CHILDREN'S OXYGEN CONSUMPTION RESPONSES TO SELF-PACED ACTIVE PLAY AND PRESCRIBED TREADMILL EXERCISE

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ABSTRACT

The intensity and intervals of physical activity (PA) are important in eliciting dose-dependent physical and physiological adaptations. The objectives of the study were to examine the VO_2 responses plus accelerometer-based PA of children (n=8; 9.0 ± 1.3 yrs) and young adults n=5; 21.7 ± 2.4 yrs) during exercise and recovery from continuous, successive bouts of intermittent ordered versus intermittent random prescribed treadmill exercise (TM) at 4, 6, 8 and 10 km/hr (0%grade) and children's (n=12; 9.6 ± 1.5 yrs) self-paced active play (AP). Children showed a faster VO_2 recovery ($p<0.05$) for the random TM. During AP, there was a poor relationship (r= -0.02) between accelerometer-measured PA and relative VO_2. AP resulted in a 2.1:1 moderate-vigorous (MVPA):light-rest (LPA) intensity PA interval. In conclusion, the variable intensities and the MVPA:LPA ratios identified for AP may serve as a viable alternative to prescribed or paced exercise programs for promoting physiological benefits.

ACKNOWLEDGEMENTS

I want to thank Driftwood Community Center for allowing the active play program to take place there, and linking us with children that were recruited for the study. I also want to thank my supervisor, Dr. Angelo Belcastro, and the graduate and undergraduate students that assisted with the project. Thank you to Dr. Veronica Jamnik, Dr. Christopher Perry, and Dr. Pillai-Riddell for their work in diligently examining and providing feedback on the manuscript.

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LIST OF ABREVIATIONS

ACC	Accelerometer
ACC-PA	Accelerometer-Measured Physical Activity Level Expressed as Vector Counts/10 secs
AP	Self-paced Active Play
AUC	Area-Under-The-Curve for Oxygen Consumption
Cont	Prescribed Continuous Treadmill Exercise
HIIT	High Intensity Intermittent Training
Int	Prescribed Intermittent Treadmill Exercise
LPA	Light Intensity Physical Activity
MVPA	Moderate-to-Vigorous Intensity Physical Activity
PA	Physical Activity
PCr	Phosphocreatine
PDC	Pyruvate Dehydrogenase Complex
Phos	Glycogen Phosphorylase
Rand	Prescribed Intermittent Random Exercise
TCA	Tricarboxylic Acid
TM	Treadmill
VPA	Vigorous Intensity Physical Activity
VO_2	Oxygen Consumption Expressed as $mLO_2 \cdot kg^{-1} \cdot min^{-1}$

Chapter 1 - Introduction:

The benefits of high intensity intermittent training (HIIT) are associated with enhanced endurance performance and exercise tolerance (Gibala et al., 2006; Hawley et al., 1997). HIIT can be subdivided into two categories, sprint interval training (SIT) consisting of all out sprints for a short period (work:rest ratio 30sec:4mins) such as the Wingate Test, and high intensity aerobic training (HIAT) at 80-95% maximal oxygen consumption (VO_2max) and a longer period (work:rest ratio of 3 mins:4 mins) such as the 3x3 Test (Matsuo et al., 2013). Previous studies have observed that both types of HIIT raise VO_2max albeit through different physiological targets, SIT increases oxidative capacity in the peripheral muscles, while HIAT focuses on improving cardiac function (Matsuo et al., 2013). These functional improvements from both types of HIIT ultimately relate to up-regulation of aerobic and anaerobic metabolism contributing to the energy resynthesis within active muscles and an enhanced reliance on aerobic metabolism (Laursen and Jenkins, 2002). For example, it has been established that a short two-week HIIT consisting of 4 to 6 x 30-s maximal efforts with 4-min passive recovery (1:8 work to rest ratio) can induce training adaptations. Improvements included increased mitochondrial function, muscle buffering capacity (estimated from titrimetric determination of muscle homogenate from muscle biopsy) and exercise performance (e.g. time trial, time to exhaustion) (Burgomaster et al. 2005, 2006; Gibala et al. 2006). Following short HIIT programs in children the changes in VO_2max are equivocal with some studies reporting no change (Burgomaster et al. 2005, 2006) and others reporting an increase of approximately 9% (Bailey et al. 2009; Hazell et al. 2010; Astorino et al. 2012). Generally, it appears that increases in VO_2max occur after a longer training intervention (such as, 4 to 6 weeks) (Burgomaster et al. 2008; Trilk et al. 2011; Macpherson et al. 2011; Zelt et al. 2014). This suggests that longer duration (in weeks) HIIT

programs could be a good strategy to induce cardiorespiratory and metabolic adaptations. Past research has reported cardiorespiratory and metabolic adaptations following HIIT programs, including a 9-12% improvement in VO_2max and increases in skeletal muscle blood flow, lactate transport capacity and sarcoplasmic reticulum function (Burgomaster et al. 2005, 2006, 2007; Gibala et al. 2006; Babraj et al. 2009; Hazell et at. 2010; Macpherson et al. 2011). However, the generalizability and/or the relative ease of translation to children's community/school-based exercise/physical activity programs are debatable.

Recently, Lambrick et al. (2015) conducted a school-based physical activity (PA) program consisting of six children's games lasting six minutes each at an average heart rate of 175 bpm (85% of measured HRmax-age predicted) with two-minute passive recovery period between each game. The results demonstrated a 6% improvement in VO_2max and a 5% increase in peak running speed (treadmill) for children (8-10yrs) following the 6-weeks of high intensity intermittent training. In addition, a significant 5% increase in VO_2max follows an eight-week community based self-paced active play (AP) (self-paced age-appropriate games) program with children (8-12yrs) (Meyerovich et al., *manuscript in preparation*). Therefore, it is clear that on average, improvement in children's cardiorespiratory fitness occurs after self-paced high intensity games-based PA program (6-8wks). Whether these results are due to a faster rate of VO_2 at onset of exercise and/or a faster recovery time following exercise remains uncertain (Lambrick et al., 2015). Furthermore whether the large range of changes (-19% to +5%) is related to VO_2 kinetics or other factors is speculative. It is known that children's aerobic fitness level, body composition, body mass, stage of maturity, and/or the characteristics of the exercise programs, such as intensity, recovery time and work:rest ratios will impact adaptation (Rowlands et al, 1998). What is less clear from the literature is if the faster recovery time coincides with a

faster recovery oxygen consumption (VO_2) response post exercise for children, which might be related to energy demand and work/play-rest intervals.

To characterize VO_2 responses to intermittent or random PA, Turner et al. (2006) employed four intermittent exercise sessions each with a different work:rest cycle (10:20s;30:60s;60:120s;90:180s) and therefore different maximal work rate. As with continuous exercise, the VO_2 response to intermittent exercise is intensity dependent. Specifically, the higher workloads were associated with increased lactate production that appeared similar to heavy and maximal exercise in continuous exercise. In contrast, the very short intermittent work cycles (10:20s; 30:60s) did not generate sustained lactate concentration. The short PA cycles, and corresponding measured VO_2 values, deviated from those predicted from constant work exercise. Another study in adults (28.8±5.0 yrs) examining intermittent activity found VO_2 and lactate responses were higher for intermittent exercise than continuous exercise of the same total time and power output (25-50% of maximal exercise power output) (Edwards et al., 1973).

For intermittent exercise of ordered intensity and/or random exercise intensity, it is unclear whether the aerobic or anaerobic metabolic contributions have any influence. The VO_2 profile of a soccer match was assessed, and compared to VO_2 responses from a continuous protocol of the same average speed (Drust et al., 2000) in order to better understand the influence(s) of intermittent exercise with random intensity (i.e. exercise demands presented in non-sequential order). Although the mean VO_2 associated with the continuous exercise group mimics the average VO_2 during the intermittent random activity, the range of VO_2 responses (2.0-3.6 $L \cdot min^{-1}$) were significant and large. It therefore becomes apparent that engaging in intermittent random intensity PA would have different physiological responses than that of continuous and/or order intermittent exercise.

The mechanism(s) underlying cardiovascular and metabolic adaptations to HIIT exercise for children (and young adults) are continually emerging. It is proposed that the greater contribution from aerobic metabolism and less contribution from anaerobic metabolism in meeting the ATP demands for subsequent bouts of high intensity exercise are important (Laurens and Jenkins, 2002). The precise mechanism(s)/factor(s) responsible for these adaptations are unknown; however the accumulated VO_2 required to achieve a steady state, the magnitude of the oxygen deficit and/or the fast and slow phases of VO_2 during recovery have been implicated (Laurens and Jenkins, 2002; Falk and Dotan, 2006). Each of these factors, alone or in combination would influence the contribution of sources for aerobic metabolism and anaerobic metabolism for the ATP generation required when starting a second bout of exercise (Linossier et al., 1993). Regardless of the mechanism(s), when the net effect of these factors is to increase the VO_2 level prior to performing a second bout, the suggestion is that this 'primes' the aerobic metabolic system to contribute more to the subsequent exercise bout with less reliance on anaerobic metabolism (Laurens and Jenkins, 2002; Lambrick et al., 2015). To date much of our understanding underlying the physiological mechanisms for HIIT stems from laboratory-based and/or well-controlled HIIT programs, and organized sports. Little evidence exists as to whether the VO_2 response(s) to exercise demand and recovery associated with-and-between self-paced active playing of games follows a similar pattern to controlled exercise protocols. Although an examination of VO_2 kinetics associated with different modes and intensities of PA are well documented (see Appendix II), their feasibility with self-paced active play is questionable (Fawkner and Armstrong, 2003).

It has been suggested, that a greater contribution of aerobic metabolism and less anaerobic metabolism accompanying random intensity short burst intermittent exercise (as with

children's self-paced active play) may be due to a modified VO_2 responses during recovery (Falk and Dotan 2006). Moreover, the impact of random intensity short burst intermittent exercise on O_2 deficit during successive bouts of exercise has not been investigated in children. The observations that children's games, which are sporadic (intermittent) in nature, and which elicit a wide range of exercise intensities requiring between 4 - 8 metabolic equivalents (MET) (Belcastro et al., 2012; Howe et al 2010) suggests that recovery processes may be important in children. In addition it was been reported that the short recovery intervals (~2min) between repeated bouts of moderate and/or high intensity games-based programs are associated with improvements in aerobic power (Lambrick et al., 2015). Although the physiological responses elicited during self-paced game-based PA are unclear, the ability for children to respond to increasing loads and/or repeated short burst activity may help elucidate the mechanism(s) underlying adaptations/accommodations to subsequent bouts of vigorous-intensity exercise.

In summary, the characteristics of the VO_2 responses for exercise and recovery of self-paced intermittent non-laboratory based children's active play (games) remain uncertain. Studies on children's playing of active games should consider the magnitude of the VO_2 response (cumulative VO_2 and accumulated oxygen deficit) during HIIT-intervals and the shape and time course of the VO_2 responses during the brief recovery period. Describing and understanding the basic VO_2 responses in a community-based active play environment is important, since unlike adults, children's PA behaviours are very intermittent and characterized by rapid changes from rest to moderate-to-vigorous intensity PA (MVPA) (Malina et al., 2004). As well, active play based programs may promote greater PA participation and/or adherence to self-paced active play programs by children (Belcastro et al., 2015; Lambrick et al., 2015).

Purpose:

The purpose of this study is to determine the VO_2 responses of children and young adults during exercise and recovery from prescribed treadmill (TM) exercise performed over four incremental speeds using three protocols: Continuous (Cont), Intermittent (Int) (same order of increasing speeds but with five-minute rest intervals) and Intermittent Random (Rand) (random order of speed with five-minute rest intervals between each speed). As well as an assessment of children's VO_2 responses during participation in an self-paced active play (AP) program. There are two objectives for this study are: 1) to assess the area under the curve (AUC); accumulated O_2 deficit; and excessive post exercise oxygen consumption (EPOC) fast and slow EPOC components for children (8-12 yrs) during Cont, Int and Rand TM exercise at 4, 6, 8 and 10 km/hr. In addition, responses by young adults participating in the TM protocols will enhance our understanding of the VO_2 responses, AUC, O_2 deficit and EPOC to Int and Rand exercise and 2) to determine and compare the effect(s) of community-based AP (games) on AUC, O_2 deficit and EPOC for children.

Hypotheses:

1. For children the accumulated VO_2 response (AUC), the accumulated O_2 deficit and the fast and slow phases of EPOC associated with exercise at each of 4, 6, 8 and 10 km/h will be higher for the Rand TM protocol compared to the Cont and/or Int protocols.
2. For children the VO_2 responses to low and high MET will be higher for the TM protocols compared to AP.
3. For young adults the accumulated VO_2 response (AUC), the accumulated O_2 deficit and the fast and slow phases of EPOC associated with exercise at each of 4, 6, 8 and 10 km/h will be higher for the Rand TM protocol compared to the Cont and/or Int protocols.

Chapter 2 - Methodology:

The study was divided into two components one using structured TM exercise (n=16; 8 children and 8 young adults) and one using AP (n=14 children) (See Appendix I). Children (7-12 years; boys and girls) were recruited from a summer camp program held at a local community center. The young adults (males and females, n=5) undergraduate kinesiology student volunteers. York University's Human Participant Research Committee approved all procedures and processes. All necessary questionnaires and informed consent documents were signed by each participant and in the case of children by parents/guardians.

Study Design and Protocols:

Prescribed Treadmill exercise:

Participants (8 children and 5 young adults) were randomly assigned to each of three TM protocols; i) Cont, ii) Int, and iii) Rand. Prior to the start of the TM exercise participants were explained the protocols and fixed with Polar heart rate monitor; an ActiGraph GT3X+ accelerometer and the portable oxygen analyzer (COSMEDFitmate™, Image Monitoring Mississauga, Ontario). The familiarization period (10 min) also served to stabilize the analyzers. Once the familiarization was complete, the participants sat quietly for resting VO_2 measures (minimum of 5 minutes or longer). The participants performed each TM speed/workload for 3 minutes regardless of the protocol with steady state verified by less than an average ±2 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$ in the last 30 seconds (Roston et al, 1987). Following the baseline collection, the participants randomly selected one of the three TM protocols. The TM protocols included;

- Cont: 4, 6, 8, 10 km/hr at 0% grade for 3 minutes at each workload. Following the protocol, participants sat for at least five-minutes.

- Int: treadmill speeds performed in the same order as with the Cont protocol but with 5-minute rest in between each workload.
- Rand: treadmill speeds performed intermittently but delivered in random order - with a 5-minute rest in between each workload.

Self-Paced Active Play:

Children (n=14) participating in a one-hour PA session as part of a summer camp program at a local community center were assessed once over a period of two weeks (four play sessions). The AP session incorporates five-six children's age-appropriate cooperative games played by ~25 children in 55 minutes (Moghaddeszadeh et al., 2017). During the AP, experienced Kinesiology undergraduate students served as positive role models. The participants were also fitted with a heart rate monitor, accelerometers and a portable oxygen analyzer prior to the games and for an average of 30 minutes per session.

Assessments:

Oxygen consumption (VO_2) was assessed using breath-by-breath capture with the COSMEDFitmate™ portable oxygen analyzer. The Fitmate™ is valid and reliable for assessing VO_2 in adults with no significant differences observed when compared to the Douglas Bag Method (Lee et al., 2011; Brisswalter et. al, 2014; Nieman et. al, 2006). Moreover, the manufacture's flow rate specifications (0-50 L/min) and previous reports indicate that the Fitmate™ is capable of accurately assessing VO_2 consumption during exercise with children (Inselman et al., 1993; Howe et al., 2010). In our laboratory Fitmate™ values are within ±4% when compared to open circuit spirometry and/or metabolic cart.

Accelerometry (Actigraph GT3X+) quantified PA levels of the participants during each of the protocols (Romanzini et. al, 2014; Hänggi et. al., 2013). Accelerometer-measured PA

combined vector counts were quantified using 10sec epochs (Bonomi et al, 2009). Where necessary, energy expenditure and MET from laboratory-derived equations were estimated (Belcastro et al., 2012).

Measurements:

To measure the contribution of aerobic metabolism of the exercise protocol and intensity, AUC was determined (Mann et al, 2014). Briefly, AUC involved separating the curve into equidistant data points on the x-axis (i.e. trapezoids of equal width) from the 10-second time points, and adding up the components corresponding to the section of the exercise protocol being examined. The number of data points incorporated into the AUC calculation was standardized by taking the first 14 points for the exercise portion and the initial 28 points for the recovery portion to ensure the same number of data points was used in cases where uncontrollable between-subject variation occurred (i.e. time stepping onto/off treadmill).

To measure the contribution of anaerobic metabolism, the accumulated oxygen deficit was determined for each condition, as recommended and validated using steady state values to quantify the total oxygen cost of the exercise (Medbo et al 1998). Briefly, the steady-state VO_2 provides an oxygen demand for the activity, from which each exercise VO_2 data point (in 10sec capture) are subtracted from the oxygen demand value to obtain oxygen deficit. The oxygen deficit at each 10sec data point summed to produce a total oxygen deficit for each condition. The total anaerobic and aerobic contributions calculated as a percentage of total oxygen consumption by total oxygen deficit and total oxygen uptake, respectively.

Recovery VO_2 responses characterized by measuring the slopes and intercepts of the fast and slow components of the VO_2 response during recovery provides a valid estimate of the EPOC (Gaesser and Brooks 1984). In addition to the rates of change, the VO_2 responses for fast

phase EPOC and slow EPOC were determined based on visual inspection of the plotted VO_2 curve. Regression analysis of the recovery VO_2 data (EPOC phases) to determine the slopes and intercepts was used.

For the measurement of VO_2 variables with AP (unstructured activity), children's games were assessed by standardizing to both MET and ACC-PA vector counts. This was completed by initially calculating the MET for the VO_2 values at each of the 10 second intervals of the Int (structured activity), and then averaging MET and ACC-PA vector counts for the entire bout in each workload. Two-three minute sections of the VO_2 response to the games were then selected which closely matched the average MET for a given workload of the TM exercise. Each of the MET classifications and their corresponding total AUC for the AP was calculated. The VO_2 and MET responses across the range of ACC-PA vector counts compared the relationships between the two variables.

Statistics:

Two-way analyses of variance (ANOVA) with repeated measures for TM exercise and speed were assessed by SPSS v23. Each of the main effects for the dependent variables, AUC exercise, accumulated O_2 deficit and the intercepts and slopes for the recovery curves were compared. Group Interactions were compared using Tukey's post-hoc analysis. Statistical significance corresponds to a p-value <0.05.

Chapter 3 - Results:

Participants:

Characteristics of the children (n=8) and young adults (n=5) completing the laboratory based TM exercise protocols are listed in Table 1. The age for children and young adults were 9.0 ± 1.3 yrs and 21.7 ±2.4 yrs, respectively ($p<0.05$). As expected body mass, height and load-limited VO_2peak (determined by Cosmed Fitmate™ from 10 km/hr workload in TM protocol) were different between children and young adults (Table 1). The children in the TM group did not participate in the AP sessions, a separate group was recruited. Overall, the children and young adults had anthropometric characteristics within published norms for their age group (Malina et al., 2004).

Characteristics of the children (n=14) completing the AP are reported in Table 2. The children's age of 9.6 ± 1.5 yrs was similar compared to those in the TM group ($p>0.05$). Similarly, the body mass and height were not different.

Table I: Subject Characteristics of Children and Young Adults Participating in the Treadmill Protocols. Values are Expressed in Mean±SD.

	N	Age (yrs)	Weight (kg)	Height (cm)	VO_2 Peak* ($mLO_2 \cdot kg^{-1} \cdot min^{-1}$)
Children	**8**	**9.0 ± 1.3**	**37.5 ± 9.9**	**134.9 ± 16.1**	**42.9 ± 1.2**
Young Adult	**5**	**21.7 ± 2.4**	**58.5 ± 12.7**	**162.8 ± 8.3**	**36.7 ± 3.3**

*** VO_2peak power was estimated from Cosmed Fitmate™ software during incremental tests.**

Table II: Subject Characteristics of Children Participating in the Self-Paced Active Play Program. Aerobic Power was Estimated from the 20m Multi-Stage Shuttle Run. Values are Expressed in Mean±SD.

	N	Age (yrs)	Weight (kg)	Height (cm)	Estimated VO_2max ($mLO_2 \cdot kg^{-1} \cdot min^{-1}$)
Child	**14**	**9.6 ± 1.5**	**41.7 ± 10.8**	**139.6 ± 16.5**	**45.6 ± 6.2**

Children's Physical Activity and Oxygen Consumption Responses to Treadmill Exercise:

Children's physical activities during the Cont, Int and Rand TM exercise protocols are presented in Figure 1. As expected an increase in three-minute accelerometer outputs (vector counts/10sec) were observed for each protocol. Although the Cont protocol had the higher ACC-PA output at each TM speed, there were no statistical differences across protocols ($p>0.05$). The average three-minute VO_2 response to the Cont protocol was linearly related to the TM speed from 4 to 10 km/hr, whether assessed by VO_2 (mL $O_2 \cdot kg^{-1} \cdot min^{-1}$) (Figure 2) and/or AUC in mL $O_2 \cdot 3min^{-1}$ (Figure 3) ($p<0.05$). The average AUC of the highest workload was 672 ± 50 $mLO_2 \cdot kg^{-1} \cdot min^{-1}$, 629± 80 $mLO_2 \cdot kg^{-1} \cdot min^{-1}$, and 657 ± 50 $mLO_2 \cdot kg^{-1}\, min^{-1}$ for each of Cont, Int, and Rand TM protocols respectively, $p>0.05$).

Qualitative Assessment of Children's Oxygen Consumption Responses to Cont, Int and Rand TM Exercise:

The qualitative patterns and time courses of children's VO_2 responses were observed for Cont, Int (stops and starts between workloads) and Rand (stop and start with Rand workloads) TM protocols. The ACC-PA outputs and VO_2 responses to increasing TM exercise were captured in10-sec Intervals. The Cont protocol showed a typical increase in VO_2 with increasing speed, reaching an average of 39.2 ± 7.1 $mLO_2 \cdot kg^{-1} \cdot min^{-1}$ at 10 km/hr (Figure 4 – top). The typical increases and decreases in VO_2 for exercise and recovery, respectively, are evident when the Int (Figure 4 - middle) and Rand (Figure 4 - lower) protocols are observed,. Across all TM protocols the average ACC-PA and VO_2 were 1000 ± 300 counts/10sec and 32.6 ± 6 $mLO_2 \cdot kg^{-1} \cdot min^{-1}$, respectively, for 10 km/hr. At the onset of exercise the Int protocol had a relatively quicker increase to plateau than the Rand protocol, which was observed for all 4 TM workloads. Furthermore, during recovery VO_2 it appears that the Int protocol had a steeper decrease to

baseline than Rand and Cont for all workloads. Finally, the increase in VO_2 with each successive TM load appears to be more consistent in the Rand protocol as compared with the Int procedure, where a disproportionately large increase occurs for the final workload of 10 km/hr.

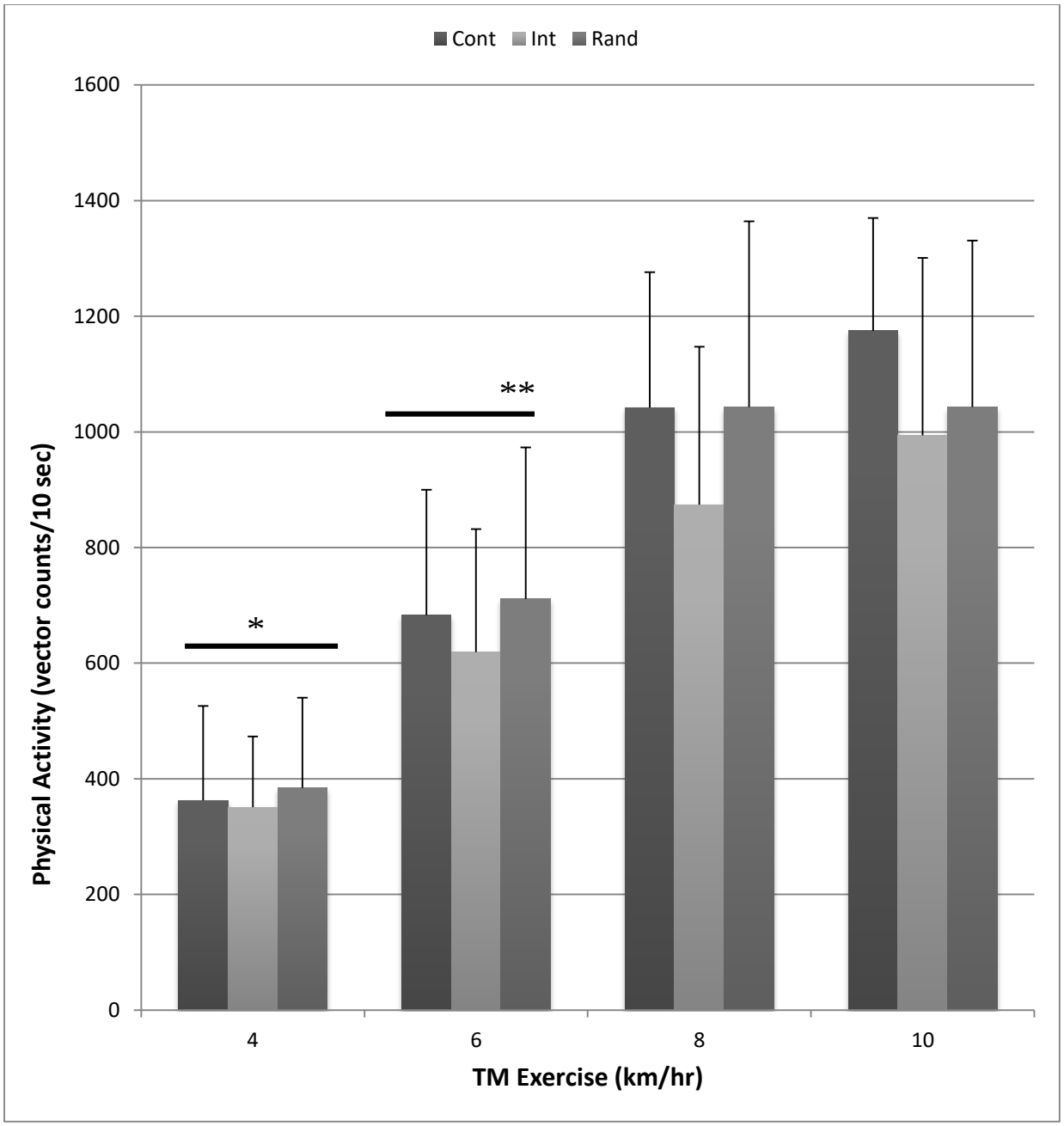

Figure 1: Children's (n=8) Acclerometer-Measured Physical Activity Responses (vector counts/10sec) to Three Minutes of Treadmill Running at each of 4, 6, 8 and 10km/hr (0%grade) for Continuous (Cont), Intermittent (Int) and Random (Rand) Protocols. The Rest Interval for Int and Rand Protocols was Five Minutes. No Statistical Differences Were Observed Across Protocols By Speed ($p>0.05$). Statistical Differences (*) between 4 vs 6, 8 and 10 km/hr; and Statistical differences (**) between 6 vs 8 and 10 km/hr) ($p<0.05$) are shown. Values are Expressed in Mean±SD.

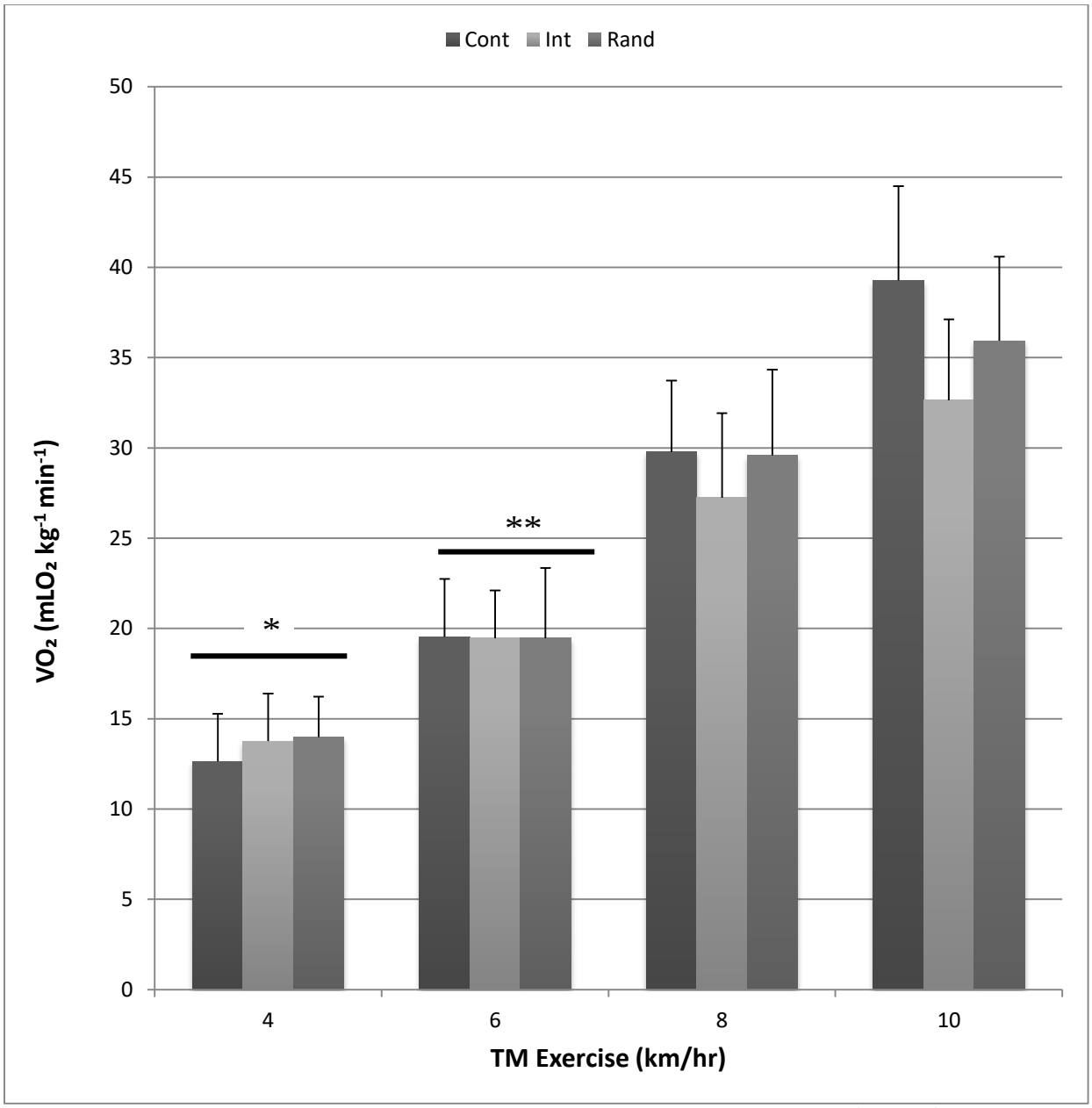

Figure 2: Children's (n=8) Oxygen Consumption Responses ($mLO_2 \cdot kg^{-1} \cdot min^{-1}$) to Three Minutes of Treadmill Running at each of 4, 6, 8 and 10km/hr (0%grade) for Continuous (Cont), Intermittent (Int) and Random (Rand) Protocols. The Rest Interval for Int and Rand Protocols was Five Minutes. No Statistical Differences Were Observed Across Protocols By Speed ($p>0.05$). Statistical Differences (*) between 4 vs 6, 8 and 10 km/hr; and Statistical Differences (**) between 6 vs 8 and 10 km/hr ($p<0.05$) are shown. Values are Expressed in Mean±SD.

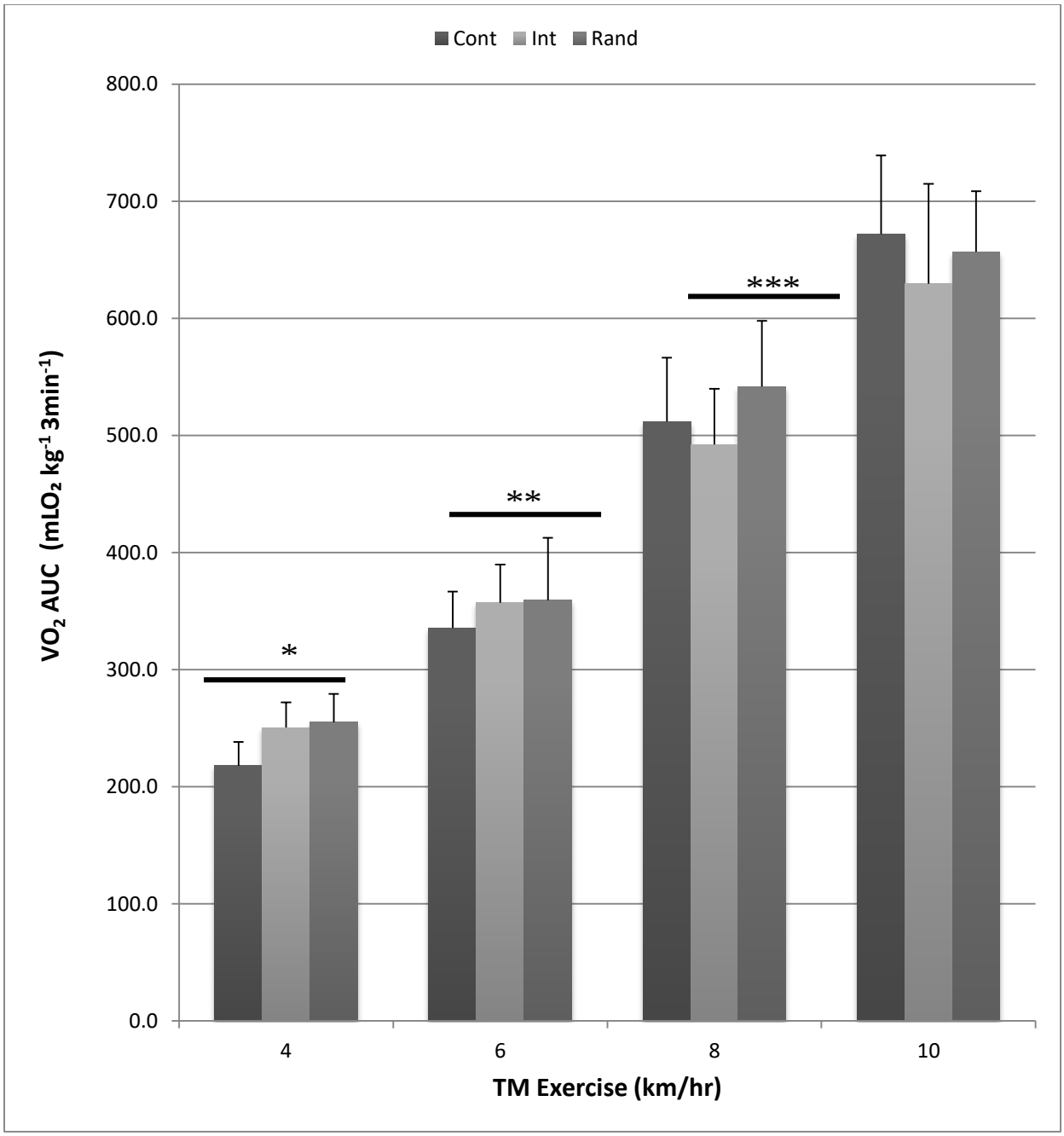

Figure 3: Children's (n=8) Total Area-under-the-Curve (AUC) ($mLO_2 \cdot kg^{-1} \cdot min^{-1}$) for Three Minutes of Treadmill Running at each of 4, 6, 8 and 10km/hr (0%grade) for Continuous (Cont), Intermittent (Int) and Random (Rand) Protocols. The Rest Interval for Int and Rand Protocols was Five Minutes. AUC Calculated from Addition of 10 Second Raw VO_2 Intervals over Three Minute Workload. No Statistical Differences Were Observed Across Protocols By Speed ($p>0.05$). Statistical Differences (*) between 4 vs 6, 8 and 10 km/hr; Statistical Differences (**) between 6 vs 8 and 10 km/hr; and Statistical Differences (***) between 8 and vs 10 km/hr ($p<0.05$) are shown. Values are Expressed in Mean±SD.

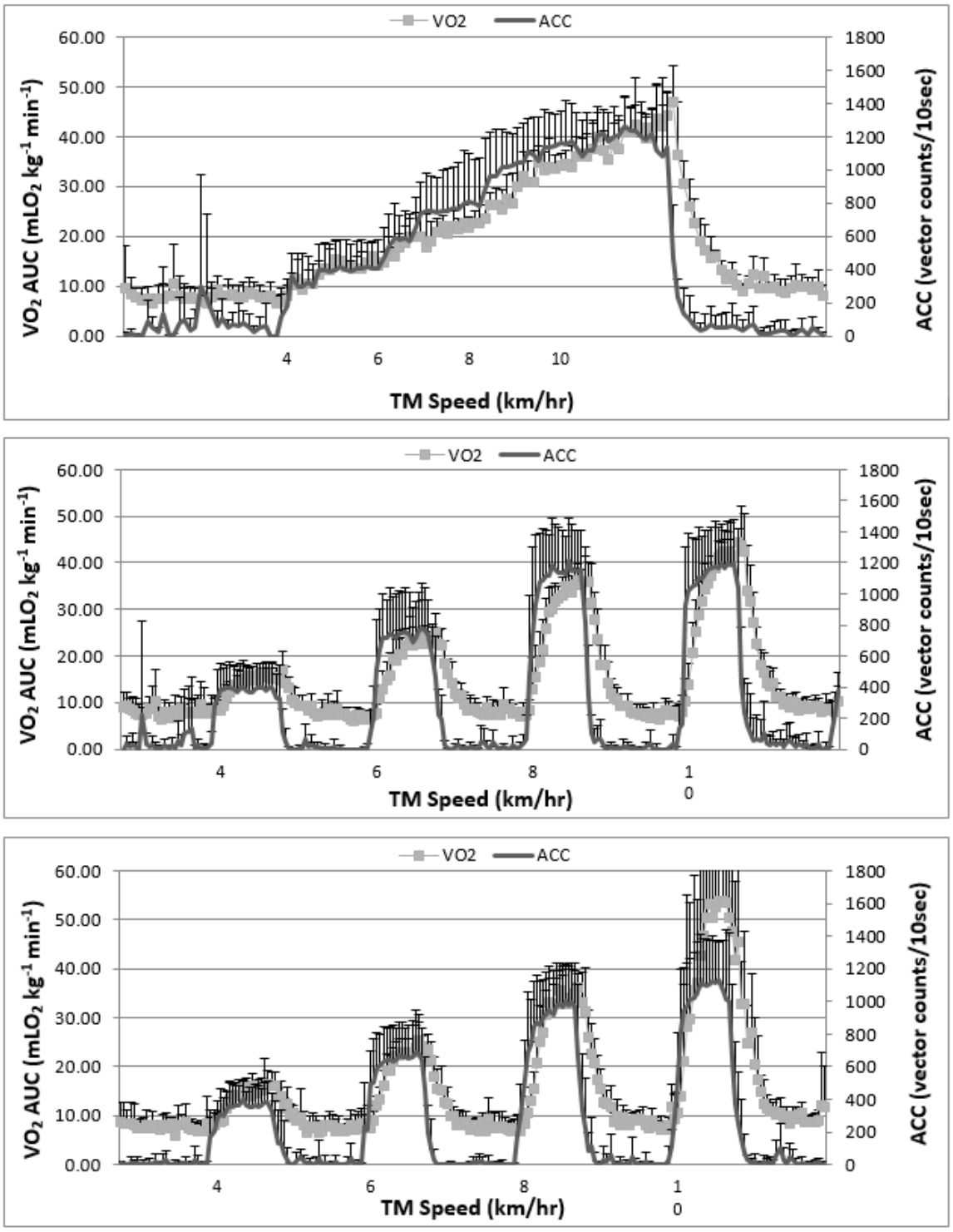

Figure 4. Children's (n=8) Accelerometer-Measured Physical Activity (ACC-PA vector counts/10 sec) and Oxygen Consumption ($mLO_2 \cdot kg^{-1} \cdot min^{-1}$) Responses at 4, 6, 8, 10km/hr (3min each; 0%grade) and Recovery for Continuous (Top), Intermittent (Middle) and Random (Lower) TM Protocols. Rest Intervals were Five Minutes. Values are Expressed in Mean±SD.

Quantitative Assessment of Children's Oxygen Consumption Responses to Int and Rand Treadmill Exercise Workloads:

Treadmill Exercise:

To address the question – is there a difference in the oxygen deficit at the onset of exercise during Int versus Rand PA– children's O_2 deficit (mL O_2 kg^{-1}) values were determined for VO_2 responses for each TM workload (4, 6, 8 and 10 km/hr) and protocol (Figure 5). The analysis revealed that there is an increase in oxygen deficit with increasing TM speed for both Int and Rand protocols. Interestingly, the Rand protocol produced a lower O_2 deficit across the workloads as compared to Int, but both were greater than Cont TM exercise ($p<0.05$). The highest workload of 10km/hr had an oxygen deficit of 5.5 ± 4 mL O_2 kg^{-1}, 12.9 ± 3 mLO_2 kg^{-1} and 12.01 ± 4 mLO_2 kg^{-1} for Cont, Int and Rand protocols, respectively. Oxygen deficit was higher for 8 and 10 km/hr compared to 4 and 6 km/hr in Int and Rand protocols ($p<0.05$).
In addition to oxygen deficit, the percentage contribution of aerobic and anaerobic energy sources was calculated using the difference in estimated steady state oxygen cost minus the measured oxygen cost during exercise for each protocol (Figure 6). The percent aerobic energy source was greater for Cont exercise vs Int and Rand exercise across all three protocols ($p<0.05$). The percent anaerobic energy sources for Int exercise was higher compared to Cont exercise ($p<0.05$), with the Rand protocol showing a higher proportion of anaerobic sources at the 10 km/hr workload compared to Cont ($p<0.05$) (Figures 6).

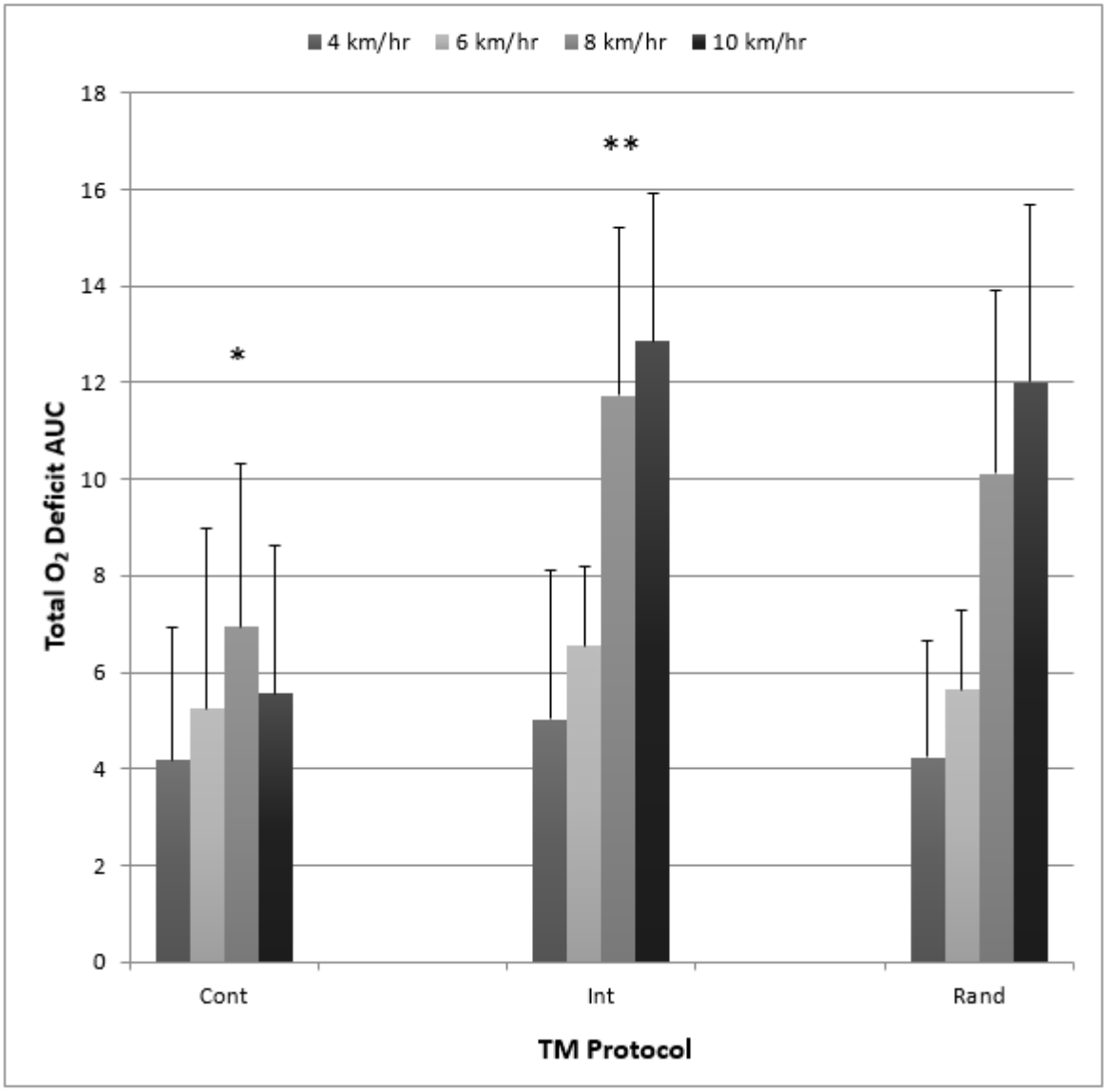

Figure 5. Children's (n=8) Oxygen deficit ($mLO_2\ kg^{-1}$) to Three Minutes of Treadmill Running at each of 4, 6, 8 and 10km/hr (0%grade) for Continuous (Cont), Intermitent (Int) and Random (Ramd) Protocols. The Rest Interval for Int and Rand Protocols was Five Minutes. O_2 Deficit Was Determined from the Difference from Estimated Steady-State O_2 Cost of Activity to the Measured VO_2 (in 10 sec). Statistical Differences (*) between 6 and 8 km/hr in Cont vs Int and Rand ($p<0.05$); and Statistical Differences (**) between 8 and 10 km/hr vs 4 and 6 km/hr for Int and Rand ($p<0.05$), No differences existed between 4,6,8 and 10 in Cont ($p>0.05$). Values are Expressed in Mean±SD.

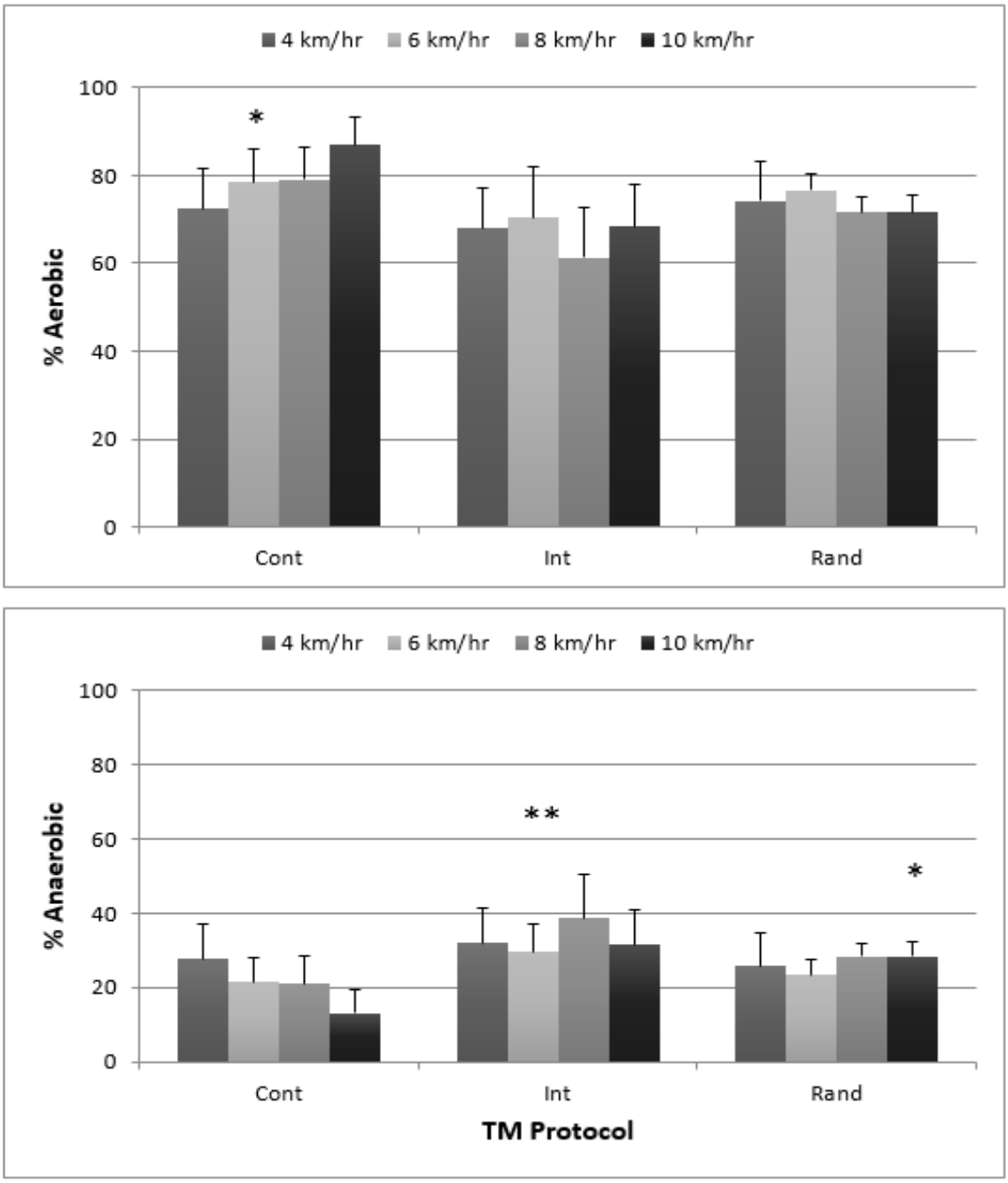

Figure 6. Children's (n=8) Proportions of Aerobic (Top) and Anaerobic (Lower) Energy Contribution to Continuous (Cont), Intermittent (Int) and Random (Rand) Treadmill Exercise at 4, 6, 8 and 10km/hr (3min; 0%grade). The Rest Interval for Int and Rand was Five Minutes. Proportions Were Determined as the Ratio of VO_2 for Total Estimated Steady State VO_2 and Measured VO_2 (Expressed as a Percentage). % Aerobic Statistical Differences (*) between Cont vs Int and Rand for all workloads ($p<0.05$). For % Anaerobic Statistical Differences (*) between 10 km/hr in Rand vs Cont ($p<0.05$); and Differences (**) between Int vs Cont for all workloads ($p<0.05$). Values are Expressed in Mean±SD.

Recovery:

The total VO_2 response during the recovery periods were determined by calculating the AUC ($mLO_2 \cdot kg^{-1} \cdot 5min^{-1}$). The total VO_2 during recovery increased linearly with increasing workload ($p<0.05$) (Figure 7). At the 10km/hr the total VO_2 for Int exercise was greater than that for the Rand and Cont protocols ($p<0.05$).

To determine the impact of the three-protocols on the time course of VO_2 responses during recovery, the rapid and slow phases were partitioned out and subjected to linear analysis for slope and intercept comparisons. When considering the rapid phase, the rate of decrease in VO_2 was related to the TM workload; the larger the demand the greater the rate of decrease (i.e., larger negative slope) (Figure 8). This pattern of response during the fast recovery phase was similar for the Int and Rand protocols. For the workloads at 4, 6 and 8 km/hr the Rand protocol had consistently faster rate of decrease for VO_2 by 7%, 23% and 11%, respectively. For the 10 km/hr workload the slopes for the rapid phase were -4.5 ± 2.5 and -3.9 ± 1.2 $mLO_2 \cdot kg^{-1} \cdot min^{-1}$ for the Int and Rand protocols, respectively, ($p>0.05$). This decrease in VO_2 was faster for the Int protocol compared to Cont protocol (-3.6 ± 0.9 $mLO_2 \cdot kg^{-1} \cdot min^{-1}$) ($p<0.05$) but not the Rand protocol ($p>0.05$). As anticipated the rate of VO_2 decrease for the slower phases were consistently less than the fast phase regardless of workload and/or protocol ($p<0.05$) (Figure 8). In addition to having significantly slower rates of VO_2 decrease, the slope values did not appear to be related to exercise intensity at 4, 6 and 8 km/hr for either the Int or Rand protocols (Figure 8). At 10 km/hr the slopes for the slow phase were higher compared to the other exercise loads ($p<0.05$), resulting in a faster recovery. This apparent faster time to recovery (for the slow phase) at 10 km/hr was similar for both the Int and Rand protocols, which were both much higher than the slope reported for the Cont protocol ($p<0.05$).

The intercept values determined for both the rapid and slow phases of recovery VO_2 for the three protocols are represented in Figure 9. During the Int and Rand protocols the intercept values for the fast phase of recovery increased with increasing workload ($p<0.05$). Although the largest intercept differences among all TM protocols were noted at the 10 km/hr workload, these means were not statistically different ($p>0.05$), which may have been due to the large standard deviation. The intercept values for recovery VO_2 of the slow phase during Int and Rand TM protocols did not change as a function of increasing TM workload at 4, 6 and 8 km/hr ($p>0.05$). Furthermore the differences determined across the TM protocols were not statistically different ($p>0.05$). The intercept values at 10 km/hr were greater than those for the lower workloads ($p<0.05$), and no statistical difference was observed across the three TM protocols ($p>0.05$) (Figure 9).

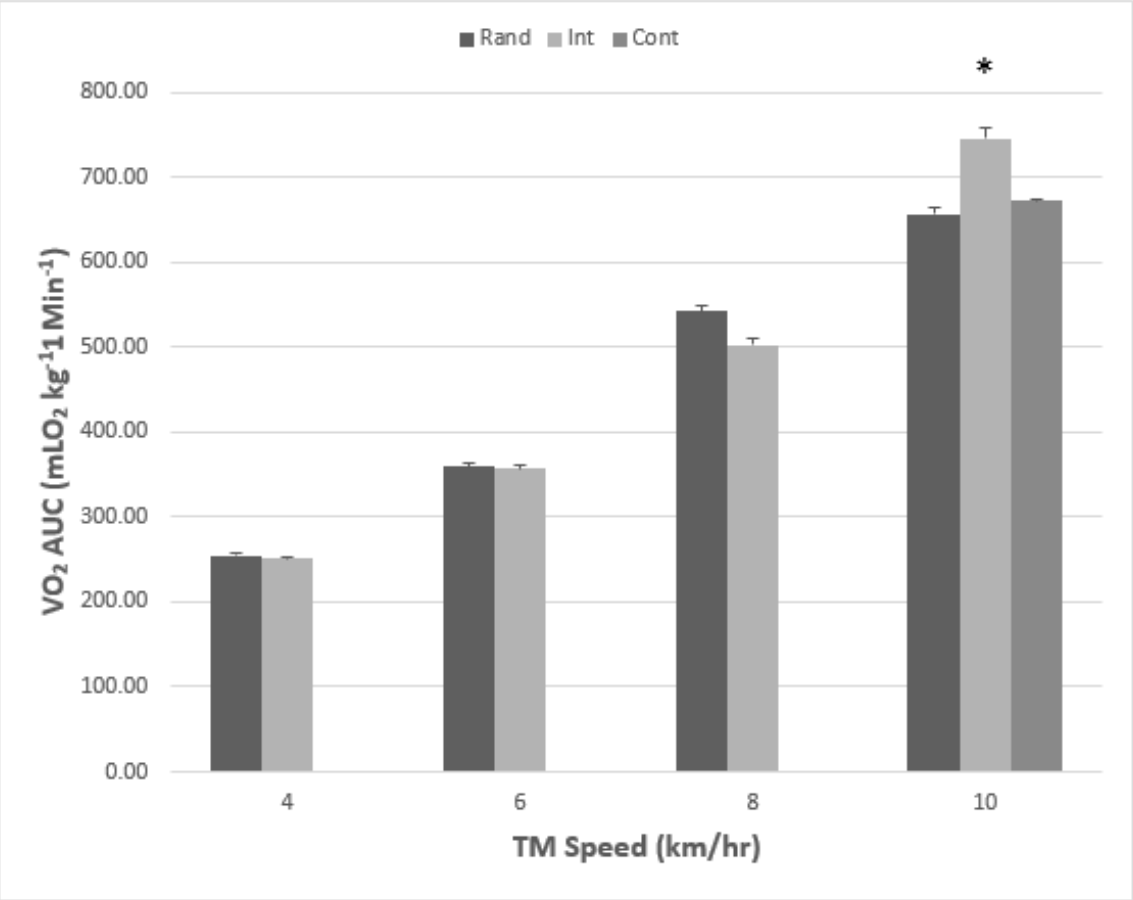

Figure 7. Children's (n=8) Recovery VO_2 Area-Under-the-Curve ($mLO_2 \cdot kg^{-1} \cdot 5\ min^{-1}$) at 4, 6, 8, 10km/hr (3min each; 0%grade) for Continuous (Cont), Intermittent (Int) and Random (Rand) Treadmill Protocols. Recovery Intervals were Five Minutes. Differences (*) Int vs Cont and Rand at 10km/hr. Values are expressed in Mean±SD.

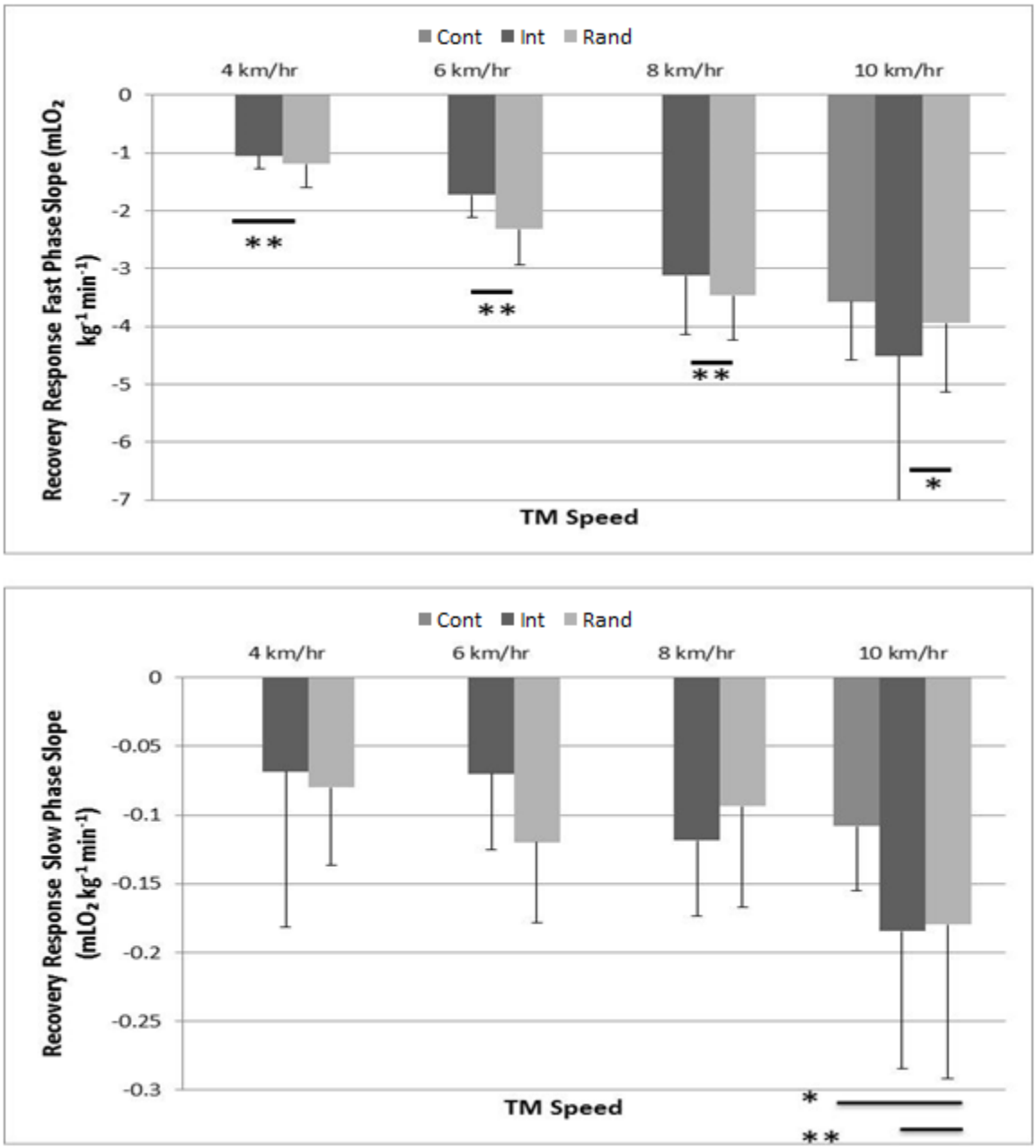

Figure 8. Children's (n=8) Fast Phase Slope of Recovery (Top) and Slow Phase of Recovery (Lower) following Treadmill Running at each of 4, 6, 8 and 10km/hr (3min; 0%grade) for Continuous (Cont), Intermittent (Int) and Random (Rand) Protocols. The Rest Intervals for Int and Rand were Five Minutes. Linear regression was applied to determine slope in each phase. Fast Phase Statistical Differences (*) Between 10 km/hr for Int vs Cont; and Statistical Differences (**) Between 4,6 and 8 km/hr in Rand vs Int ($p<0.05$). For Slow Phase Statistical Differences (*) Between 10 km/hr and 4,6 and 8 k/hr for Int and Rand, and Statistical Differences (**) Between 10 km/hr for Cont vs Int and Rand ($p<0.05$). Values are Expressed in Mean±SD.

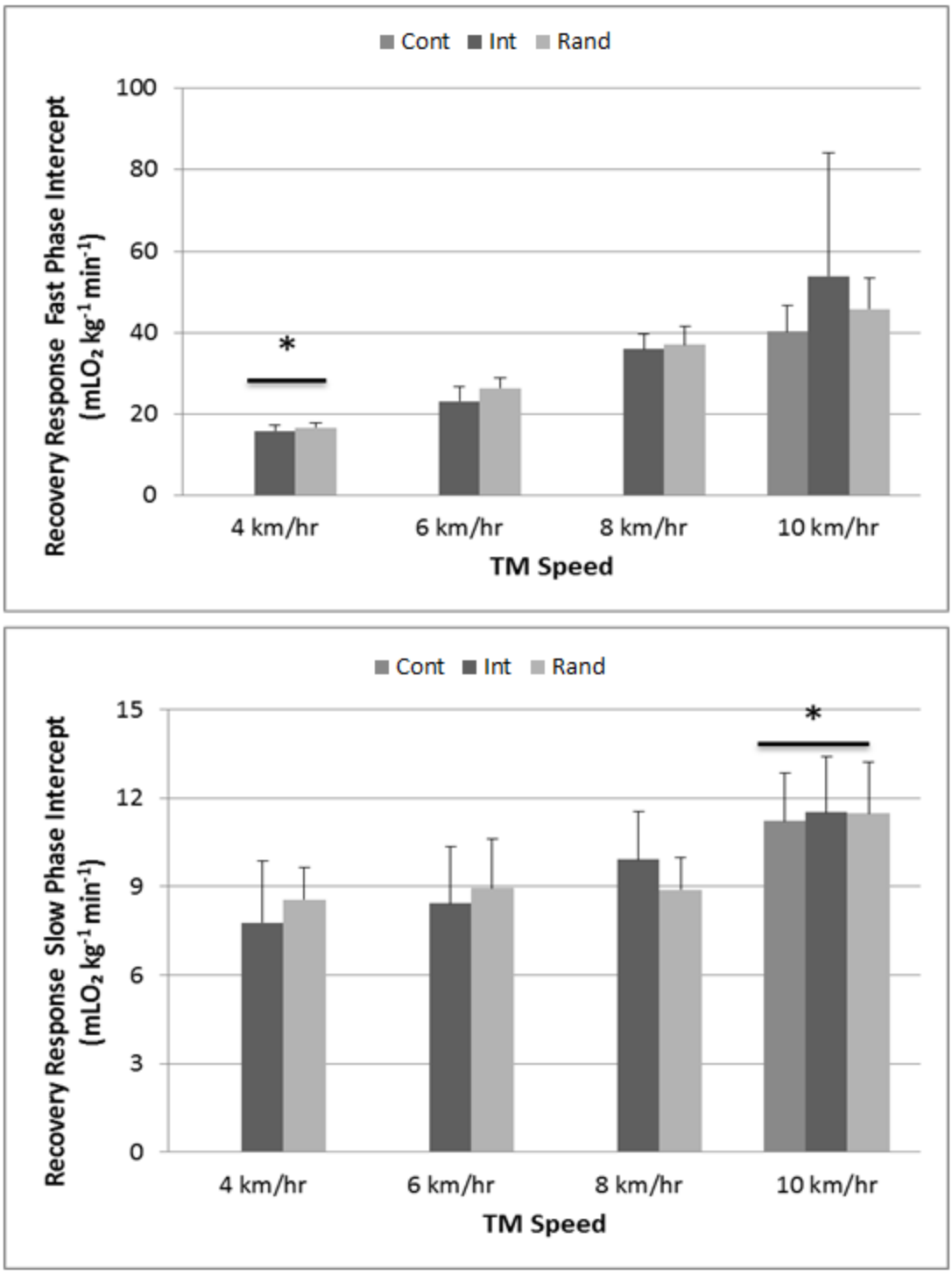

Figure 9. Children's (n=8) Fast Phase Intercept (Top) and Slow Phase Intercept (Lower) for Recovery following Treadmill Running at each of 4, 6, 8 and 10km/hr (3min; 0%grade) for Continuous (Cont), Intermittent (Int) and Random (Rand) Protocols. The Rest Interval was Five Minutes. Linear regression was applied to determine Intercept in each phase. Statistical Differences in Fast Phase (*) between 4 vs 6, 8 and 10 km/hr in Int and Rand ($p<0.05$); and Statistical Differences (*) in Slow Phase 10 vs 4,6, and 8 Km/hr ($p<0.05$). Values are Expressed in Mean±SD.

Classifying Children's Treadmill Exercise Intensity into Low, Moderate, and Vigorous Intensities Using Metabolic Equivalents (MET):

When assessing PA in the field or non-laboratory settings the MET has become the variable of choice in expressing the intensity of PA/exercise. Freedson et al., 2005 have reported that by using MET classifications, PA can be partitioned into low, moderate and vigorous intensity. To this end, the VO_2 values for the three TM protocols were transformed to MET and the proportions of light, moderate and vigorous intensity PA (%LPA, %MPA and %VPA) were determined. The results for the Int, Rand and Cont protocols showed a similar pattern with minor variations in MET values (Figure 10). In general, with increasing TM workloads (4, 6, 8, and 10 km/hr) there is a linear decrease in %LPA, a linear increase in %VPA, and an "inverted U" for %MPA. Specifically workload of 4 km/hr showed over 90% light, with nearly negligible contributions of moderate and vigorous intensities. At a workload of 10 km/hr all TM protocols averaged 90 %VPA, with the remaining 10% coming from light and moderate intensities.

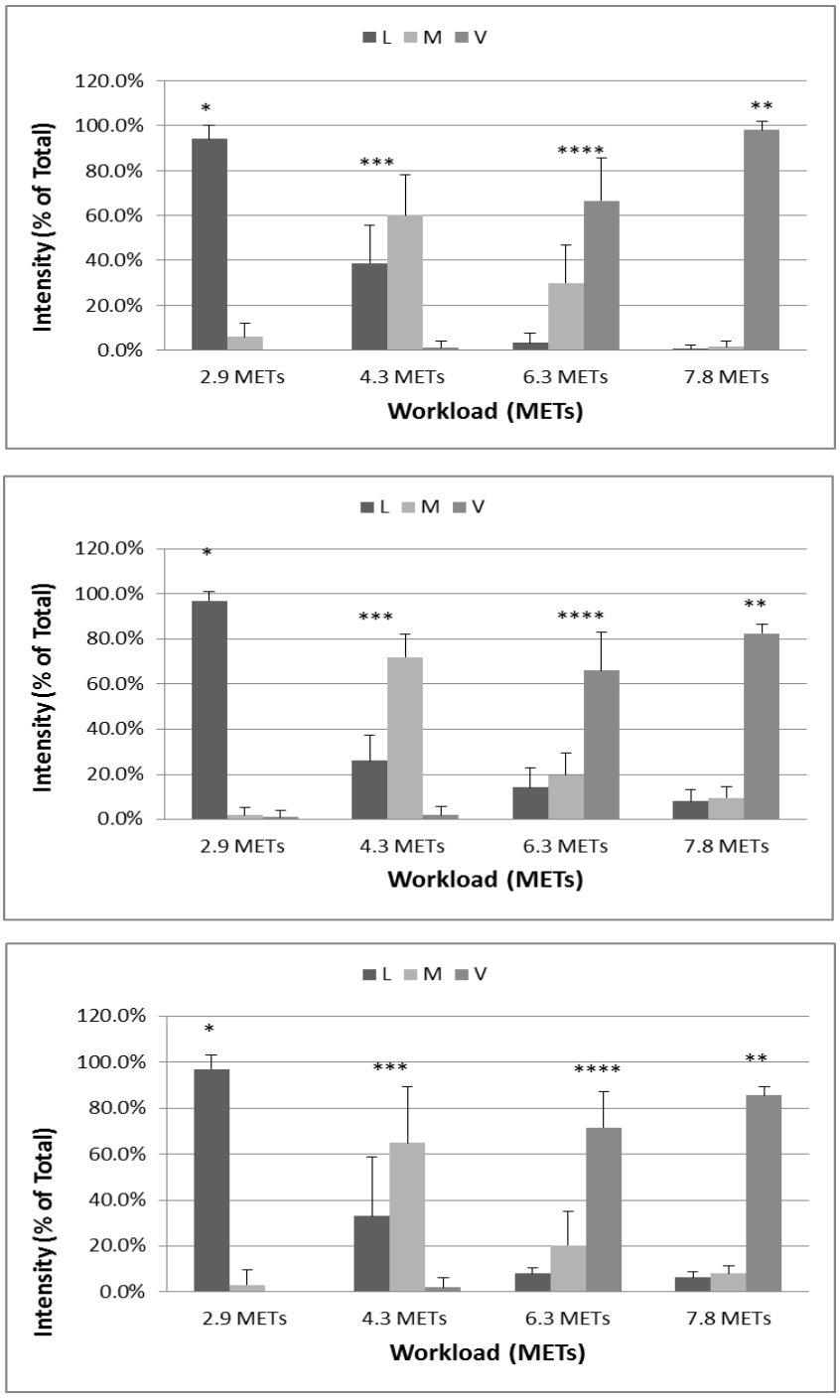

Figure 10. Children's (n=8) Proportion of MET characterized in Light (L), Moderate (M), and Vigorous (V) Physical Activity Intensities for Treadmill Running at each of 4, 6, 8 and 10km/hr (3min; 0%grade) for Continuous (Top), Intermittent (Middle) and Random (Lower) Protocols. All Statistical Differences (*) between L in 2.9 MET vs 4.3, 6.3 and 7.8 MET; Statistical Differences (**) between V in 7.8 MET vs 2.3, 4.3, and 6.3 MET; Statistical Differences (***) between M in 4.3 MET vs 2.3, 6.3, and 7.8 MET; and Statistical Differences (****) between M in 6.3 MET vs 2.3, 4.3, and 7.8 MET ($p<0.05$). Values are Expresses as Mean±SD.

Qualitative Assessment of Young Adult's Oxygen Consumption Responses to Int and Rand Exercise Workloads:

Young adult PA during the Cont, Int and Rand TM protocols is illustrated in Figure 11. As anticipated the ACC-PA outputs and VO_2 responses for the young adults showed typical patterns during all three TM protocols. Although Cont had the highest ACC-PA at the higher speeds versus Int and Rand TM protocols minimal differences were observed between the protocols (<0.05). In regards to the pattern of the VO_2 response, the Int TM protocol appears to have a sharper increase to steady state than the Rand TM protocol with each workload. Furthermore, the general pattern of recovery VO_2 shows the Int TM protocol to have a steeper decrease to baseline than Rand or Cont for all TM workloads.

Quantitative Assessment of Young Adult's Oxygen Consumption Responses to Int and Rand Treadmill Workloads:

Treadmill Exercise:

The Cont protocol had the largest total VO_2 values compared to the Int and Rand patterns. The total VO_2 assessed by AUC response for Cont exercise at 10 km/hr was 581.5 ± 5 $mLO_2 \cdot kg^{-1} \cdot min^{-1}$, which was higher than the total VO_2 of 450.8 ± 8 $mLO_2 \cdot kg\text{-}1 \cdot min^{-1}$ and 487.6 ± 10 $mLO_2 \cdot kg\text{-}1 \cdot min^{-1}$ for Int and Rand, respectively, ($P<0.05$) (Figure 12). Anecdotally, the movement patterns for the Cont TM protocol were exaggerated (i.e. more arms and side-to-side movement) compared Int and Rand, which may have increased the AUC value for Cont.

Recovery: To determine the impact of the three TM protocols on the time course of VO_2 responses during recovery, the rapid and slow phases were partitioned out and subjected to linear analysis for slope and intercept comparisons. When considering the rapid phase, the rate of decrease in VO_2 was related to the TM workload; the larger the demand the greater the rate of

decrease (i.e., larger negative slope) (Figure 13 - Top). This pattern of response during the fast recovery phase was similar for the Int and Rand protocols. In the fast phase, the 10 km/hr TM speed exhibits the steepest slopes for all 3 protocols with values of -2.40 ± 0.7, -2.60 ± 0.5, -2.25 ±0.6 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$ in the Cont, Int and Rand TM protocol, respectively, ($p>0.05$). The Cont TM protocol contains only one slope as there is only one recovery period within that protocol and it is only slightly larger than the Rand, however there is little variation amongst the slopes in the three TM protocols. Overall, the standard deviation is large throughout the workloads of the fast slope of all 3 protocols as illustrated in Figure 13 - Top. The slow phase slope increases with TM speed were -0.19 ± 0.12, -0.21 ± 0.08 and -0.12 ±0.05 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$ for the Cont, Int and Rand protocols, respectively. As anticipated, with higher TM speeds (8 and 10 km/hr) much larger slopes were determined compared to the lower speeds (4 and 6 km/hr) ($p<0.05$), highlighting the differing physiological response to higher workloads (Figure 13- Lower).

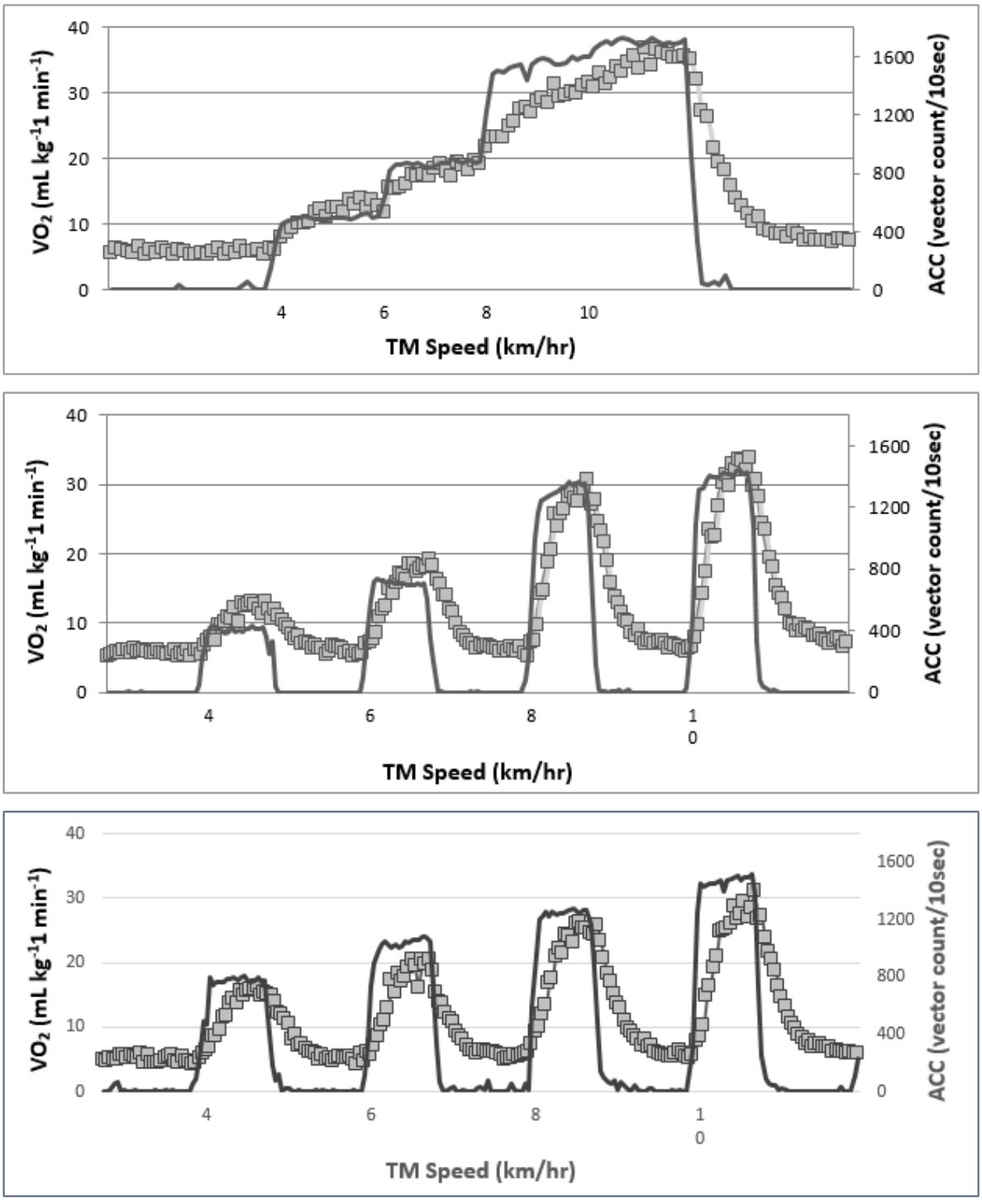

Figure 11: Young Adults (n=5) Accelerometer-Measured Physical Activity (vectors counts/10 sec) and VO_2 ($mLO_2 \cdot kg^{-1} \cdot min^{-1}$) Responses for Continuous (Top), Intermittent (middle) and Random (Lower) Protocols for Treadmill Running at each of 4, 6, 8 and 10km/hr (3min; 0%grade). Rest/Recover Intervals were for 5 Minutes. Values are expressed in Mean±SD.

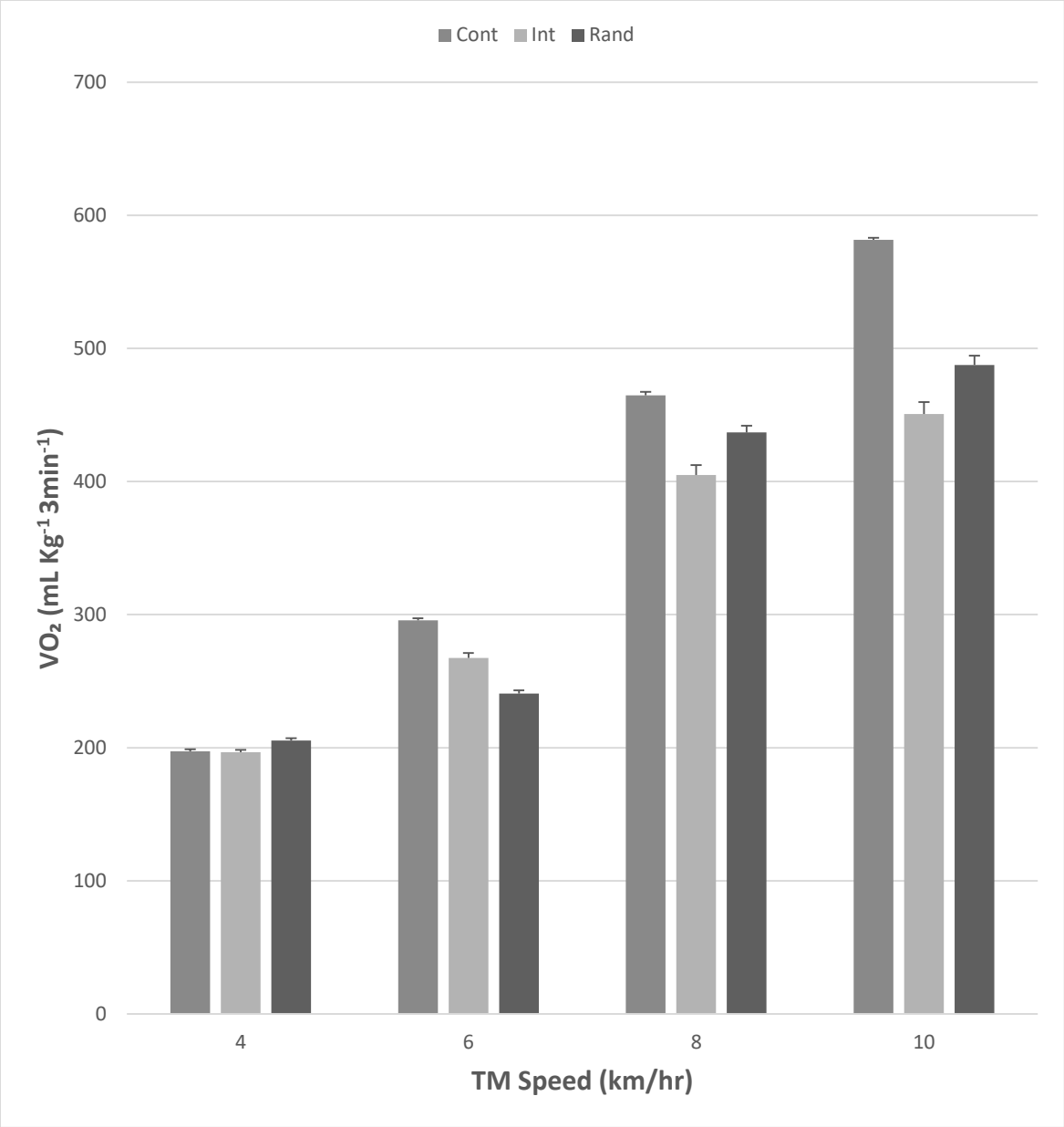

Figure 12: Young Adults (n=5) Total AUC to Three Minutes of Treadmill Running at each of 4, 6, 8 and 10km/hr (0%grade) for Continuous (Cont), Intermittent (Int) and Random (Rand) Protocols. The Rest Interval for Int and Rand Protocols were Five Minutes. Values are expressed in Mean±SD.

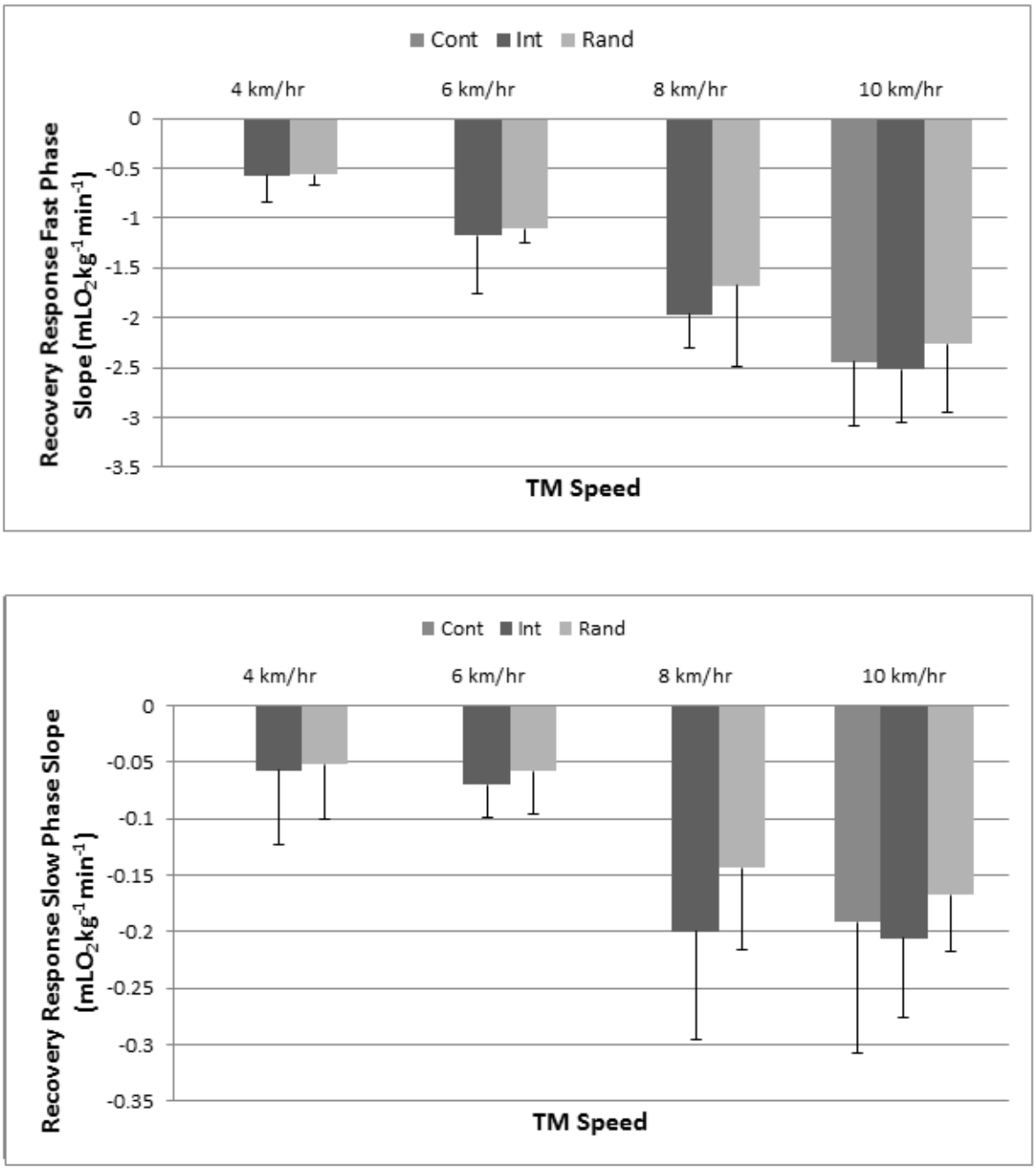

Figure 13. Young Adults (n=5) Fast Phase Slope (Top) and Slower Phase Slope (Lower) Recovery Following Treadmill Running at each of 4, 6, 8 and 10km/hr (3min; 0%grade) for Continuous (Cont), Intermittent (Int) and Random (Rand) Protocols. The Rest Interval for Int and Rand Protocols were Five Minutes. Linear Regression was applied to determine Slope in Each Phase. Values are Expressed in Mean±SD.

The intercept values corresponding to the rapid and slow phases of the recovery VO_2 are represented in Figure 14. The intercept values for the fast phase increased with each increasing TM speed in Rand and Int protocols ($p<0.05$) with the 10 km/hr having recorded the highest intercept value. (Figure 14 – Top). No statistically significant differences in the intercept values for the fast phase of recovery were determined at 10 km/hr with 34.0 ± 6, 32.0 ± 9, 31.0 ± 6 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$ in the Cont, Int, and Rand protocols, respectively, (Figure 14 – Top). In the slower phase of recovery the greatest speed had a value of 10.2 ± 1.5 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$, and Int was closely matched with 10.4 ± 3 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$ ($p>0.05$). The Rand protocol contained a lower value of 8.8 ± 2.4 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$compared to the other two protocols (Figure 14 – Lower).

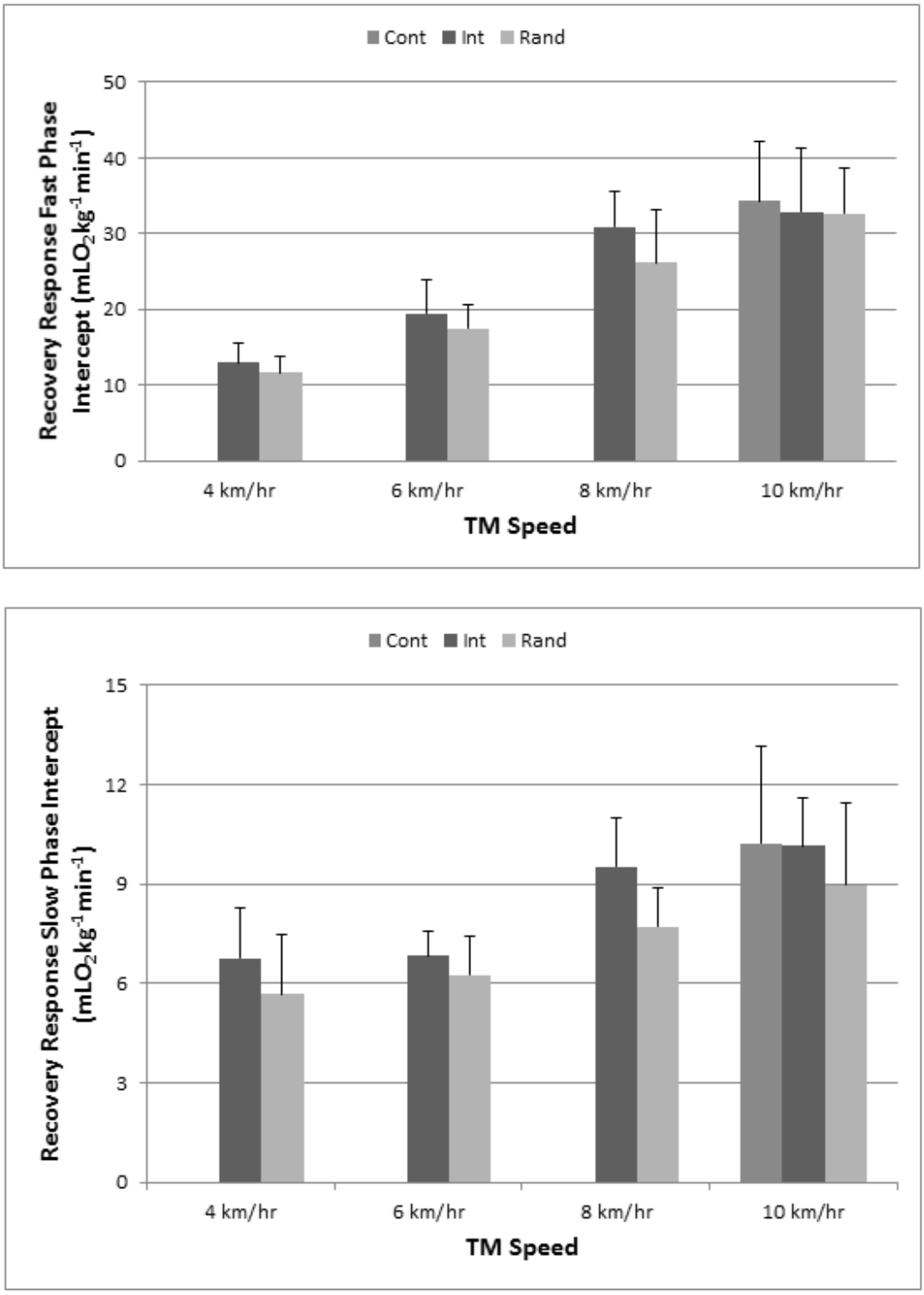

Figure 14. Young adults (n=5) Fast Phase Intercept (Top) and Slow Phase Intercept (Lower) for Recovery Following Treadmill Running at each of 4, 6, 8 and 10km/hr (3min; 0%grade) for Continuous (Cont), Intermittent (Int) and Random (Rand) Protocols. The Rest Interval for Int and Rand Protocols were Five Minutes. Linear Regression was applied to determine Slope in Each Phase. Values are Expressed in Mean±SD.

Children's Physical Activity and Oxygen Consumption Responses to Active Play:

Physical activity participation was assessed for all children during the PA portion of a summer camp (7 weeks; 4days/week) using ACC-PA (vector counts/10 sec epoch). The variables (VO_2, ACC-PA), MET and proportion of time at light, moderate and vigorous intensity were assessed during AP over four sessions (~55 minutes each) within a community recreation centre environment. Each session consisted of children (~25-35) participating in 6-7 self-paced age-appropriate cooperative games. Fourteen children agreed (with parental informed consent) to participate in the VO_2 assessments. VO_2 responses during the four AP sessions were collected on a total of 12 children (data was lost on two children) for an average time of 29.3±7.9 min with a range of 17.8 to 44.0 minutes. The children were not assessed during the full 55 minutes since session time was required to place and remove the Fitmate™.

Qualitative Assessment of Children's Physical Activity and Oxygen Consumption Responses to Active Play:

The children's individual PA responses to AP were varied for both ACC-PA and VO_2 with many peaks and valleys. Prior to any further analysis of AP, it was important to identify the patterns of PA participation between children wearing and not wearing the Fitmate™ portable oxygen analyzer. Figure 15 illustrates the PA responses of children (n=7) not wearing the portable system to children (n=3) wearing the portable system during a portion of one of the four sessions. It is apparent that the wearing the Fitmate™ portable oxygen analyzer did not dramatically alter the pattern of PA participation during the AP of games. A similar observation was noted for the other three sessions (data not shown). The only exception was in one session where one specific game (fishes and whales) was approximately 20% less ACC-PA output due to

several transitions from standing to lying down to standing. This game (~5min) was played in only one session out of four and was only one out of twenty-four games, and as a result it was included in subsequent analysis.

The VO_2 responses during AP followed similar patterns to those observed for ACC-PA. The children's individual and average responses (n=12) were quite varied over the four sessions (Figure 16). The individual VO_2 values showed a much larger range of differences compared to the mean values. For example, individual VO_2 responses ranged from of 5 $mLO_2 \cdot kg^{-1} \cdot min^{-1}$ to 45 $mLO_2 \cdot kg^{-1} \cdot min^{-1}$ throughout the games, while the highest VO_2 in the averaged data was 27.9 $mLO_2 \cdot kg^{-1} \cdot min^{-1}$. It may be argued that using the mean of physiological variables to characterize AP masks the impact/magnitude of changes noted with individual responses.

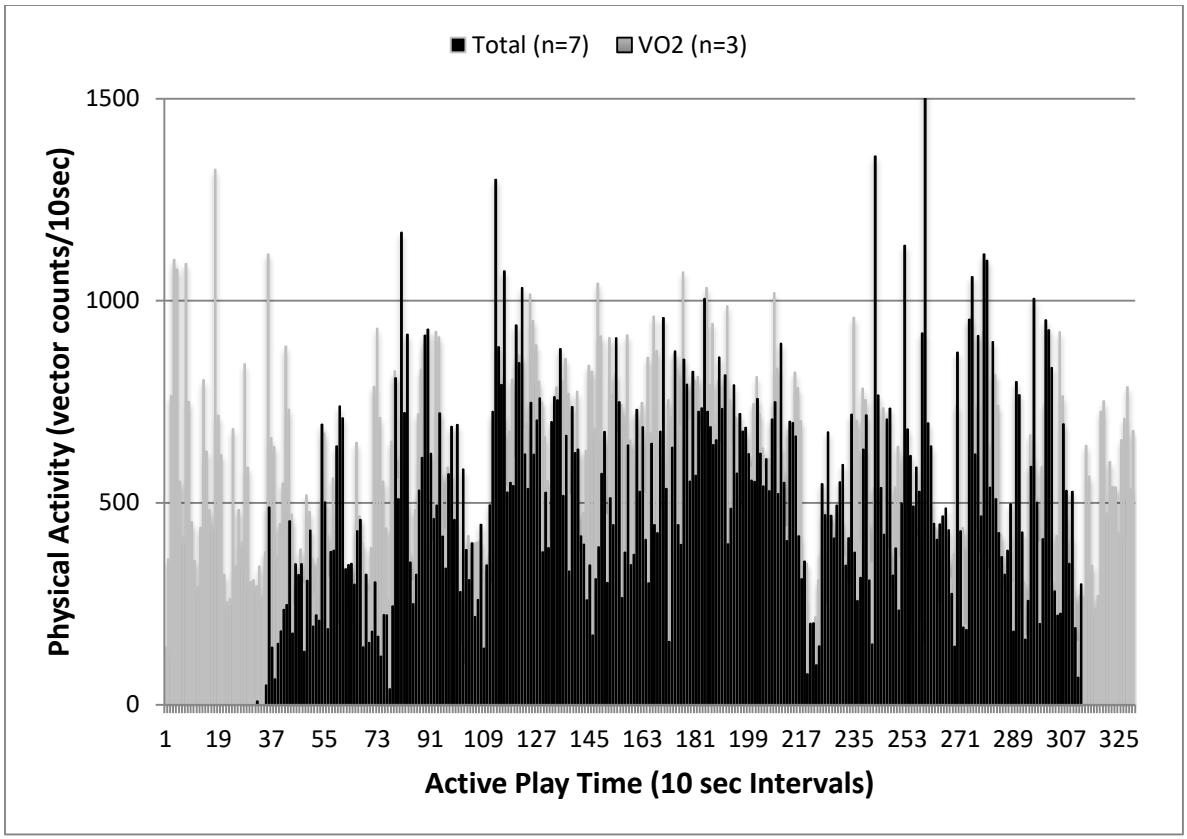

Figure 15. Children's (n=10) Accelerometer-Measured Physical Activity Response to 60 Minutes of the First Active Play Session by All Children, Only Children Wearing Fitmate™ Portable Oxygen Analyzer (n=3) and Only Children Without (n=7).

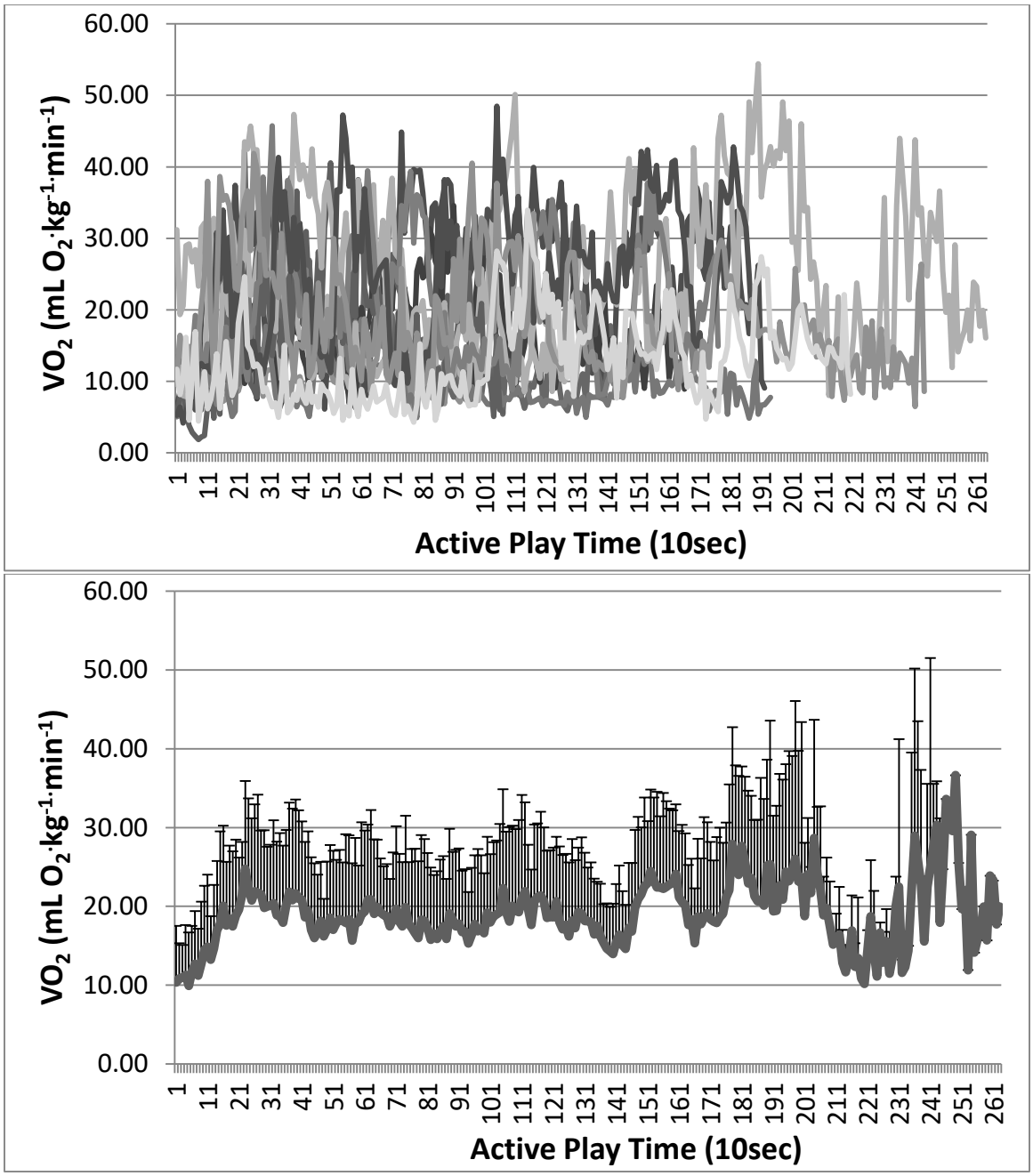

Figure 16. Children's VO_2 Responses for Individuals (n=12) (Top Panel) and Average VO_2 (Mean±SD) (Lower Panel) During Self-Paced Active Play. The Average Time of Play was 29.3±7.9 min With a Range of 17.8 To 44.0 minutes. Children's VO_2 was determined with a Fitmate™ Portable Oxygen Analyzer.

Quantitative Assessment of Children's Oxygen Consumption Responses to Active Play:

To compare the relationship between increasing ACC-PA and VO_2 responses during AP a scatterplot between these two variables over each of the four days are provided in Figure 17. Each panel represents 2-4 children participating in the same games in the order they were delivered. The customary linear relationship identified for ACC-PA vector counts and VO_2 with TM exercise was not observed during AP. When the children's AP data are averaged across the four days the relationship assessed by Pearson Coefficient (r) was -0.02. A similar pattern was noted for ACC-PA vector counts/10sec and MET (data not shown).

A second analysis was performed to assess whether the above relationships between PA and VO_2 and ACC-PA and MET was a function of the order in which the games were played. The ACC-PA outputs were rank ordered from low to high and compared to VO_2 and MET values. When ACC-PA outputs are ordered from low to high, the relationship with either VO_2 and/or MET values remain variable across the ACC-PA outputs (Figure 18). What does become clear is that a linear response for metabolic variables with ACC-PA outputs does not exist with the AP. That is at low ACC-PA outputs (<200 vector counts/10sec) the VO_2 and MET values may be as high as 20 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$ and 4.5 MET, respectively. At higher ACC-PA outputs (>700 counts/10sec) the VO_2 and MET responses are similar. These results suggest that the metabolic cost of PA performed during an AP session vary for a given ACC-PA output, or in other words that the efficiency might be different across a range of ACC-PA outputs with AP. Presumably, low PA and high metabolic cost would be associated with lower (light or resting) PA that occurs between bouts of AP, where the active portion of the AP would have high ACC-PA outputs and high metabolic costs. To assess the magnitude of the variable metabolic costs with AP, ratios for VO_2/ACC-PA outputs and MET/ACC-PA outputs were determined. The

result of this analysis quantifies and confirms that the metabolic costs associated with a specific ACC-PA vector counts and/or over a range of ACC- PA vector counts are variable during AP (Figure 18). This may imply that the movement patterns associated with AP elicits different metabolic efficiencies and/or effectiveness during AP. Interestingly during AP as the ACC-PA output increases the ratio of MET/ACC-PA outputs and VO_2/ACC-PA outputs decreases in a linear function from approximately 350 vector counts/10sec to approaching 1100 vector counts/10sec. In contrast the increase in VO_2 and/or MET are not necessarily associated with an increase in ACC-PA output (Figure 19). Although these responses are intriguing, it is unclear whether the range, sequence and magnitude of the metabolic cost variations (i.e. MET/ACC-PA outputs) during AP are sufficient for increasing short- or long-term physiological benefits. In other words, are the PA intensities and the nature of the intermittent movement patterns that accompany AP within the levels associated with traditional training programs and/or is all AP beneficial?

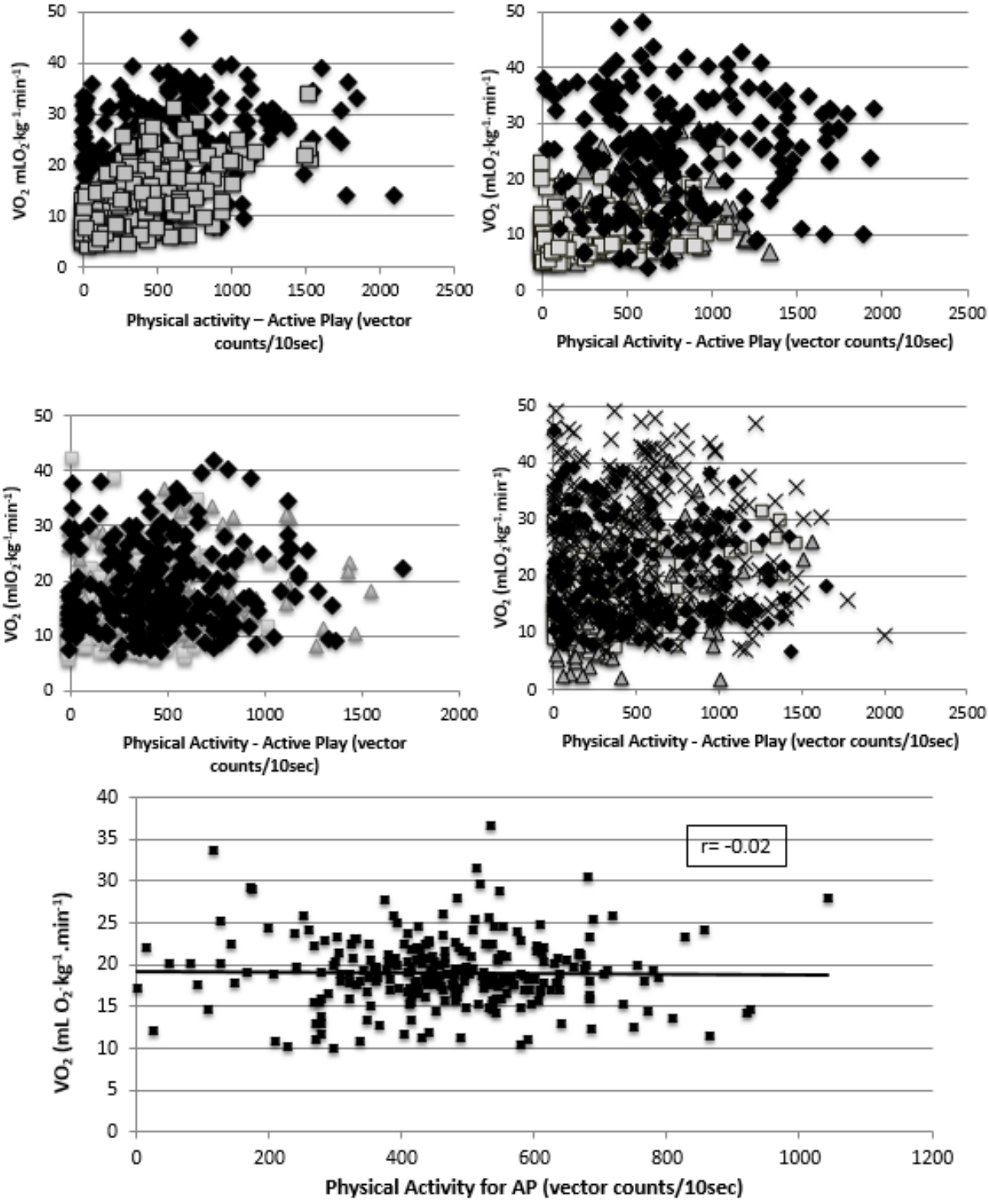

Figure 17. Relationship between Children's Accelerometer-Measured Physical Activity and Oxygen Consumption (VO_2) Individual Responses to Self-Paced Active Play Distributed over Four Days (Top Four Panels). The Relationship (r= -0.02) for the Average

Accelerometer-Measured Physical Activity and VO_2 Responses of Children (n=12) are in the Lower Panel.

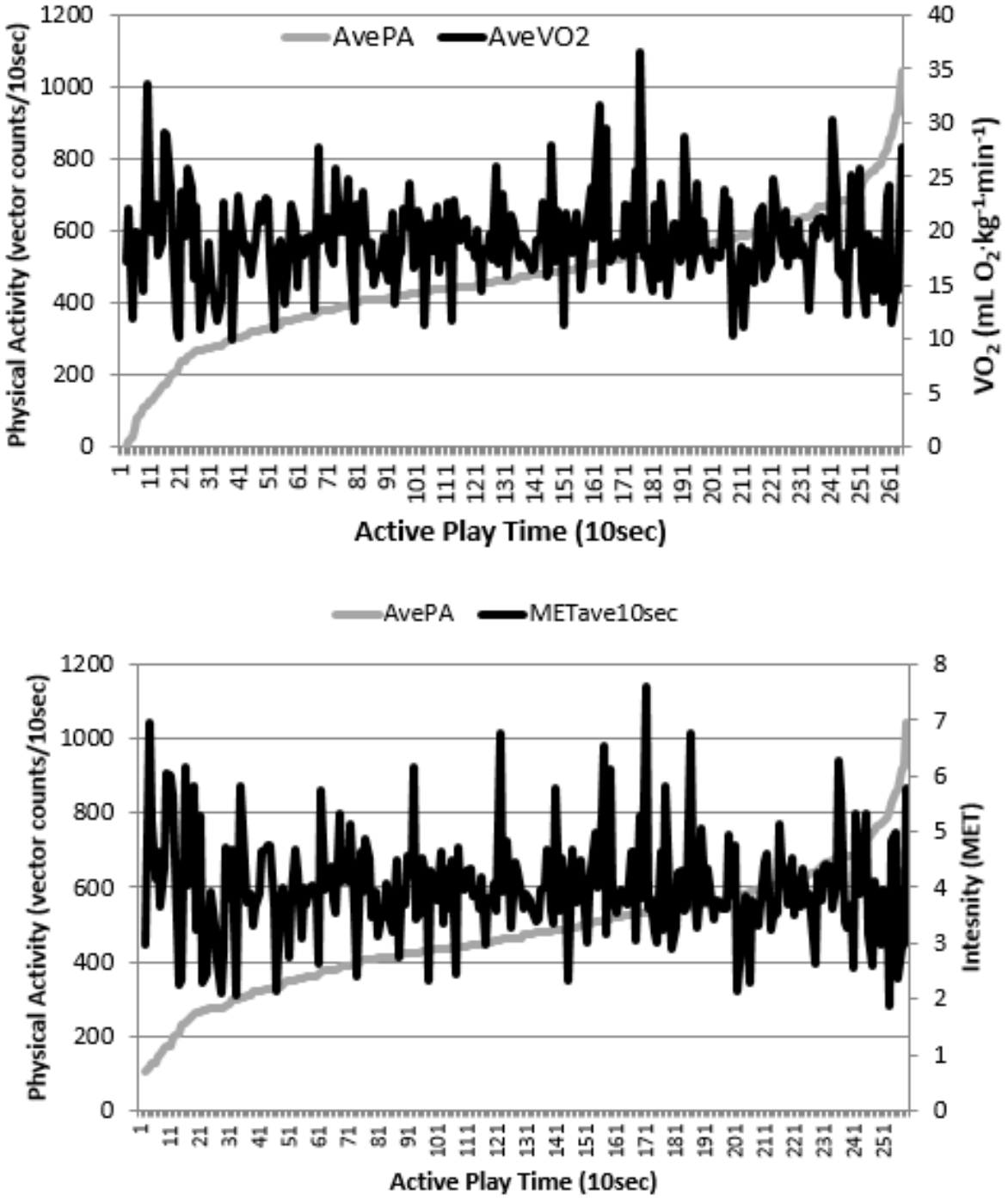

Figure 18. Children's Accelerometer-Measured Physical Activity and Oxygen Consumption Responses to Active Play Distributed by Rank Order of Accelerometer-Measured Physical Activity Output.

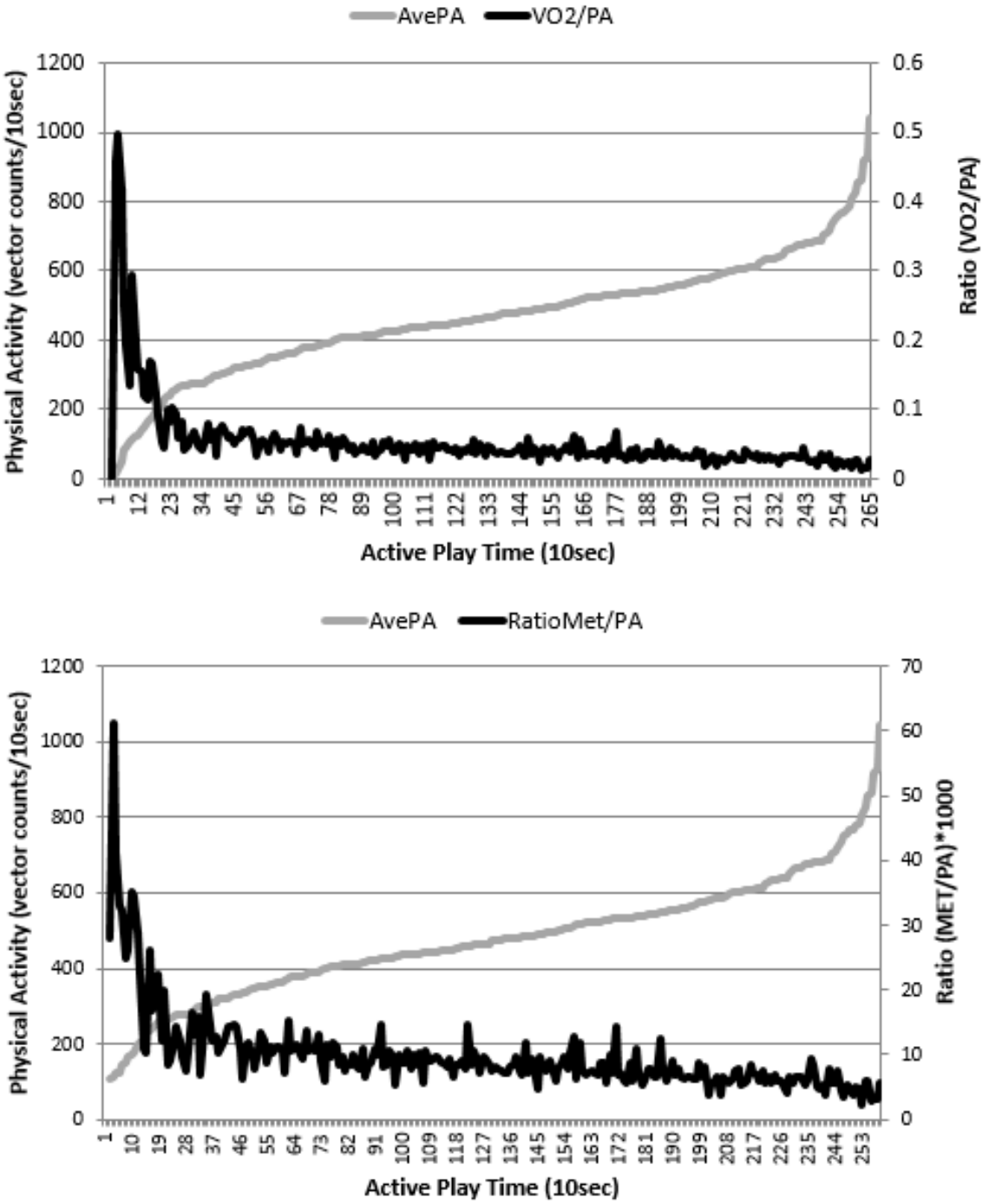

Figure 19. The Ratio of Children's Oxygen Consumption to Accelerometer-Measured Physical Activity Responses (Top Panel) and Metabolic Equivalents (MET) to Accelerometer-Measured Physical Activity Level (Lower Panel) During Active Play Distributed by Rank Order of Accelerometer-Measured Physical Activity Output.

To shed further light on the potential of physiological improvements to occur during an AP program, an analysis of the proportion of time spent at moderate-vigorous (%MVPA) intensity and the range of Int movement patterns (ratio of light-to-moderate-vigorous intensity PA) was undertaken. To address the issue of intensity, two approaches were used: a) to determine the proportion of MET classified as rest/light (L), moderate (M) and vigorous (V) intensities; and b) to determine the total VO_2 for the ACC-PA level and then distribute the proportional AUC found for each of light, moderate and vigorous intensity ACC-PA levels. To determine and classify the MET values, the VO_2 values for all children and all sessions were used to generate individual MET values for every 10sec interval (1 MET = 4.82 mL $O_2 \cdot kg^{-1} \cdot min^{-1}$). Next the MET values were classified into rest/light (1.00-3.99MET), moderate (4.00-5.99MET) and vigorous (>6.00MET) intensities and expressed as a percentage of the total PA level (i.e. %LPA, %MPA and %VPA). The proportions of MET determined across the four sessions were 37% LPA, 33% MPA and 30% VPA (63%MVPA) (Figure 20 – Top). In regards to the determining the proportion of total VO_2 from the AUC associated with each intensity; first the average total VO_2 for the AP session (43.3 min) was determined (i.e, 699.5±300.4 $mLO_2 \cdot kg^{-1} \cdot 3min^{-1}$) (Figure 20 - Lower). Next, the proportion of VO_2 responses during the 43 minutes of AP were partitioned into MET and the corresponding VO_2 summed to provide a proportion of AUC at L, M and V intensity PA (Figure 20 – Lower). In general, the proportion of AUC classified as moderate-vigorous intensity PA was determined to be 63%, which is likely sufficient to promote physiological benefits for children.

Although the magnitude of the intensity requirement during AP seems sufficient to promote positive adaptations, it was important to identify the Int nature (sequence) of AP. Therefore, the intervals/ratios of light and moderate-vigorous intensity accompanying AP were

determined. This was accomplished by identifying the sequence(s) of intensity at rest/light PA (% LPA), moderate (%MPA) and vigorous (%VPA) using the proportion of total VO_2–AUC as depicted in Figure 21. Clearly, there are several intervals of rest/light versus moderate and/or vigorous intensity activities with reported peaks of 63% and 73%, for MPA and VPA respectively. When viewing the pattern of vigorous intensity PA over the session it is clear that VPA does occur throughout and is not confined to the earlier phases of the program (as might be expected if fatigue/tiredness was present). Moreover, the vigorous intensity PA was achieved over several bouts showing that vigorous intensity PA is evident in AP from the start to finish of the AP (Figure 22). In short the children's %VPA contributing to the AP is variable and the sequence or Int nature of VPA within the AP is distributed over the entire AP format.

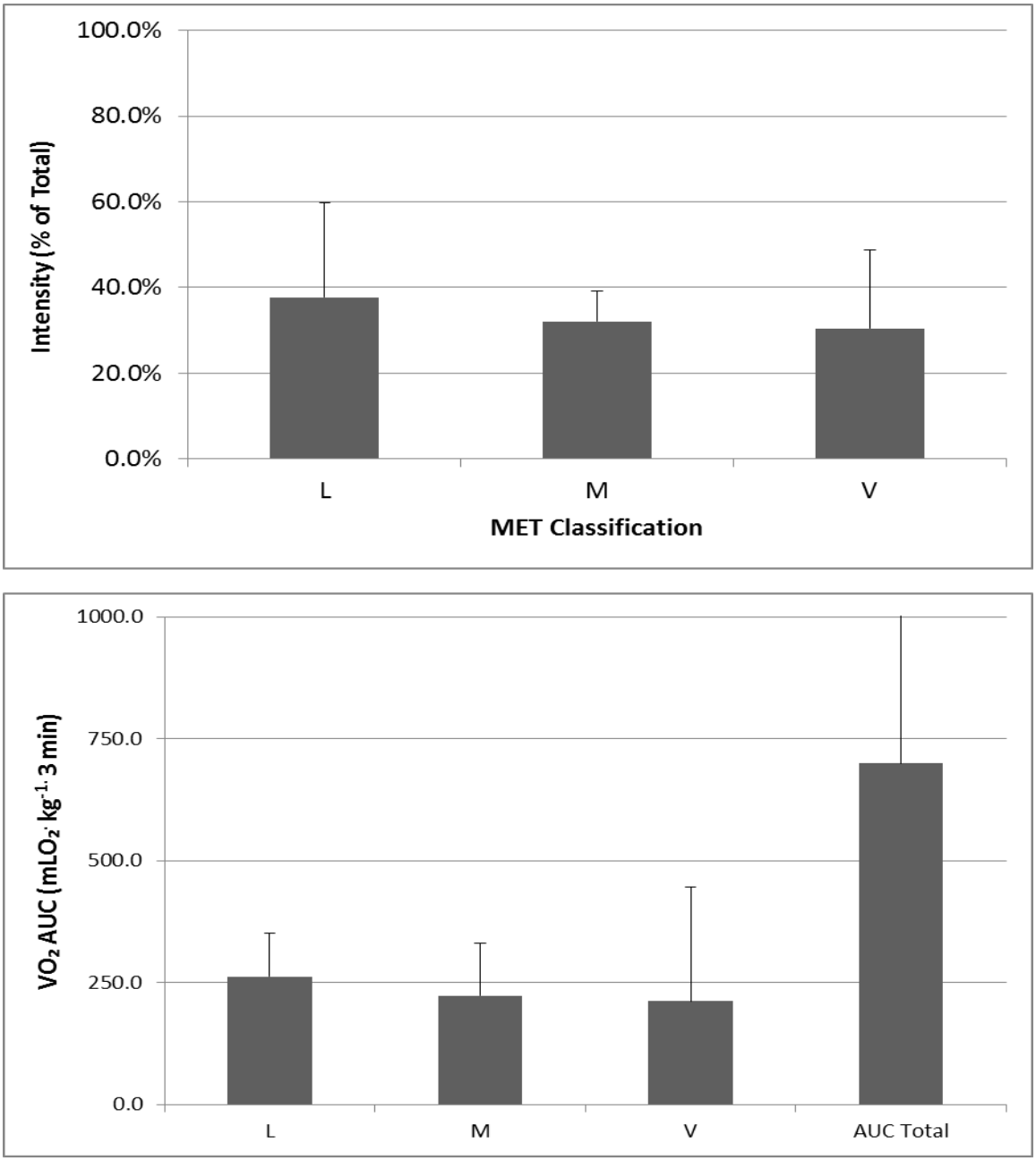

Figure 20. Intensity of Self-Paced Physical Activity During Active Play for Children (N=12) Using the Proportion of MET (Top) and Percent of Total VO_2 (AUC) (Lower). TOP: MET were Classified as Rest/Light (L) (1.00-3.99MET), Moderate (M) (4.00-5.99MET) and Vigorous (V) (>6.00MET). Lower: The Proportion of AUC Assigned to MET Values Were Summed and Distributed Among the L. M and L Categories. Values are Expressed as Mean±SD.

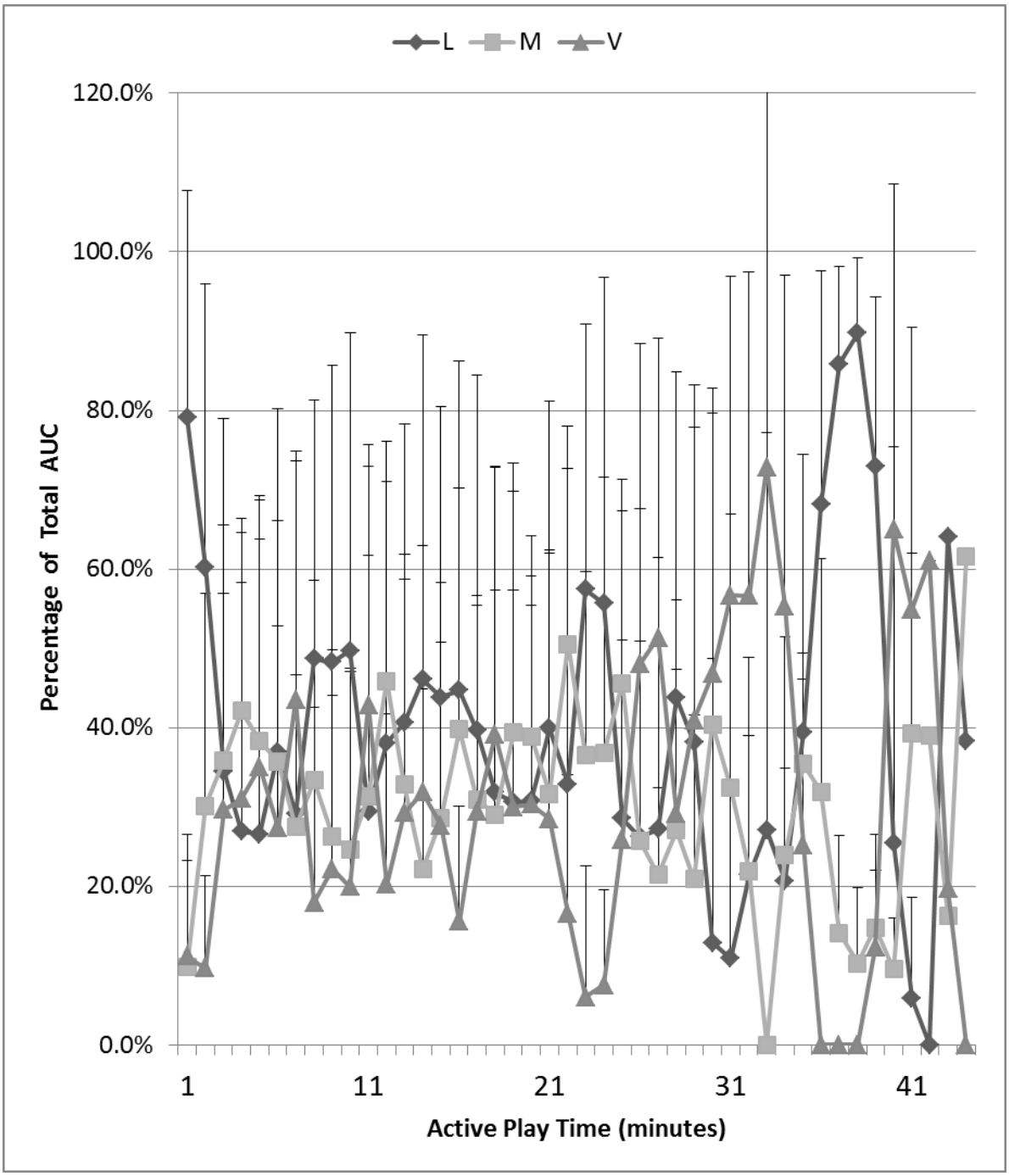

Figure 21. Children's (n=12) Percentage of Total Area Under the Curve (AUC) previously Categorized as Low, Moderate, and Vigorous Intensity Category to 43 minutes of Self-Paced Active Play. Values are Expressed as Mean±SD.

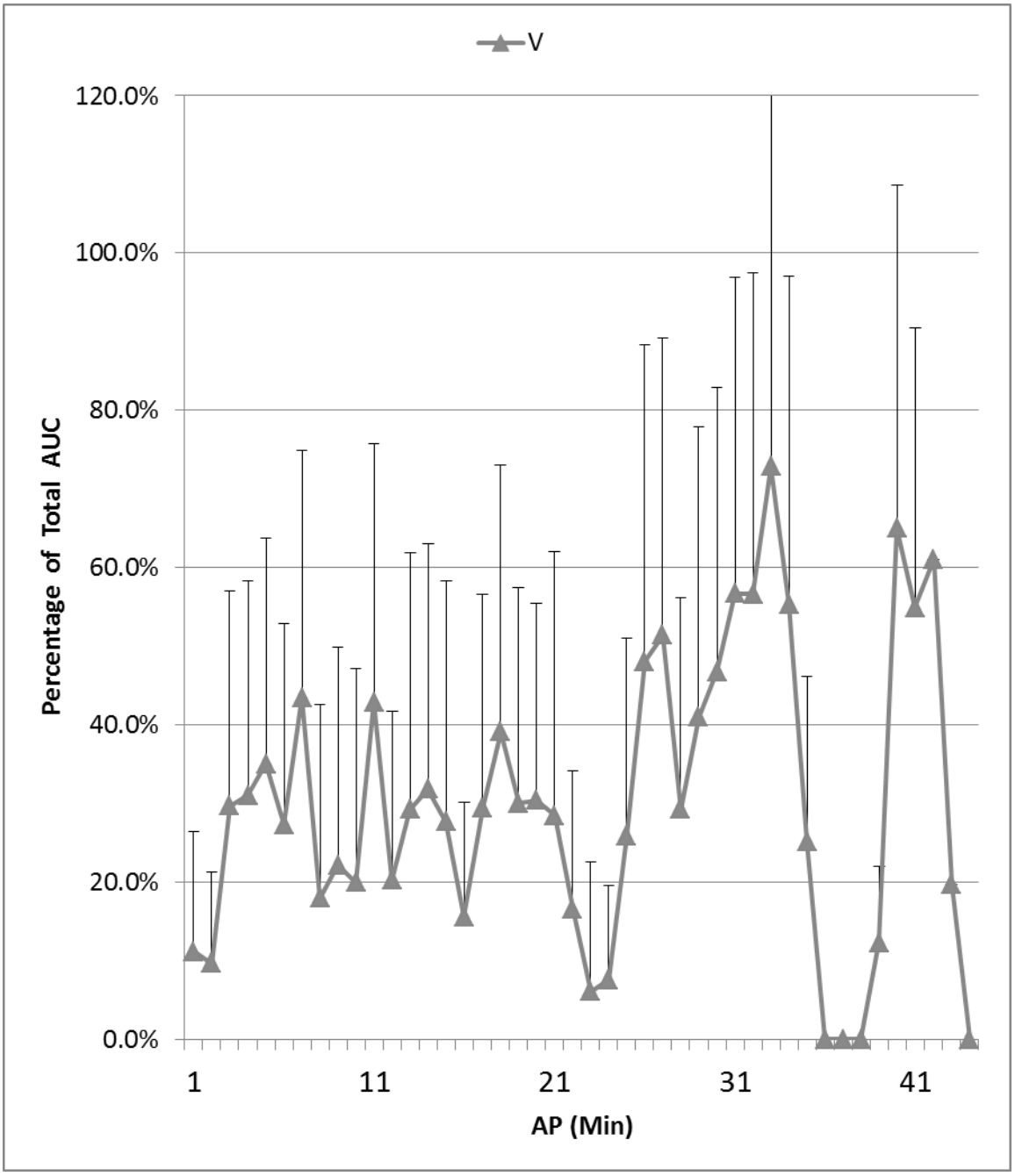

Figure 22. Children's (n=12) Percentage of Total Area Under the Curve (AUC) previously Categorized as Vigorous Intensity Category to 43 minutes of Self-Paced Active Play. Values are Expressed as Mean±SD.

To further assess the Int nature of AP the periods of moderate-vigorous intensity PA and light-rest intensity PA were evaluated by quantifying the ratio of MVPA to LPA (including resting) for each child Figure 23 -Top. The results demonstrate that when children are engaged in PA there is a range in work:rest intervals, with the MVPA:LPA interval ranging from 0.2:1 to 6:1 work:rest intervals with an average of 2:1 work:rest intervals. Figure 23 - Lower illustrates the ratio of VPA to LPA PA with an average of 1.4:1 work:rest intervals. The intermittent nature of AP associated with moderate-to-vigorous intensity PA appears to replicate some of the training principles reported in traditional training studies.

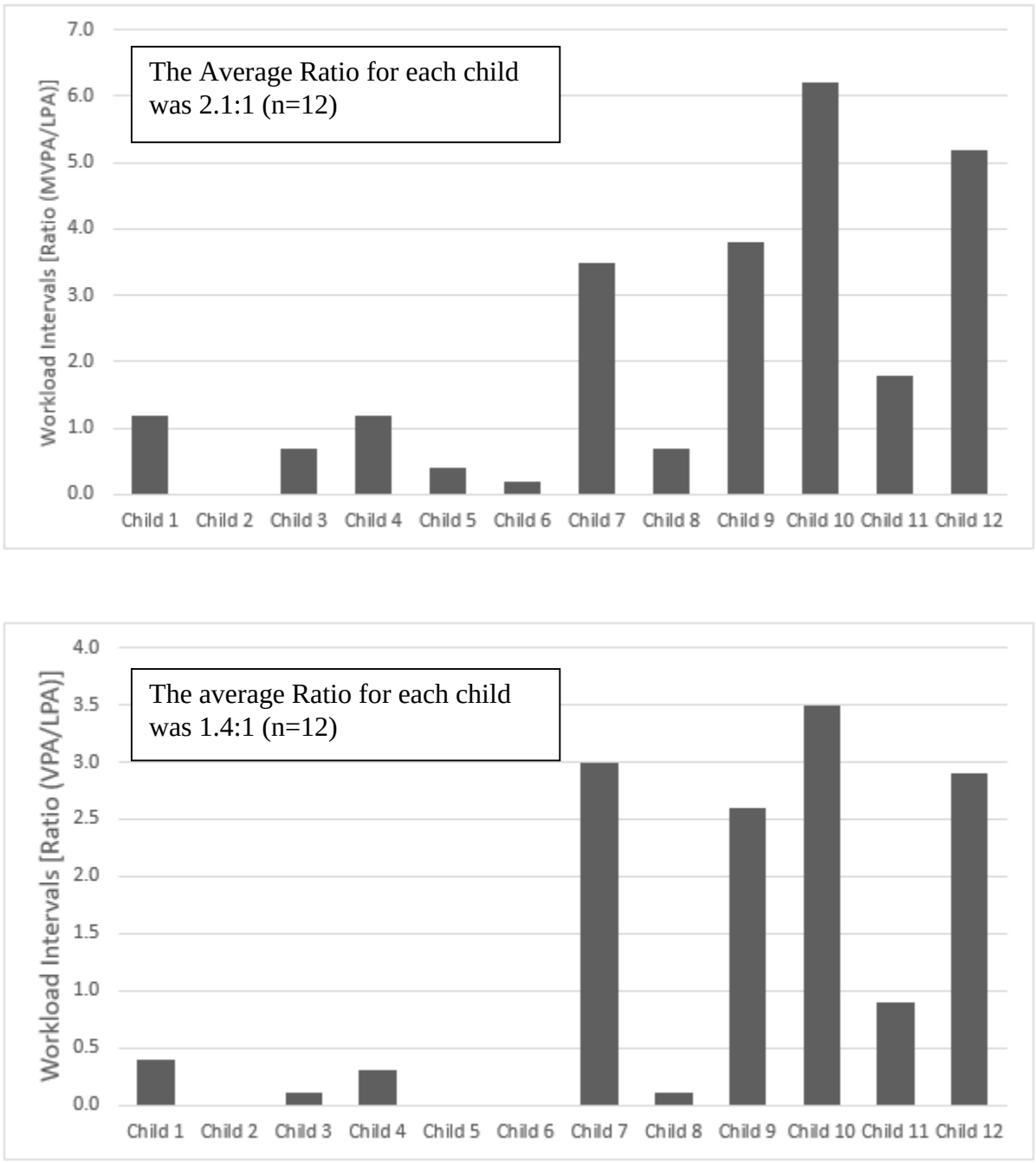

Figure 23. Children's (n=12) Physical Activity Ratios for Moderate-Vigorous (MVPA):Light-Rest (LPA) Intensity (Top) and Vigorous (VPA): LPA Intensity (Lower) over 17 to 43 minutes of Self-Paced Active Play.

Chapter 4 - Discussion:

Rand exercise is comprised of non-sequential variable intensity bouts of PA, which is the foundation of many activities of daily living, occupational tasks and AP. The significance of the high intensity and/or sprint Int exercise in promoting physiological adaptations has gained considerable attention (Trilk et al., 2007; Sperlich et al, 2010). As a result, the physiological responses to intermittent exercise have been well documented in controlled and/or laboratory settings; however the physiological characteristics of PA associated with children's AP is not well understood. The purposes of the present study were to examine children (n=8) and young adults (n=5) qualitative and quantitative responses to different TM running protocols (Cont, Int and Rand) and children (n=12) during AP. A novel finding in this study is that during AP the PA does not demonstrate a classic linear relationship between ACC-PA vector count outputs and VO_2 and/or MET responses. Rather PA is associated with variable metabolic costs and movement efficiencies during AP when performed at the same ACC-PA (vector counts/10sec). As well, it was observed that the proportions of light, moderate and vigorous intensities for PA with AP across a range of MET values (2-8 MET) are similar to those identified for TM exercise. Another important finding is that the combination of PA intensities (MVPA and VPA) and the ratio of high intensity-to-rest intensity intervals in AP approaches those observed for more traditional training studies; albeit there is a larger inter-child variability during AP. Interestingly for AP the larger variability observed across individual measures by child is masked when the data for ACC-PA outputs, VO_2 and MET are averaged over time. Finally, the children's physiological responses to structured Int and Rand TM exercise, showed no statistical differences for the oxygen deficit and the percent aerobic/anaerobic energy sources. Overall, the results of this study expand our understanding of how AP may serve as a viable alternative to

prescribed or paced exercise programs for promoting physiological benefits. Future work should target specific outcomes that occur following AP programs.

The strong linear regression between exercise and VO_2 forms the bases for many prediction equations used to estimate energy expenditures (EE) and/or fitness levels. Many of these are associated with a high degree of predictability and accuracy when used to assess EE in a controlled laboratory environment for TM, cycle and other work related ergometers (Crouter et al., 2006). When laboratory TM generated equations are used to predict activities of daily living and or children's AP, the standard error of measurement has been reported to be as high as 40% (Easton et al., 1998). Despite many attempts to improve estimates of EE by using multiple sensors and/or multiple regression for non-laboratory settings significant improvements have not be forthcoming (Tikkanen et al., 2014). Recently, it has been hypothesized that the nature of the ACC-PA outputs (vector counts determination) and/or the characteristics of the PA associated with AP may account for the large standard error of the mean (Moghaddaszadeh et al., 2017). In this study the poor relationship between ACC-PA vector counts and VO_2 or MET ($r= -0.02$) during AP partially supports this hypothesis. Although the precise nature of the relationship remains unknown, the poor relationship might be linked to a changing efficiency of movement (the ratio of VO_2/ACC-PA vector counts or MET/ACC-PA) that occurs with increased PA levels during games-based AP. As the level of ACC-PA increases the metabolic cost decreases with AP. However caution must be taken when expressing PA levels with ACC-PA, as recent research has reported that factors such as body mass and height may impact intensity of ACC-PA vector counts, and therefore a size-dependency may exist. The impact of changing metabolic efficiency during AP, ranging from an efficiency ratio of approximately 10 to 3 (MET/ACC-PA*1000) over vector outputs from 400 to 1100 counts/10sec, requires further investigation. In

summary, AP represents a novel pattern of self-paced PA that is unique and presents a variable metabolic drive for physiological adjustments and/or benefits.

As the PA with AP is self-paced in nature, there are large inter-child variations in VO_2 and ACC-PA outputs . It is clear from the results of this study that when preparing averaged/group data for both ACC-PA outputs and VO_2 responses the group data shows much smaller range of PA and/or VO_2 values. The range of individual children VO_2 responses goes from 5-to -45 $mLO_2\ kg^{-1}\ min^{-1}$ or approximately 1 to 9 MET, which may be sufficient intensity to evoke physiological benefits (Malina et al., 2004). In contrast, the average data shows a maximal value of 17 $mLO_2\ kg^{-1}\ min^{-1}$ or 3.5MET. Thus, averaging individual AP data is not suitable to effectively describe the metabolic stimulus for physical benefits; and an individual inter-child analysis must be considered.

The use of MVPA/LPA or VPA/LPA ratios with AP may be considered a 'real-world' replacement of work:rest ratios used in more controlled prescribed exercise programs. These results allow for a meaningful assessment of AP and associated physiological responses. The use of these ratio variables has two advantages, first the individual classification and separation of MET data by child is feasible. Secondly, forming the MET ratios can be modified to include either moderate-vigorous intensity or just vigorous intensity PA, which has important implications for meeting PA guidelines and/or training targets (Janssen et al., 2002). This latter point is particularly salient in demonstrating that an AP program has the exercise stimulus necessary to induce adaptations by comparing MVPA/LPA ratios with work:rest ratios used in structured programs. The results of this study shows average ratios of 2.1:1 MVPA/LPA and 1.4:1 VPA/LPA, which are comparable to previously reported intervals ranging from 1:1 to 1:3 (Baquet et al., 2010; De Araujo et al., 2012; McManus et al., 1997; 2005). When individual

inter-child ratios are considered it is clear that some children perform more bouts of higher intensity PA (i.e. 6:1MVPA/LPA) versus some children with lower ratios (0.25:1 MVPA:LPA). In summary, the use of MET ratios seem to represent a viable method of assessing the intensity of AP in children.

In general, the pattern of children's VO_2 and metabolic responses to AP do not follow the linear relationships seen with increasing workloads on laboratory TM exercise. The extent to which the self determined intermittent time intervals and/or self determined random intensity of AP contributes to the poor relationship between workload and metabolic responses is unclear. To help address this question and understand the mechanism(s) contributing to the variable VO_2 and metabolic responses children's responses to Int and Rand TM protocols were assessed. The TM exercise results support the suggestion that despite identical the TM speeds/workloads, the order in which workload is presentation influences the physiological responses. At the higher workloads, 8 and 10 km/hr, the oxygen deficit was significantly lower in Rand than Int TM protocols, which had an impact on the recovery oxygen consumption (EPOC). As well as a lower O_2 deficit, the Rand TM protocol had a faster slope of VO_2 recovery following TM speeds of 4, 6, and 8 km/hr. This relationship has been reported in the literature for adults (Gaesser & Brooks, 1984), which seems to remain consistent with children. In short, the lower O_2 deficit at the onset of TM exercise, the faster the decrease in VO_2 during recovery at comparable workloads delivered in a Rand manner indicating a greater O_2 availability (aerobic metabolism) at the onset promoting therefore a faster return to baseline and a quicker recovery. These results of this study not only support the hypothesis that children's' greater aerobic contribution to subsequent bouts of exercise and recovery are quicker (Falk and Dotan, 2006), but extends the concept that children's greater aerobic contribution with Rand PA will influence the metabolic efficiency of

movement during AP. The mechanism(s) underlying the improved metabolic efficiency associated with greater activation of aerobic metabolism from preceding bouts of exercise as with children's AP is not available in the literature. Previous reports for adults have shown that intermittent workloads prime the aerobic system for the next bout by building-up pyruvate dehydrogenase (PDH) Intermediates and electron transport chain (ETC) metabolites (Peters, 2003; Gurd et al., 2006). It is possible that the PDH activation may have influenced the metabolic efficiency with increasing ACC-PA outputs in this study, particularly at the higher intensities (i.e., VPA) and VPA/LPA ratios.

Limitations:

There are several limitations to the present study that must be considered. One important limitation is the use of accelerometers to quantify PA levels, as the devices have poor discrimination above 1500 vector counts/10 seconds and the proportion of each axis (horizontal, vertical, perpendicular) influences the vector calculations. Additionally, ACC-PA is not size-independent and therefore the anthropometric parameters of the children may have impacted their ACC-PA vector counts and subsequent metabolic efficiency calculations. Another limitation is the use of the term EPOC to describe the recovery portion of exercise in the present study, which deviates from previous definitions described in the literature. Typically EPOC involves the excess VO_2 (above baseline/resting levels) that occurs when steady state exercise has ceased, however in the AP protocols the recovery was not always passive and did include periods of active recovery, limiting generalizability with other studies. Therefore, a better term to describe the recovery oxygen consumption in this study might be EPOC-like. Furthermore, TM work: rest intervals were limited to 3:5min for all TM protocols. It is probable that the interval was too long, and much of the oxygen debt was recovered, and thereby the aerobic system

activation attenuated. Perhaps a rest interval of 2-3 minutes between workloads in future studies will expose differences needed to reach significance between the Int and Rand groups. Another limitation exists in the impact of the equipment on the PA behavior of children. It was previously demonstrated that the PA behavior of children not wearing the Fitmate™ closely matched those that did. However, this was not always the case, a game involving many instances of sitting and standing such as "Fishes and Whales" did produce a variation. It is suggested that this is a very minor limitation, as the vast majority of games did not involve many cycles of sitting and standing. Rather this point is a consideration for future self-paced physical activity studies employing the Fitmate™ system.

Finally an adult group (n=5) completed the same TM protocols as the children in a separate part of the system to find any differences. Although more statistical power is required, there appears to be a trend of little difference between the young adult and child groups in terms of response to the three TM protocols. It is hypothesized that a lack of difference may occur in AP analysis, however a direct comparison of children vs young adults was not the focus of this study and is recommended for future investigations.

In conclusion, the variable intensities and the MVPA:LPA ratios identified for AP have the appropriate characteristics necessary to elicit physiological benefits. Moreover, the poor relationship noted for ACC-PA outputs (workload) versus VO_2 and/or MET was accommodated for by an improved metabolic efficiency with increasing ACC-PA vector counts. The improvements in metabolic efficiency could be related to the intermittent manner and random intensity associated with AP, since the TM exercise showed a lower O_2 deficit and faster rate of recovery with increasing intensities when delivered in a Rand protocol. These mimic the patterns of PA associated with AP. The results of this study expand our understanding of how

AP may serve as a viable alternative to prescribed or paced exercise programs for promoting physiological benefits. Future work should target cardiorespiratory improvements that may occur following AP programs for children.

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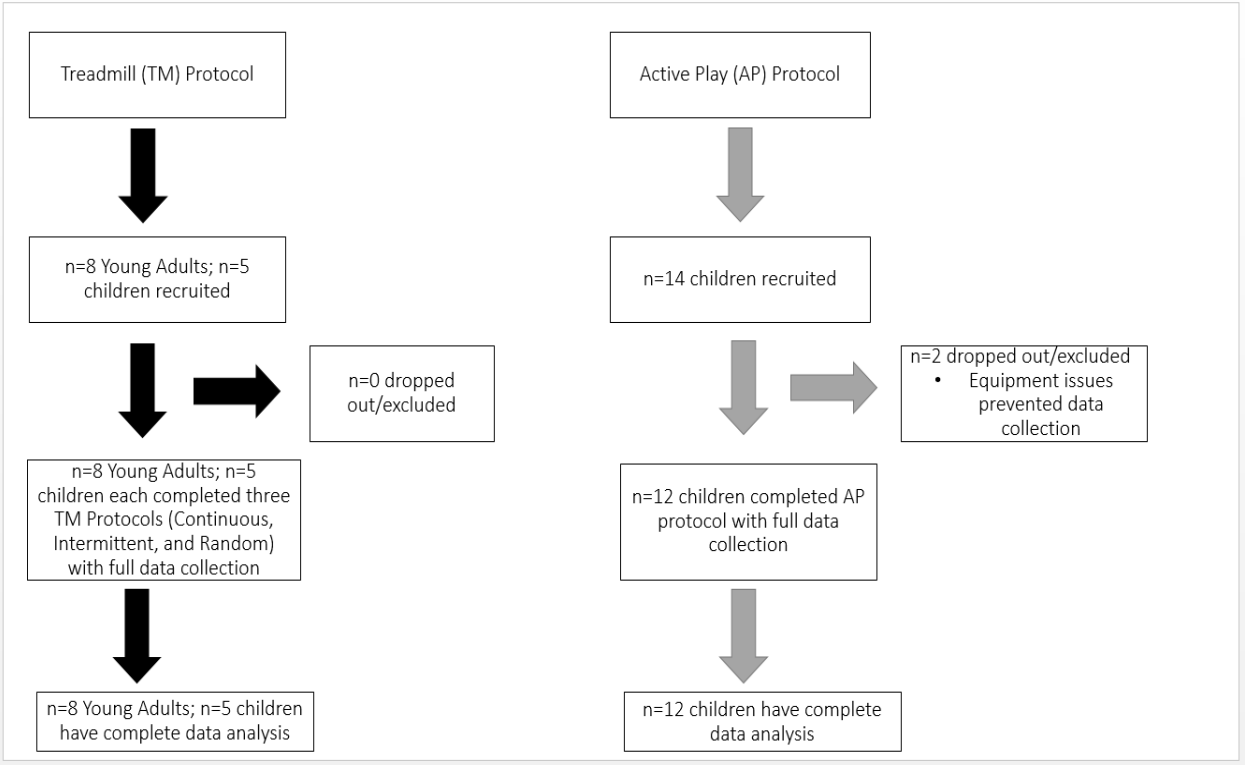
Appendix A: Consort Diagram for Study Protocols:



Appendix B: Oxygen Consumption Kinetics

Section I: Characterizing Oxygen Consumption During Exercise:

At the very general level, the oxygen consumption response to an exercise stimulus follows a generic pattern illustrated in Figure S1. From resting (baseline) levels oxygen consumption will rise exponentially in response to the presentation of an exercise stimulus. The VO_2 will then reach a plateau termed "steady-state" in oxygen consumption kinetics. Once the exercise stimulus is removed recovery begins with an immediate and exponential decline back to baseline levels of oxygen consumption. This recovery portion of oxygen consumption has been termed the excess post-exercise oxygen consumption (EPOC). The portion of the oxygen consumption response when an exercise stimulus is present refers to the ON kinetics and the period without physical activity refers to OFF kinetics (EPOC). Following a brief Introduction of the representative components of the VO_2 responses to exercise, a more detailed explanation/understanding of the physiological mechanisms is covered.

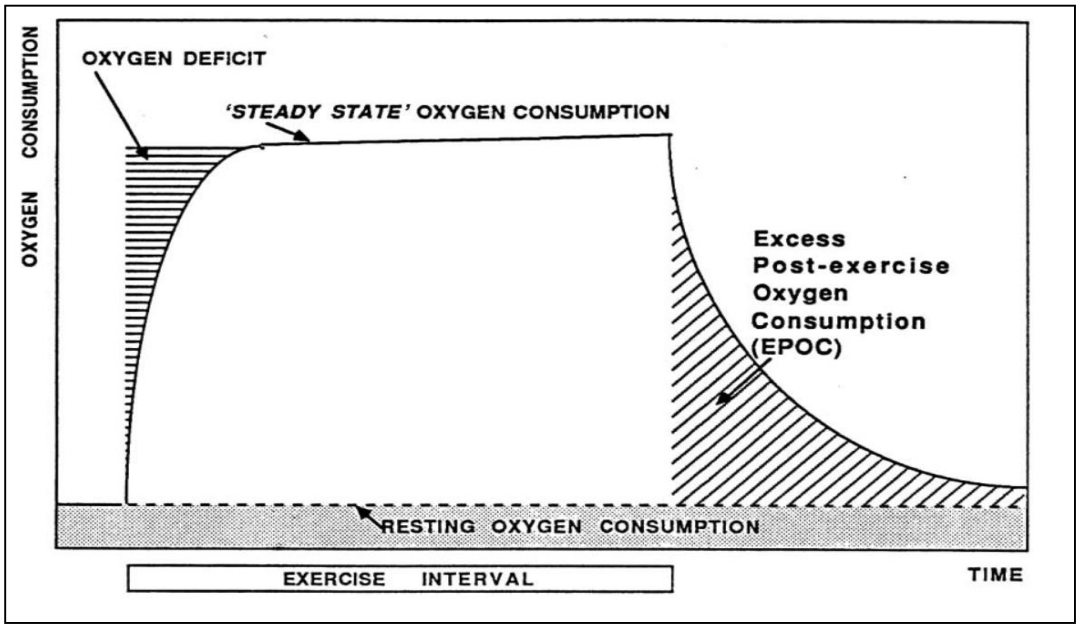


Figure S1: General Oxygen Consumption (VO_2) Response to Continuous Exercise (LaForgia et al., 2006)

Oxygen Consumption Kinetics:

Three distinct phases explain the startup period for VO_2, these include; phases I, II, and III. Phase I, known as the cardio-dynamic phase, is primarily driven by an instantaneous increase in cardiac output (Q) resulting in a rapid increase in pulmonary blood flow (Baum et al.,1992; Burnley et al., 2000) (not depicted in Figure S1). The quick rise in Q has been attributed to the rapid withdraw of vagal nervous input and resetting of the arterial baroreflex by the motor cortex and mechanical pumping of the contracting muscles (Hughson et al, 2001) . There is debate over whether or not this increase occurs in the presence of altered venous oxygen content, with some studies suggesting that there is not enough time in this transient phase (15-25 seconds) for changes in muscle venous O_2 to reach the lungs and impact phase I (Xu and Rhodes, 1999). This suggests that the kinetics of pulmonary VO_2, due to rapid changes in Q, are quicker than VO_2 of the exercising muscle. Therefore, the oxygen consumption measured at the mouth does not correspond to VO_2 kinetics at the muscle for this phase (Fawkner and Armstrong, 2003). As a result phase I will not be discussed in any great detail in the remainder of this review.

Phase II, reflects a change in the VO_2 response to exercise primarily due to changes/flux of muscle metabolites/metabolism. In this phase, the oxygen delivered to the muscle cell is changing rapidly to support the flux of metabolites entering the mitochondria for ATP generation and subsequent force generation to meet the exercise demand. In phase II oxygen consumption increases exponentially until a steady-state is achieved (Fawkner and Armstrong, 2003. Previous research has observed a linear relationship between VO_2 response and the constant-work-rate of the exercise. In other words, oxygen consumption increases according to intensity of the exercise (when it is below lactate threshold) (Xu and Rhodes, 1999). Therefore, time spent in phase II is primarily dependent on the nature of the exercise stimulus.

Phase III is characterized by a steady state VO_2 and is present at roughly 2-3 minutes into exercise. The uptake of oxygen matches the changes/flux of muscle metabolites/metabolism, which contributes to the VO_2 plateau. The magnitude of the VO_2 increase in phase III, has been demonstrated to have a linear relationship with work rate, and an increase of 9-11 mL O_2/watt/min in adults (Xu and Rhodes, 1999; Burnley et al., 2000) (Figure S2).As a result the VO_2 responses are classified as a function of exercise intensity: moderate (below AT); heavy (between AT and Critical Power); severe (above Critical Power); and supramaximal (above VO_2max.).

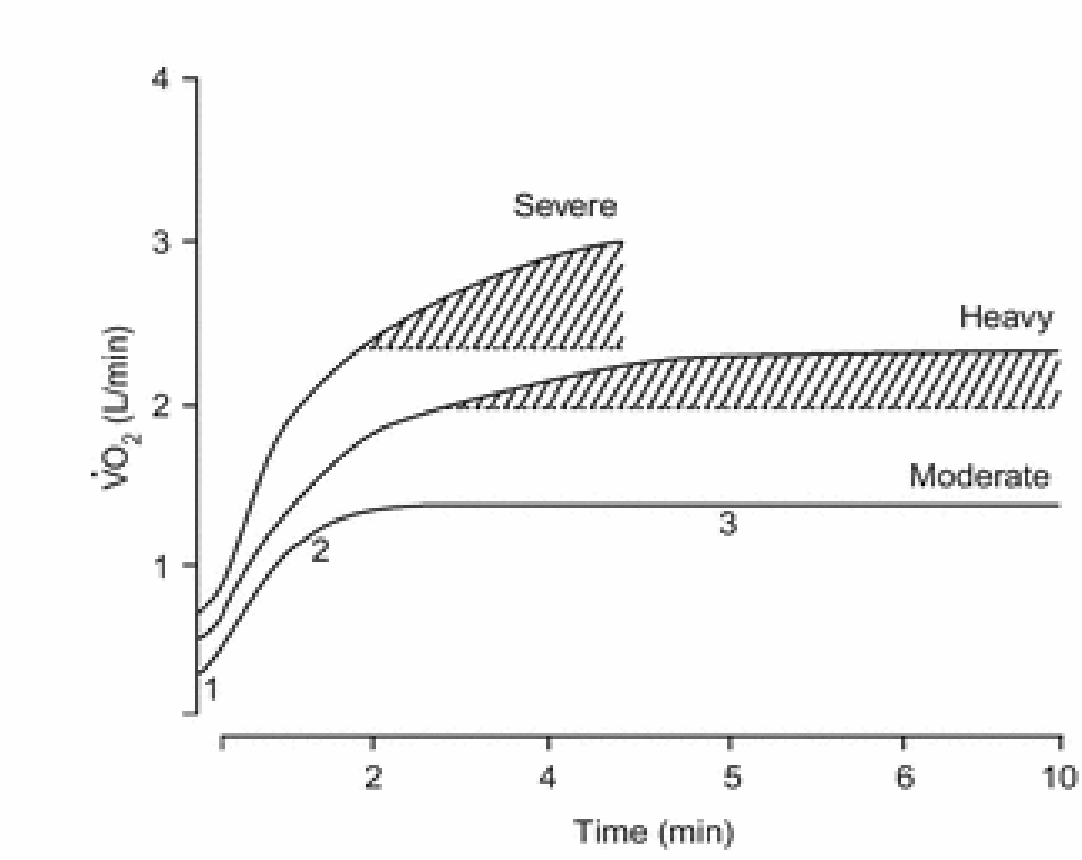


Figure S2: Initial Response of VO_2 to Exercise by Intensity (Xu and Rhodes, 1999).

Moderate Intensity:

The primary feature of the moderate-intensity classification relevant to this discussion is the lack of significant lactate production, with the intensity of exercise below the Lactate Threshold (LT) or Anaerobic Threshold (AT) (Xu and Rhodes, 1999). In addition, the metabolic equivalents (MET) associated with this classification ranges from 3.0 - 5.99 MET.

Heavy Intensity:

The primary feature of the heavy-intensity classification relevant to this discussion is the sustained increase in lactic acid that escalates above its rate of clearance, with the intensity above the LT or AT (Xu and Rhodes, 1999). Although blood lactate will continue to rise, as long as the exercise intensity is below the maximum lactate steady state (MLSS) the levels will plateau (Poole and Jones, 2012). In addition, the metabolic equivalents (MET) associated with this classification ranges from 6.0 - 7.99 MET.

As a result, the presence of a "slow component" appears with heavy exercise between phase II and III leading to VO_2steady state. There have been a number of reports, which attempt to identify/quantify this slow component; however, several methodological limitations exist (Casaburi et al., 1989; Xu and Rhodes, 1999), which prevents it usefulness in describing contributions to VO_2 responses for non-continuous steady state exercise, as with self-paced physical activity.

Severe Intensity:

Severe intensity exercise is categorized as exercise that elicits a maximum VO_2 response. The key feature associated with severe exercise is the lack of attainment of steady state, where VO_2 continues to rise throughout the exercise until the VO_2 plateaus despite further increases in exercise demand and/or the participant can no longer continue due to fatigue. There is a maximal

increase in lactic acid that is significantly above the clearance rate for lactate. In addition, the metabolic equivalents (MET) associated with this classification is >10.0 MET for adults.

Regulation of the Oxygen Consumption:

In discussing the regulation of oxygen consumption during physical activity, one needs to consider the Fick principle:

VO_2= (Heart Rate x Stroke Volume) X (A-VO_2 difference) of muscle cell

The two main factors making up this equation are cardiac output (HR x SV) and oxygen extraction (A-VO_2). As mentioned previously, the introduction of an exercise load introduces a strain on the physiological systems of the body, specifically this burden translates Into an ATP demand. The need for more ATP, the fuel that powers muscle contraction, in turn triggers an increase in oxygen consumption. This rise in VO_2 to meet this demand is achieved by changes in the parameters making up cardiac output (Q) and oxygen extraction. When an exercise stimulus is introduced, heart rate and stroke volume will increase in proportion to the intensity of the exercise in order to meet the rise in ATP demand by the working muscle (at phase I and II). These two parameters result in an increased Q, which translates into a higher blood flow. In addition, a higher oxygen extraction is triggered, and the myocyte is able to abstract more oxygen per volume of blood. Thus, the increased Q and oxygen extraction facilitate increased oxygen delivery to the cell, where the O_2 can be consumed to produce ATP via oxidative phosphorylation. The aforementioned parameters will increase, along with VO_2 according to the Fick equation, until a steady state is achieved (at phase III). Steady state represents when oxygen consumption has increased to the level where enough ATP is produced to balance the demand from the exercise load. Thus the parameters, HR, SV, oxygen extraction, will attenuate to result in oxygen consumption leveling-off at this stage.

It is important to distinguish that the parameters listed in the Fick equation represent just the oxygen delivery side of oxygen consumption. Once the oxygen is delivered to the mitochondria, it still must be utilized in oxidative phosphorylation to produce ATP. This process is heavily regulated through several metabolites including ADP (increased at the on-set of exercise) and multiple enzymes (Hughson et al). Without utilization of the oxygen that was delivered to the working myocyte the ATP demand cannot be met, subsequently driving oxygen consumption higher. Similarly, without adequate oxygen delivery the cell cannot produce ATP as oxygen serves as the final electron acceptor in the electron transport chain of the oxidative phosphorylation process. Thus, oxygen consumption kinetics are driven by the balance between oxygen delivery and oxygen utilization and cooperative their role in providing adequate ATP to sustain exercise (Fawkner and Armstrong, 2003). It is important to note that oxygen delivery and oxygen utilization differ considerably between individuals and can be modulated by training, disease states, and nutritional status (Xu and Rhodes, 1999). Hence, the VO_2 kinetics will vary from one person to the next for the exact same exercise load. Numerous studies have attempted to quantify oxygen consumption kinetics in order to make comparisons possible, often by calculating time constants and slopes for each phase in conjunction with complex modelling procedures (Burnley et al., 1999). There is little standardization in the literature for quantification of the oxygen consumption response, leading to several questions of validity and generalizability. This problem is exacerbated when examining unstructured activity where VO_2 deviates greatly from the typical curve just described, making these previous quantification methods neither effective nor practical. A more viable approach is to quantify the area-under-the-curve (AUC) of the oxygen consumption curve for the entire exercise from on-set to recovery. This method quantifies the net VO_2 response incorporating slopes of the phases, lengths of the

phases, and the recovery without need for distinctions on where they are occurring. This would allow for valid comparisons between one individual's VO_2 kinetics to another for the same exercise and during different exercise types (i.e. structured vs. unstructured activity).

Limitations:

The parameters behind oxygen delivery and oxygen utilization in most cases facilitate exercise by meeting the ATP demand, however in some extreme exercises they can impose limitations. There is much controversy in the literature as to whether the oxygen delivery or oxygen utilization side of the VO_2 kinetics is primarily responsible. Typically, at maximal or near-maximal intensities oxygen delivery can become insufficient for oxidative phosphorylation, with several physiological mechanisms suggested, including oxygen-dependent changes in metabolites and variations in microvasculature perfusion. On the oxygen utilization side there has been strong evidence that this factor can both limit and regulate oxygen consumption. This is apparent when examining the impact of previous exercise, a factor relevant in characterizing Int or Rand activity.

Section II: Oxygen Consumption Responses to Int and Rand (Self-Paced) Activity:

Few studies have attempted to characterize oxygen consumption kinetics to intermittent exercise of random intensity, limiting available information. One study by Turner et al. (2006) had subjects complete 4 Int cycles varying from 20W-120% of maximal work rate and in different work:rest cycles (10:20s;30:60s;60:120s;90:180s). The authors observed that as with Cont (constant-load) exercise the oxygen consumption response to Int exercise is Intensity dependent. Specifically, the higher workloads were associated with increased lactate production that appeared similar to the moderate, heavy, and maximal domains in Cont exercise. A slow

component was suggested due to elevated lactate levels, however only in the very heavy work cycles of 60:120s and 90:180s. This was only suggested, not demonstrated The very short Int work cycles (10:20s; 30:60s) did not produce sustained lactate production as it was suggested that clearance/utilization rates were able to match lactate production. This was not the case for the higher work cycles of 60:120s ad 90;180s where an elevated concentration of lactate was detected. It has been observed from this study, that the short duty cycles, producing oxygen consumption values below that associated with the lactate threshold, deviated in terms of kinetics from that predicted from constant work exercise. However, the specific mechanism behind this discrepancy needs further investigation. Another study examining intermittent activity and oxygen consumption responses (Edwards et al., 1973), observed that oxygen consumption and lactate was higher for intermittent exercise than continuous exercise of the same total time and power output. Literature on random intensity intermittent activity is limited, defined as intermittent activity but in a non-sequential order. In order to understand the mechanism behind random intensity intermittent exercise, the impact of previous exercise on the oxygen consumption kinetics must be examined.

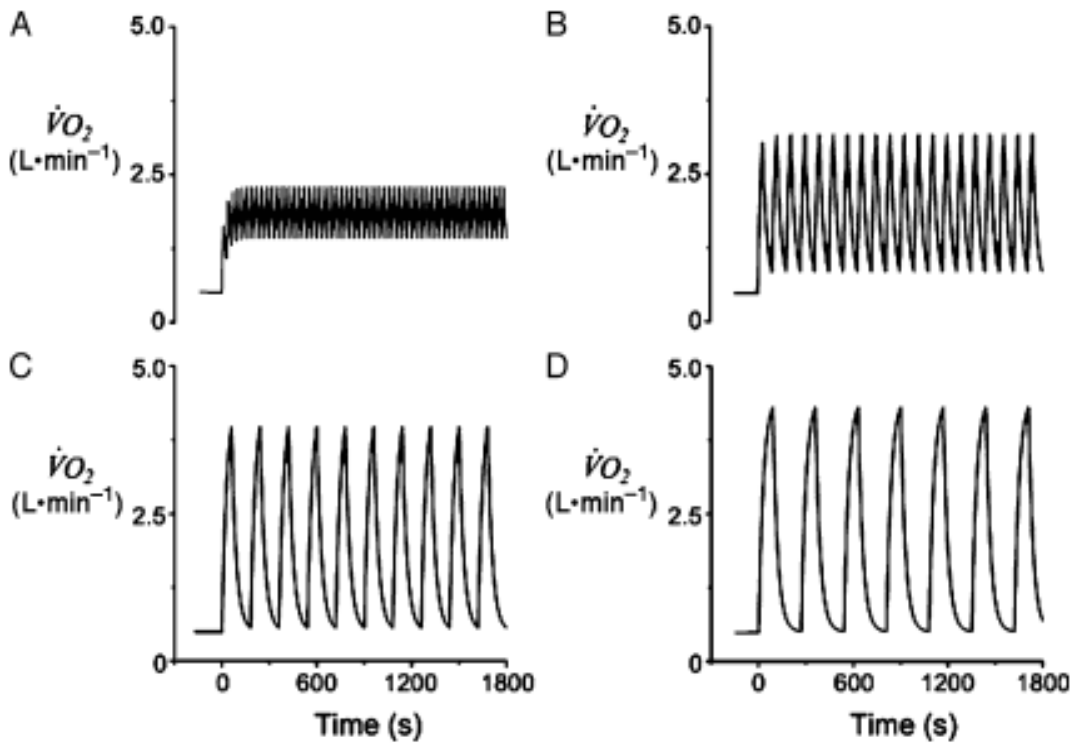


Figure S3: First-Order Model Simulations of Phase 2 VO_2 Responses to the Four Intermittent Tests. a) 10-s:20-s test; b) 30-s:60-s test; c) 60-s: 120-s test; d) 90-s:180-s Test. (Turner et al., 2006)

Onset of Exercise:

Examining the effect of previous exercise on oxygen uptake kinetics has important physiological relevance as it offers insights into the mechanisms behind Phase II oxygen kinetics. Many early studies examining the impact of previous exercise on the oxygen uptake kinetics of subsequent exercise have concluded that the Phase II kinetics in heavy exercise are speeded up by the presence of a previous bout of heavy exercise. This is in contrast to the results of a later study by Burnley et al. (1999) that demonstrated no speeding up of Phase II kinetics in response to heavy exercise. This discrepancy was attributed to differences in mathematical curve fittings of the oxygen consumption response over exercise time. Originally, Phase II was treated with monoexponential curve fittings and this led to the description of the Phase II and the slow

component by one curve. A poor fit can be examined in Figure S4 see below for a monoexponential model. However, Burnley et al. 1999, used a better fitting model including three exponentials terms demonstrating a better fit in that same figure. This latter procedure elucidated an important result, that the previous bout of heavy exercise did not speed up the Phase II kinetics of the subsequent bout, but rather reduced the amplitude of the slow component leading to lower end-exercise oxygen consumption. In fact, the previous bout reduced the slow component by 63%. Thus, the contradicting results stem from differences in modelling procedures, which have important ramifications for understanding oxygen uptake kinetics.

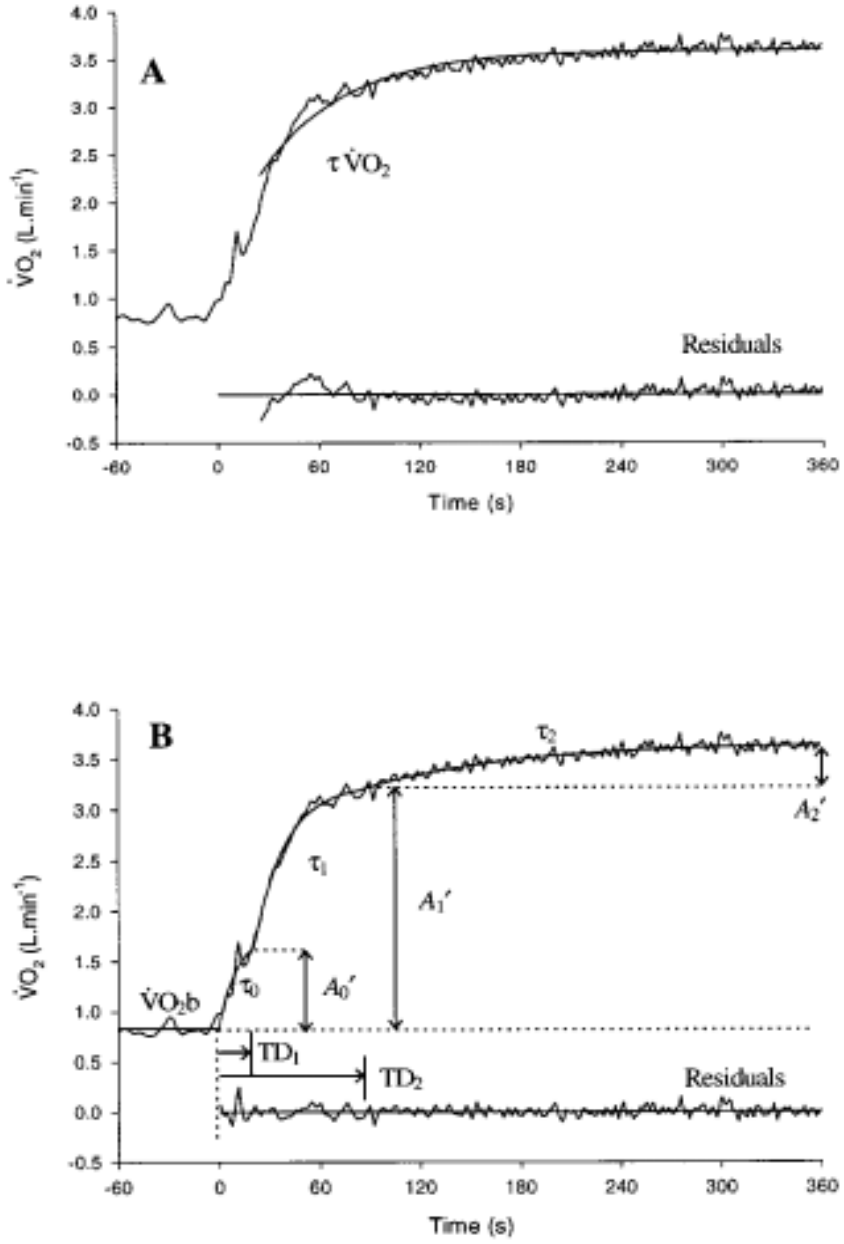


Figure S4: Curve fitting procedures using a Monoexponential vs Biexponential Model for Oxygen Consumption Kinetics of Heavy Intensity Exercise (Burnley et al., 1999)

Changes in the slow component observed from the effects of previous exercise on subsequent exercise highlights how oxygen utilization can regulate oxygen consumption kinetics, specifically phase II. Returning to the previous discussion on the mechanisms behind the slow component, the recruitment of the larger and faster muscle fiber types, Type II fibers,

have been implicated as a slow component mechanism by a substantial amount of evidence. Heavy exercise (>LT or AT) is associated with the recruitment of the Type II fibers, so to find a reduction in the amplitude of the slow components after prior heavy exercise suggests a difference in the recruitment patterns of motor units. It seems plausible that the first bout of heavy exercises may have shifted important substrates and activation of key enzymes in the TCA and pyruvate dehydrogenase complex leading to a quicker establishment of Intracellular hemostasis in the second bout of heavy exercise (Hughson et al., 2001; Grassi, 2001). In order to explore this physiological mechanism further, the key regulators and metabolites of oxidative phosphorylation must be introduced.

In the complex of stages that make up oxidative phosphorylation in the muscle cell there are several rate-limiting steps that may regulate the pathway. One such step is that of pyruvate dehydrogenase complex (PDC) which catalyzes the decarboxylation of pyruvate into acetyl-CoA. Acetyl-CoA is then fed into the Kreb's cycle or the Tricarboxylic Acid cycle. Regulation of PDC occurs by two enzymes in a reversible phosphorylation, a phosphatase that activates and a kinase, which inactivates PDC. PDC is also influenced by several allosteric factors. The kinase regulating PDH is inhibited by pyruvate, and high ratios of,CoA-SH:Acetyl-CoA, NAD+:NADH and ADP:ATP, all leading to activation of PDC. The phosphatase is activated by Ca2+ leading to activation in PDC. Previous research has uncovered that the initial release of Ca2+ from the myocyte activates 60% of PDC through the phosphatase (in rat cardiac muscle), and the remainder of PDC is activated by changes in the concentrations of the aforementioned allosteric regulators (Parolin et al., 1999). Previous studies have also uncovered a lag in PDC activation causing a stall in oxidative phosphorylation and a subsequent reliance on anaerobic means of

energy production such as PCr. In fact, PDC activation kinetics have been closely linked to exercise Intensity.

In addition to the metabolites previously described, another factor requires mentioning, glycogen phosphorylase (Phos). Phos is the rate-limiting enzyme for gylcogenolysis, the biochemical process of freeing glucose from glycogen which can enter glycolysis to produce pyruvate. As glycogen is the main substrate in high-Intensity exercise, it provides the pyruvate used by PDC. If there is an imbalance between pyruvate production and utilization by PDC, the pyruvate gets converted to lactate. As exercise progresses Phos becomes inactivated leading to a reduction in lactate production. Together, changes in the activity of Phos and PDC throughout exercise change the metabolic inertia to subsequent exercise. In other words, the first bout primed the second bout, allowing the aerobic system to respond more efficiently to the next workload. Ultimately, the result is fewer Type II muscle fibers are necessary to be recruited and a lower amplitude slow component will correspond. Further validation comes from the lower blood lactate observed in the second heavy bout of exercise consistent with fewer Type II fibers recruited, which produce more lactate than Type I fibers. Thus, by impacting key regulators of gylcogenolysis and PDC, the first bout of exercise acted to prime the aerobic system for the next bout leading to a more rapid oxygen consumption response.

An additional factor may further contribute to the difference in VO_2 kinetics between the first and second bout of heavy exercise. The first bout may have caused a slight acidosis in blood flow due to the presence of lactate. This acidosis as previously discussed, could impact oxyhaemoglobin dissociation leading to a higher delivery of oxygen. However, as oxygen delivery was not limiting in the first place this factor would not likely be of primary importance in reducing the amplitude of the slow component. This was evidenced by the lack of increase in

speed for overall Phase II kinetics observed in the study. Interestingly, the same study observed no priming effects of previous moderate-Intensity exercise on subsequent heavy exercise or previous moderate-Intensity exercise on subsequent moderate-intensity exercise oxygen uptake kinetics. This once again offers insight into the kinetics of oxygen uptake as the slow component is typically not found in moderate-Intensity exercise, further suggesting that lower Intensities have different impacts on the aerobic mechanisms of the working muscle and hence did not have a priming effect.

It therefore becomes apparent that engaging in an intermittent type of activity would have a different physiological response than that of continuous physical activity. This is quite important as the impact of exercise is typically characterized under a continuous protocol in the laboratory, and under steady state. As previously mentioned, activity in the real-world in both children and adults is very rarely of just the continuous type, with a high proportion of intermittent activity. Additionally, it is imperative to reiterate that the very nature of the way children play is high intensity bursts of activity, followed by periods of low-moderate activity/rest (Bailey et al., 1995). This play is mainly intermittent activity and there are rarely long periods of continuous activity observed among children playing, as the current literature bases its models. This continuous model is therefore invalid in making conclusions on real-world physiological responses to similar intensities, as it does not account for the Int nature of these activities.

Consequently, in order to be able to accurately describe and predict the physiological recovery of Int or Rand activity such as children playing games, there needs to be a new model, which can incorporate the impact of different physical activity types. Proper characterization of oxygen consumption kinetics will be important for understanding its role as an indicator of

fitness and health, and elucidating the physiology behind performance recovery. With the significant evidence in the literature presented for the contribution of EPOC in unregulated self-paced physical activity (intermittent nature), a new model would need to account for this variable and in doing so would require characterization of what influences EPOC.

Recovery from Exercise:

Once an exercise stimulus is removed, which marks the start to recovery, oxygen consumption remains elevated for a period before returning to baseline/resting levels. However, the VO_2 response kinetics during recovery follows similar patterns as when exercise is present in the low-moderate domain exercise kinetics. In fact they are symmetrical, with a biphasic VO_2 curve with a rapid phase (Phase I) followed by a slower phase (Phase II). On the other hand, when the exercise was of heavy intensity there is the typical slow component present in the kinetics during exercise, however no slow component is observed in the recovery kinetics. It therefore appears that in the heavy domain the slow component may be combined together with Phase II during recovery (Poole et al., 2012).

Excess Post-Exercise Oxygen Consumption (EPOC) – "Oxygen Debt":

During exercise energy production for force generation relies on stored sources of fuels/substrates (e.g. phosphocreatine (PCr), ATP; glycogen) present in the muscle. As a result, with progressive exercise the initial stored substrates are metabolized without oxygen - anaerobic pathways (Hughson, 1984). Christensen et. al, (1960) suggested that a deficit in these substrates/fuels metabolites occurs which needed to be repaid during recovery, termed "oxygen debt". The relevance of this with regards to exercise is that if the recovery is not complete and substrate levels are not returned to baseline levels by the next bout, a subsequent strain is placed on the physiological machinery of the muscle in the next bout. In this way, the previous bout of

exercise is not mutually exclusive from the next bout. The "Oxygen debt" is therefore carried on, where the muscle has a lower capacity to initially buffer the exercise stimulus with those metabolites, and must therefore engage the ATP generating pathways. With oxygen being a key requirement in the generation of that ATP, in addition to its role in re-paying that oxygen debt, there is an obvious impact on oxygen uptake and the circulatory system (e.g. heart rate).

A number of characteristics of exercise have been implicated in determining the level and duration of EPOC in the literature. However, there is no agreement as to which factors or the size of their contribution. To make this increasingly unclear, studies from the past few decades examining EPOC often used limited numbers of subjects, unstandardized exercise protocols (illustrated in Table 1), and indirect measurements of energy expenditure and metabolic rate that may be inaccurate, and the lack of control for confounding variables (e.g. food Intake, resting levels). There are, however, patterns emerging which suggest key factors impacting EPOC, these include; Intensity; duration; mode; and training status.

Intensity:

Perhaps the most established factor in determining both the level and duration of EPOC is that of the exercise intensity. A number of studies have established that when keeping other parameters constant, increasing the intensity of exercise has a significant impact on EPOC. The correlation between exercise intensity and EPOC is moderated by the categorization of intensity, leading to a curvilinear overall relationship. Bahr and Sejersted (1991), observed that lower intensity exercise (i.e. <40% VO_2max.), results in minimal increases in EPOC that do not significantly contribute to overall energy expenditure. Moderate intensity exercise (approximately 50% VO_2max.) has a moderate correlation to resultant EPOC level and duration. However, when exercising at higher intensity (i.e. >75% VO_2max.) there is a linear relationship

between EPOC and exercise intensity, representing approximately 15% of total energy expenditure. This suggests that the relevance of exercise intensity to EPOC shifts with a change in category of intensity. In the context of children, this finding is particularly important as children participate in short bursts of moderate-high Intensity activity and therefore further justifies research of EPOC in this demographic (Bailey et al., 1995). Additionally, this finding also gives clues into the mechanisms behind EPOC. The anaerobic threshold occurs in most untrained individuals around 50% VO_2 max. (Bahr and Sejersted, 1991) and this level would represent the activation of the

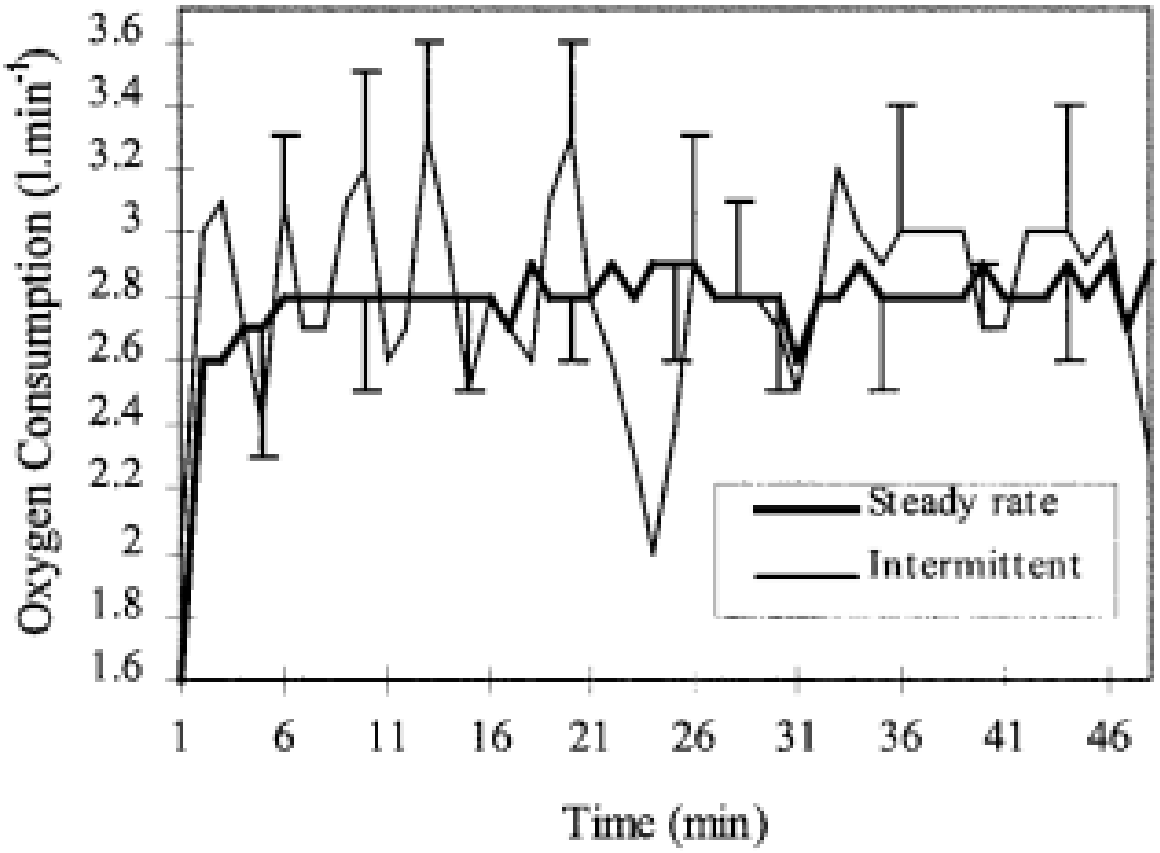


Figure S5: Oxygen Consumption for The Soccer-specific Intermittent and Steady-rate Exercise Protocols (Mean±SD). (Drust et al., 2000)

metabolic processes previously discussed (i.e. anaerobic metabolism, oxidation of fatty acids, etc.). One particular metabolite of interest associated with anaerobic energy production is lactate. In a study by Frey et al. (1993), subjects completed both a high (80% VO_2max.) and moderate intensity (65% VO_2max.) exercise protocol with lactate levels monitored before, during, and after the exercise. EPOC remained significantly elevated for the high intensity protocol as compared to the low intensity protocol for up to 20 minutes after the end of exercise. Similarly, lactate levels remained significantly elevated at the end of exercise with higher lactate observed in the high intensity protocol. Lactate levels remained significantly elevated past the 60 minutes recovery period for high, and for 40 minutes in the low. These results were in agreement with another study examining lactate levels following moderate intensity exercise (60-70% VO_2max.) which also found elevated lactate for up to 40 mins before returning to baseline levels (Maresh et al., 1992). Although the value of lactate clearance may be coincidental rather than causal of EPOC, it is a strong indicator of the strain of exercise and the efficiency of the recovery process (Baker et al., 2012). Additionally, part of the purpose of EPOC has been to clear lactate (80% is converted to glycogen in the Cori cycle in the liver, 20% is metabolized in Kreb's cycle), exemplified in the high correlation between EPOC and lactate levels. Therefore, the Intensity of exercise has been established as a major determinant in the level and duration of EPOC.

Duration:

The duration of exercise is another primary factor that has been hypothesized to impact length and level of EPOC. Although much of the mechanisms previous discussed would support duration as a key factor, very little experimentation has been done in the literature of this factor in isolation. The few studies that have investigated duration of exercise on EPOC have suggested a near linear relationship given exercise at above anaerobic threshold levels. Studies conducted at

a low exercise Intensity (40-50% of VO_2max.) have observed little relation between EPOC and duration of exercise (Bahr et al., 1987, Maehlum, Sverre, et al., 1986, Borsheim and Bahr, 2003). The same mechanisms suggested to explain the relationship between exercise Intensity and EPOC describe the influence of duration, however the evidence is stronger here. Blood lipid analysis during exercise has revealed that in a typical adult population approximately 20 minutes of moderate exercise (60% VO_2max.) is required to increase blood plasma levels of fatty acids and this increases progressively over a 3 hour period of exercise (Bahr et al., 1987). Increases in blood fatty acid levels require the usage of ATP in the triacylglycerol-fatty acid substrate cycle in adipose tissue, reflecting an increase in oxygen uptake. This coincides with the change in the RER during increasing exercise durations, reflecting a shift to the use of fats as fuels both during exercise and in the recovery. In vivo studies with rats have not just supported this assertion, but have also demonstrated the influence of catecholamine's in raising the rate of this cycle (Bahr et al., 1987). Studies investigating the impact between exercise duration and EPOC have observed corresponding increases in catecholamine levels. However, there are limited numbers of studies examining exercise duration and EPOC in isolation, therefore more studies are needed to further establish this relationship.

Mode:

The mode of exercise (i.e. aerobic or resistance) has also been demonstrated to be a factor in the magnitude and duration of EPOC. Direct comparisons of aerobic and resistance (circuit weight; 60-70% of one-rep max; 8-12 reps) training protocols has demonstrated a higher EPOC in the resistance procedure (Borsheim and Bahr, 2003). Resistance exercise has been reported to have higher lactate levels, ventilation, perceived exertion, heart rate, and Respiratory Exchange Ratio (RER) than treadmill exercise (aerobic training) (Braun et al., 2005). All of these factors imply a

differing sympathetic response to exercise, resulting in a discrepancy in energy expenditure. It is suspected in these studies that although the aerobic energy expenditure of the two modes was closely matched, the anaerobic energy components differed. The higher ventilation rate demonstrated a need to remove CO_2 during the exercise and demonstrating a higher anaerobic contribution than in the treadmill training protocols. This corresponds to a higher RER during exercise in the resistance training protocol, significantly higher than the aerobic procedures. However there is a shift in RER during the recovery period, which eventually becomes significantly lower than the treadmill protocol (Braun et al., 2005). The reason for this shift is still debated, and no clear mechanism has been offered in the literature for this shift. Two explanations given have been the retention of CO_2 for the purposes of bicarbonate stores, and an increase in fat oxidation to spare glycogen reserves during recovery. Unfortunately, further investigations into this shift in RER have not been undertaken and therefore the mechanisms behind the differences between resistance training and aerobic training are not well understood. Additionally, relationships between Intensity and duration of resistance protocols and resulting EPOC have not been determined. What is suggested by the research at this poInt is that there are differing mechanisms between resistance and aerobic protocols, which are resulting in different overall energy expenditures and thus impact EPOC differently (Borsheim and Bahr, 2003). Further investigation is necessary.

Training Status:

Past research in this area is limited, and several studies have methodological flaws such as short observation periods or insufficient exercise Intensities during testing. Nonetheless, there is a general trend in studies examining the EPOC of individuals with and without training that there is a decrease in EPOC with training (Short and Sedlock, 1997, Borsheim and Bahr, 2003).

Specifically, the decrease is in the duration of EPOC and not in the initial magnitude. The decreasing in EPOC with training observation is not particularly surprising given the adaptations one receives from exercise training. Aerobic training is associated with increased muscle blood flow and changes in substrate utilization, all factors in oxygen uptake recovery. It is therefore obvious why the overall duration is shorter in trained subjects, but the reason for magnitude differences in not entirely clear. One possible explanation in a study by Short and Sedlock was that the trained subjects exercised with higher oxygen uptake as the trained and untrained group were match by relative Intensities. This procedural approach is repeated in other studies, and this would leave the trained group at higher starting EPOC magnitude than the untrained group. However more investigation is needed to uncover this relationship, as training and EPOC have not been well examined.

Summary:

The term oxygen debt has in more recent decades become part of an umbrella term called "Excess Post-Exercise Oxygen Consumption" or EPOC. EPOC refers to the general elevation in oxygen consumption that continues once an exercise stimulus has ceased. The elevation encompasses oxygen debt as well as other factors, but for the purposes of this research oxygen debt will be the main mechanism considered behind EPOC. Furthermore EPOC and oxygen debt will be used as Interchangeable terms in this discussion. The significance of EPOC in terms of practical considerations have not well documented, however previous reviews on EPOC have identified a 7-14 % component of total oxygen consumption in a given activity being attributed to EPOC necessary to stimulate health and fitness adaptations (LaForgia et al., 2006). The typical oxygen consumption kinetics characterized up to this point are accurate in characterizing structured or continuous (endurance) type exercise, but what about other activity types such as

intermittent activity? If the oxygen consumption kinetics differ across activity types, then the model just described may not adequately predict the physiological response to exercise, and evidence in the literature suggests a basis for this discrepancy.

Section III: Oxygen Consumption Responses During Exercise for Children vs. Adults:

VO_2 Responses:

Research on the kinetics of oxygen consumption in children is sparse as much of the literature contains studies on adults. However, it has been noted that children go through the same three phases as adults do, and it has been found that the overall VO_2 response is faster in children then it is in adults (Xu and Rhodes, 1999; Fawkner and Armstrong, 2003).

An important difference has been observed between children and adults in Phase I VO_2 kinetics. The length of time for Phase I VO_2 kinetics remains similar for children across a wide range of intensities. However, this was not the case with adults where one study comparing boys and men found that at exercise intensities greater than 50% of the peak oxygen uptake (approaching the heavy domain of exercise) were associated with a shorter Phase I component (Hebestreit et al., 1998). There are also gender differences apparent. Although moderate exercise does not show any significant differences between boys and girls, maximal exercise shows substantial differences. In a study by Fawkner and Armstrong (2004), boys had a shorter VO_2 response than did girls when matched for growth and development. This finding is difficult to interpret given limited data on gender differences in children with regards to VO_2, but may be a product of lower overall fitness in the girls vs boys. Although the VO_2 max is often reported lower in girls than boys the potential for elucidating a sex difference is compounding by the fact that many early studies group boys and girls together or have a significantly higher proportion of

boys than girls.An additional explanation is that boys may have a higher proportion of Type I (oxidative type) muscle fiber types than girls although this remains a theory, and has not been determined experimentally. In order to determine a mechanism to account for the differences in responses for adults and children one must compare the physiological parameters between adults and children. In this initial phase there is a muscle-lung transit delay so therefore the relevant Fick equation parameters are with the cardiac output. Children Contain smaller hearts than adults and stroke volume increases with growth (Malina and Bouchard, 1991). Although children have a significantly higher heart rate than adults do, the overall cardiac output is still lower by comparison. However, when comparing Contribution of Q to absolute percentage of steady-state VO_2, adults Contribute more than children. This may suggest the potential for a shorter cardiodynamic (Phase I) for children, however there is conflicted data for the length of this component of VO_2 kinetics in children vs adults.

In moderate exercise the oxygen consumption kinetics have been observed to be linear in response to exercise Intensity as they are in adults. Although many studies used differing methodologies, especially a lack of standardization in exercise protocols (many did not match exercise Intensity appropriately between participants), there generally appears to be an overall faster VO_2 response in children during moderate-Intensity exercise. This suggests that the aerobic potential in children may be greater than that of adults (Fawkner and Armstrong, 2003).

When examining higher Intensity exercise bouts including both heavy and severe Intensity exercise it has been observed that children also have a shorter oxygen consumption response than adults do. Furthermore, It has also been demonstrated that the pattern of VO_2 kinetics is different in children than adults. Children have faster rate of VO_2 at the on-set of exercise. This is reflected in the significantly lower magnitude of the slow component and its

relative Contribution to total amplitude in boys when compared to men. In some studies a slow component was completely absent from the Phase II response to heavy exercise in children (Williams et al, 2001). However, the end exercise oxygen cost has been determined to be higher for children in these experiments than adults. This is consistent with the notion that children are very efficient in aerobic means of energy production, however their anaerobic systems are underdeveloped and therefore have much lower capacity for anaerobic energy production. This is further evidenced by the lack of lactate production observed in boys exercising at the heavy and severe exercise domains, a result indicative of lower employment of anaerobic pathways (Barstow, 1994).To further this point, studies examining molecular substrates of anaerobic energy production have demonstrated that at high exercise intensities adults exhibit a more rapid change in pH and PCr throughout exercise, indicating that adults have higher glycolytic ability than do children (Fawkner and Armstrong, 2003; Kuno et al., 1995). These findings did not differ even when comparing children that had received aerobic training, which suggests that this is a developmental effect. It is important not to overlook the significance of lactate differences between children and adults at high exercise Intensity levels. As mentioned in the section on mechanisms behind the slow component, lactate production is inferred as being coincidental rather than a cause of the slow component. Most evidence points to the difference in fiber types recruited at the higher work rates, and the larger muscle fiber types are associated with a higher production of lactic acid. The lower production of lactate suggests that the larger fiber types are not recruited and it is mainly the oxidative Type I fibers that are recruited with little lactate produced in comparison. This finding is particularly Interesting because a larger proportion of Type I fibers recruited in higher Intensity exercise is something observed in elite athletes and after high levels of aerobic training (Fawkner and Armstrong, 2003). Therefore, it appears that

children's VO_2 response patterns and physiology, at least from an oxidative potential point of view, closely resemble that of elite athletes and this could have profound implications for response to exercise Interventions. However, caution must be made as this has not been experimentally established at this point in time. It is unknown if fiber type distribution changes as an individual becomes more biological mature.

During rested muscle, elevated levels of NADH lead to allosteric inhibition of the Tricarboxylic acid (TCA) cycle, a key component in oxidative phosphorylation. The TCA cycle is further inhibited by low Intracellular calcium, indicating that this processes will not be activated unnecessarily during rest. During exercise the Contraction of muscle leads to an initial drop in ATP causing a spike in ADP levels. ADP stimulates the oxidation of NADH, ultimately stimulating the TCA cycle. The rise in calcium associated with muscle Contraction further activates TCA by increasing activity of acetyl coenzyme A and an additional two enzymes from the TCA cycle (Hughson et al., 2001).

The critical PO_2 required for maximal oxidative phosphorylation with sufficient substrate has been demonstrated to be 0.5 mmHg. It has also been observed that over the majority of work rates Intracellular PO_2 can fall to 3-5 mmHg. It would therefore seem unlikely that oxygen would be a key regulator of oxidative phosphorylation. However a study by Arthur et al., (1992), revealed that rates of oxidative phosphorylation can be maintained under high rates when levels do fall below this amount, however the other substrates involved must alter their concentrations to make this possible. For example, in the case of reduced PO_2 to achieve the same rate as with abundant O_2, the concentrations of NADH and ADP may need to increase.

Additionally, Haseler et al., (1998), found that breathing in mixtures Containing either a high or a low concentration of oxygen altered the levels of phosphocreatine (PCr) during

exercise of a constant workload. At the on-set of exercise the oxidative phosphorylation machinery necessary in the production of ATP is too slow to activate. As a result, other systems of producing ATP are necessary to provide energy to exercising muscle, mainly glycolysis and creatinine kinase reaction. The latter is the quickest system generating ATP by acting as a shuttle of high energy phosphate, and therefore provides an important source of ATP for the on-set of exercise. The changes in PCr concentrations during exercise when breathing in oxygen of varying levels has important implications. This result demonstrates that phosphorylation potential is altered in response to changes in oxygen delivery. Additionally, previous exercise has been demonstrated to alter some of the concentrations of substrates in the pyruvate dehydrogenase complex (PDC), which may having implications for overcoming metabolic inertia when activating the system in the next bout as described by Arthur's model (Grassi, 2001). This will be explored further in a subsequent section, but taken together the evidence suggests the VO_2 kinetics may be primarily limited by metabolic inertia and secondarily limited by oxygen delivery in healthy individuals during normal conditions.

Differences between Adult and Child Recovery from Exercise (VO_2 Responses):

It was observed that children have a faster VO_2 recovery at any work intensity. Additionally at any effort, whether maximal or submaximal body mass-relative power output is much higher in adults when compared to children. Even though children have a lower relative difference between maximal VO_2 and resting VO_2, their recovery is much faster. This has important implications for the EPOC as exercise generates a deviation from homeostasis and the recovery process reflects the body's return to balance. There are several parameters such as lactate, heart rate, and maturity status which all play a role in the faster VO_2 recovery observed in children compared to adults.

Lactate differences:

The metabolic systems of children is geared towards a preference for aerobic energy generation as opposed to anerobic methods. This is evident in the lack of phosofructose kinase activity in children compared to adults, the rate-limiting enzyme in glycolysis. Additionally, enzymes involved in aerobic generation of energy such as succinic dehydrogenase and isocitiricdehydogenase are higher. With a preference towards using alactacid aerobic exercise in children, less lactic acid is produced. This was discussed previously in the slow component mechanisms section of the VO_2 kinetics discussion where muscle fiber recruitment patterns differ between adults and children. Smaller oxidative muscle fibers, Type I, lead to lower production of lactate. Further evidence for differing recruitment patterns comes from regeneration of PCr. As mentioned previously PCr is an important substrate needed to provide immediate sources of ATP when the oxidative system is not yet activated. Children experience shorter recovery times for PCr levels back to pre-exercise levels compared to adults, which is indicative of less reliance on anaerobic system and a greater capacity for aerobic means of production. It has also been demonstrated that children reach peak lactate values much quicker than adults do with implications for removal. A possible mechanism accounting for lactate peaking earlier in children is with shorter cardiovascular circulation distances corresponding to shorter circulation times (Falk and Dotan, 2006). An additional factor has been suggested to also influence lactic acid production during exercise. Studies of insulin-deficient diabetics have revealed lowered lactate production suggesting a correlation (Wirth, Alfred, et al., 1978). In exercising children, lower levels of insulin are produced compared to adult populations. These insulin levels may therefore contribute to lactate levels and rates of splanchnic removal of lactate. However this is controversial, and not all studies have observed a difference in lactate

removal between children and adults. The significance of a higher lactate concentration was discussed in the characterization of different exercise types. Therefore, this difference in lactate production would change the capacity of recovery for children in comparison to adults.

Heart Rate:

In addition to differences in lactate levels there is also a greater contribution of the heart rate in increasing oxygen uptake than stroke volume, in children compared to adults. It may be suggested that the differing contribution of the heart rate may have an impact on recovery from exercise. Heart rate recovery from physical activity is poorly characterized in children and is only recently receiving attention. One study investigating heart rate recovery in children observed that recovery decreases with age (Singh et al., 2008). It is suspected that dominance of parasympathetic vs sympathetic nervous input may be modulating this heart rate recovery. It is unclear at this point how age-related changes play a role in the modulation of nervous input and subsequently impact heart rate, but the research is suggesting a correlation with age. With heart rate having more of a contribution to oxygen uptake levels in children than adults, this serves as an indication that their recovery mechanism may be different and thus cannot be compared on the same basis. Additionally in that same study, it was found that boys had a better recovery of heart rate then girls but no mechanism was offered for explanation, this may require further investigation.

Limitations:

There is an appreciable difficulty in interpreting results from studies involving children due to inherent methodological flaws such as accounting for equipment signal noise in VO_2 measurements, large range in the age of participants, and not accounting for maturity levels. The amplitude of the VO_2 response in children is smaller compared to adults and this creates a large

amount of signal noise in the data. In order to reduce signal noise it has been suggested by several authors to average out multiple transitions from one workload to another during exercise (Ozyener et. al, 2001, Lamarra et al., 1987). In adults, this method has shown to narrow confidence intervals and better identify changes in phases. However, the signal-to-noise ratio differs between adults and children considerably and this method may no longer apply. The ultimate result is difficulty in assessing transitions from one phase to another in children's VO_2 data (Ozyener et. al, 2001).

Recovery Kinetics Children vs Adults Summary:

In summary, it therefore appears that there is substantial difference in the VO_2 response kinetics both during exercise and in the recovery stage in adults versus children, and there are many gaps present in our knowledge of oxygen consumption kinetics. This is especially visible in the discussion of VO_2 recovery kinetics, of which limited data is available. Data is even further limited in the discussion of physiological differences in the EPOC portion of the VO_2 response in children vs adults. With the EPOC component demonstrated to be a considerable proportion of total oxygen consumption and imperative in the discussion of intermittent exercise where another bout is introduced before the recovery of a subsequent bout of exercise, more research needs to be done. As mentioned previously, unstructured activity such as children playing games is highly intermittent in nature. Continuous activity is generally structured (forced-paced). Therefore, in order to understand VO_2 responses during unstructured activity one will need to characterize and investigate the responses (mechanisms) behind the recovery portion of oxygen consumption.

Limitations of the Oxygen Consumption Response:

What regulates VO_2 kinetics has been the subject of much controversy over the past few decades, with evidence implicating many parameters surrounding the nature of oxygen consumption responses to exercise. This is not surprising given the vast range in methodologies used to assess and measure parameters associated with VO_2. However, the bulk of evidence has been associated with two key factors: a) oxygen delivery; and b) oxygen utilization/metabolic inertia (Poole et al., 2008). One of the central controversies has been determination of which of these factors is the primary limitation behind VO_2 kinetics. However, with previous research demonstrating few scenarios where either determinant could be shown to be a limitation, such as in a disease state or at the very high extremes of exercise intensity, the focus has shifted to their role in regulation. The second controversy has been the physiological mechanism underlying how each factor can regulate oxygen consumption kinetics. In terms of oxygen delivery, that debate has surrounded oxygen-regulated metabolites vs. lack of blood flow in the microvasculature. With oxygen utilization, there has been discrepancies over the proportion of regulation at specific steps of oxidative phosphorylation. Each of these factors will be discussed in detail next.

Oxygen Delivery:

There is evidence showing limited oxygen delivery by inducing hypoxia, using beta-blockades, and during supine exercise. An important series of experiments by Hughson and Morrissey exhibited faster oxygen consumption kinetics when moving from rest to 40% of AT than from 40% to 80% of AT (Hughson et al., 1982). The study authors linked this to a reduction in cardiac output. From the Fick equation cardiac output is a product of heart rate and stroke volume. Therefore, it became necessary to determine the contribution of each. As stroke volume

reaches a peak at low Intensity of exercise, an increase in heart rate has been found to account for higher cardiac outputs necessary at heavy exercise (Higginbotham et al., 1986). Based on this finding, it was suggested that the slower heart rate response limited cardiac output (Hughson, 1990). Thus from these initial studies, it was inferred that this drop in cardiac output (Q) limited oxygen delivery leading to prolonged kinetics. However, there is large doubt in implicating Q as a physiological mechanism for oxygen delivery limitation as increases in Q associated with the on-set of exercise greatly outpace that of VO_2, a phenomenon consistent across several exercise Intensities

Additional evidence for oxygen delivery as a limitation is provided in supine (lying down, face up) vs upright exercise, where VO_2 kinetics are significantly slower in the former vs the latter. This is intuitive, as in the supine position gravity would resist blood flow to the lower regions of the body. This would interfere with sufficient oxygen delivery and ultimately limit oxygen consumption. Further evidence comes from the improvement in the oxygen consumption kinetics when blood flow is restored to the lower regions by applying negative pressure to the legs (Hughson et al., 1993; Koga et al., 1999). Some studies have found that breathing in hyperoxic air compared with normal air has also been found to increase oxygen consumption kinetics, although this result has not always been replicated, in fact many studies have found just the opposite result (Xu and Rhodes, 1999). This may be due to the varying degrees of oxygen delivery as a limitation to VO_2 depending on exercise Intensity, which will be discussed next. An important note is that much of the evidence for oxygen delivery as a limitation to oxygen consumption kinetics comes from work at heavy-maximal Intensity. Key in this consideration is that oxygen delivery involves more than simply cardiac output, which is merely one component. The blood pumped by the heart still needs to arrive at the working myocyte. Thus blood flow and

oxygen extraction represent two additional components in oxygen delivery. The main factors behind blood flow have been resistance of the vasculature, blood pressure and blood viscosity. To understand the development and Controversy of two central mechanisms for oxygen delivery requires examination of a key study. Hortsman et al., (1976) conducted a study involving alterations of oxygen delivery on isolated dog gracilis muscle, and offered a physiological mechanism to help explain oxygen delivery limitations. It has been well documented in previous studies that electrical stimulation on isolated muscle cells at frequencies simulating high Intensity muscle contractions, will result in a finite maximum blood flow. This maximal blood flow was then found to coincide with a plateau in oxygen consumption and the production of lactate. In other words, blood vessels are able to increase blood flow, but only up to a certain point where maximal blood flow occurs. The main assumption here was that blood vessels act as a rigid tube (Poiseuille's law), where vasodilation can drop resistance and improve blood flow. Vasodilation can be stimulated by a drop in blood pressure (baroreceptor reflex), and this was apparent in low stimulation rates. However, at high stimulation rates the authors observed that lowering the blood pressure had no impact on vasodilation, indicative of maximum dilation. Therefore, the authors suggested that at low rates of stimulation (moderate-intensity exercise) the blood flow can be increased as needed by vasodilation, which is sufficient to offset the drop in oxygen delivery from resting blood flow rates. Still, the blood vessels cannot keep dilating infinitely and at higher stimulation rates (heavy or maximal-intensity exercise) the blood flow results cannot deliver oxygen as required, resulting in hypoxia. However, it has been demonstrated previously that even at the highest intensities of physical activity, oxygen saturation does not drop below 1 mmHg (Grassi, 2001). The minimum oxygen saturation necessary for optimal functioning of the oxidative phosphorylation system is 0.5 mmHg.

Therefore, it would appear at least initially that oxygen delivery could not limit or regulate VO_2 kinetics. Two Controversial theories to reconcile this finding have since been offered: oxygen-dependent changes in metabolites by Hughson (Hughson, 2005) and differences in microvasculature perfusion by Barstow (Poole et al., 2008).

Hughson developed a model in which varying degrees of oxygen supply to the working myocytes can impact concentrations of key metabolites (Hughson, 2005). Specifically, concentrations of ATP and PCr (high-energy phosphometabolites) represent the "energy state" of the myocycte, and their proportions change throughout exercise. At the onset of exercise these phosphometabolites are used up in proportion to demands placed by the activity, and as such the energy state of the cell is reduced. It has been observed in several studies that the energetic state can be reduced by varying O2 supply at the same work rate (ATP demand) during moderate exercise (Linnarsson et al., 1974; Haseler, et al, 1998). It is important to note that this is not the result of on-set kinetics, as this occurred during hypoxia and hyperoxia at the same submaximal Intensity workload and during steady state. It is suggested the by lowering the energy state (concentrations of PCr and ATP) during reduced oxygen saturation will keep the ATP supply matching the demand. PCr is of particular importance in regulating not just substrate-level phosphorylation, but also oxidative phosphorylation. As Creatine Kinase is present both in the cystol (close to myosin ATPase) and is present in the mitochondria, the cycling of PCr and Cr between those two locations may provide a signaling link between the utilization and production of ATP (Haseler, et al, 1998). It has also been demonstrated that drops in oxygen present in the cell shift cystolic [ATP]/[ADP][Pi]and intramitochondrial [NAD+]/[NADH], all of which serve as signals to change the rate of mitochondrial respiration. None of these aforementioned metabolite regulators (PCr, ADP,Pi, ATP/ADP*Pi) has been observed to be rate limiting, but

rather it is the balance of their concentrations that set the rate of oxidative phosphorylation (Wilson et al., 1977, Haseler, et al, 1998). Although these PCr differences during hyperoxia and hypoxia were found to be minimal at the submaximal rates of exercise, it is possible that they may be much greater at high intensities of exercises, thereby regulating oxygen consumption kinetics.

On the other side of the physiological mechanisms behind oxygen delivery debate is the of discrepancies of the blood flow between the arteries and capillaries. In studies that have assessed blood flow during varying exercise conditions, such as in the Hortsman study, they have used either specific sites distant from the capillaries (conduit arties), capillaries from an isolated section of muscle, or taken a measurement of blood flow across a muscle. The main reasoning for this method was that a lack of technology existed to measure capillaries directly in the working muscle. It was then a key assumption that the proxy measures of blood flow matched what was occurring at the level of the capillaries. However, with the emergence of near-infrared spectroscopy (NIRS) this assumption was brought Into question. Valid estimates of Q at the capillaries could be done by rearranging the Fick equation to solve for blood flow, with the oxygen consumption from phase II as the VO_2 muscle and using hemoglobin saturation from NIRS to represent (A-V) O_2. Studies that have directly compared the Q capillary values to the Q values of conduit arties has found that they are not comparable (Harper et al., 2006). In fact, the conduit capillary has been observed to have faster kinetics than the capillary, with the kinetics matching or in some cases slower than those of VO_2 at the muscle (Ferreira et al., 2005; Harper et al., 2006). Therefore, the blood flow kinetics at the level of the capillary can present an oxygen delivery limitation.

Both physiological mechanisms for oxygen delivery have evidence and are plausible, and it can be inferred that the true regulation may not be an either/or, but rather a combination of the two in various degrees. The oxygen-dependent regulation of metabolites has not been well assessed in terms of its direct impact on the oxygen uptake curve, with some studies indicating that faster overall kinetics with hyperoxia and slower with hypoxia (Poole et al., 2008; Grassi, 2001; Hughson, 2005). However, the physiological mechanism has strong implications for oxygen utilization, and therefore better examined in conjunction with the factors in the next section on that topic. In terms of the capillary blood flow dynamics side of oxygen delivery, it appears that the kinetics of Q at the capillary have been found to coincide closely with sections of the oxygen consumption curve, allowing for determinations of where regulations are manifested.

It has been observed that capillary blood flow kinetics follow a biphasic profile, similar to that of oxygen consumption during exercise (Harper et al., 2006). The first phase is a rapid phase and occurs during the first 5 seconds of exercise where vasodilation increases blood flow to the working muscle. This is referred to as the "muscle pump" and is indiscriminant, meaning that the entire muscle experiences vasodilation. Phase II of capillary blood flow kinetics is a slower phase and is regulated partly by several factors including H+, adenosine, ATP, nitric oxide, potassium, and prostaglandins. These are all metabolites associated with metabolic activity in exercising muscle, hence this second phase is tightly coupled with oxygen demand from the active myocytes. These two phases resemble that of phase II and III of oxygen consumption kinetics and in fact, the dynamics of blood flow at the capillaries has been found to correlate with muscle VO_2 dynamics (Poole et al., 2008; Harper et al., 2006). When muscle begins contracting the capillary blood flow increases to match rising oxygen demand, eventually

beginning to plateau. At this point, oxygen extraction increases significantly to compensate for the slowing rise in blood flow and once again meet demands of the muscle, however this increase plateaus within seconds. Although oxygen extraction plateaus quickly, Q capillary begins to rise yet again, indicating that blood flow is still increasing and it has been suggested that there must be some sort of oxygen sensing mechanism to stimulate this increase in Q capillary (Poole et al., 2008). This area of research is still lacking and it is possible that a metabolite such as NO released in response to the drop in arterial oxygen saturation, stimulating this further increase in Q capillary (Allen and Piantadosi, 2006; Kindig et al., 2002). However, what has been elucidated thus far is the close coupling between the final increase in Q capillary and VO_2 of the muscle at phase III of oxygen consumption kinetics. The plateau in phase III of VO_2 represents a matching between oxygen required for ATP supply and ATP demand by muscle, and the final increase in Q capillary mediated by an oxygen sensor may represent the fine-tuning of oxygen delivery to suite this need. Thus, it would appear that the regulation of oxygen consumption due to oxygen delivery dynamics manifests itself at phase III of the VO_2 response curve. Further evidence for a phase III regulation comes from studies investigating oxygen consumption kinetics during hyperoxic and hypoxic conditions. In an experiment by MacDonald et al., (1997) that examined human oxygen consumption kinetics after breathing in a hyperoxic gas mixture during cycle ergometer exercise, it was found that at exercise Intensities above the ventillatory threshold, the slow component was reduced. However, at Intensities below VT there was no change in oxygen consumption kinetics, highlighting the importance of exercise Intensity in regulation by oxygen delivery. Similarly, Engelen et al. (1996) found that human subjects breathing hypoxic air had overall slowed VO_2 kinetics when performing heavy exercise, however there was no change in the slow component when compared to room air conditions.

Differing modelling procedures in that study revealed that the hypoxic condition did not alter phase I kinetics but rather the slowing occurred after the rise in phase I. Whether or not the slow component changes with oxygen delivery remains controversial, and may be a result of differing methodologies, such as breathing in hypoxic gas vs altitude for introducing the oxygen delivery restriction. Overall, it would appear that an oxygen delivery problem would manifest itself as a slowing of phase III kinetics. This slowing of Phase III may become particularly difficult to discriminate at very high Intensities as this phase ends with a plateau, and the subject is likely to fatigue quickly. In fact this is particular visible in maximal-(or supra-maximal) exercise or in children where phase III may not be present.

Evidence for oxygen utilization as a limitation behind VO_2 kinetics begins with the observation in some studies that with an increase in O_2 delivery there is not a corresponding increase in VO_2 kinetics (Grassi et al., 1998). Thereby, researchers began seeking another mechanism principally limiting VO_2 kinetics, which is suggested to be oxygen utilization. Oxygen utilization refers to the rate of oxidative phosphorylation, a process in which oxygen in the muscle is converted to ATP. Oxidative phosphorylation involves a number of steps transferring energy stored in electrons from glucose before using oxygen as a final electron acceptor to generate ADP from ATP. It is possible that the availability of some substrates and enzymes will limit oxidative phosphorylation therefore reducing ATP available to the cell. Without aerobic means of energy the muscle will need to refer to anaerobic means of energy production leading to lactate production and fatigue. Therefore, energy utilization can limit oxygen consumption kinetics.

In the complex of steps that make up oxidative phosphorylation in the muscle cell there are several rate-limiting steps that may regulate the pathway. One such step is that of pyruvate

dehydrogenase complex (PDC) which catalyzes the decarboxylation of pyruvate Into acetyl-CoA. Acetyl-CoA is then fed Into the Kreb's cycle or the tricarboxylic acid (TCA) cycle. Regulation of PDC occurs by two enzymes in a reversible phosphorylation, a phosphatase that activates and a kinase which inactivates PDC. PDC is also influenced by several allosteric factors. The kinase regulating PDH is inhibited by pyruvate, and high ratios of,CoA-SH:Acetyl-CoA, NAD+:NADH and ADP:ATP, all leading to activation of PDC. The phosphatase is activated by Ca2+ leading to activation in PDC. Previous research has uncovered that the initial release of Ca2+ from the myocyte activates 60% of PDC through the phosphatase (in rat cardiac muscle), and the remainder of PDC is activated by changes in the concentrations of the aforementioned allosteric regulators (Parolin et al., 1999). Previous research has uncovered a lag in PDC activation causing a stall in oxidative phosphorylation and subsequent reliance on anaerobic means of energy production such as PCr. In fact PDC activation has been linked to exercise intensity in previous research.

Strong evidence for oxygen utilization, specifically through PDC, as a limitation of oxygen kinetics comes from studies on the impact of previous exercise on subsequent bouts of exercise. Although there is an entire section devoted on this topic, it has been observed that previous activity of sufficient Intensity will activate the PDC complex and if another bout of high Intensity activity is Introduced during that activation, VO_2 kinetics are sped up. This is precisely the protocol behind high Intensity Int training (HIIT). There is also a change observed in several metabolites, such as less lactate and increased PCr sparring in subsequent bouts. These metabolites closely correlate with the slow component observed in phase II of the oxygen consumption response curve. This finding implies that the oxygen utilization limitation manifests itself in phase II, and this has been validated in past studies where improving oxygen utilization

has reduced the slow component. This will be explored more in-depth in a further section, however It is important to note that PDC is not the only complex in the oxidative phosphorylation pathway which can limit VO_2 kinetics. It has also been observed in a study by Kindig et al. (2002) that the removal of nitric oxide (NO) in the electron transport chain of horse mitochondria by an inhibitor (L-NAME), sped up VO_2 kinetics during moderate exercise. This is important to note as no slow component is elicited in moderate exercise. However, as is apparent in the below figure, phase II was effected.

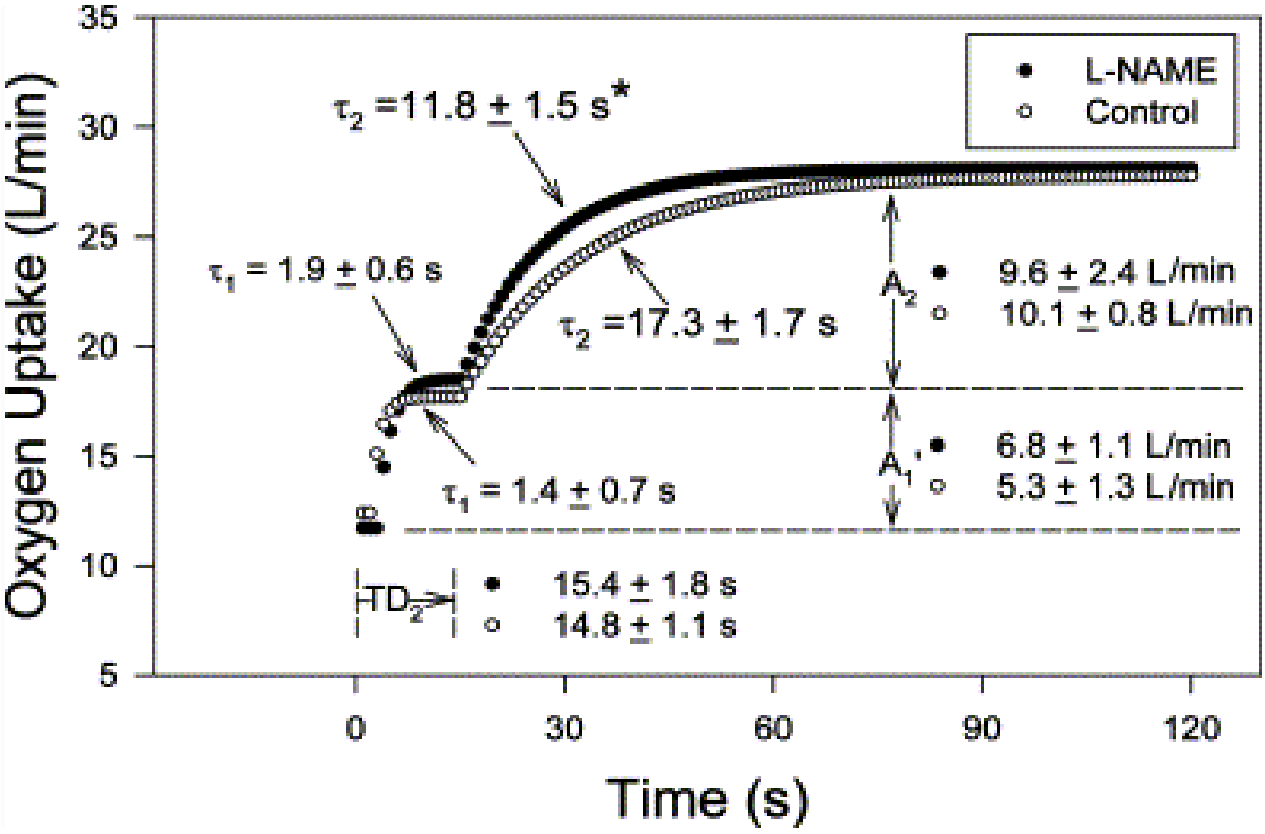


Figure S6. Mean Oxygen Uptake Kinetic Responses Modeled for all Five Horses under Control and L-NAME Conditions (Kindig et al., 2002)

As oxygen delivery limitations have been confined to phase III and oxygen utilization limitations to phase II, the question becomes on how to quantifying these limitations on the oxygen consumption response curve. There are several ways this can be done, such as measuring the slopes, intercepts, and amplitude of the curve in each phase. However, this may become ineffective when the oxygen consumption response does not produce the typical curve. The three phases may be indiscriminate or simply not present. This is typical of intermittent exercise, which involves much stop–and-go type of movement generating an oxygen consumption curve deviating from the typical response. It may therefore be more practical to take the area-under-the-curve of the oxygen consumption in order to quantify the response. In this way, oxygen consumption can be quantified and compared over a range of different exercise types as a whole.