise.

Hypothesis A: Young adults would have higher RER than children after moderate exercise.

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[1] The author is unaware of any articles presenting VO_2 data at one-hour post exercise. Articles show the data graphically and display end results, but do not present the data at one hour. Thus, the author used existing data to calculate what would be considered significant at one hour post-exercise. Tarah et al investigated 679 young adults**53.** Tahara Y, Moji K, Honda S, et al. Fat-free mass and excess post-exercise oxygen consumption in the 40 minutes after short-duration exhaustive exercise in young male Japanese athletes. *J.Physiol Anthropol.* 2008;27(3):139-143.**53.** Tahara Y, Moji K, Honda S, et al. Fat-free mass and excess post-exercise oxygen consumption in the 40 minutes after short-duration exhaustive exercise in young male Japanese athletes. *J.Physiol Anthropol.* 2008;27(3):139-143.**53.** Tahara Y, Moji K, Honda S, et al. Fat-free mass and excess post-exercise oxygen consumption in the 40 minutes after short-duration exhaustive exercise in young male Japanese athletes. *J.Physiol Anthropol.* 2008;27(3):139-143.. The main outcome variable was EPOC measured for 40minutes after an exhaustive exercise bout (45-105 seconds on a treadmill set at 5 degrees). The article does not mention VO_2 at 40min, however the authors do give data that allows calculation of that value, although with not a much certainty. After 25 minutes post-exercise the participants had consumed 86.1% of total EPOC for 40min leaving 13.9% left to be consumed in the last 15 minutes of the rest period. Total EPOC consumption was 142.7 ml/kg for 40 minutes, 13.9% of which was over the last 15 minutes. 13.9% of 142.7 is 19.8353 ml/kg divided by 15 minutes = 1.322 ml/kg/min.

Hypothesis B: Young adults would have a higher RER than children after vigorous exercise.

6) Examine if any differences exist in RER between the post-exercise period of moderate and vigorous in children and young adults at minutes 1, 5, 10, 15, and 20.

Hypothesis: The vigorous exercise would elicit a higher RER post-exercise when compared to moderate intensity exercise for children and young adults.

7) Examine if any differences exist in RER between pre-exercise and post-exercise (moderate and vigorous) in children and young adults.

Hypothesis A: Children's pre-exercise RER would be lower than RER from moderate and vigorous values at minutes 1, 5, 10, 15, and 20 post-exercise.

Hypothesis B: Young adults' baseline RER values would be lower than moderate and vigorous values at minutes 1, 5, 10, 15, and 20 post-exercise.

Hypothesis C: RER values one hour post-exercise would be similar to baseline in both children and young adults.

Chapter 2 – Literature review

History and definition of EPOC

In humans oxygen consumption is vital to cellular respiration at rest, submaximal exercise (also activities of daily living), and maximal exercise. Following an acute bout of physical activity or exercise, an individual's metabolic rate remains elevated above resting levels then decays exponentially for a period of time depending on the intensity and duration of the workload. This phenomenon has been observed for a century[17], and the mechanisms by which it operates have been under considerable debate[8,22].

The understanding of this elevated metabolic rate after the cessation of activity is rooted in classic work of A. V. Hill, who demonstrated not just an increase in heat produced after muscle contraction[17], but that the heat that was produced after the cessation of contraction was equal to or greater than heat freed during the contraction itself[18]. These experiments were followed by the work of Hill and Lupton, who observed the elevated oxygen consumption after an exercise bout and labeled it "oxygen debt"[54]. The proposed mechanism of this "oxygen debt" was due to the metabolism of a fraction of the lactic acid produced in the activity, specifically the conversion of lactic acid to glycogen in a 1:4 ratio, which was observed earlier by Meryehof in 1920[22]. Margaria et al.[55] noted in 1933 that the delayed oxidation of lactic acid alone was an inadequate explanation for the phenomenon because previous work observed three key discrepancies between recovery O_2 and lactic acid: 1) much of the lactic acid produced, in the isolated muscle, occurs after the contraction is over, 2) at lower intensities the changes in lactic acid concentration are meager compared to the corresponding oxygen debt, and 3) the re-synthesis of glycogen from lactic acid was not the only oxidative process happening during this recovery period. These discrepancies, in addition to the observation that a fast and slow component of the oxygen debt existed, led Margaria and associates to propose an amendment to the current oxygen debt theory[55]. They suggested two mechanisms for oxygen debt: the initial, rapid decline of oxygen post exercise where lactic acid is not contributing to the excessive oxygen consumption called “alactacid” and the prolonged elevation of oxygen post exercise in which lactic acid is responsible for the elevated metabolic rate which they called “lactacid”[22,55].

In 1984 Gaesser and Brooks[22] proposed that the oxygen debt hypothesis, as suggested by Margaria and associates, was “too simplistic” reporting that no causal relationship had been established between lactate metabolism and elevated oxygen consumption post-exercise. Furthermore, they stated that there are a host of other factors potentially “loosen the coupling between oxidation and phosphorylation” contributing to increased metabolism post-exercise. These other factors include, but are not limited to: elevated temperature, fatty acid mobilization, the commandeering of Ca+ by mitochondria[56], and increased sympathetic drive[22,57]. Beyond these issues, lactic acid can fuel the recovery process by directly supplying the Krebs Cycle with substrate instead of using glucose or glycogen for recovery metabolism[57]. Therefore, in order to avoid incorrectly implied mechanisms in the name “lactacid” and “alactacid” oxygen debt, Gaesser and Brooks suggested that the elevated level of oxygen consumption during recovery should be referred to as excess post-exercise oxygen consumption or EPOC. For the purposes of this review, EPOC is defined as “the VO_2 above resting requirements after the cessation of exercise”[8].

Review of the Literature

Several review papers on EPOC have been published[8,22,24,58], but none have focused on children or adolescents. The purpose of this review is to compile the studies that have investigated EPOC in children and adolescents and identify where more research is needed. Given the limited information in youth, the following sections are included and reflect a blend of what is known from literature involving adults and children: measurement of EPOC using different protocols, influence of exercise intensity and duration on EPOC, age- and sex-associated variation in EPOC, adult-child differences in EPOC, EPOC in obese individuals, implications of EPOC in the context of TDEE, substrate utilization, and methodological considerations for investigating EPOC.

Measurement of EPOC

Although there is general agreement in the definition of EPOC, there is no consensus in literature on the most appropriate protocol to assess EPOC. The assessment of EPOC varies widely and includes several protocols, ranging from unloaded pedaling for 10 minutes after exercise[20] to time to 50% VO2peak[47]. Some of the methodologies are described in the following text.

Adult literature concerning EPOC following aerobic exercise focuses on recovery from an exercise bout lasting several minutes or more. Children, on the other hand, rarely attain steady state during daily physical activity. In order to study pediatric oxygen kinetics in a more realistic environment, Zanconato et al.[20] used exercises lasting one minute. Oxygen kinetics, onset and recovery, was analyzed for five separate exercise intensities ranging from moderate to supramaximal. Prior to the exercise bout, subjects cycled at an unloaded resistance for 3 to 5 minutes; the readers are left to assume that at this time "pre-exercise" measurements of oxygen consumption were recorded to compare to post-exercise measurements. After the one-minute of exercise, participants actively recovered for 10 minutes by unloaded cycling (~7 – 12 W) while expired gases and HR measurements were obtained. After a bout of supramaximal effort, unloaded cycling will aid in recovery from exercise; however, it also creates artifact when attempting to quantify EPOC such that the metabolism post-exercise is not simply a measure of the body's requirement to recover, but also the requirement to move the lower limbs. In this study, EPOC was assessed while participants actively recovered from various workloads ranging from moderate to supramaximal, but it was justified by comparing post-exercise oxygen consumption to measured oxygen consumption of unloaded cycling pre-exercise[20]. Children's physical activity patterns must be considered when assessing EPOC, as children typically do not partake in exercise bouts lasting thirty minutes or more. Thus, shorter exercise bouts may be more appropriate when considering EPOC in children.

Exercise recovery, specifically early oxygen consumption post-exercise, has been used to assess the disease severity in individuals with chronic chest diseases (CCD). Measuring early recovery is designed to aid diagnosing CCD, but it also captures the onset of EPOC. Unfortunately, measuring recovery for a short period of time is unable to fully answer the question of volume or duration of EPOC. Stevens et al.[47] studied 27 children with CCD and compared them to 27 healthy controls. A graded exercise test to exhaustion on an electronically braked cycle ergometer was used to establish the participants' peak oxygen consumption. Expired gas measurements were recorded using a 10-second moving average throughout the pre-exercise, during exercise, and post-exercise time periods. Prior to the exercise bout, oxygen consumption was measured in the upright position. Immediately after the test the children were instructed to sit quietly on the cycle ergometer while oxygen consumption was measured. The recovery period was recorded as the time required for the participants to reach 50% of their VO2peak and was labeled "early recovery VO2." Oxygen consumption was recorded for 10-minute post-exercise, but these results were not reported. These authors cited previous studies using the early recovery VO2 method in adults with COPD as a measure of functional capacity. Early oxygen uptake recovery, in this case time to reach 50% of exercise VO2, is an accepted method for measuring recovery[47]. The benefit of this assessment technique is that the authors' measurement time post-exercise is short and the information that is gained is highly valuable for their specific research aim: to assess recovery time in CCD patients. However, by only assessing the initial fast phase of recovery they fail to assess the slow component of recovery and, therefore, the total volume and duration of EPOC is unknown in this population. The authors admitted that more appropriate measures need to be established to best represent recovery[47].

The majority of the investigations of EPOC include a comparison of the post-exercise period with a period of time that represents resting metabolic rate (RMR). Some investigators have used a control day to measure oxygen consumption throughout the time that the exercise trial and EPOC assessment would take place[23,59-62]. Conducting a control day increases the amount of time a participant must dedicate to the research study and is typically a drawback in terms of feasibility; however, a control day substantially increases the strength of the study. Others have used the convenience of the time period before the exercise trial to assess RMR[26,28,35,63-65]. However, the anticipatory response prior to the exercise test can be enough to elevate the metabolic rate above normal resting values and must be taken into consideration when reporting the data as an underestimation of EPOC could have occurred[24]. Typically, EPOC research is separated into two schools of thought when comparing to RMR values: a control day to assess RMR or assess RMR just prior to exercise bout.

Most of the investigations that have been performed in the pediatric population use a period of time just prior to the exercise bout to assess RMR. The period of time before exercise has varied from three minutes[20] to 30 minutes[66]. The positions in which children were placed for this pre-exercise time period varied significantly as well: cycling with little-to-no resistance[20,47], resting in a seated position[19], resting in a recumbent position[21], and also resting supine[66]. These different time periods and postures were chosen for reasons related to the different research aims of each study. Further, not all of the studies were designed for the assessment of the volume and duration of EPOC. However, to appropriately assess EPOC an accurate assessment of RMR is necessary so that oxygen consumption above RMR post-exercise can be measured.

In a recent review of EPOC in adults, the authors suggested that the most appropriate way to assess a post-exercise measurement is to record pre-exercise measurements on a control day where no exercise will take place in order to establish a proper comparison[8]. In addition, since the statistical analysis of recovery data has not been standardized, the authors provided a detailed method of statistical analysis. Most EPOC studies consist of measuring participants during recovery and in a control state. These serial measurements can be effectively analyzed using repeated-measures analysis of variance (RMANOVA) if homoscedasticity "exists among different time points and treatments." LaForgia et al. also suggested using a Dunnett's post hoc test, used from comparisons between control and treatment, if recovery and control VO2 result in a statistically significant F-ratio. Appropriate comparisons of EPOC to a control day must be made in order to have an accurate representation of the elevated metabolism that is induced by the specific exercise protocol. Furthermore, by using RMANOVA investigators are able to appropriately compare the control and recovery states.

Investigators have assessed EPOC in different ways based on the particular aims of each study. Excess post-exercise oxygen consumption has been thoroughly researched in adults and the volume and duration of EPOC has been described with different intensities, durations, and modes of exercise in many different populations. However, the studies previously described in this section focus on the pediatric population with research aims not directed toward describing EPOC, but using the measurements taken in the recovery period as surrogates for other, more invasive, measurements. There exists a need in the literature to describe EPOC in children using methodology that will capture the volume and duration of EPOC.

Effects of intensity and duration on EPOC

Investigators have previously reviewed the effects of exercise intensity and duration on EPOC in adults and will be briefly summarized[8,24]. Studies which have specifically investigated EPOC have varied the duration of the exercise from one minute to more than 160 minutes[24,67,68]. Exercising at or lower than 30% VO_2max, no matter the duration, result in similar and negligible EPOC values[24]. The research also suggest a threshold exists at approximately 50-60% VO_2max, above which EPOC increases linearly with increasing exercise duration[24,37]. Furthermore, exercise intensity has been investigated thoroughly in adults. Intensities have ranged from 25-108% VO_2max[67,69]. Evidence points to EPOC increasing exponentially in magnitude as exercise intensity increases[8,24,37]. In their review, Børsheim and Bahr discuss that the intensity of an aerobic exercise explained nearly 46% of the variation in EPOC while the duration explained a mere 9% of the variation in measured EPOC[24,37].

LaForgia, et al. also reviewed EPOC studies that investigated iso-energetic exercise bouts and found mixed results with regard to volume of EPOC. Several reports indicated that EPOC doubled after higher intensity than what was observed in a lower intensity bout[33,38,39]. Other studies did not support this claim[25,29]. However, the studies that did not observe a significant increase in EPOC due to intensity also had some potential methodological issues (ex. lack of a cross-over design). The evidence supports that exercise intensity must be closely controlled in studies investigating magnitude and duration of EPOC. As exercise intensity increases the magnitude of EPOC subsequently increases exponentially. Exercise duration is an important consideration as well, but seemingly only when the exercise intensity exceeds 50 – 60% VO_2max, the apparent exercise intensity threshold for the effect of duration on EPOC. None of the preceding discussion on the effects of intensity or duration has been investigated in children.

EPOC in children and adolescents

Although investigators have conducted a significant amount of work on the oxygen uptake response during exercise, there is limited information on children's and adolescents' metabolic responses immediately post-exercise. Given the lack of studies in this area, a more detailed account of each study will be provided in the following text.

A multitude of factors are responsible for driving EPOC, such as maturation, environment, aerobic fitness, and body composition. Considering the role that biological changes during adolescence play in influencing oxygen consumption during recovery is important, since adolescence represents a time of great changes due to growth and maturation. The only way to appropriately measure variables with regard to changes in maturation is to conduct a longitudinal study. Thus, Paterson et al.[19] followed 19 boys from age 11 to 15 years in an attempt to quantify the changes in anaerobic capacity (AnC) by measuring "oxygen debt" as a function of chronological and biological age. Unfortunately, due to the multitude of known and unknown factors influencing the elevated oxygen consumption post exercise, as mentioned previously, researches have argued measuring EPOC is insufficient for estimating anaerobic metabolism during exercise[57]. Aware of these issues, Paterson and colleagues stated that there is no standardized measurement or assessment of AnC and the results should be understood in context. However, the data gathered are still relevant to this review and constitutes the bulk of what is known about changes in EPOC with regard to maturation. Paterson's study began with measuring resting O_2 consumption for 5-min with subject seated prior to the exercise. The exercise test employed was on a treadmill at 20% grade at a speed designed to elicit exhaustion in 45 – 90 seconds. Following the maximal effort, VO_2 was measured for 15-min in seated position. The results showed that recovery O_2 increased over subsequent testing periods of the study from 1.65 to 4.92 L and increased by 80% when expressed per unit body mass (45.6 to 81.9 ml/kg). Furthermore, height and weight were significantly correlated with recovery O_2 consumption at all ages. When taking into consideration each age group, the results showed that age and recovery O_2 were correlated through 13.9 years, but height and recovery O_2 consumption were correlated throughout the study (from ages 11 to 15). Thus, body size is an important predictor of recovery O_2 during growth and maturation. To account for changes that would occur over time, the workloads participants were required to perform increased over subsequent years of the study so as to stress the anaerobic system. Subjects were able to perform more work (kJ) as the study progressed except for the last year when the protocol increased substantially in difficulty. In the last year the average work was nearly half that from the previous year. This issue in methodology could be a possible explanation of the lack of significance found between age and recovery O_2. This longitudinal study performed by Paterson and associates addresses the question concerning maturation's effect on recovery. Although age was not found to be a significant factor related to EPOC, the authors cited references indicating that these particular adolescents had yet to obtain adult recovery O_2 values (expressed in L/min and ml/kg/min). These results suggest that there is indeed a maturational effect on EPOC. The threshold to attain adult status had yet to be reached by these adolescents.

Environmental factors influence EPOC, specifically altitude is known to reduce aerobic capacity and it is intuitive to conclude that recovery from exercise is negatively affected by altitude as well. Similar to Paterson's study, Fellmann and colleagues[21] used EPOC as a surrogate for AnC and investigated the differences between the AnC of children residing at altitude (3700 meters above sea level) and sea level habitants (330 meters above sea level). Data from males and females were grouped together for analysis although maturation was not taken into consideration. Resting VO_2 was measured for 5 minutes after a 10-20min rest in recumbent position on the day of the supramaximal test. Participants cycled at 115% of "maximal aerobic power" for 1.5min. Ten total expired air samples were taken via Douglas bag: one sample in the last 30 seconds of the exercise bout and then nine samples during the 30 minute recovery. The oxygen consumption of all participants had reached the pre-exercise measures by the twentieth minute of the recovery phase. The researchers compared the slopes "O_2 debts" when expressed as a percentage of VO_2max and found that the slopes were similar between high altitude and sea level population. However, absolute "O_2 debt" was significantly higher in the high altitude population. Furthermore, there was a linear relationship between "O_2 debt" and lactate concentrations which were similar between the altitude and sea level populations. Excess post-exercise oxygen consumption, relative to percent VO_2max, was similar between high and low altitude children suggesting that although altitude effects the absolute magnitude of EPOC other factors significantly modify oxygen consumption post-exercise.

This review has only briefly alluded to the duration of EPOC. Fellmann's study indicated participants recovered by the twentieth minute post-exercise. Few studies consider the duration of EPOC, which is an important piece of information when considering a study using multiple exercise tests in a single visit or recent exercise of an individual prior to participation in a study. Stevens et al.[47], in a study described earlier, reported that children who were the healthy controls reached 50% VO_2max in 50 ± 7 seconds after the cessation of a maximal bout of exercise. However, there was no indication of how long the elevated O_2 consumption lasted after the cessation of exercise.

Furthermore, no results discussed age/maturity status or sex. In a study investigating the recovery of individuals with cystic fibrosis (CF), Wideman et al.[66] studied 20 youth and young adults 10-22 years of age. Ten of the subjects had CF and 10 healthy controls (Con) were matched for age, BMI, sex, and maturation. The investigation consisted of RMR, which was measured via indirect calorimetry two hours after eating while the participants were supine in bed for 30 minutes. After resting measures were taken, the participants performed a peak exercise test on an electronically braked cycle ergometer. After the peak test the participants performed a two-minute cool down, and then rested quietly in a chair for 20 minutes while expired gases were collected. Two to three hours after the peak test (and two hours after eating a standardized snack) participants performed a submaximal exercise test for 20 minutes at 50% VO_2peak then again sat quietly for 20 minutes while recovery oxygen consumption was measured. A smaller subgroup came back to the lab several days later to perform an exercise bout at the same absolute intensity. The results indicated that VO_2peak, HRmax, Power Output (PO), and total exercise EE was lower in the CF group compared to the controls. Time to reaching resting VO_2, after VO_2peak test, was shorter in the CF group vs C, 7.7min vs 10.9min, respectively. EPOC (2.7 L vs. 2.1 L of O_2) and time to resting VO_2 (7.5min vs 8.8min) was similar between the CF vs C, respectively, when exercising at the same relative intensity (50% VO_2max); however, when exercising at the same absolute intensity EPOC was greater in the group with cystic fibrosis (2.79 L vs 1.46 L). Interestingly, time to reach resting VO_2 was the similar between the two groups after the same absolute exercise intensity was performed (5.9 min vs 4.9 min). The results of these studies indicate that the duration of EPOC is less than what others have previously reported in adults; however the exercise intensity used in the pediatric studies must be taken into consideration as most of the adult research on EPOC occurs above 60% VO_2max. Furthermore, these pediatric investigations highlight a potential for multiple exercise tests in a single visit given the rest period is adequate with consideration for duration and intensity of the exercise.

Investigators have not extensively studied correlations between EPOC and different anthropometric and physiologic variables within the pediatric/adolescent literature. Tahara et al.[53] investigated EPOC in 250 Japanese adolescents/young adults between the ages of 16 and 21. Participants, using a treadmill, underwent a protocol designed to elicit exhaustion in 45 – 105 seconds. Oxygen consumption was measured via the Douglas bag method for 40min of recovery. They observed after the recovery period that the participants' EPOC was 33% above RMR. In addition, they observed EPOC was significantly correlated with fat free mass (FFM), body weight, height, and body mass index. The participants' VO_2max was correlated to the volume of EPOC; however, after controlling for FFM the relationship between aerobic capacity and EPOC was no longer significant. These authors observed EPOC being most strongly related to an individual's FFM.

Only Wideman and associates reported exact time to reach pre-exercise measurements: 7.7 minutes for CF children and 10.9 minutes for healthy controls. They used an exercise intensity of 50% VO_2max, which is considered to be close to the threshold where duration of the exercise is significantly related to the magnitude of EPOC as reported by LaForgia[8]. However, considering the CF population that was being studied the exercise intensity seems appropriate.

Duration and intensity of the exercise significantly affects the volume of EPOC; this finding is especially true in adults. The information that can be drawn from the pediatric literature is not as conclusive, only because the variables of duration and intensity have not specifically been investigated in this population. However, with the research that is available, it is seems that children follow a similar patterns of recovery of adults (just at a different rate): at intensities above a certain threshold, such as 60% VO_2max in adults, the volume and duration of EPOC increases linearly with the duration of the exercise and exponentially with the intensity of the exercise. More research is required to understand the effect of duration and intensity of a particular bout of exercise on EPOC in children.

Summary of studies involving children

Based on the available literature in children, there is an increase in EPOC with age (both absolute and relative measurements). However, there is not enough evidence to support any differences in EPOC between males and females, even though a sex difference in oxygen kinetics has been reported[70]. The evidence presented also indicates that there is a lower absolute amount of oxygen consumed post-exercise in children living at high altitude than those who live at sea level and when analyzed as percent VO_2max, the difference between the groups is eliminated. Further research needs to document the age- and sex-associated variation in EPOC of the growing and maturing child.

EPOC in obese individuals

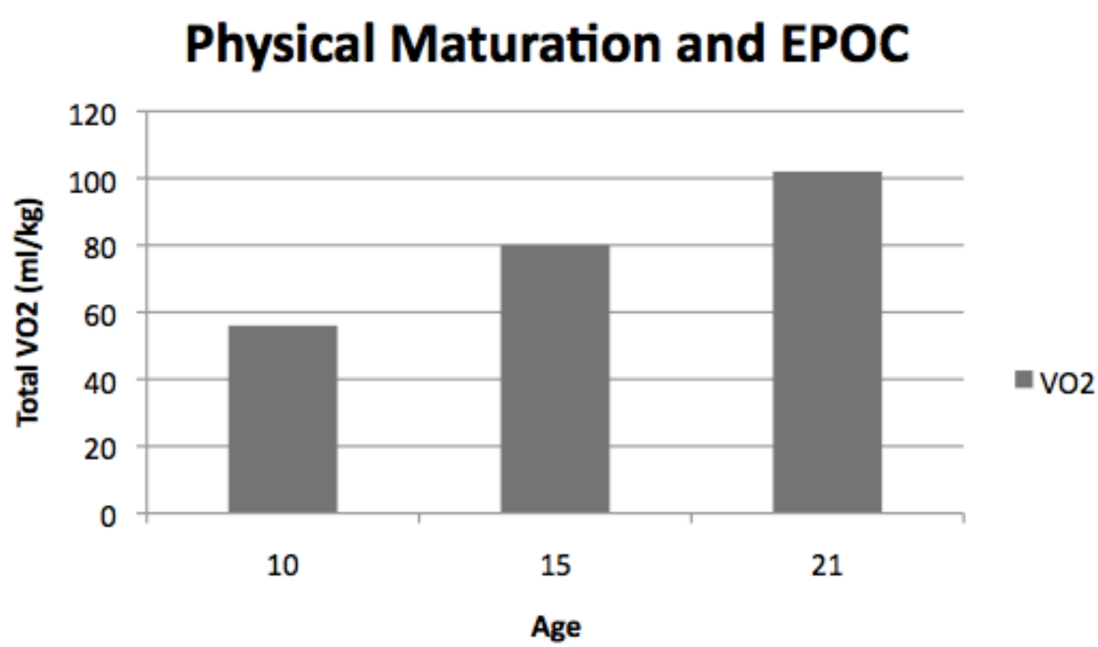
Considering the epidemic of childhood obesity and the emphasis on caloric expenditure, studies investigating the impact of EPOC on overweight vs normal weight children are warranted, but rare in the literature. Investigators conducted a study on EPOC in obese and lean women and reported no differences between the two populations after an iso-energetic bout of cycling at 60-65% VO_2max. Aerobic fitness between the groups was not significantly different[26]. Researchers studying EPOC differences between lean and obese men found that the obese men experienced less EPOC and a greater reliance on CHO during recovery even though there was no difference in aerobic fitness (relative to FFM) between the groups[49]. These seemingly paradoxical findings illustrate the complexity of EPOC. If a population of obese, aerobically unfit children were compared to a sex, age, and maturation matched population of lean, aerobically healthy children it could be speculated that the obese children would require a greater absolute volume of oxygen to recover, after an exercise bout of the same relative intensity, indicating a greater caloric expenditure. However, the clinical significance of the speculated elevation of EPOC is uncertain. No matter the result, quantifying EPOC in children will allow for a better understanding of metabolism in this population and could further the understanding of caloric balance.

Adult-Child differences in EPOC

According to the available literature, children have faster oxygen uptake kinetics[7,46,70] during exercise and recover faster than adults after exercise[46,71-74]. These data point to a potential difference in EPOC as well. A sparse number of studies have investigated adult-child comparisons of recovery oxygen consumption; two studies are discussed in the following text.

Paterson et al. compared the results of their longitudinal child and adolescent study[19] to adult studies of similar design[75,76]. The authors described that at 15 minutes post-exercise, recovery O_2 (both absolute and relative measures) was lower in children than in adults (see *Figure 1*); however the exercise bouts were designed to elicit exhaustion within 45 to 90 seconds. Therefore, absolute energy expenditure would not be controlled. It would stand to reason then that those who were able to expend more energy during the exercise bout would have a different volume of EPOC than those that expended less energy. Paterson and associates observed an increased "anaerobic performance" with an increase in age as defined by an increase in the volume of recovery oxygen consumption, but even at 15 years old the children had yet to obtain "adult status" from an anaerobic capacity standpoint[76].

Figure 1. Physical maturation and EPOC



Zanconato, et al.[20] administered a one minute exercise bout at different intensities based on AT and VO_2max and found that there was a non-statistically significant trend for the time constant for EPOC to be faster in children than adults at all exercise intensities above 80% of anaerobic threshold (AT), it was only at 125% of VO_2 max that this variable reached significance. However, there were no significant differences in VO_2 10 minutes post-exercise between children and adults.

Figure 2 – Time constant of VO_2 in children vs adults after different exercise intensities. Graph provided by Zanconato, et al.[20]

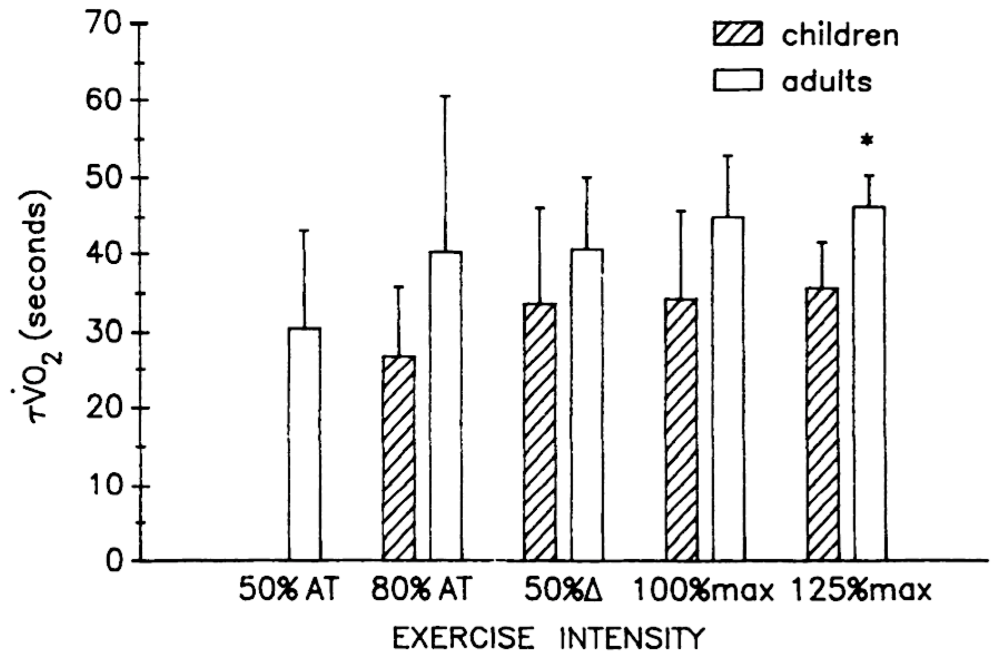


Figure 2 depicts the time constant for O_2 recovery (τVO_2) in adults and children. Values are mean ± SD. In adults τVO_2 increased significantly from 50 to 80% AT ($P < 0.01$), but we found no effect of work intensity on τVO_2 in children. At 125% max τVO_2 was significantly higher in adults than in children ($P < 0.001$).*

Based on these studies, it appears that adults recover more slowly than children and that adolescents' up to 15 years of age still have not fully matured anaerobically, as measured by volume of EPOC. Furthermore, children seem to have lower recovery oxygen consumption than adults with increasing intensities. It is also possible that there exists a threshold in which there are no differences in EPOC between adults and children. However, these claims cannot be stated with much certainty due to the lack of systematic studies investigating adult/child comparisons of EPOC. It is possible that the differences shown in the literature could be a result of different protocols to assess EPOC – e.g., sitting quietly in a chair immediately after the exercise test[19] or cycling at an unloaded resistance for 10 minutes post exercise[20]. Clearly, an accepted protocol to measure EPOC needs to be devised.

Substrate utilization post-exercise

A review was written regarding substrate utilization in children during submaximal exercise[15]. Reproducing the current literature regarding oxidation of fats and carbohydrates during exercise is beyond the scope of this review; however, there are particular issues regarding substrate utilization during exercise that are applicable to the post-exercise period. The most widespread method of assessing of substrate utilization is accomplished by measuring expired gases via indirect calorimetry and using the ratio between the production of carbon dioxide (VCO_2) and consumption of oxygen (VO_2) resulting in respiratory exchange ratio (RER), which is considered to be equal to non-protein RQ, if protein oxidation is insignificant[77]. It is important to know that RER is strongly influenced by changes in breathing rate, especially when exercising at intensities above the ventilatory threshold. Hyperventilation, either voluntary or involuntary, removes stored CO_2, increasing RER, and leading to error in estimating substrate utilization[15]. In addition, exercising at high intensity increases $[H^+]$ in the blood which is buffered by the bicarbonate ion and results in the production of CO_2 apart from the metabolic processes, further increasing RER. Thus, RER does not always accurately reflect substrate utilization; however, considering the technique is valid, relatively simple, and non-invasive it is considered to be one of the more acceptable ways to assess substrate oxidation.

Currently the literature addressing substrate oxidation post-exercise has focused mainly on the adult population. It has long been reported that that children rely more on fat oxidation during rest and submaximal exercise than adults do, as measured by RER[16,52,78]. Others, though, have reported no difference in RER at the same absolute[79,80] or relative exercise intensity[80]. It is difficult to make any conclusions regarding children's substrate utilization post-exercise because the research in this area is lacking. However, Crisp, et al.[81] have recently published a study addressing the issue of substrate utilization post-exercise in pre-pubertal boys with the addition of comparing weight status, overweight versus normal weight. Crisp, et al. observed no differences between overweight and normal weight boys' substrate utilization before or during exercise; however, for minutes 5 - 10 after the cessation of exercise overweight boys' RER was higher than normal weight boys[81]. When expressed in absolute terms, overweight boys' expended more energy than normal weight boys during minutes 5 – 10 post-exercise, but was similar between the two groups when expressed relative to FFM[81]. Crisp and associates concluded that overweight children relied more on CHO and used more energy post-exercise than normal weight indicating that overweight children may have a reduced capacity to oxidize fats post-exercise. The majority of the literature regarding substrate utilization post-exercise has focused on the adult population. Understanding substrate utilization post-exercise in children could lead to further understanding of the obesity crisis.

Some researchers have postulated that a decreased ability to oxidize fats has been associated with the development of obesity[51]. Substrate utilization post-exercise provides insight into a change in oxidation of fats and CHO. Previous research has demonstrated that fat oxidation increases post-exercise in adults[38,49,82]. Furthermore, populations experience differently the benefits of the post-exercise increase in fat oxidation[49,66]. Obese adults are unable to utilize fats during recovery as well as lean adults[49]. Crisp et al observed a similar trend in overweight boys[81]. One could hypothesize a study on obese children would observe similar results as these investigations; however, currently no research is available to confirm this conclusion.

Nutritional considerations for assessing substrate utilization and EPOC

Dietary intake is an important factor when considering substrate utilization. The consumption of different macronutrients is a significant variable in the oxidation of substrates[83,84]. It has been widely accepted for many years, in adults, diets high in carbohydrates yield higher oxidation rates of CHO (as measured by RER), whereas diets high in fat do not yield a concomitant increase in fat oxidation[83-89]. Additionally, increased intake of fat does not seem to further increase energy expenditure leading to the promotion of energy storage[85,87]. It seems that the type of fat that is consumed is influential in the rate of fat oxidation where monounsaturated fat significantly increases the postprandial fat oxidation over saturated fat[90]. Others have found no significant difference in postprandial fat oxidation among isocaloric meals of polyunsaturated fats, monounsaturated fats, or saturated fats[91]. Consumption of specific macronutrients significantly affects substrate oxidation in adults; therefore, dietary intake should be considered when investigating substrate utilization.

The literature investigating the effect of nutritional intake on substrate utilization in healthy and obese children is not as clear as the adult research. Treuth and associates investigated the 24 hour response of children and adolescents to high carbohydrate and high fat diet using direct calorimetry. They observed that substrate utilization in healthy, normal weight children is not affected by pubertal status, total body fat, intraabdominal fat, or aerobic fitness, but that these children adapted appropriately to the different diet compositions as measured[92]. Furthermore, it seems that dietary intake does not affect a healthy, normal weight, child's total energy expenditure[92]. However, results of some investigations showed that obese children oxidized fat at a higher rate postprandially than lean children[93,94]. Maffeis and colleagues made the argument that even when FFM was statistically controlled, FM still explained an additional 8.1% of the variance in fat oxidation rates[94]. Therefore, it seems that although FFM is the metabolically active tissue in the body, increased FM in obese children contributes to the mechanisms responsible for fat oxidation[94]. In another study Maffeis and associates observed that obese girls had a lower postabsorptive respiratory quotient, but a similar postabsorptive resting energy expenditure; however, REE was expressed as an absolute value (kJ/min) and obese girls had significantly greater FFM than the lean girls[2]. Others have observed that fat mass is highly positively correlated ($r > 0.8$) with endogenous and exogenous fat oxidation after a mixed meal containing 51% CHO, 40% fat, and 9% protein[95]. With regard to CHO oxidation, obese and lean children showed similar oxidation rates of glucose when expressed in absolute terms and also relative to FFM[94]. The literature concerning children's postprandial substrate utilization response to different meal compositions is not as clear as the adult literature. However, it seems fat oxidation is higher in obese children than lean children, and that lean and obese children oxidized CHO similarly after a meal when expressed relative to FFM.

In addition to the issues previously discussed, it is well established that the TEF affects metabolic rate[23]. A two hour fast from food is a typical time frame that researchers use before any metabolic data are collected in order for there to be minimal interaction with the TEF[96]. Also three and four hour fasts have been used in an attempt to limit diet-induced thermogenesis[35,64]. The studies addressing the reproducibility of RMR in children have used a 10 to 12 hour fasts prior to the assessment laboratory visit[97,98]. Segal and colleagues addressed the question of the duration of the TEF in lean and obese adults[99]. The lean and obese participants in this study were matched for age, height, and FFM. Furthermore, RMR was similar between groups. Segal and associates observed that after three hours postprandial both lean and obese individual's metabolic rates were significantly elevated above resting levels and after six hours most, but not all, individuals had reached RMR[99]. The investigators concluded that it is possible that six hours may not be enough to assess the total volume of the TEF in adults. Additionally, Reed and Hill[100] performed an intense study consisting of 131 measurements of the TEF in adults and found that after five hours metabolic rate was only slightly elevated above resting values; the authors concluded that the TEF should be assessed for at least five hours. The literature is less clear concerning the duration of TEF in the pediatric population; however, elevated metabolism has been observed three hours postprandial[101].

The thermic effect of food can increase RMR and since the quantification of EPOC involves subtracting the pre-metabolic rate from the post-exercise metabolic rate; it is important to consider the TEF when assessing the volume of EPOC. The evidence is sparse and not definitive in children concerning the duration of diet-induced thermogenesis; however, a fast of greater than three hours seems desirable when attempting to reduce the effect of the TEF in children.

Summary of the literature

The current literature concerning EPOC is sparse in children, and the information that does exist is spread among various topics. Thus adult literature has been used to supplement where the pediatric literature falls short. Although it seems that the volume of EPOC does not make up a large percentage of TDEE and is, therefore, seemingly not significant in the context of weight management, one cannot ignore the importance of the accumulated benefit of EPOC. Beyond this, insight into metabolic control is gained through the transition from one phase of activity to another[102]; thus, the transition from exercise to rest is an opportunity to investigate the control of metabolism from a perspective that has not been thoroughly studied in children. Furthermore, substrate utilization has been shown to be an important aspect of the metabolic profile of an individual due to the observation that lower oxidation of fats can be an determining factor for obesity[51]. It has been observed that obese pubescent children oxidize fat at a lower rate than lean children during exercise[103], although that observation has been contested by others[81], little is known concerning the oxidation of fat after exercise in obese children.

The duration and intensity of an exercise are major factors for determining the volume of EPOC. Investigators have found that EPOC in adults is exponentially related to the intensity and linearly related to the duration of the exercise as long as the exercise is performed above 60% VO_2max[8]. However, this information has not been demonstrated in the pediatric population. According to the EPOC literature that has been performed on children, it seems that with a moderate exercise of 50% aerobic capacity for twenty minutes healthy children were able to reach pre-exercise metabolic rate in just under nine minutes and consume 1.46 L of O_2[66]. The longest duration of EPOC recorded in children is around twenty minutes after a 1.5 minute bout of supramaximal exercise (125% of VO_2max)[21].

A large portion of EPOC is dependent on the RMR, which is determined before the exercise trial begins, or during a control day. As discussed previously, nutritional status carries a significant impact on RMR and therefore EPOC. Furthermore, the composition of the diet has been shown to greatly affect the TEF. Thus, investigators must take into account caloric consumption and macronutrient composition when measuring RMR and EPOC.

The preceding section summarized the literature available concerning EPOC and substrate utilization in the pediatric population. Unfortunately, little is known concerning the difference between children and adults metabolism post-exercise. More research must be performed to understand the differences between these two populations.

Chapter 3 – Methods

Subjects

Forty-two individuals, 20 children, 11 boys and 9 girls, (ages 7-9 years) and 22 young adults, 11 men and 11 women, (ages 20-25) were recruited from youth programs, medical programs, and other research studies by emails, posted flyers, college classes and word of mouth. Children showed no overt signs of onset puberty based on parental verbal confirmation. Any potential participants who were not able to complete regular physical activity, similar to what would be experienced in a physical education class at school, were excluded. Participants visited the laboratory on three separate occasions after at least a three-hour fast in order to help reduce the effects of the thermic effect of food[100]. Each visit was separated by at least 24 hours. The Michigan State University Institutional Review Board approved this study. Written informed consent and assent were obtained from the adult participants and parents and child participants, respectively.

Measures

The different measures included anthropometric measurements, macronutrient composition, caloric consumption, RMR, VO_2peak (aerobic fitness), substrate utilization, and EPOC. For anthropometry, standing height, weight, and skinfolds were assessed. Height and weight measurements were taken in duplicate using standardized procedures and averaged for a final value. Height was measured using a wall-mounted stadiometer (Harpenden). If measurements differed by more than half a centimeter a third measurement was obtained. Weight was measured via a digital scale (Seca 770). If height or weight measurements differed by more than 0.5kg a third measurement was taken, and the three were averaged. Body composition was assessed using skinfold thicknesses via the subscapula and triceps folds with a Harpenden caliper using methods as described by Lohman et al. for children[104]; for adults, chest, abdominal, and thigh were assessed in men; and triceps, supraillium, and thigh were assessed in women. Body fat estimation was derived using the Slaughter equation[105] for children and the Jackson and Pollock equations for adults[106,107].

Dietary data were obtained using two 24-hour dietary recalls, prior to visits two and three, to assess caloric consumption, macro nutrient composition and the intake of nutrition supplements of participants in order to account for the TEF. The recalls were administered by trained dietetic students using the validated USDA multiple pass method[108,109]. The procedure took approximately 20-30 minutes to complete, and the participants were informed about assessment procedures at the time of consent as well as prior to the recalls being conducted. A quick list of foods and beverages consumed within the prior 24-hour period was first collected. The interviewer used probes for foods forgotten during the quick list with open-ended questions and mention of frequently missed foods. Mealtime and eating occasions for each food were collected and reviewed with the participant. Detailed descriptions of the foods and their preparation and serving methods with portion sizes were obtained. The participants were asked if the day recalled was a typical day pertaining to eating habits. Any dietary supplements, vitamins/minerals or herbal/ home remedies were recorded and the interviewer used a final probe for anything else that was consumed and double checked for incomplete items.

To obtain energy expenditure during resting, pre-exercise, exercise, and post-exercise time periods, expired gases were collected and analyzed using a Parvo Medics'

TrueOne® 2400 metabolic cart. The metabolic cart was calibrated before each visit for volume, using a 3 liter syringe, and gas concentration using ambient air (of known temperature, pressure, and humidity) and known gases: 16.00% O_2, 4.00% CO_2, and balanced N_2 before each of the exercise tests. Previously, this metabolic system has been found to be a valid measurement of oxygen consumption and carbon dioxide production as only small differences were found when compared to the Douglas Bag method (F_EO_2 = 0.0004, F_ECO_2 = -0.0003, and VO_2= -0.018 L/min)[110]. Substrate utilization was estimated indirectly using the widely accepted method of the analysis of respiratory exchange ratio: VCO_2/VO_2. As previously mentioned in this paper, there are shortcomings to this technique, however, it has been found to be a valid, non-invasive, method for estimating the whole body use of macronutrients and has been used in numerous studies[14,60,111,112].

Aerobic fitness was assessed using a graded exercise test to exhaustion on an electronically braked cycle ergometer. The Godfrey protocol[46] was used for the children, and the adults used an incremental protocol tailored to their needs, as some adults required a more aggressive exercise test than others. Both children and adult maximal exercise tests were designed to last 10 – 15 minutes. Each stage lasted one minute. For children the initial power output began at 10W with 10W increase per stage each minute. Initial power output began at 50W and increased by 25W each minute for adult maximal exercise test. Some adults required increases of 10W each minute toward the end of test in order to attain exhaustion in 10 – 15 minutes. Expired gases were collected continuously and examined in 15-second intervals and heart rate was collected continuously throughout the duration of the test for both adults and children. No reliability tests of the equipment were performed; however previous studies have shown the ParvoMedics TrueOne® 2400 metabolic cart to be valid[110,113].

Excess post-exercise oxygen consumption (EPOC) was calculated using the data from ParvoMedics TrueOne® 2400 metabolic cart. EPOC is considered as the accumulated VO_2 during the post-exercise period minus the accumulated resting VO_2 during the same time period, which previous studies showed to be an appropriate method of determining EPOC[49,65]. This study did not have a control visit and, therefore, could not compare the post-exercise VO_2 to resting VO_2 of the same time period. Instead post-exercise oxygen consumption was compared directly to pre-exercise oxygen consumption of the same day. Duration of EPOC was calculated by recording the time when post-exercise expired gases reached baseline. EPOC was considered to have reached baseline when two, 15-second intervals, were less than or equal to baseline measures.

Substrate utilization was estimated by the ratio between the production of carbon dioxide (VCO_2) and consumption of oxygen (VO_2) collected in expired gases. It has been established that RER does not accurately reflect substrate utilization when individuals are transitioning from one workload to another, moving from rest to exercise, or exercise to rest[15]. Thus, RER immediately post-exercise cannot be assumed to be reflective of substrate use; however, it can be used as an indication of metabolic disturbance. Despite limitations, post-exercise RER has been used in numerous research studies[38,49,59,61,65,81,114,115]. Respiratory exchange ratio was statistically analyzed by averaging VCO_2/VO_2 over one minute, in 15-second intervals, for minutes 1, 5, 10, 15, and 20 post-exercise.

Procedures

The first visit was composed of anthropometric measurements and aerobic fitness testing. The aerobic fitness test was assessed via a graded exercise test to exhaustion on an electronically braked cycle ergometer (Lode B.V. Corival Pediatric for children and Sensormedics for adults) in order to establish working loads for the second and third laboratory visits. Attainment of at least two of the following three criteria were needed for the exercise test to be considered peak: 1) volitional fatigue 2) heart rate (HR) $\geq$95% age predicted maximum 3) RER > 1.00. If criteria were not met the peak test was repeated; however, only one test needed to be repeated. Furthermore, ventilatory threshold (VT) was estimated using the data from the peak test by plotting data to find the moment just prior to the disproportionate increase in VE/VO_2 when compared to VE/VCO_2.

The second and third visits consisted of participants cycling at moderate and vigorous intensities visit after a three-hour restriction from exercise and nutrition. The order of these intensities (moderate and vigorous) was randomized and counter balanced. Prior to each exercise test, pre-exercise oxygen consumption (baseline) was assessed. To obtain this measure, participants rested for 30 minutes in a comfortable, reclining chair placed in a semi-recumbent position while expired gases were collected using a Hans-Rudolph, oro-nasal mask. In order to ensure pre-exercise measurements were as close to basal metabolism as possible, the lowest and most consistent 10 of the last 20 minutes of the rest period were used to establish baseline oxygen consumption measures. Once this baseline measure was established, the same time period was also used for the baseline RER measure. Participants were instructed to remain awake, still, and quiet for the duration of the test. They were allowed to watch a movie (standard for all children) during this process to help keep them from becoming impatient and fidgeting. Other researchers have also employed the technique of using television or a movie to help control the subjects' movements while resting and have found that there are negligible, non-significant differences between resting measurements with and without television viewing[96,116].

Children typically are not continuously physically active for extended periods of time and rarely do they reach a steady state; therefore, the participants in this study exercised on an electronically braked cycle ergometer in two-minute, square wave intervals – two minutes of exercise followed by two minutes of static rest while remaining on the ergometer. Heart rate (HR) and expired gases were recorded continuously throughout the exercise trials, although HR was not used in statistical analyses. The moderate intensity exercise test required the participant to work at 35% of his or her maximal power output (attained in the aerobic capacity test) in two-minute intervals for a total of 60 minutes. The participant accumulated 30 minutes of moderate intensity exercise over the 60-minute period. The vigorous intensity exercise test required the participant to pedal at 70% of his/her maximal power output attained in the maximal aerobic capacity test in two-minute intervals for 30 minutes. In this laboratory visit the participant accumulated 15 minutes of vigorous activity. The nature of the exercise tests did not allow participants to maintain a steady state of exercise. It is commonly accepted that children are able to reach a metabolic steady state during exercise faster than adults[117]. Some have reported that children can reach steady state within two minutes[46,118], whereas adults may require three to four minutes before reaching a steady state of exercise[119]. The criterion for achieving steady state is typically HR ± 5 bpm, VO_2 ± 10%, and VCO_2 ± 10% for three consecutive 20-s intervals[80]. Since analyzing steady state VO_2 to describe exercise was not an option we averaged the four peak 15-second VO_2 measurements of the last three exercise bouts, which turned out to be the twelve highest, 15-second VO_2 measurements of the exercise. By averaging the peaks of VO_2 during the intermittent exercise, we were able to establish an estimate of the metabolic cost of the exercise intensities and durations. This method could potentially result in an overestimation of the exercise VO_2 so we also averaged all exercise VO_2 measurements.

Immediately upon cessation of the exercise the participant was moved to the semi-recumbent position where s/he remained still, quiet, and awake as expired gases were discontinuously monitored for one hour. During this hour the participants were allowed to continue the standard movie. At the end of the first 20 minutes the mask was removed and the participants remained still, quiet, and awake for the remainder of the hour. This method was implemented for two main reasons: 1) current research suggests that recovery in children is very rapid and only a few minutes of post-exercise measurements are needed and 2) we believed it to be overly demanding to have study participants wear a mask for a longer period of time. A major drawback to only capturing expired gases for the first 20 minutes after exercise would be the inability to measure EPOC if participants required more time to recover. According to the available literature, it seemed unlikely that children would experience more than 20 minutes of EPOC. In the last ten minutes of the hour the mask was placed back on the participant's face and expired gases were collected.

Analysis

A power analysis was conducted using the results of studies of oxygen kinetics (during exercise and recovery) differences between children and adults[20,120]. Zanconato et al[20] is only major investigation to compare oxygen consumption post-exercise between children and adults. The main outcome variable was duration of EPOC as the results provided the most conservative estimate (i.e., smallest effect size) for sample size determination. The results of the power analysis indicated a sample size of 16 total participants for assessment by one tail t-test, assuming an expected difference of 14 seconds, power of 0.8, alpha level of 0.05, and effect size of 1.1. Previous research focusing on anaerobic metabolism has shown children's VO_2 returning to baseline faster than adults[20], thus a one tailed test is an appropriate statistical method. A second power analysis was performed using substrate utilization as the main outcome variable. Expected effect sizes for RER differences were greater than those for EPOC differences, indicating sample size needed to detect differences would be smaller than the first power analysis (4-8 participants total). Therefore, the selection of a sample size of 16 participants for each group was considered sufficient for the substrate utilization variable.

The analytical plan involved performing descriptive statistics, t-tests, correlations, and various forms of ANOVA such as repeated measures ANOVA and ANCOVA to examine differences between children and young adults for each exercise condition.

The aims and analytical plan of this study were the following:

1) To describe the metabolic profile (oxygen consumption pre-, during and post-exercise) of children and young adults.

This aim required use of descriptive statistics and t-tests for comparing pre-exercise values from the moderate and vigorous intensity visits. Oxygen consumption during exercise was described as a percent of VO_2peak in children and VO_2peak in adults as well as in relation to ventilatory threshold (VT) and percent of VT.

2) To examine differences in EPOC between young adults and children after moderate and vigorous intensity exercise.

Hypothesis A: Young adults would experience greater volume of EPOC than children after moderate intensity exercise.

Hypothesis B: Young adults would experience greater volume of EPOC than children after vigorous intensity exercise.

Hypothesis C: Young adults would experience longer duration of EPOC than children after moderate intensity exercise.

Hypothesis D: Young adults would experience longer duration of EPOC than children after vigorous intensity exercise.

The dependent variables were the volume (L) and duration (seconds) of EPOC. The independent variable was age level (young adult or child). ANOVA was used to analyze age level differences in volume and duration of EPOC separately after moderate intensity and vigorous intensity exercise. ANCOVA was also used to investigate the differences in EPOC between adults and children while controlling for sex, caloric consumption, and macronutrient composition, fitness, and body composition. Separate analyses were run for those who reached baseline and for those who did not.

3) To examine differences in EPOC between moderate and vigorous intensity exercise in children and young adults.

Hypothesis A: Vigorous exercise would elicit a greater volume of EPOC than moderate exercise in children and young adults.

Hypothesis B: Vigorous exercise would elicit a longer duration of EPOC than moderate exercise in children and young adults.

Repeated measures ANOVA was used to analyze the effect of exercise intensity on volume and then duration of EPOC with age level as a factor. A single analysis was performed for Aim 2 and 3. Another analysis controlling for sex was performed. Since controlling for fitness, body composition, caloric consumption, and macronutrient composition in Aim 2 analyses had no effect on outcome, those variables were not included as covariates in these analyses.

4) To examine if differences existed between pre-exercise oxygen consumption and one-hour post-exercise (moderate and vigorous) in children and young adults.

Hypothesis A: Children's oxygen consumption would not be significantly different ($<$1.3ml/kg•min^{-1})[53] between pre-exercise and one-hour post-exercise for moderate or vigorous exercise.

Hypothesis B: Young adults' oxygen consumption would be significantly elevated ($>$1.3ml/kg•min^{-1})[53] one-hour post exercise when compared to pre-exercise values.

T-tests were used to compare pre-exercise to post-exercise values in both children and young adults for moderate and vigorous visits.

5) To examine if any differences existed in RER between young adults and children after moderate and vigorous intensity exercise at minutes 1, 5, 10, 15, and 20 post-exercise.

Hypothesis A: Young adults would have higher RER than children after moderate exercise.

Hypothesis B: Young adults would have a higher RER than children after vigorous exercise.

The dependent variables were RER recorded at minutes 1, 5, 10, 15, and 20 post-exercise. The independent variable was age level. Separate analyses were performed for moderate and vigorous intensity exercise. Multivariate ANOVA (MANOVA) was used to analyze for any RER differences between children and adults after moderate exercise and then again after vigorous exercise. ANCOVA, controlling for sex, caloric consumption, and macronutrient composition, fitness, and body composition, was also performed.

6) To examine if any differences exist in RER between the post-exercise period of moderate and vigorous in children and young adults at minutes 1, 5, 10, 15, and 20.

Hypothesis: The vigorous exercise would elicit a higher RER post-exercise when compared to moderate intensity exercise for children and young adults.

Repeated measures ANOVA was used to investigate the differences in RER between moderate and vigorous intensity exercise with age level (young adult vs. child) as a factor. Separate analyses were used for each minute (1, 5, 10, 15, and 20). A single analysis was performed to examine Aims 5 and 6. Since controlling for caloric consumption, and macronutrient composition in Aim 2 analyses had no effect on outcome, these variables were not covariates in these analyses.

7) To examine if any differences existed in RER between pre-exercise and post-exercise (moderate and vigorous) in children and young adults.

Hypothesis A: Children's pre-exercise RER would be lower than moderate and vigorous measurements at minutes 1, 5, 10, 15, and 20 post-exercise.

Hypothesis B: Young adults' baseline RER values would be lower than moderate and vigorous post-exercise measurements.

Hypothesis C: RER measurements one hour post-exercise would be similar to baseline in both children and young adults.

Paired t-tests were used to analyze within young adult and within child differences between pre-exercise and post-exercise RER in moderate and vigorous intensity visits.

All analyses were adjusted for fitness. All results were considered statistically significant at an alpha level of $p \leq 0.05$.

Significance of the results

The duration and volume of EPOC in children has been quantified following moderate and vigorous exercise. Prior studies investigating recovering from exercise in children have used either tests of very short duration (around 1 minute) or steady state exercise. This study was the first of its kind to use an exercise protocol that resembles the daily intermittent physical activity of children.

It is estimated by the U.S. Census Bureau that nearly 20 million children 7 – 11 years old and 25 million adolescents 12 – 17 years old participate in sporting activities[121]. According to the National Federation of State High School Associations, sports participation has increased for the 22nd consecutive school year to more than 7.6 million participants nationwide[122]. Understanding recovery metabolism and substrate utilization could provide information for children who are participating in interval training for sports as well as consideration for recovery nutrition.

Pediatric researchers sometimes administer multiple exercises tests in a single visit in order to save time and reduce the load on participants[21,123-126]. A greater understanding of recovery metabolism will enable pediatric researchers to appropriately administer multiple exercise tests in a single visit.

Chapter 4 – Results

Physical characteristics

The physical characteristics of the sample are depicted in *Tables 1 and 2*. Nineteen children and 22 young adults: 11 boys, 8 girls, 11 men, and 11 women (ages 8.0 (mean) $\pm$0.9 (sd), 8.5$\pm$1.0, 21.5$\pm$0.8, and 21.2$\pm$0.7 years, respectively) volunteered to participate in this study. No significant differences ($p<0.05$) were found between boys and girls in age, weight, height, BMI, BMI%ile, body fat percentage, peak power, or VO_2peak; thus boys' and girls' data were combined and are referred to as the group 'children' in the following results and discussion *(see Table 1)*. No significant differences were found between men and women in age, weight, height, or BMI. However, men had significantly lower body fat percentage (6.9$\pm$3.0 vs 23.6$\pm$5.0 percent respectively, t = -9.419, $p < 0.001$, d = 4.21), higher peak power (243$\pm$31 vs 202$\pm$53 W respectively, t = 2.206, p = 0.04, d = 0.98), and higher VO_2peak (47.0$\pm$11.0 vs 35.8$\pm$5.5 ml/kg•min^{-1} respectively, t = 3.018, p = 0.007, d = 1.35) than women. Since the aims of this study were to investigate differences between children and young adults, men and women's data were combined and are referred to as 'young adults' in the following results and discussion. As expected, significant differences were found between children and young adults in the following areas: age, height, weight, BMI, and peak power. Similarly, it was also expected and observed that no significant differences were found in body fat percentage and relative VO_2peak (ml/kg•min^{-1}) between children and young adults. However, when absolute VO_2peak was analyzed children's VO_2 was found to be significantly lower than young adults (1.12 $\pm$ 0.21 vs. 2.97 $\pm$ 0.75 L/min, t = 10.691, p <0.001, d = -3.382, children and young adults, respectively). The following were observed to reject the null hypothesis for Levene's test for Equality of Error Variances: weight, height, BMI, BF%, peak power (significance = 0.001, 0.024, 0.013, 0.039, and <0.001, respectively). Levene's test assesses the assumption that the samples are of equal variance. Failure to reject the null hypothesis for Levene's test shows evidence of unqual variance between the groups[127]. Laboratory temperature and barometric pressure averaged 22.7±1.0° Celsius and 740±4mmHg, respectively.

Table 1. Combined physical characteristics.

	Children mean±sd (min, max)	Young Adults mean±sd (min, max)
N	19	22
Chronological Age (years)	8.2±0.9 (7.1, 9.9)	21.3±0.7* (19.9, 22.7)
Weight (kg)	27.9±6.2 (22.5, 50.4)	71.8±10.5* (55.4, 88.8)
Height (cm)	131.3±5.4 (123.1, 144.7)	172.4±8.1* (159.4, 188.1)
BMI (kg/m^2)	16.1±2.3 (13.8, 24.1)	24.2±3.6* (18.4, 32.8)
BMI Percentile	45±27 (5, 97)	NA
Body Fat (%)	14.5±5.2 (9.6, 28.0)	15.3±9.4 (3.9, 30.8)
Peak Power (W)	88.0±13.9 (60, 110)	222.5±47.9* (145, 305)
Peak VO_2 (L/min)	1.12±0.21 (0.72, 1.48)	2.97±0.75* (1.92, 4.62)
Peak VO_2 ($ml/kg \bullet min^{-1}$)	40.6±7.3 (28.4, 54.8)	41.4±10.2 (28.4, 70.8)

∗ p-value < 0.05 from t-test comparing to children

Table 2. Physical characteristics

	Boys mean±sd (min, max)	Girls mean±sd (min, max)	Men mean±sd (min, max)	Women mean±sd (min, max)
N	11	8	11	11
Chronological Age (years)	8.0±0.9[c,d] (7.1, 9.9)	8.5±1.0[c,d] (7.5, 9.9)	21.5±0.8[a,b] (19.9, 22.7)	21.2±0.7[a,b] (20.1, 22.4)
Weight (kg)	27.9±3.3[c,d] (23.6, 34.3)	28.1±8.9[c,d] (22.5, 50.4)	73.9±9.6[a,b] (59.7, 88.8)	69.6±11.3[a,b] (55.4, 88.3)
Height (cm)	131.4±3.1[c,d] (126.9, 137.7)	131.1±7.5[c,d] (123.1, 144.7)	175.8±7.1[a,b] (163.9, 188.1)	169.0±7.9[a,b] (159.4, 184.1)
BMI (kg/m^2)	16.1±1.3[c,d] (14.7, 18.7)	16.1±3.3[c,d] (13.8, 24.1)	24.0±3.3[a,b] (18.4, 28.3)	24.4±4.0[a,b] (19.1, 32.8)
BMI %ile	51±21 (22, 88)	37±33 (5, 97)	NA	NA
Body Fat (%)	14.3±5.4[c,d] (9.6, 24.2)	14.8±5.3[c,d] (11.2, 28.0)	6.9±3.0[a,b,d] (3.9, 12.9)	23.6±5.0[a,b] (15.1, 30.8)
Peak Power (W)	90.5±14.0[c,d] (60, 110)	85.0±14.0[c,d] (70, 109)	243.2±31.5[a,b,d] (195, 305)	201.8±53.6[a,b] (145, 300)
Peak VO_2 ($ml/kg•min^{-1}$)	42.3±7.4 (30.1, 54.8)	38.6±7.1 (28.4, 48.4)	47.0±11.0[d] (33.6, 70.8)	35.8±5.5 (28.1, 46.1)

[a] p-value < 0.05 from t-test comparing to boys
[b] p-value < 0.05 from t-test comparing to girls
[c] p-value < 0.05 from t-test comparing to men
[d] p-value < 0.05 from t-test comparing to women

Nutrition – caloric consumption and macronutrient composition

Dietary recalls were analyzed to determine the amount of calories consumed and the macronutrient composition of the diet 24 hours prior to each exercise test. No significant differences were observed in caloric consumption or macronutrient composition between the moderate and vigorous visits in children or young adults. The data are further described in *Table 3*.

Table 3. Caloric consumption and macronutrient composition

Children

	moderate visit mean±sd	vigorous visit mean±sd	t	d
kcals	1509±667	1780±580	-1.481	-0.4422
%CHO	58.9±10.8	60.0±9.0	-0.349	-0.1156
%protein	13.8±4.8	13.4±4.2	0.524	0.0919
%fat	28.9±9.3	28.7±7.1	0.72	0.0226

Young Adult

	moderate visit mean±sd	vigorous visit mean±sd	t	d
kcals	1909±752	1992±812	-0.565	-0.0349
%CHO	51.7±13.3	52.8±12.7	-0.648	-0.1719
%protein	19.1±7.1	18.5±6.7	0.407	0.0943
%fat	31.0±11.4	27.3±8.5	1.252	0.0438

Note: CHO = carbohydrates

Pre-exercise Oxygen Consumption

Pre-exercise VO_2 measurements (pre-exercise measurements are referred to from this point on as baseline) for children revealed no significant differences between moderate and vigorous visits: 6.5 $\pm$ 1.1 and 6.3 $\pm$ 1.0 ml/kg•min^{-1}, respectively ($t = 0.919$, $p = 0.370$, $d = 0.17$) and were significantly correlated ($r = 0.713$; $r^2 = 0.508$; $p < 0.001$). Adult baseline VO_2 measurements were also not significantly different between the moderate and vigorous visits: 3.4 $\pm$ 0.4 and 3.5 $\pm$ 0.5 ml/kg•min^{-1}, respectively ($t = -0.539$, $p = 0.596$, $d = 0.02$) and were also significantly correlated ($r = 0.569$; $r^2 = 0.323$; $p < 0.006$). Children had significantly higher pre-exercise VO_2 than young adults for both moderate and vigorous visits (6.5 $\pm$ 1.1 vs. 3.4 $\pm$ 0.04 ml/kg•min^{-1}; $F = 157.245$, $p < 0.001$, $d = 3.96$ and 6.3 $\pm$ 1.0 vs. 3.5 $\pm$ 0.5 ml/kg•min^{-1}; $F = 133.231$, $p < 0.001$, $d = 3.657$). However, when considering absolute values, young adults showed greater pre-exercise VO_2 before the moderate exercise (0.176 ± 0.02 vs. 0.243 ± L•min^{-1}; $F = 56.893$, $p < 0.001$, $d = -2.39$; children and young adults, respectively) and before vigorous exercise (0.173 ± 0.25 vs 0.246 ± 0.008 L•min^{-1}; $F = 51.949$, $p < 0.001$, $d = -2.28$; children and young adults, respectively).

Exercise oxygen consumption

During exercise children and young adults cycled at 35% and 70% of their peak power output. In order to capture the peaks of oxygen consumption during the square-wave cycling protocol, the highest 12 samples (15 second samples) of VO_2 during exercise were averaged. Children exercised at 60.2 $\pm$ 9.7 and 88.8 $\pm$ 13.9% of VO_2peak for moderate and vigorous exercise, respectively ($t = -16.530$, $p < 0.001$, $d = 2.44$). Young adults' VO_2 during exercise was 46.1 $\pm$ 5.9 and 73.0 $\pm$ 6.2% of VO_2peak for moderate and vigorous exercise, respectively ($t = -27.530$, $p < 0.001$, $d = 4.57$). It is possible that the technique of choosing the highest VO_2 samples would exaggerate the actual VO_2 during exercise, so the data were also analyzed by averaging all the VO_2 gathered during the exercise trial – including the rest intervals between exercise bouts. These data showed VO_2 during moderate exercise was significantly less than that of vigorous exercise in both children and young adults. The VO_2 data also showed the energy expenditure of the vigorous exercise was not a two-fold increase over the moderate exercise as anticipated, even though the power output was a two-fold increase. The exercise bouts were designed to elicit similar caloric expenditure through increasing the exercise intensity by 100% and reducing the time by 50% during the vigorous exercise when compared to the moderate exercise. In reality, children only experienced a 48% (using the 12 highest VO_2 measurements) or 40% (using the average of all VO_2) increase in VO_2 during the vigorous exercise when compared to the moderate exercise.

Adults experienced a 60% (using the 12 highest VO_2 measurements) or 50% (using the average of all VO_2) increase in VO_2 during the vigorous exercise when compared to the moderate exercise. These data are presented in *Tables 4 and 5.*

Table 4. Child: moderate vs vigorous

	moderate mean±sd	vigorous mean±sd	t	d
%VT	81.0±13	118.7±16.9	-17.731*	0.73
%VO_2peak	60.2±9.7	88.8±2.4	-16.53*	2.44
Avg. 12 Highest VO_2 15-sec ml/kg•min^{-1}	24.0±3.0	35.6±5.4	-15.161*	-2.6556
Avg. VO_2 ml/kg•min^{-1}	14.7±2.0	20.6±3.3	-12.391*	-2.1946

* p-value <0.001

Table 5. Young adult: moderate vs vigorous

	moderate mean±sd	vigorous mean±sd	t	d
%VT	68.5±12.7	108.3±15.4	-23.971*	2.89
%VO_2peak	46.1±5.9	73.0±2.3	-27.53*	4.57
Avg. 12 Highest 15-sec VO_2 ml/kg•min^{-1}	18.7±2.9	29.9±5.7	-14.751*	-2.4767
Avg. VO_2 ml/kg•min^{-1}	11.3±1.5	17.0±2.9	-14.938*	-2.5811

* p-value <0.001

As previously stated, exercise intensity plays a significant role in post-exercise metabolic measurements. Although the exercise trials were set at the same percentage of peak power output for all participants (35% and 70% of peak power output), children and young adults differed in their relative intensity (as measured by percent of VO_2peak) during the exercise bouts. In order to understand how participants responded metabolically to the power output being set at a percentage of their peak, a post-hoc examination relative to exercise intensity was performed. After calculating ventilatory threshold (VT) from VO_2peak tests, the average VT occurred at 30 ± 5 ml/kg•min (74 ± 11% of VO_2peak) for the children and 28 ± 7 ml/kg•min (68 ± 9% of VO_2peak) for young adults. There were no significant differences found for VT between children and young adults as expressed relative to body mass (30 $\pm$ 5 vs. 28 $\pm$ 7 ml/kg•min; F = 0.859, p = 0.360, d = 0.30) or relative to VO_2peak (74 $\pm$ 11% vs. 68 $\pm$ 9% of VO_2peak, children and young adults respectively; F = 3.334, p = 0.76, d = 0.59). Absolute power output was significantly different between children and young adults at moderate (30.8 $\pm$ 4.9 vs. 77.9 $\pm$ 16.7 W, children and young adults respectively; t = 12.104, p <0.001, d = -3.83) and vigorous exercise intensities (61.6 $\pm$ 9.7 vs. 155.75 $\pm$ 33.5 W, children and young adults respectively; t 12.104, p<0.001, d = -3.83). Percent of VT was significantly less in the moderate intensity visit when compared to the vigorous intensity visit in the children (81.0 $\pm$ 13.0% vs 118.7 $\pm$ 16.9%; t = -17.731, p < 0.001, d = 0.73); see *Table 4*. Similarly in young adults, percent of VT was significantly less in the moderate intensity visit when compared to the vigorous intensity visit (64.5 $\pm$ 12.7% vs 108.3 $\pm$ 15.3%; t = -23.971, p < 0.001, d = 2.89); see *Table 5*. When relative intensities were compared for moderate and vigorous visits children exercised at a significantly higher percentage of VT than young adults (81.0 $\pm$ 13.0% vs. 68.5 $\pm$ 12.7%; F = 9.862, p = 0.003, d = 1.02 and 118.7 $\pm$ 16.9% vs. 108.3 $\pm$ 15.3%; F = 4.264, p = 0.046, d = 0.66, respectively); see *Tables* 6 *and 7*. The results indicating greater percentage of VT for the vigorous exercise visit when compared to the moderate exercise visit were expected and provided researchers with the confidence that the exercise tests selected placed the participants in two distinct exercise environments. Children performed at a greater percentage of their VT than young adults during both the moderate and vigorous exercise. Children and young adults did not differ significantly in their VO_2peak or in their VO_2 at VT.

Table 6. Children versus young adults during moderate exercise

	Child mean±sd	Young Adult mean±sd	F	d
%VT	81.0±13	68.5±12.7	9.862*	1.02
$\%VO_2$peak	60.2±9.7	46.1±5.9	33.336**	1.7769
Avg. 12 Highest 15-sec VO_2 ml/kg•min^{-1}	24.0±3.0	18.7±2.9	33.226**	1.7979
Avg. VO_2 ml/kg•min^{-1}	14.7±2.0	11.3±1.5	40.802**	1.9972

* p-value <0.01
** p-value <0.001

Table 7. Children versus young adults during vigorous exercise

	Child mean±sd	Young Adult mean±sd	F	d
%VT	118.7±16.9	108.3±15.4	4.264*	0.66
$\%VO_2$peak	88.8±2.4	73.0±2.3	23.104***	6.729
Avg. 12 Highest 15-sec VO_2 ml/kg•min^{-1}	35.6±5.4	29.9±5.7	10.779**	1.0253
Avg. VO_2 ml/kg•min^{-1}	20.6±3.3	17.0±2.9	13.990**	1.1799

* p-value <0.05
** p-value <0.01
*** p-value <0.001

Excess post-exercise oxygen consumption

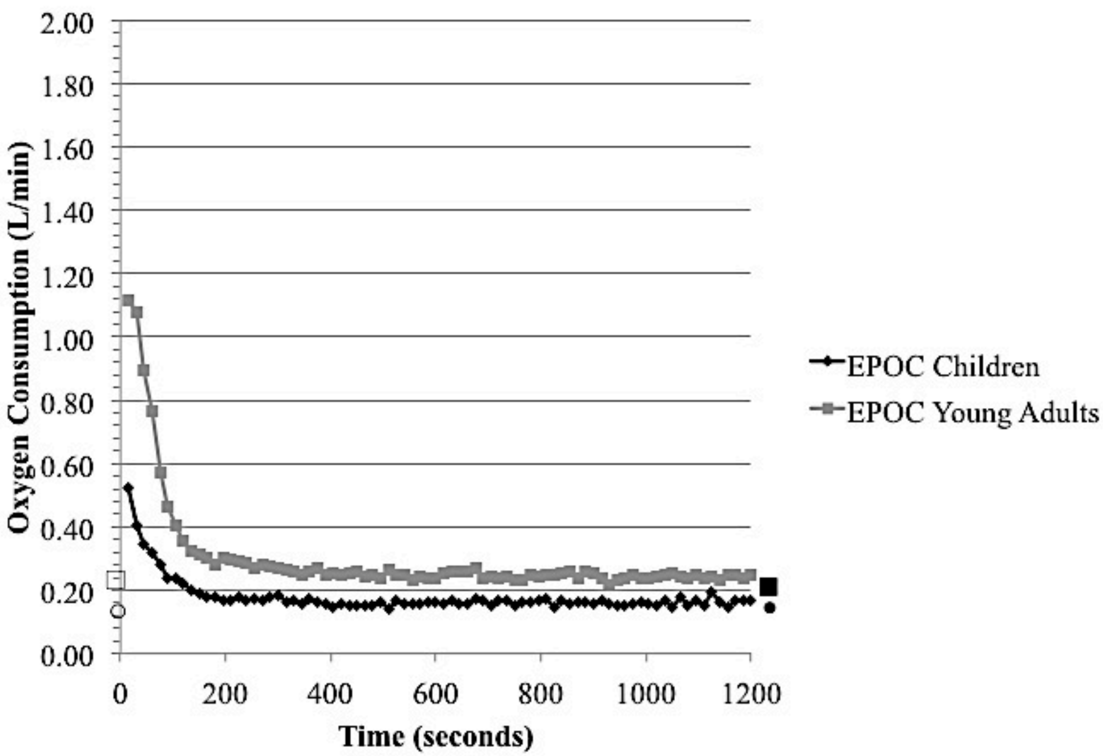
Oxygen consumption was measured continuously for twenty minutes post-exercise. Twenty minutes of post-exercise measurements was not enough time to evaluate complete EPOC as twelve participants, two children and 10 adults, failed to reach baseline in the period post-exercise measurement across both visits. Concerning the moderate visit: all children reached baseline, but three young adults (two men and one woman) did not reach baseline. Concerning the vigorous visit: two children (both girls) and seven young adults (four men and three women) did not reach baseline. Data from those who reached baseline were analyzed separately and in combination with those who did not and were reported as such and will be further addressed later. There was an inherit limitation to the approach of only measuring for twenty minutes post exercise as the data from participants whose VO_2 did not return to baseline were naturally truncated by the cessation of measurement. Data from all participants at minutes 19 and 20 post-exercise were analyzed in order to establish if the children and young adults had reached baseline. There was no significant difference between pre-exercise VO_2 measures and the last two minutes of the post-exercise in either children or young adults (6.47±1.07 vs. 6.05±1.17 ml/kg•min^{-1}; $t = 1.637$, $p = 0.118$, $d = 0.38$ for children's pre- vs post-exercise; and 3.40±0.38 vs. 3.41±0.50; $t = -0.023$, $p = 0.982$, $d < -0.01$ for young adults' pre- vs. post-exercise), indicating EPOC, in this sample population and on average, had ended in both children and young adults and they had indeed reached baseline, see *Table 8*.

Table 8. Pre-exercise vs. post-exercise VO_2 in children and young adults – total sample

Children (n=20)	mean	sd	t	d
Pre-Ex VO_2 (ml/kg•min^{-1})	6.47	1.07	1.637	0.3813
Post-Ex VO_2 (ml/kg•min^{-1})	6.05	1.17		
Young adults (n=22)				
Pre-Ex VO_2 (ml/kg•min^{-1})	3.40	0.39	-0.023	0.0003
Post-Ex VO_2 (ml/kg•min^{-1})	3.41	0.50		

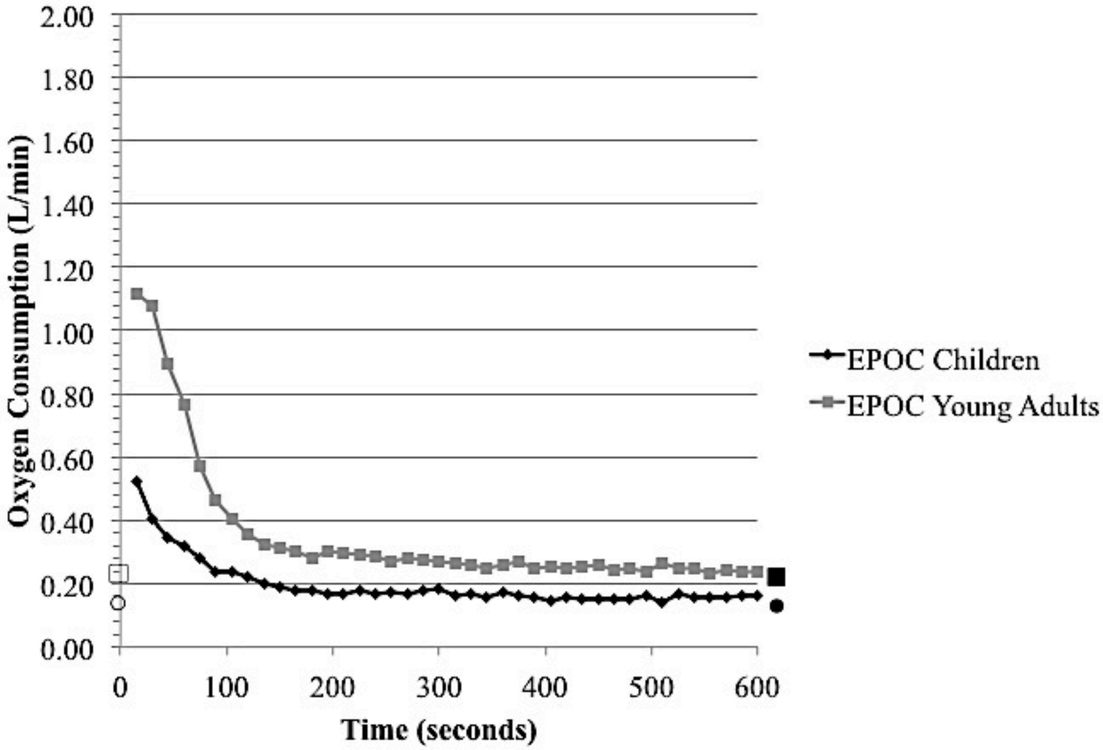
Children's EPOC after both the moderate and vigorous exercise tests was significantly less than young adults' (0.30 $\pm$ 0.13 vs. 1.18 $\pm$ 0.38 L; $F = 19.609$, $p < 0.001$, $d = 0.31$ for moderate and 0.71 $\pm$ 0.26 vs. 2.46 $\pm$ 0.95 L; $F = 59.73$, $p < 0.001$, $d = 2.44$ for vigorous exercise). Results did not differ after controlling for sex, aerobic fitness, body composition, and/or caloric consumption. Figures 3 – 6 depict EPOC in children and young adults after moderate and vigorous intensity exercise, along with pre-exercise (baseline) and end-exercise VO2. *Figure 3* depicts oxygen consumption taken in 15-second intervals of young adults and children for 20 minutes following the moderate exercise protocol. *Figure 4* depicts oxygen consumption taken in 15-second intervals of young adults and children for 10 minutes post-exercise following the moderate exercise protocol. By showing only 10 minutes of measurements the reader has a closer image of the period during which EPOC occurred. *Figure 5* depicts oxygen consumption taken in 15-second intervals of young adults and children for 20 minutes following the vigorous exercise protocol. *Figure 6* depicts oxygen consumption taken in 15-second intervals of young adults and children for 10 minutes post-exercise following the vigorous exercise protocol.

Figure 3. EPOC volume after moderate exercise: children and young adults (20 minutes).



Note: ☐*Mean pre-exercise* VO_2 *in young adults*
○ *Mean pre-exercise* VO_2 *in children*
■ *Mean end-exercise* VO_2 *in young adults*
● *Mean end-exercise* VO_2 *in children*

Figure 4. EPOC volume after moderate exercise: children and young adults (10 minutes).



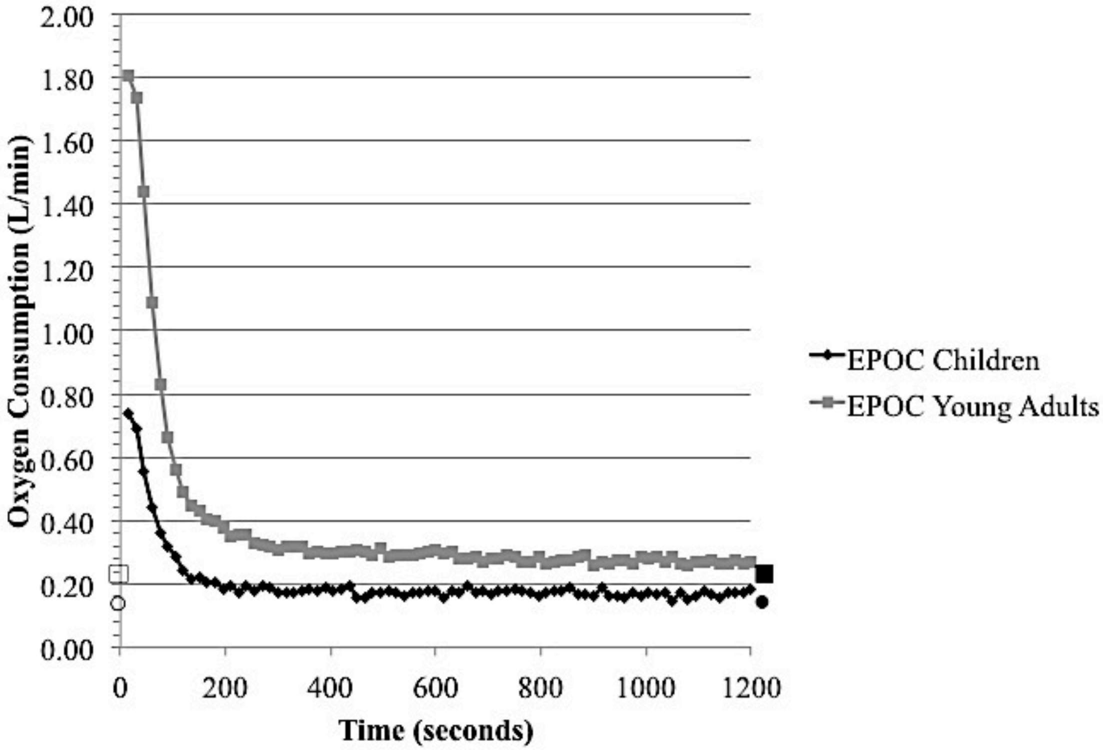
Note: □*Mean pre-exercise* VO_2 *in young adults*

○ *Mean pre-exercise* VO_2 *in children*

■ *Mean end-exercise* VO_2 *in young adults*

● *Mean end-exercise* VO_2 *in children*

Figure 5. EPOC volume after vigorous exercise: children and young adults (20 minutes).



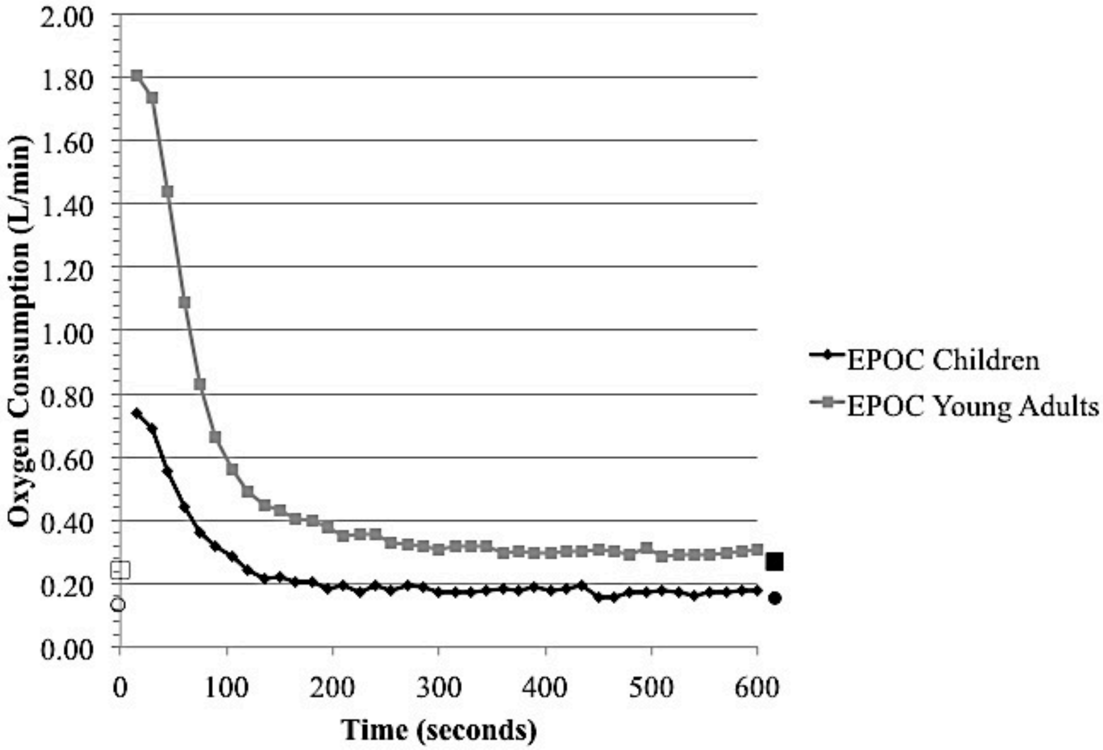
Note: □*Mean pre-exercise VO_2 in young adults*

○ *Mean pre-exercise VO_2 in children*

■ *Mean end-exercise VO_2 in young adults*

● *Mean end-exercise VO_2 in children*

Figure 6. EPOC volume after vigorous exercise: children and young adults (10 minutes).

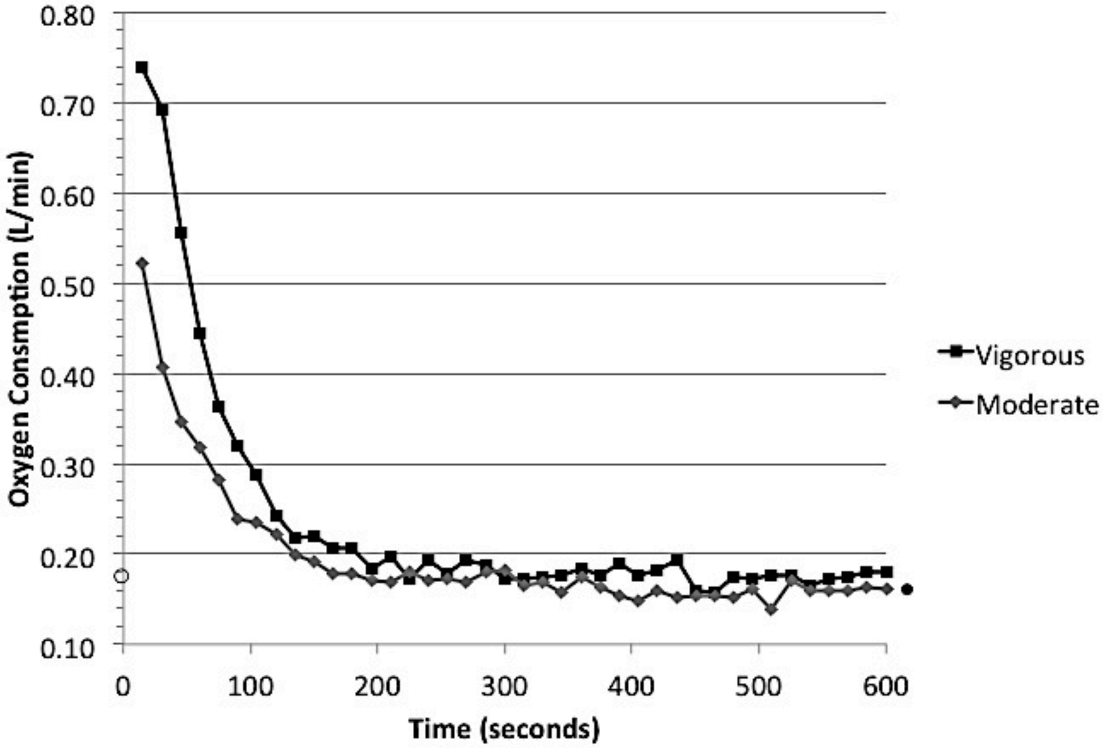


Note: □Mean pre-exercise VO_2 in young adults
○ Mean pre-exercise VO_2 in children
■ Mean end-exercise VO_2 in young adults
● Mean end-exercise VO_2 in children

In each classification (children and young adults) and in both groups (those who attained baseline and those who did not) the vigorous exercise bout elicited a significantly greater EPOC when compared to the moderate intensity exercise, even though equal energy expenditure for both exercise bouts was anticipated. The VO_2 of the vigorous exercise, which was 50% of the duration of the moderate exercise, would need to have increased by 100% above the moderate exercise for caloric expenditure to be similar between exercises. However, as previously mentioned, the increase was less than 100% resulting in greater energy expenditure in the moderate exercise than the vigorous exercise. The volume of EPOC for children who reached baseline was 0.30 ± 0.13 L for the moderate intensity exercise compared to 0.64 ± 0.15 L for the vigorous exercise.

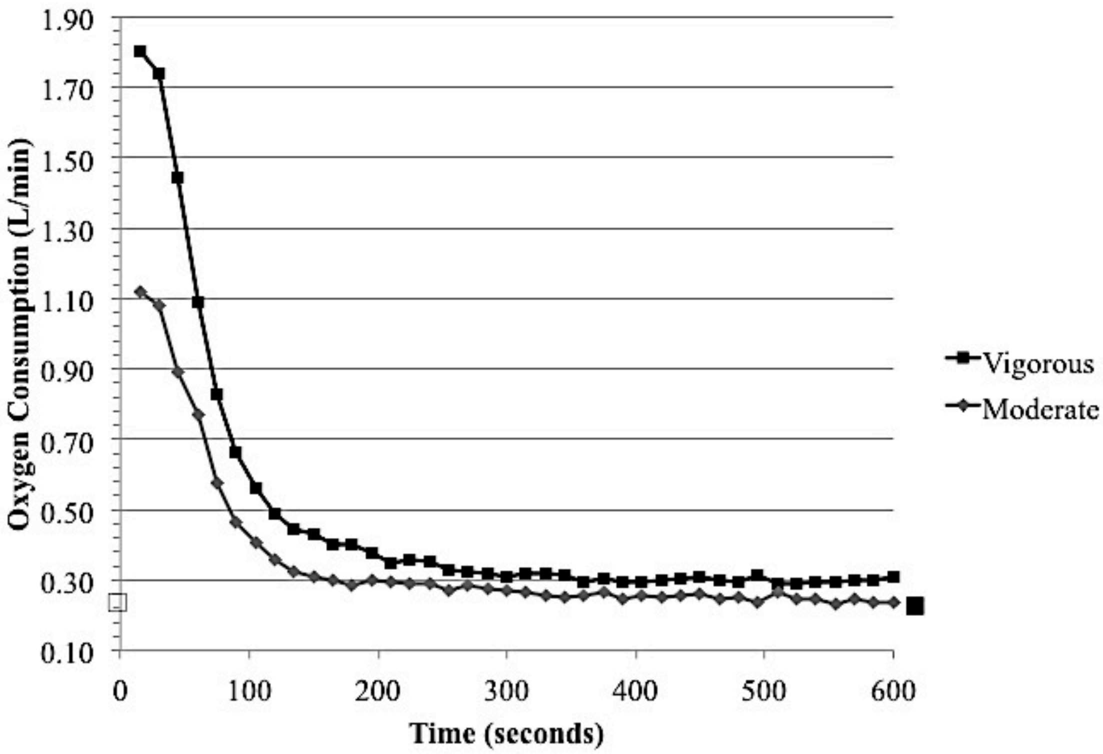
Since only two girls failed to reach baseline, the results remained similar when their data were included: 0.30 ± 0.13 L and 0.71 ± 0.26 L for moderate and vigorous exercises, respectively ($F = 30.37$, $p < 0.001$, $d = 0.22$). For young adults who reached baseline, EPOC was 1.07 ± 0.21 L for the moderate intensity exercise and 2.13 ± 0.31 L for the vigorous exercise. When the data from young adults who failed to reach baseline were included in the analysis, the averages were higher for the moderate and vigorous exercises 1.18 ± 0.38 L and 2.46 ± 0.95 L, respectively ($F = 59.94$, $p < 0.001$, $d = 0.48$). Thus, the vigorous intensity exercise elicited a greater EPOC volume than the moderate exercise visit in both children and young adults even though caloric expenditure was planned to be similar. Figures 7 and 8 address EPOC data compared between moderate and vigorous exercise in children and young adults, respectively.

Figure 7. Children's EPOC: comparison between moderate and vigorous exercise (10 minutes).



Note: ○ Mean pre-exercise VO_2 for both moderate and vigorous exercise
● Mean end-exercise VO_2 for both moderate and vigorous exercise

Figure 8. Young adult's EPOC: comparison between moderate and vigorous exercise (10 minutes).



Note: □ Mean pre-exercise VO_2 for both moderate and vigorous exercise
■ Mean end-exercise VO_2 for both moderate and vigorous exercise

Data regarding the duration of EPOC are reported in *Tables 9 and 10*. *Table 9* describes the duration for all subjects, whereas *Table 10* reports the duration of EPOC for only those subjects who reached baseline in the twenty minutes post-exercise. When considering all subjects' data (regardless of returning to baseline; see *Table 9*), the observed difference between young adults was significant after moderate (237 $\pm$ 104 vs. 552 $\pm$ 325 seconds, respectively, $F = 16.413$, $p < 0.001$, $d = 1.30$) and vigorous exercise (424 $\pm$ 345 vs. 925 $\pm$ 340 seconds, respectively, $F = 21.912$, $p < 0.001$, $d = 1.50$). Results did not differ after controlling for sex, caloric consumption, aerobic fitness, and/or body composition. Children returned to baseline significantly faster after the moderate exercise than the vigorous (237 $\pm$ 104 vs. 432 $\pm$ 345; $t = -4.184$, $p < 0.031$, $d = 0.19$). Young adults also returned to baseline significantly faster after the moderate exercise than vigorous (552 ± 325 vs. 925 ± 340, $t = -4.184$, $p < 0.001$, $d = 1.15$). Significance did not change when considering the data from only those who reached baseline (see *Table 10)*.

Table 9. Descriptive statistics for EPOC duration, in seconds (data from all subjects).

	Children mean±sd (min, max)	Young adults mean±sd (min, max
Moderate	n = 19	n = 22
	237±104 (135, 495)	552±325* (165, 1200)
Vigorous	n = 19	n = 22
	429±336[a] (120, 1200)	925±340*[a] (240, 1200)

* p-value < 0.05 from t-test comparing children

[a] p-value < 0.05 from t-test comparing to moderate

Table 10. Descriptive statistics for EPOC duration, in seconds (data represent only those who reached baseline within the twenty minutes of continuous measurements).

	Children mean±sd (min, max)	Young adults mean±sd (min, max)
Moderate	n = 19	19
	237±104 (135, 495)	450±205* (165, 930)
Vigorous	n = 17	n = 13
	338±210[a] (120, 915)	734±325*[a] (240, 1140)

* p-value < 0.05 from t-test comparing to children

[a] p-value < 0.05 from t-test comparing to moderate

Expired gases were compared to baseline measures after one-hour of recovery from exercise. It was determined children's baseline measures prior to the moderate exercise were significantly higher than one-hour post-exercise, see *Table 11*. It should be noted this was a statistically significant differences that may not correspond to biological significance. No other significant difference was found between baseline and one-hour post-exercise VO_2 measurements in either children or adults, see *Table 12*.

Table 11. Pre-exercise vs post-moderate exercise VO_2 in children and young adults

Children (n=20)	mean	sd	t	d
Pre-Ex VO_2 (ml/kg•min^{-1})	6.47	1.07	2.257*	0.2458
One-hour Post-Ex VO_2 (ml/kg•min^{-1})	6.21	1.10		
Young Adults (n=22)				
Pre-Ex VO_2 (ml/kg•min^{-1})	3.40	0.39	1.994	0.4089
One-hour Post-Ex VO_2 (ml/kg•min^{-1})	3.21	0.57		

Note: Pre-Ex = pre-exercise; Post-Ex = post-exercise

* p-value <0.05

Table 12. Pre-exercise vs post-vigorous exercise VO_2 in children and young adults

Children (n=20)	mean	sd	t	d
Pre-Ex VO_2 (ml/kg•min^{-1})	6.30	1.03	1.082	0.1367
One-hour Post-Ex VO_2 (ml/kg•min^{-1})	6.16	1.15		
Young Adults (n=22)				
Pre-Ex VO_2 (ml/kg•min^{-1})	3.45	0.50	0.501	0.0517
One-hour Post-Ex VO_2 (ml/kg•min^{-1})	3.43	0.49		

Note: Pre-Ex = pre-exercise; Post-Ex = post-exercise

During exercise respiratory exchange ratio

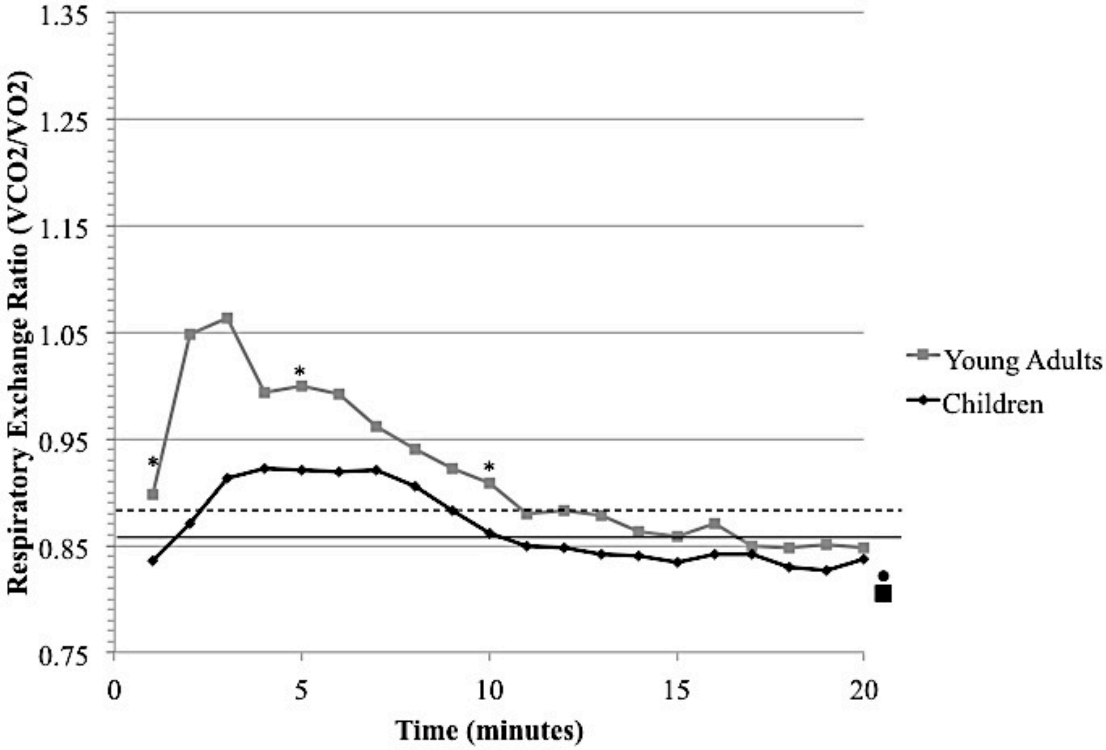
The exercise bouts were too short to lend themselves for any steady state analysis of variables. However, in the interest of describing relative intensity of participants the RER during exercise was analyzed. Immediately following each exercise bout during the exercise tests, RER increased substantially due to hyperventilation of the participants; thus, all RER data throughout the exercise tests were averaged. Furthermore, since steady state was rarely attained, if at all, it can be concluded that these data do not represent substrate utilization, but are merely another indication of relative intensity. Children's RER was significantly lower than young adults' RER during moderate exercise

(0.85±0.04 vs. 0.94±0.05, F = 34.419, $p < 0.001$, d = -1.8572; children and young adults, respectively) as well as vigorous exercise (0.97±0.05 vs. 1.08±0.05, F = 50.795, $p < 0.001$, d = -2.213; children and young adults, respectively). Children's RER was, on average, lower than young adults' during both moderate and vigorous exercise bouts.

Post-exercise respiratory exchange ratio

Adult-child comparison revealed that for the first, fifth, and tenth minute after moderate exercise children's RER was significantly lower than young adults (0.84 $\pm$ 0.05 vs. 0.90 $\pm$ 0.05, F = 15.470, $p < 0.001$, d = 1.28; 0.93 $\pm$ 0.05 vs. 1.00 $\pm$ 0.08, F = 10.887, p = 0.002, d = 1.05; and 0.86 $\pm$ 0.03 vs. 0.91 $\pm$ 0.05, F = 10.643, p = 0.002, d =1.05; respectively). Results did not differ after controlling for sex, caloric consumption, aerobic fitness, and/or body composition. However, no significant differences between children and young adults were found in fifteen or twenty minutes post-exercise (0.84 $\pm$ 0.04 vs. 0.86 $\pm$ 0.04, F = 3.374, p = 0.074, d = 0.59 and 0.84 $\pm$ 0.04 vs. 0.84 $\pm$ 0.06, F = 0.612, p = 0.439, d = 0.26; respectively, *Figure 9)*. Results did not differ after controlling for sex, caloric consumption, aerobic fitness, and/or body composition Figure 9 shows all RER data between young adults and children after moderate exercise; however, only minutes 1, 5, 10, 15, and 20 were statistically analyzed. In Figure 9, the dashed line represents the baseline RER measurements for young adults and the solid line represents the baseline RER measurements for children.

Figure 9. Comparison of RER after moderate exercise.



Note: All data are shown. Statistical analyses were only performed on minutes 1, 5, 10, 15, and 20. Dashed line represents baseline for young adults and solid line represents baseline for children.

■ *Mean end-exercise RER in young adults*

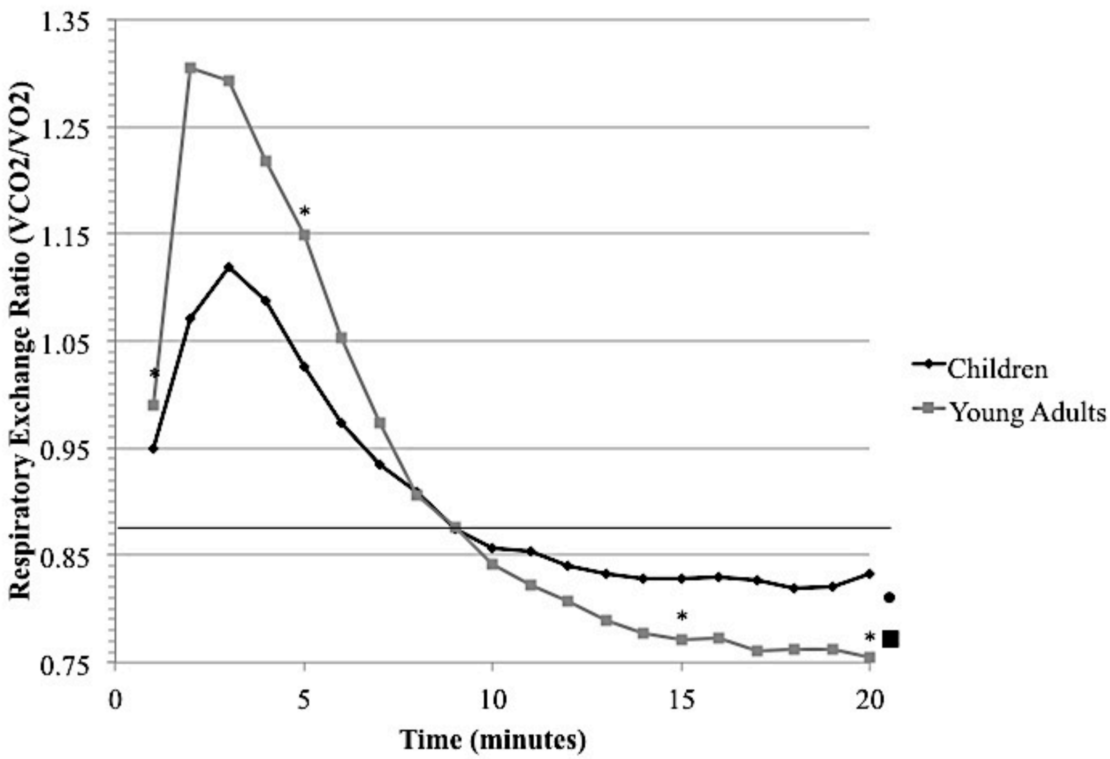
● *Mean end-exercise RER in children*

* p-value < 0.05 from t-test comparing to children

The results after the vigorous intensity exercise showed more pronounced adult-child differences than moderate intensity exercise (*see Figure 10).* Figure 10 shows all RER data between young adults and children after vigorous exercise; however, only minutes 1, 5, 10, 15, and 20 were statistically analyzed. In Figure 10, the dashed line represents the baseline RER measurements for young adults and the solid line represents the baseline RER measurements for children. Children's RER in the first and fifth minute was significantly lower than young adults (0.96 ± 0.05 vs. 0.99 ± 0.05; $F = 6.232$, $p = 0.017$, $d = 0.82$; and 1.02 ± 0.06 vs. 1.15 ± 0.11; $F = 19.375$, $p < 0.001$, $d = 1.41$).

However, there were no significant differences between children and young adults in the tenth minute (0.86 $\pm$ 0.04 vs. 0.84 $\pm$ 0.07; $F = 0.722$, $p = 0.415$, $d = 0.27$). Children's RER in the fifteenth and twentieth minute post-exercise of exercise was significantly higher than young adults' (0.83 $\pm$ 0.05 vs. 0.77 $\pm$ 0.06; $F = 13.204$, $p = 0.001$, $d = 1.18$; and 0.83 $\pm$ 0.05 vs. 0.75 $\pm$ 0.06; $F = 20.113$, $p < 0.001$, $d = 1.43$, respectively). Results did not differ after controlling for sex, caloric consumption, aerobic fitness, and/or body composition.

Figure 10. Comparison of RER after vigorous exercise.

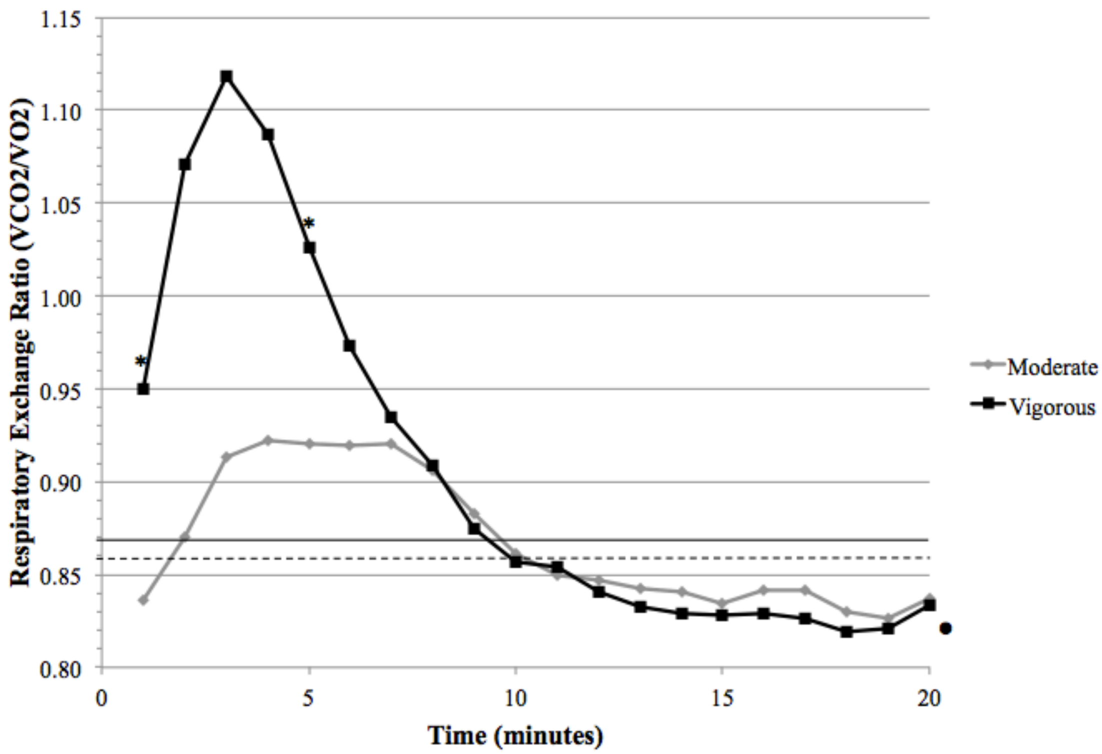


Note: All data are shown. Statistical analyses were only performed on minutes 1, 5, 10, 15, and 20. Solid line represents baseline for both children and young adults.
■ *Mean end-exercise RER in young adults*
● *Mean end-exercise RER in children*
* p-value < 0.05 from t-test comparing to children

Respiratory Exchange Ratio was examined for any differences between moderate and vigorous exercises (*see Figures 11 and 12)*. Figure 11 displays all the post-exercise

RER measurements for both moderate and vigorous visits in children; however only minutes 1, 5, 10, 15, and 20 were statistically analyzed. The dashed line in Figure 11 represents the baseline RER for the moderate visit and the solid line represents the baseline RER for the vigorous visit. Children experienced a significantly higher RER post-vigorous exercise when compared to post-moderate exercise in the first (0.84 $\pm$ 0.05 vs. 0.95 $\pm$ 0.05; $t = -10.098$, $p < 0.001$, $d = 2.30$) and fifth (0.92 $\pm$ 0.06 vs. 1.03 $\pm$ 0.06; $t = -8.137$, $p < 0.001$, $d = 2.28$) minutes, but no significant differences were found in minutes ten (0.86 $\pm$ 0.04 vs. 0.86 $\pm$ 0.04; $t = 0.307$, $p = 0.762$, $d = 0.10$), fifteen (0.83 $\pm$ 0.04 vs. 0.83 $\pm$ 0.05; $t = 0.513$, $p = 0.614$, $d = 0.03$), or twenty (0.84 $\pm$ 0.04 vs. 0.83 $\pm$ 0.05; $t = 0.267$, $p = 0.792$, $d = 0.016$), moderate and vigorous exercise, respectively *(see Figure 11)*.

Figure 11. Children's RER after moderate and vigorous exercise.



Note: All data are shown. Statistical analyses were only performed on minutes 1, 5, 10, 15, and 20. Dashed line represents baseline for the moderate visit and solid line represents baseline for vigorous visit.

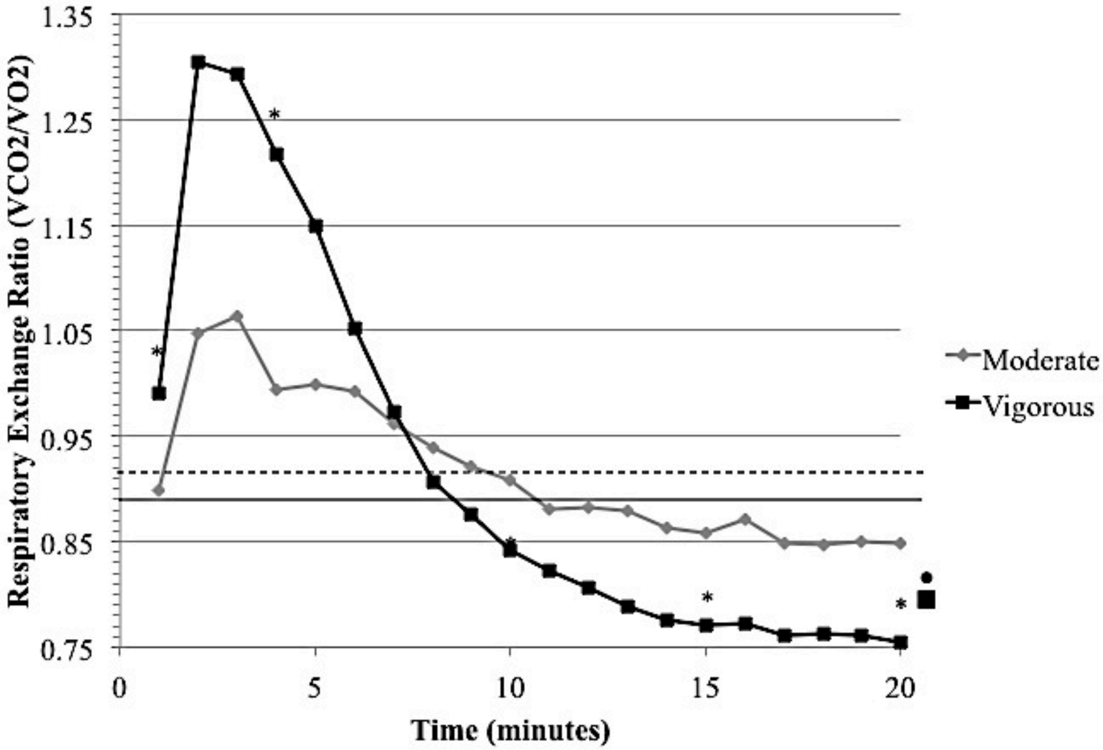
● *Mean end-exercise RER in both moderate and vigorous visits*

* p-value < 0.05 from t-test comparing to moderate

Young adults showed a more exaggerated RER response than children, particularly after the vigorous exercise; young adults experienced a sharp increase in RER at the cessation of exercise, followed by a steep decline, resulting in a post-exercise RER below the moderate post-exercise RER values. There were significant differences in each of the post-exercise measurements of RER: first (0.90 $\pm$ 0.05 vs. 0.99 $\pm$ 0.05; t = -10.314, p < 0.001, d = 2.05), fifth (1.00 $\pm$ 0.08 vs. 1.15 $\pm$ 0.11; t = -0.6392, p < 0.001, d = 1.60), tenth (0.91 $\pm$ 0.05 vs. 0.84 $\pm$ 0.07; t = 3.785, p = 0.001, d = 1.17), fifteenth (0.86 $\pm$ 0.04 vs. 0.77 $\pm$ 0.06; t = 6.839, p < 0.001, d = 0.64), and twentieth minutes (0.85 $\pm$ 0.06

vs. 0.75 $\pm$ 0.06; $t = 6.009$, $p < 0.001$, $d = 0.46$) for moderate and vigorous exercise, respectively *(see Figure 12)*. Figure 12 displays all the post-exercise RER measurements for both moderate and vigorous visits in young adults; however only minutes 1, 5, 10, 15, and 20 were statistically analyzed. The dashed line in Figure 12 represents the baseline RER for the moderate visit and the solid line represents the baseline RER for the vigorous visit. Thus, no significant differences were observed in RER between the post-moderate and post-vigorous exercises in children after the fifth minute, whereas young adults were observed to have significant differences in RER post-moderate and post-vigorous exercises throughout the continuous measurement period.

Figure 12. Young adults RER after moderate and vigorous exercise.



Note: All data are shown. Statistical analyses were only performed on minutes 1, 5, 10, 15, and 20. The dashed line represents baseline for the moderate visit and solid line represents baseline for vigorous visit.
■ *Mean end-exercise RER after vigorous visit*
● *Mean end-exercise RER after moderate visit*
* p-value < 0.05 from t-test comparing to moderate

Respiratory exchange ratio was also analyzed for any differences from baseline. After moderate exercise, children's RER was significantly lower than baseline (0.86 $\pm$ 0.05) for the first minute post-exercise (0.84 $\pm$ 0.05, t = -2.123, p = 0.047, d = 0.42), but was significantly elevated above baseline at the fifth minute (0.92 $\pm$ 0.06, t = 6.061, p < 0.001, d = 1.24). At the tenth minute post-exercise, there was no significant difference when compared to baseline RER (0.86 $\pm$ 0.04 vs. 0.86 $\pm$ 0.05, t = 0.551, p = 0.588, d = 0.12). The steady decline in RER continued into the fifteenth (0.83 $\pm$ 0.04 vs 0.86 $\pm$ 0.05, t = -3.504, p = 0.002, d = 0.50) and twentieth minutes and was significantly lower than baseline (0.84 $\pm$ 0.04 vs 0.86 $\pm$ 0.05, t = -3.270, p = 0.004, d = 0.47).

The baseline RER for the vigorous visit (0.87 $\pm$ 0.4) was not significantly different than baseline for the moderate exercise (0.86 $\pm$ 0.05) (t = -1.222, p = 0.237, d = 0.09). Children's RER was significantly greater than baseline at minute 1 and 5 post-vigorous exercise (0.95 $\pm$ 0.05 vs. 0.87 $\pm$ 0.4, t = 7.877, p < 0.001, d = 1.95 and 1.03 $\pm$ 0.06 vs. 0.87 $\pm$ 0.4, t = 12.956, p < 0.001, d = 0.90, respectively). At ten minutes post-exercise there was no significant difference from baseline RER measure (0.86 $\pm$ 0.04 vs. 0.87 $\pm$ 0.4, t = -1.110, p = 0.281, d = 0.25). RER continued its decline and was significantly lower than baseline RER at the fifteenth and twentieth minutes post-vigorous exercise (0.83 $\pm$ 0.05 vs. 0.87 $\pm$ 0.4, t = -5.044, p < 0.001, d = 0.99, and 0.83 $\pm$ 0.06 vs. 0.87 $\pm$ 0.4, t = -4.006, p = 0.001, d = 0.91, respectively).

Baseline RER measure for young adults after the moderate exercise visit was 0.88 $\pm$ 0.05. After one minute post-exercise, RER was not significantly different from baseline (0.90 $\pm$ 0.5 vs. 0.88 $\pm$ 0.05, t = 1.743, p = 0.096, d = 0.35). At minutes five and ten RER was significantly greater than baseline (1.00 $\pm$ 0.08 vs. 0.88 $\pm$ 0.05, t = 6.700, p < 0.001, d = 1.84, and 0.91 $\pm$ 0.05 vs. 0.88 $\pm$ 0.05, t = 2.243, p = 0.36, d = 0.56, respectively). RER was not significantly different from baseline at minute fifteen (0.86 $\pm$ 0.04 vs. 0.88 $\pm$ 0.05, t = -2.032, p = 0.055, d = 0.48), but was significantly lower than baseline at minute twenty (0.85 $\pm$ 0.06 vs. 0.88 $\pm$ 0.05; t = -2.53, p = 0.023, d = 0.61).

Young adults' baseline RER for the vigorous exercise was 0.87 $\pm$ 0.06 and was not significantly different from the baseline RER of the moderate visit (0.88 $\pm$ 0.05, t = 1.022, p = 0.318, d = 0.24). After the vigorous exercise, RER at minutes one and five were found to be significantly greater than baseline (0.99 $\pm$ 0.05 vs. 0.87 $\pm$ 0.06, $t = 8.867$, $p <0.001$, $d = 2.33$, and 1.15 $\pm$ 0.11, $t = 9.824$, $p < 0.001$, $d = 3.18$, respectively). RER declined after the fifth minute and was found to not be significantly different from baseline at the tenth minute (0.84 $\pm$ 0.66 vs. 0.87 $\pm$ 0.06; $t = -1.553$, $p = 0.135$, $d = 0.40$). After the tenth minute RER continued to decline and was observed to be significantly lower than baseline at the fifteenth and twentieth minutes post-exercise (0.77 $\pm$ 0.06 vs. 0.87 $\pm$ 0.06, $t = -6.689$, $p < 0.001$, $d = 1.60$, and 0.75 $\pm$ 0.06 vs. 0.87 $\pm$ 0.06, $t = -8.854$, $p < 0.001$, $d = 1.90$, respectively).

Substrate utilization one-hour post-exercise

Substrate utilization was significantly different from the baseline values after nearly an hour of rest post-exercise in both children and young adults. Children's baseline RER for the moderate trial was 0.86 $\pm$ 0.05. In the last ten minutes of post-moderate exercise measurements (minutes 50-60) RER was significantly lower than baseline (0.82 $\pm$ 0.3, $t = 4.438$, $p < 0.001$, $d = 0.80$). Children's baseline RER for the vigorous trial was 0.87 $\pm$ 0.04, these data were not significantly different than the moderate trial, $p = 0.237$. Fifty minutes post-vigorous exercise RER was significantly lower when compared to baseline values (0.83 $\pm$ 0.03 vs. 0.87 $\pm$ 0.04, $t = 5.913$, $p < 0.001$, $d = 1.09$). RER during minutes 50 – 60 after the moderate trial was not significantly different to RER during minutes 50 – 60 after the vigorous trial in children (0.82 $\pm$ 0.3 vs. 0.83 $\pm$ 0.03; $t = -0.658$, $p = 0.519$, $d = 0.15$).

Young adult data showed a lower RER during minutes 50 – 60 of the post-exercise measurement period than at baseline. For the moderate trial, baseline RER values were 0.88 $\pm$ 0.05 and was significantly lower during minutes 50 – 60 post-exercise at 0.81 $\pm$ 0.05; $t = 5.499$, $p < 0.001$, $d = 1.37$). For the vigorous trial, baseline RER values were 0.87 $\pm$ 0.06 (not significantly different than moderate baseline RER, $t = 1.022$, $p = 0.318$, $d = 0.23$) and declined significantly to 0.78 $\pm$ 0.05 for minutes 50-60 post-exercise ($t = 9.973$, $p < 0.001$, $d = 1.62$). RER values during minutes 50-60 post-exercise were significantly higher in the moderate trial than the vigorous trial (0.81 $\pm$ 0.05 vs. 0.78 $\pm$ 0.05; $t = 3.257$, $p = 0.004$, $d = 0.72$). Children's RER during minutes 50 – 60 after the moderate trial's post-exercise measurements were not significantly different than young adults (0.814 $\pm$ 0.5 vs. 0.824 $\pm$ 0.03; $F = 0.725$, $p = 0.400$, $d = 0.26$), but children's RER was significantly higher than young adults' during minutes 50 – 60 after the vigorous trial (0.83 $\pm$ 0.03 vs. 0.78 $\pm$ 0.05; $F = 12.866$, $p = 0.001$, $d = 1.13$).

Chapter 5 – Discussion

Major findings

Children's EPOC was found to be significantly less in volume and duration than young adults' after both moderate and vigorous exercise. Furthermore, vigorous exercise elicited greater EPOC volume and duration in both children and young adults when compared to moderate exercise. Children were observed to have significantly lower RER than young adults for ten minutes after moderate exercise and no differences were observed in minutes fifteen or twenty post-exercise. After vigorous exercise children had lower RER for minutes one and five, but after fifteen minutes and through twenty minutes post-exercise children's RER was significantly higher than young adults.

Interpretation of findings

In general, children have less mass than young adults, which was the case in this study as all the children were prepubertal. Greater body mass implies greater absolute volume of air during rest and exercise and, therefore, a greater volume of oxygen consumed while exercising at similar intensities. This study described oxygen consumption pre- and during exercise in a manner widely accepted in the literature: relative to body mass (ml/kg•min^{-1}). Young adult baseline values measured in this study were similar to what is typically considered a normal resting value: 3.5 ml/kg•min^{-1}. Relative to body mass, children's baseline VO_2 in this study was considerably higher than young adults, which is expected considering the higher metabolic demands of the growth process in children and evidence from previous literature[128-130]. However, with respect to exercise VO_2, for non-weight bearing exercises (cycle ergometry) an argument can be made to use absolute values ($L \cdot min^{-1}$) to express oxygen consumption, but should be done with the understanding that individuals with greater mass (specifically in the legs) will have a greater capacity to produce power on a cycle ergometer than individuals with lesser mass.

Percent of peak power output was used to establish workload, as it would have been difficult to keep participants at a particular percentage of VO_2peak due to nature of the exercise protocol. Although children and young adults both performed the exercise bouts at 35% and 70% peak power, children's VO_2 during exercise averaged 24.0 ± 3.0 $ml/kg \cdot min^{-1}$ and 35.6 ± 5.4 $ml/kg \cdot min^{-1}$ or when expressed as a percentage of VO_2peak: 60% and 88%, respectively. It is possible the method of establishing VO_2 during exercise inflated the true value: thus VO_2 was also expressed as the averaged across the exercise bout (including non-exercise periods): 14.7 ± 2.0 and 20.6 ± 3.3 $ml/kg \cdot min^{-1}$ or 35% and 49% of VO_2peak, for moderate and vigorous exercise respectively. Young adults performed during exercise at 18.7 ± 2.9 and 29.9 ± 5.7 $ml/kg \cdot min^{-1}$ or 46% and 73% of VO_2peak, for moderate and vigorous exercises, respectively. When VO_2 was expressed as the averaged for the duration of the exercise (including non-exercise periods) the result was 11.3 ± 1.5 and 17.0 ± 2.9 $ml/kg \cdot min^{-1}$ or 29% and 44% of VO_2peak, for moderate and vigorous exercises respectively. This finding is not surprising as others have found relative oxygen consumption to be significantly higher in children at the same absolute exercise intensities (40 and 60 Watts)[80]. Another contributing factor to this difference in percent VO_2 during exercise could be children's immature anaerobic system, which causes them to rely more on aerobic metabolism[76,131,132].

Several hypotheses were formulated concerning the nature of EPOC in the young adult and pediatric population. In aim 2, hypothesis A and B stated that young adults would experience a greater volume of EPOC than children after moderate and vigorous intensity exercise. The results, as expected, supported the hypothesis; that young adults would have greater EPOC after moderate and vigorous intensity exercises. It is important to note EPOC was expressed as absolute liters (L), which is limited by not factoring in the body mass of the participants. However, an absolute measurement of EPOC was specifically chosen due to the descriptive nature of the study as literature in this field is lacking and the fact that cycling is a non-weight-bearing exercise. Data exist from this study to investigate allometric scaling of EPOC; however, those issues will be covered in future research to most appropriately express EPOC in the pediatric population.

Early studies investigating the volume of EPOC has emphasized the 'extra calories burned' after exercise[39,133]. As mentioned previously, extra calorie burn this was not the focus of this study, and it became clear that caloric expenditure during EPOC was minimal[63]. However, for the children whose EPOC lasted less than 20 minutes they expended an extra 1.5 and 3 kilocalories for moderate and vigorous exercise, respectively (as estimated by using 1 L of oxygen consumed equals 5 kilocalories). Adults were found to 'burn' approximately 5 and 10 'extra' kilocalories after moderate and vigorous exercises, respectively. The amount of energy used post-exercise is minimal, but is consistent with other findings[63].

Aim 2, hypothesis C and D stated that young adults would experience a longer duration of EPOC than children after both moderate and vigorous intensity exercise. The results of this study provide evidence supporting the hypothesis; young adults required more time to reach baseline than children after moderate and vigorous exercise. Unlike the volume of EPOC, which can be scaled using a variety of factors such as body mass, duration of EPOC has not been previously viewed as something that should be scaled. Thus direct comparisons between children and young adults can be made with confidence. Although few investigations address oxygen consumption post-exercise in the pediatric population, some addressed heart rate recovery and showed children recovered faster than adults at multiple intensities[134]. The time constant of children's HR was approximately 51 seconds faster than adults after a single minute sprint at 100% VO_2peak and approximately 56 seconds faster than adults after a single minute sprint at 125% of VO_2peak[134].

Cardiovascular variables are not in the scope of this study, but for comparison to previous HR recovery research a repeated measures analysis on heart rate (HR) variables was performed to determine when participants' HR reached baseline post-exercise for both moderate and vigorous exercise visits. After moderate exercise children's HR was significantly higher than baseline for 16 minutes ($p \leq 0.05$). After vigorous exercise children's HR did not reach baseline in the 20 minutes post-exercise measurement period. Adults' HR did not reach baseline in the 20 minutes post-exercise measurement period for either the moderate or the vigorous exercise visits. At one-hour post-moderate exercise children's HR had reached baseline measures, but adults' HR was significantly lower than baseline. However, one-hour post-vigorous exercise neither children nor adults HR had returned to baseline. These results may seem counterintuitive, but since participants immediately returned to the semi-recumbent position following the exercise bout it stands to reason venous return was no longer aided by the action of the muscle pump, decreasing ventricular filling causing an increase in heart rate to compensate for reduced stroke volume in order to maintain cardiac output. This study can only confirm children's faster cardiovascular recovery in the context of moderate exercise as recovery time was not long enough after the vigorous exercise to indicate a difference in age groups.

Insight into the duration of EPOC can be found in the study of the onset of exercise. At the onset of exercise, oxygen kinetics have been shown to be faster in children than adults[135-137]; this is especially true as exercise intensity increases[7]. Thus, the fact that children's (both boys and girls) metabolism, as measured by oxygen consumption, reached baseline significantly faster than young adults at both exercise intensities was expected. Although the physiological conditions post-exercise are not the same as the onset of exercise, an observation made abundantly clear by Gaesser and Brooks[22], it is important to note the possible mechanisms that govern faster uptakes at the onset of exercise as they may provide insight into recovery oxygen kinetics. Researchers have proposed children's faster O_2 kinetics at the onset of exercise may be due to their "limited ability to generate ATP anaerobically" paired with their preferential use of aerobic pathways to supply energy[136]. Furthermore, children's faster O_2 kinetics could also be reflective of a "greater relative capacity for oxygen utilization" and/or greater efficiency of oxygen transport, when compared to the adult population[135]. Gaesser and Brooks noted that core temperature is a major contributing factor explaining the volume and duration of EPOC[22]. Research in thermoregulation has shown that if the environmental temperature is below core temperature the greater surface area to body mass ratio of children provides them with the ability to radiate heat to the environment more effectively than adults[138]. Children's ability to cool faster than adults after exercise provides a portion of the explanation as to the differences in recovery. These proposed mechanisms may provide insight into the shorter EPOC duration of children when compared to young adults. However, we did not assess skin temperature in the current study so we cannot definitively corroborate this.

Aim 3, hypotheses A and B stated that vigorous exercise would produce a greater volume and duration of EPOC than moderate exercise in children and young adults. The data from this study support the hypothesis; higher intensity exercise elicited a greater volume and duration of EPOC. In this study moderate exercise was performed at 35% of peak power output accumulating thirty minutes of exercise, whereas the vigorous exercise bout was performed at 70% of peak power output and accumulated fifteen minutes of exercise. Thus, theoretically, the caloric expenditure was similar between the two exercise bouts. However, as reported concerning the oxygen consumption during exercise, it was observed that children's VO_2 was approximately 60% of VO_2peak during the moderate exercise (or 35% of VO_2peak if all VO_2 for the duration of the exercise is averaged) and 88% of VO_2peak during the vigorous exercise (or 49% of VO_2peak if all VO_2 for the duration of the exercise is averaged). Since expired gases were not collected for the duration of the exercise bout, the total caloric cost of the trial was unknown. However, using all expired gas data collected during the exercise bouts an estimate can be extrapolated. Children's estimated exercise energy expenditure was 123 ± 11 and 84 ± 9 kilocalories for the moderate and vigorous exercises, respectively ($t = 18.414$, $p < 0.001$, $d = 3.931$). Adults' estimated exercise energy expenditure (EE) was 243 ± 37 and 184 ± 33 kilocalories for the moderate and vigorous exercises, respectively ($t = 15.101$, p

< 0.001, $d = 1.732$). The duration of EPOC was significantly longer after the vigorous trial than the moderate trial in children. It was previously observed in adults that exercise bouts at greater than 60% VO_2max will produce EPOC exponentially related to intensity and linearly to duration[8]. However, no study has investigated if or when a threshold exists below which no appreciable rise in EPOC occurs in children. Although EPOC increases linearly with exercise duration in adults (above 60% VO_2max), it is unknown the impact of exercise duration on the volume of children's EPOC. Thus, further research is warranted if investigators desire to understand children's metabolism as well as adult metabolism.

Aim 4, hypothesis A stated that children's VO_2 one-hour post-exercise would not be significantly different ($<1.3 ml/kg•min^{-1}$) from pre-exercise (baseline) VO_2. The results support the hypothesis. Children did not experience significantly elevated VO_2 after one-hour recovery from exercise in either the moderate or vigorous exercise trials. Previous studies have used time constant[20] (time required to reach a particular percentage of the difference between peak and resting VO_2) for oxygen recovery in order to shorten the measurement time necessary post-exercise. Assessing recovery via a time constant has its advantages as the load on the participant is significantly reduced. However, using time constant does not allow for the assessment of the complete volume or duration of EPOC. No study has previously investigated energy expenditure after one-hour recovery time in children. Results from the children in this study are as expected, but not previously documented: children reach baseline oxygen consumption well before one-hour post-exercise.

Aim 4, hypothesis B stated young adults VO_2 would be significantly elevated (>1.3ml/kg•min^{-1}) one-hour post-exercise. The results do not support this hypothesis as no significant difference (statistical or biological) was found between baseline and one-hour post-exercise in either the moderate or vigorous exercise visits. This result is supported by a few studies observing the duration of EPOC to last less than one hour[33,39,40]. However, others have observed EPOC to last several hours[34-38]. Intensity and duration of the exercise as well as protocols surrounding the exercise bout are of vital importance to determine duration of EPOC. Square-wave exercise protocols are rarely implemented in adult research since adults typically use steady state intensities for daily exercise. The rest periods between exercising likely reduced EPOC one-hour post-exercise. However, in order to compare to children it was necessary to use the same square-wave protocol. According to this study, using a square-wave exercise protocol does not contribute to increased metabolism one-hour post-exercise for moderate or vigorous exercise in young adults.

Aim 5, hypotheses A and B stated that young adults would have a higher RER after exercise than children for both moderate and vigorous intensity exercise. The results of this study do not completely support that hypothesis. After moderate exercise, young adults of this study did have a higher RER than children for ten minutes, however from minute fifteen and following there was no significant difference between young adults and children. Furthermore, young adults had a statistically significant higher RER after the vigorous exercise - only for five minutes. At minute ten there was no significant difference between young adults and children, and from minute fifteen and on children were using more CHO than young adults. When considering using RER as a surrogate measurement of substrate utilization it is important that the individual in question is at a steady state. It is recognized when an individual is not at a steady state of activity many variables, such as hyperventilation and blood pH, may affect the use of RER as a representation of substrate utilization[114]. Thus, it is important to note the duration of EPOC in order to understand when RER can be used as an appropriate measure of substrate utilization post-exercise. Since the rapid component of EPOC is not steady state, the use of RER is not an appropriate estimate of substrate utilization upon the cessation of activity; however, it is still beneficial for describing the physiological conditions of the individual and becomes more appropriate surrogate for substrate utilization as steady state approaches.

Fat oxidation has been reported to be relatively greater in children than adults at rest and during exercise, indicating children's greater reliance on fat to fuel metabolism[15,16,78]. Although sparse muscle biopsy evidence exists in children due to ethical considerations, the majority of these studies have indicated lower muscle glycolytic enzyme activity when compared to adults, indicating a potential contributing mechanism for the lower CHO oxidation rate in children[15]. However, no study has compared substrate utilization post-exercise between children and adults. Contrary to existing literature[7,8,71], there were no significant differences between children's and young adults' RER prior to exercise. It is possible that anxiety could have been a factor influencing the children's substrate utilization to be similar to the young adults. However, great care was taken to control for any anxiety that the children may have experienced as the exercise tests were on their second and third visits (the first visit was a familiarization to the lab), the baseline measures were administered only after the children had been in the lab for approximately thirty minutes, and the children had been in a semi-recumbent position for approximately ten minutes while watching a familiar movie in an environment with no interruptions. Although no data have been published concerning RER post-exercise between children and adults, there seems to be no evidence in the literature to suggest that substrate utilization post-exercise would differ from what is currently known. Available research suggests an increase in fat oxidation in normal, healthy adults post-exercise when compared to resting or pre-exercise values[34,49,61,65]. The results from the young adults of this study are consistent with the adult literature. However, currently there is a paucity of literature concerning children's metabolism post-exercise. One study by Wideman et al[66] reported children's RER twenty minutes after a peak aerobic capacity test for healthy controls and those with cystic fibrosis (average of minutes six through twenty) was 0.89 for both groups (no standard deviation reported, but a range of 0.76 to 0.98 and 0.78 to 0.95 for those children with cystic fibrosis and healthy controls, respectively). The children's RER participating in this study at minute twenty post-exercise was 0.83 $\pm$ 0.05 for vigorous exercise. However, the children's RER values were higher than the young adults of this study, suggesting that children have a reduced capacity to oxidize fats fifteen and twenty minutes post-exercise when compared to young adults. This difference in RER post-exercise was observed even when statistically controlling for 24-hr caloric consumption and macronutrient composition as well as three hour fast prior to the laboratory visit.

The aim 6 hypothesis stated that RER values would be higher after vigorous exercise when compared to moderate exercise in children and young adults. The results do not fully support the hypothesis. Initially, children had a higher RER after the vigorous exercise, but minute ten through twenty RER was not significantly different between moderate and vigorous exercise. It was also observed young adults to have an initially a significantly higher RER upon cessation of the vigorous exercise when compared to the moderate exercise bout. By minute ten through twenty, RER after the vigorous exercise was significantly lower than after the moderate exercise. Initially, these results are surprising since children exhibited a higher percentage of VO_2peak during exercise; however, children also have a lower anaerobic capacity and are not capable of large disturbances in acid-base balance as seen in adults[139]. Thus, the greater acid-base disturbance in young adults would elicit a greater hyperventilation response during rest periods and significantly increase RER when compared to children.

The rapid component of EPOC is a time of high ventilation with little skeletal muscle activity; this translates to high CO_2 production with rapidly declining O_2 consumption resulting in initially high RER values, which decline as the rapid component of EPOC comes to an end. Children's ability to oxidize fats after exercise did not differ between moderate and vigorous exercise bouts. It is postulated a glycogen sparing effect, as a result of the heavy reliance on aerobic metabolism, allows children to quickly return to baseline after a bout of activity without having to replenish much muscle glycogen post-exercise. Thus, fat oxidation post-exercise would not necessarily be much different from other exercise intensities. Adults have higher muscle glycogen storage as well as a greater ability to utilize stored glycogen via anaerobic metabolism when compared to children[15]. Reduced muscle glycogen has been associated with increased fat oxidation[140]. The vigorous exercise was at a high enough intensity (108% of VT) to require significant contribution from muscle glycogen. The increased use of muscle glycogen during exercise would help explain the lower RER upon recovery from the vigorous exercise when compared to the moderate exercise in young adults.

Aim 7, hypotheses A and B stated that baseline RER values would be lower than the RER measurements taken after both exercise intensities in children and young adults. The results support the rejection of this hypothesis, concluding that both exercise intensities elicited an increased fat oxidation after fifteen minutes post-exercise when compared with baseline for children and young adults. Since RER is only appropriate measure of substrate utilization when the body is at steady state, no conclusions can be made from these data regarding fat or CHO oxidation during the rapid component of EPOC[15]. Factors influencing substrate utilization during exercise continues into the post-exercise period such as increased sympathetic drive and elevated catecholamine levels, both of which increase FFA mobilization and oxidation[141]. These factors continue to stimulate fat oxidation even after the cessation of exercise and can explain the lower RER in children and young adults when compared to baseline once oxygen consumption values reached a steady stat[141]. No significant difference was found in RER between moderate and vigorous exercise in children after minute five; it is not known if this is a factor of the physiology of children or that the exercise intensities did not differ enough to elicit a varied response.

Aim 7 hypothesis C stated that RER after one hour post-exercise would be similar to pre-exercise measurements. The data do not support this hypothesis as even one hour post-exercise RER was still significantly lower in both exercise visits than RER at baseline in both children and young adults. Adult literature has shown exercise to alter metabolism for several hours post-exercise[20,27-29]. However, no literature is available on the prolonged effect of exercise on substrate utilization in children. These data support the notion that children can benefit from increased fat oxidation for an hour after moderate exercise and vigorous exercise, but it is unknown how long the benefits of exercise last in children. Children's RER were similar between the moderate and vigorous exercise trials one hour post-exercise, since VO_2 had already retuned to baseline in both visits, moderate and vigorous exercise produce similar benefits of fat oxidation an hour after exercise. From a fat oxidation standpoint, young adults have a greater benefit from higher intensity exercise than children since RER remains significantly lower in young adults than children at the end of the post-exercise measurements.

Ventilatory threshold issues

The metabolic cost of exercise is a major contributor to the physiological conditions of post-exercise. It is evident in the literature that at high exercise intensity, metabolic cost of exercise increases above what can be calculated from power output, which is referred to as the "slow component" of VO_2. The slow component occurs due to several factors including increased cost of ventilation; however, the majority of the factors which influence the slow component have its origins in the exercising muscle, specifically the higher fatigable, less oxidative muscle fibers[142]. Ventilatory threshold (VT) has been used as a marker for exercise intensity, above which will elicit a slow component response[143]. Although not predominately anaerobic, efforts above VT require significant anaerobic contribution contributing to the slow component response. In this study children and young adults had similar VO_2 at VT, and VT occurred at a similar percentage of VO_2peak/max. Although children and young adults exercised at 35% and

70% of peak power output, children exercised at a greater percentage of VT than young adults during the moderate and vigorous trial, a factor that one would expect to play a significant role in post-exercise metabolism. However, according to the data it does not seem likely that the greater slow component influenced the RER differences between children and young adults since children's RER during min 15 and 20 was similar between moderate and vigorous exercise. It is possible that young adults have a greater sympathetic response to exercise increasing circulating catecholamines, thereby increasing lipolysis and circulating free fatty acids (FFA). With an increase in circulating FFA the body would preferentially use fats as the major source of energy and resulting in a lower RER. However, this has not been substantiated and more research is warranted. More research is needed to understand the differences in children and young adults after a vigorous exercise bout, but currently these data show that children are not able to mobilize fats for fuel post-exercise as efficiently as young adults.

Effects of caloric consumption, and macronutrient composition on EPOC and substrate utilization

Results did not show caloric consumption, and macronutrient composition to be a significant contributing factor to EPOC or RER in children or young adults. Participants were required to visit the lab after a three hour fast; however, three hours was the minimum as some participants chose to fast longer due to work/school schedules. Previous adult studies have shown that the thermic effect (TEF) of food can continue for up to six hours; however, 60% of the total TEF was measured by the third hour, 78% by the fourth hour, and 91% by the fifth hour[100]. The cessation of exercise in this study occurred 4 and 4.5 hours after participants' last meal for the vigorous and moderate visit, respectively. Thus, EPOC and substrate utilization were being measured after the majority of the TEF had been accounted.

Carbohydrates are a major source of energy for exercising muscles. Muscle glycogen levels would contribute to substrate utilization post-exercise as well as the recovery time. No estimates or measurements of glycogen levels were performed for any study participants. Generally speaking, glycogen storage increases with age[144,145]. It can be speculated that the children had lower muscle glycogen levels than the young adults in this study, but this cannot be substantiated and therefore not statistically controlled. Although outside the scope of this study, the impact muscle glycogen has on children's EPOC and substrate utilization remains unknown.

Limitations

This study was not without its limitations. Expired gases were only collected continually for twenty minutes post-exercise; therefore, it is unknown the duration or magnitude of EPOC for those who did not reach baseline in the twenty minutes immediately following exercise. The unknown duration or magnitude for those who did not reach baseline is problematic because among the aims of the study were to describe the magnitude and duration of EPOC, but it is also an issue relative to statistical comparisons to include groups containing some individuals who did not reach baseline and those who did. The subjects were monitored for only an hour post-exercise as values such as RER continued to be lower at minute sixty than pre-exercise for children and adults. Thus, it is unknown how long the suppressed RER lasts post-exercise in children.

It also would have been beneficial to measure oxygen consumption throughout the exercise period in order to calculate caloric expenditure. The mask was taken off briefly (and necessarily) during the exercise due to the discomfort it was causing, which was understood when designing the study.

This study was also limited in the fact that there was no control visit. On the one hand this can be viewed as a limitation; however, the author believes that a control visit designed to establish a metabolic baseline in order to calculate EPOC on subsequent exercise visits would only reflect the metabolism of the day the measurements were obtained and would not be appropriate for assessing EPOC on each additional visit to the lab. Diurnal variation of metabolism is more accurately controlled by establishing appropriate baseline measurements pre-exercise in order to assess EPOC. For example, an individual with a day of higher metabolism (due to diet or any number or variables) would fail to reach baseline when compared to a lower metabolism control visit or vise versa. Furthermore, it did not seem feasible to add another visit to an already time-demanding study. No allometric scaling of EPOC was performed, as this study was not designed to investigate potential mechanisms contributing to EPOC, but simply to describe a normal pediatric post-exercise response. Data exist from this study to provide future publications with the ability to consider allometric scaling of EPOC in the pediatric population.

This study did not use the accepted criterion measure for collecting expired gases: Douglas Bag method (DB). However, collection and analysis of expired gases via the ParvoMedics TrueOne® 2400 metabolic cart, as used in this study, has been previously found to be a valid method of indirect calorimetry[110,113]. Bassett found significant differences, although small, between the ParvoMedics TrueOne® 2400 metabolic cart and the DB (0.04% lower in the electronic system for both inspired and expired gases)[110].

Strengths

This study had several strengths. This is the only pediatric study to investigate the post-exercise measurements for one hour. Other pediatric studies have struggled with maturation as a confounding variable[146]. The pediatric population of this study was prepubertal, thus maturation effect was minimized. Furthermore, the exercise visits were randomized and counterbalanced, reducing any order effect that might exist. When investigating EPOC it is important to control for the thermic effect of food, since it can account for three to ten percent of total daily energy expenditure[100]. Caloric intake was considered in the current study by a three-hour fast before the participants began the exercise protocol as well as a 24 hour dietary recall, which was used to statistically control for caloric consumption and macronutrient composition of the diet. The moderate and vigorous exercise bouts were designed to theoretically require the same energy expenditure, thus controlling for differences in metabolism that could result in any caloric discrepancies between exercise bouts.

Future research

Although much is known concerning metabolism post-exercise in adults, little is known in the pediatric population. The lack of literature concerning EPOC in children may be due to the difficulties associated with keeping this population still for an extended period of time. However, it is also possible that the scientific community may not fully recognize the benefits of understanding post-exercise metabolism. If volume of literature on a particular subject is any indication of its importance then metabolism is important to understand. A substantial amount literature is focused on oxygen uptake kinetics at the onset of exercise and its indication of the control of aerobic metabolism. Although initially EPOC was the focus of the benefits (or lack thereof) of caloric expenditure post-exercise, it should be viewed as an opportunity to study metabolic imbalance from another perspective, since the post-exercise state is a substantially different physiological condition than the onset of exercise. According to this study, normal weight children expended 1.5 and 3 "extra" kilocalories due to EPOC after moderate and vigorous exercise, respectively. These results support the previously established notion of EPOC not contributing significantly to EE[63]. Furthermore, if it is important to understand the pediatric response to exercise, then future studies should consider the effect maturation has on EPOC. It is difficult to compare results between populations, especially those of differing maturity status; thus, allometric scaling should be considered for future work in this area. In addition, it will be important to investigate the differences in EPOC and post-exercise substrate utilization between normal weight and overweight children, as results may be indicative of other previously unknown metabolic differences between these populations. It will be informative to study other cardiovascular responses post-exercise, such as blood pressure, in different populations and exercise modalities as blood pressure is a indication cardiovascular health and little is known concerning the immediate post-exercise effects of exercise on this health marker. Finally, the study of EPOC in pediatrics has potential to provide researchers with a vast amount of information regarding metabolism in this unique population.

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