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Section: Original Research

Article Title: Assessment of the Five-Minute Oxygen Uptake Efficiency Slope in Children With Obesity

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Journal: *Pediatric Exercise Science*

Acceptance Date: February 10, 2017

DOI: https://doi.org/10.1123/pes.2016-0248

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Title: Assessment of the five-minute oxygen uptake efficiency slope in children with obesity

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Running title: Five-minute OUES in children with obesity

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Word Count: Abstract – 197 words; Main text – 3326 words

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ABSTRACT

Purpose: Poor cardiorespiratory fitness is associated with increased all cause morbidity and mortality. In children with obesity, maximum oxygen uptake ($\dot{V}O_{2max}$) may not be achieved due to reduced motivation and peripheral fatigue. We aimed to identify a valid submaximal surrogate for $\dot{V}O_{2max}$ in children with obesity. **Method**: Ninety-two children with obesity (7–16 years) completed a maximal exercise treadmill test and entered a three-month exercise and/or nutrition intervention after which the exercise test was repeated (n=63). Participants were required to reach $\dot{V}O_{2max}$ to be included in this analysis (n=32 at baseline and n=13 at both time-points). The oxygen uptake efficiency slope (OUES) was determined as the slope of the line when $\dot{V}O_2$ (L/min) was plotted against log$\dot{V}$E. Associations between the maximal OUES, submaximal OUES (at 3, 4, 5 and 6 minutes of the exercise test) and $\dot{V}O_{2max}$ were calculated. **Results**: In the cross-sectional analysis, $\dot{V}O_{2max}$ (L/min) was strongly correlated with 5-min OUES independent of Tanner puberty stage and sex (R^2=0.80, P<0.001). Longitudinal changes in $\dot{V}O_{2max}$ were closely reflected by changes in 5-min OUES independent of change in percent body fat (R^2=0.63, P<0.05). **Conclusion**: The 5-min OUES is a viable alternative to $\dot{V}O_{2max}$ when assessing children with obesity.

Keywords: Exercise intervention, oxygen consumption, childhood obesity

Clinical trial registration: NCT01991106

INTRODUCTION

Poor cardiorespiratory fitness (CRF) is associated with increased all cause morbidity and mortality regardless of obesity status (6, 8). CRF assessment in children provides valuable information on present and future health status (14) and provides a tool to evaluate the efficacy of an exercise intervention. Assessment of CRF through maximal oxygen uptake ($\dot{V}O_{2max}$), involves exercise to exhaustion which may not be achievable in a pediatric population with obesity due to enhanced pre-test anxiety (27), a reduced ability to tolerate physical load and increased ratings of perceived exertion (5), and early peripheral fatigue (19). These limiting factors may in part be due to the increased metabolic demand of moving excess mass as well as true cardiorespiratory deconditioning (15, 18, 26). Regardless of the contributing factors, a large proportion of 'maximal' CRF assessments are likely submaximal (23) which is problematic for data interpretation.

The oxygen uptake efficiency slope (OUES), proposed by Baba et al. (1996), is objective and effort-independent, and provides information on how effectively oxygen is extracted and taken into the body (4). The OUES reflects the relationship between oxygen uptake ($\dot{V}O_2$ in L/min) and total minute ventilation ($\dot{V}E$ in L/min) (4). A steeper slope or higher OUES represents a more efficient oxygen uptake whereas a lower OUES indicates that more ventilation is required for given oxygen uptake (2). Due to the tight linear relationship throughout exercise, the OUES holds possible utility as a surrogate for $\dot{V}O_{2max}$. Maximum OUES (i.e. using VE and $\dot{V}O_2$ data to maximal exertion to calculate the OUES) has been used to determine CRF in healthy-weight children (1, 21) and children with overweight (13) and obesity (7), illustrating strong relationships with $\dot{V}O_{2max}$. Furthermore, the submaximal OUES has been validated at ventilatory threshold (VT) in children with obesity (20). While submaximal OUES was beneficial for tracking CRF, the need to determine VT may limit its clinical and practical applications (7). VT may not be identified in all participants, it is

subjective, and susceptible to intra- and inter-observer variability (4). Assessment of the OUES at a pre-determined time should be more feasible and provide prognostic information. Previous exercise-training studies in adults illustrated that longitudinal changes in the OUES were strongly associated with $\dot{V}O_{2max}$ (24, 32). To our knowledge, such investigations are yet to be completed in a pediatric population.

The aims of this study were to determine the relationship between: 1) OUES derived at 3, 4, 5 and 6 minutes of exercise and $\dot{V}O_{2max}$ and 2) longitudinal changes in 3, 4, 5 and 6-min OUES and $\dot{V}O_{2max}$.

PARTICIPANTS AND METHODS

Ninety-three children with obesity (7–16 years old, BMI ≥ percentile curves that pass through 30 kg/m^2 at age 18) (9) entered a multi-centre randomised controlled trial at the Norwegian University of Science and Technology (NTNU), Trondheim, Norway and The University of Queensland (UQ), Brisbane, Australia. The study was approved by the NTNU Regional Committee for Medical and Health Research Ethics (reference number 2009/1313-4), UQ Human Research Ethics Committee (reference number 2013000539) and The Mater Hospital Human Research Ethics Committee (reference number HREC/13/MHS/119/AM01). Participants' legal guardians approved consent, and participants provided written assent prior to participation. Details of the trial have been previously described (12). In brief, a physical examination that included height, weight, blood pressure and heart rate was completed and is detailed elsewhere (12). Pubertal status was assessed through examination by a pediatrician or self-report using the Tanner stages of puberty. CRF was quantified through a maximal graded exercise treadmill test with ventilatory expired gas analysis. While CRF assessment on a cycle ergometer may be suited for participants with obesity (3), children are less accustomed to cycling and are more likely to experience peripheral fatigue (22) compared to walking and running (27). Body composition was assessed using dual-energy x-ray

absorptiometry (Hologic, QDR Series, Massachusetts, USA) or air displacement plethysmography (BodPod, COSMED, Rome, Italy). Identical equipment was used for each individual at each assessment. Following the baseline assessments, participants were randomised to one of three intervention groups and were re-assessed following completion of a twelve-week exercise and/or nutrition intervention (12). Briefly, participants were randomised into one of three 12-week interventions: 1) high intensity interval training (HIIT) [n=31, 4 x 4-min bouts at 85–95% maximum heart rate (HR_{max}), interspersed with 3 min of active recovery at 50–70% HR_{max}, 3 times/week] and 4 – 6 nutrition consultations; 2) moderate intensity continuous training (MICT) [n=30, 44 mins at 60–70% HR_{peak}, 3 times/week]; and 4 – 6 nutrition consultations, and 3) and 4 – 6 nutrition consultations only (nutrition) [n=31]. Individual nutrition sessions were based on current Norwegian (16) and Australian (25) eating guidelines and specifically focused on healthy food choices, portion sizes and regular meal times.

Cardiopulmonary exercise testing

Participants performed a treadmill ramp protocol (22) to measure $\dot{V}O_{2max}$ using continuous breath-by-breath respiratory gas analysis (Cortex Metamax 3B, Cortex Biophysik GmbH, Leipzig, Germany, Cortex or Jaeger Oxycon Pro, CareFusion, Hoechberg, Germany) and facemask system (Hans Rudolph, KS, USA). Pressure, volume (Hans Rudolph^{TM} 3L volume calibration syringe) and gas (two-point calibration using gases of known concentrations) were calibrated according to manufacturer's instructions and testing was performed in a temperature-controlled environment. Identical equipment was used for each individual at each assessment. Direct measurements of oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), and minute ventilation ($\dot{V}E$) were obtained, allowing for calculation of the respiratory exchange ratio (RER). Measurements were stored in 10 s and 30 s epochs for data analysis. Following a three-minute rest period, participants performed a

minimum four-minute warm up at 4 km/h during which they were familiarized with treadmill walking. Participants were discouraged from using the handrails for support. Each minute, treadmill inclination was increased by 2% reaching a gradient of 12–16%. Following this, treadmill speed was increased (1 km/h each minute) until exhaustion. Heart rate was measured continuously during the test by radio telemetry (Polar, Polar Electro, Kempele, Finland).

Measures of cardiorespiratory fitness

Maximal oxygen uptake and the OUES at maximal and submaximal exercise (3, 4, 5, and 6 minutes) were calculated.

$\dot{V}O_{2max}$ criteria

A test was classified as maximal if the following conditions were met: 1) levelling off or plateau in $\dot{V}O_2$ despite increased workload using 30-second epochs, and 2) peak RER ≥ 1.05. A plateau was defined if the increase in oxygen uptake between the final two stages was $< \frac{150 mL O_2/min}{body\ mass}$ (10, 30). If $\dot{V}O_{2max}$ criteria were met, the two highest consecutive 30-second values were averaged to calculate $\dot{V}O_{2max}$. If either of these criteria were not met, peak oxygen uptake ($\dot{V}O_{2peak}$) was determined as the average of the two highest 30-second values attained. Participants were required to achieve $\dot{V}O_{2max}$ to be included in this investigation.

Oxygen uptake efficiency slope

Using the equation, $\dot{V}O_2 = a\ (\log\dot{V}E) + b$, the OUES is defined by the constant *a*, when log($\dot{V}E$) is plotted against $\dot{V}O_2$ (4). The logarithmic transformation of $\dot{V}E$ creates a linear relationship between oxygen consumption and ventilation (2). Maximal OUES ($OUES_{max}$) was calculated from the start to the end of the test using 10-second epochs. As the minimum exercise time for all participants was 6 minutes, the OUES was also calculated from the start of the protocol to six minutes resulting in 6-min OUES. The same technique

was used to calculate the 3, 4, and 5-min OUES to query the possibility of a shorter, valid exercise protocol. The four-minute warm up was excluded from OUES analyses due to hyperventilation that may occur at the onset of exercise.

Statistical analysis

Continuous data are expressed as mean ± standard deviation [SD] or median (interquartile range) [IQR] if non-normally distributed. Categorical data are expressed as median (IQR) and percentages. SPSS Statistics (Version 24.0, IBM, Armonk, NY, USA) was used to perform all statistical analyses. An independent t-test was used to determine differences between participants who achieved $\dot{V}O_{2max}$ at baseline and participants who achieved $\dot{V}O_{2peak}$ at baseline. A dependent t-test was used to test for longitudinal within group changes. Univariate linear regression modelling was used to determine the relationship between $\dot{V}O_{2max}$, 3, 4, 5 and 6-min OUES, and $OUES_{max}$ in participants who achieved $\dot{V}O_{2max}$ at baseline (n = 32). The predictive value of 3, 4, 5 and 6-min OUES was further validated by examining a subset of the cohort (n=13) who achieved $\dot{V}O_{2max}$ at baseline and 12 weeks. For this analysis, $\dot{V}O_{2max}$ was linearly normalised to body weight for consistency with previous research. OUES markers were normalised using the same method enabling comparison between both measures of CRF. Univariate linear regression modelling was used to determine the relationship between change in $\dot{V}O_{2max}$, change in 3, 4, 5 and 6-min OUES and change in $OUES_{max}$. Tanner puberty stage at baseline, and sex were considered independent variables in the multivariate linear regression model (model 1) and change in percent body fat was considered an independent variable when examining the relationship between longitudinal changes in relative $\dot{V}O_{2max}$, 3, 4, 5, and 6-min OUES, and $OUES_{max}$ (model 2). To derive a $\dot{V}O_{2max}$ prediction equation, bootstrapping was applied to the baseline multivariate linear regression model (n=32) with 1000 bootstrap samples drawn. Bland-Altman analysis was used to assess the level of agreement between the OUES markers, and

systematic bias was assessed using linear regression. All statistical tests with P value < 0.05 were considered statistically significant.

RESULTS

Participant characteristics and CRF exercise test responses

Table 1 illustrates baseline clinical and anthropometric characteristics, and exercise test responses in participants who achieved $\dot{V}O_{2peak}$ at baseline (n=60, 65%; HIIT, n=21; MICT, n=16; nutrition, n=23), participants who achieved $\dot{V}O_{2max}$ at baseline (n=32, 35%; HIIT, n=10; MICT, n=14; nutrition, n=8) and those who also achieved $\dot{V}O_{2max}$ at both assessments (n=13, 14%; HIIT, n=4; MICT, n=6; nutrition, n=3). There were no significant differences in anthropometric and cardiopulmonary exercise test responses prior to and following the three-month intervention (Supplementary table 1).

Validity of a time based OUES marker

The validity of a time based min OUES was assessed by calculating the cross-sectional associations between $OUES_{max}$, 3, 4, 5, and 6-min OUES and $\dot{V}O_{2max}$. $OUES_{max}$ was significantly correlated with absolute $\dot{V}O_{2max}$ (r=0.97, P < 0.001) (Table 2, Figure 1 A). Figure 1 B, C, D and E illustrate significant linear relationships between baseline $\dot{V}O_{2max}$, and the time based OUES markers. The most strongly correlated, in order, were 6-min OUES (r=0.90, P < 0.001), 5-min OUES (r=0.88, P < 0.001), 4-min OUES (r=0.84, P < 0.001) and 3-min OUES (r=0.75, P < 0.001). These associations were maintained when significant covariates (sex and pubertal status) were considered (Table 2). At baseline, model 1 illustrated that max OUES, pubertal status and sex were able to account for 93% of variation in absolute $\dot{V}O_{2max}$ (P < 0.001). Similarly, when 6-min OUES, pubertal status and sex were entered in the model, 83% of variation in relative $\dot{V}O_{2max}$ was accounted for (P < 0.001). Although still significant, the proportion of variation that was accounted for when 5, 4, and 3-

min OUES was entered in the model reduced progressively (R^2=0.80, R^2=0.74 and R^2=0.63, respectively).

The time based OUES markers were further validated by examining the relationship between longitudinal changes in relative $\dot{V}O_{2max}$, $OUES_{max}$ and 3, 4, 5, and 6-min OUES. Univariate linear regression revealed that changes in 4-min, 5-min and 6-min OUES and $OUES_{max}$ were significantly correlated with changes in relative $\dot{V}O_{2max}$ (r=0.74, r=0.76, r=0.82 and r=0.82, respectively; P < 0.001 for all associations). Longitudinally, the effect of body composition changes on the OUES was considered and change in percent body fat was entered into model 2. Even so, changes in $\dot{V}O_{2max}$ were significantly accounted for by changes in percent body fat and changes in relative $OUES_{max}$ (R^2=0.83, P = 0.005), relative 6-min OUES (R^2=0.74, P = 0.036) and relative 5-min OUES (R^2=0.63, P = 0.049). Changes in percent body fat and changes in relative 4-min OUES were unable to significantly account for changes in $\dot{V}O_{2max}$. The 5-min OUES was therefore chosen as the preferred time based OUES marker. The 5-min OUES requires the shortest exercise test time while illustrating significant associations with $\dot{V}O_{2max}$ across all investigations. Across the cross-sectional and longitudinal analyses, $OUES_{max}$ and 5-min OUES showed excellent agreement with no bias (Figure 2).

Prediction of $\dot{V}O_{2max}$ from 5-min OUES

A linear regression in the cohort who achieved $\dot{V}O_{2max}$ at baseline (n=32) produced the $\dot{V}O_{2max}$ prediction equation: 0.682 + 0.614 (5-min OUES) + 0.019 (Tanner stage of puberty), P < 0.001. The r value and standard error of the estimate for this equation was 0.88 and 0.37 L/min, respectively (Supplementary table 2).

DISCUSSION

Our findings demonstrate that the 5-min OUES accurately determines $\dot{V}O_{2max}$ in children with obesity. Furthermore, this is the first investigation to assess the association of longitudinal changes in the OUES with $\dot{V}O_{2max}$ in pediatric obesity.

We have shown that submaximal, 5-min OUES is highly associated with $\dot{V}O_{2max}$ in children with obesity. While this finding is novel, we also reported a strong relationship between $OUES_{max}$ and $\dot{V}O_{2max}$ which is supported by previous research in healthy-weight children and children with obesity (7, 13, 20). Several studies have shown that the OUES does not require a maximal exercise effort (2, 17, 31) and remains stable over the entirety of an exercise test (31). Importantly, the OUES is independent of participant effort, and is a reproducible measure of CRF with a low coefficient of variation (< 10%) in young, healthy adults (2). It is also capable of predicting and assessing exercise capacity in chronic disease (28, 31), and may be particularly valuable in populations limited by skeletal muscle deconditioning, pulmonary function and patient motivation (31). Past attempts to validate submaximal OUES have focused on specific percentages of total exercise time, i.e. 50% OUES, 75% OUES, 85% OUES, 90% OUES. Such studies found small differences (1.9–3.5%) between the OUES calculated at these percentages compared to $OUES_{max}$ (4, 17, 31). Similarly, we have shown that 5-min OUES, which equated to approximately 65% OUES in this cohort when related to mean exercise time, was highly associated with $OUES_{max}$. While previous findings emphasise that the OUES is independent of intensity, it does not provide a basis for a time based exercise test, which makes test termination difficult to specify. A time based exercise test can be communicated and provides a goal for the participant. Given the validity of submaximal OUES, evaluation of this marker at a given time, i.e. 5 minutes, is a plausible suggestion that is well supported by our findings.

We found that longitudinal changes in $OUES_{max}$ and 5-min OUES were strongly associated with changes in $\dot{V}O_{2max}$. In patients with chronic heart failure (24, 32) and coronary artery disease (11), the OUES was sensitive to exercise-training induced CRF change, and change in the OUES was most strongly associated with change in $\dot{V}O_{2peak}$ (r=0.61 – 0.77). Our investigation resulted in more robust longitudinal associations (r=0.82), which is likely due to all included participants achieving $\dot{V}O_{2max}$. A substantial proportion of children with obesity in our study were unable to attain $\dot{V}O_{2max}$ at one (65%) or both (86%) time-points. Therefore, a valid assessment of CRF through 5-min OUES holds particular importance. The efficacy of an exercise intervention is primarily assessed through changes in $\dot{V}O_{2max}$ and consistent pre-test preparation (diurnal variation, hydration, nutritional intake, sleep status) heightens the likelihood of observing true change. However, motivation is a psychological factor that is very difficult to replicate in two assessments separated by several months. Comparing $\dot{V}O_{2max}$ (plateau in $\dot{V}O_2$, and RER $\geq$ 1.05) to $\dot{V}O_{2peak}$ (criterion not achieved) is likely to skew results and hinder accurate interpretation of CRF change. Therefore, examination of 5-min OUES as a marker of CRF when $\dot{V}O_{2max}$ is not achieved each assessment, i.e. before and after an intervention, may provide a more valid representation of exercise training efficacy.

Examining longitudinal changes in the OUES required careful consideration of anthropometric variation. Due to participant growth over the intervention period, the OUES markers were normalised to body weight. Furthermore, change in percent body fat was entered in the multivariate linear regression model as a potential independent variable to account for longitudinal changes. It is currently unknown whether adjusting for a body composition fully equalizes the OUES. Marinov et al. (2007) examined the OUES in healthy 7–18 year old children and concluded that it was considerably dependent on anthropometric variables including height, weight, body surface area and BMI (21). These findings were

supported by Breithaupt et al. (2012) who observed that in a pediatric population with obesity, larger body mass resulted in increased absolute OUES values and linear anthropometric scaling was necessary (7). The OUES is also susceptible to changes in maturation, which is highly relevant in a pediatric population (29). Determinants of the OUES are influenced by the relative contribution of glycolytic and oxidative metabolism during exercise which varies by maturation and even with anthropometric normalisation, there was an inverse relationship between puberty stage and the OUES (29). Younger children rely more on oxidative metabolism and show enhanced oxygen extraction at the tissue level (29). In light of this, Tanner puberty stage was entered into the multivariate linear regression models as a potential independent variable.

The key strengths of this study lie in identification of a novel and valid index of CRF derived from a 5-min exercise test. The 5-min OUES allows exercise testing data to be utilised even if $\dot{V}O_{2max}$ is not achieved. Limitations are related to shortcomings inherent to the OUES, namely anthropometric and maturational confounding effects on the marker. While we attempted to account for these factors through the statistical approaches employed, there were methodological discrepancies in the assessment of body composition and Tanner stage of puberty between the two centres. However, importantly, the centres conducted identical assessments at each time-point. Secondly, as the OUES is influenced by glycolytic and oxidative metabolism, the exercise interventions, particularly HIIT, had the potential to modify metabolism and may have introduced additional variability in the statistical models. Furthermore, the time chosen for assessment of the submaximal OUES marker may not be applicable to significantly deconditioned clinical populations where individualised stress protocols are becoming more common than standardized protocols. Assessment of the 5-min OUES requires a minimum test duration of 9 minutes (if a 4 minute warm-up is used). While exercise test duration may vary with protocol and ergometer choice, the 5-min OUES should

remain applicable for exercise tests using the one-minute treadmill ramp protocol (Table 3). Given the small sample size of this study and assessment of the 5-min OUES using one exercise test protocol only, further studies are warranted to investigate the accuracy of the 5-min OUES in a large, heterogeneous paediatric and adult cohort using different exercise test protocols and modalities.

Application of the 5-min OUES treadmill protocol is highly relevant in the pediatric population, most notably for children who are sedentary, children with overweight or obesity, and children who have difficulty comprehending pre-test instructions, display behavioural problems or anxiety which may result in poor cooperation. We acknowledge that $\dot{V}O_{2max}$ remains the gold-standard assessment of CRF and children should be encouraged to reach a maximal effort. However, clinical judgement must be used to decide whether a maximal exercise test is appropriate. Coercing a child to exercise at a maximal effort could result in negative affect and may deter participation in future exercise. Clearly this could undermine any subsequent physical activity or exercise intervention and impact research outcomes or clinical practice. In this case, we propose that CRF may be assessed through a 5-min submaximal treadmill protocol. However, in our experience, a significant proportion of children are willing to attempt a maximal effort at the outset of a CRF assessment. In this situation, it would be reasonable to perform a maximal exercise treadmill protocol as outlined in Table 3, and use the 5-min OUES as the preferred marker of CRF if $\dot{V}O_{2max}$ is not attained.

CONCLUSION

In conclusion, our findings show that the 5-min OUES is a viable alternative to $\dot{V}O_{2max}$ when assessing children with obesity. The marker holds great utility in reflecting $\dot{V}O_{2max}$ in cross-sectional analyses and is sensitive to detect longitudinal changes in $\dot{V}O_{2max}$. The 5-min OUES should therefore be considered in exercise testing protocols for pediatric populations.

CONFLICTS OF INTEREST

Disclosures: Dr. Coombes reports grants from Coca Cola and Renew Corp, personal fees from Tolmar Pharmaceuticals and Novo Nordisk Pharmaceuticals, all outside the submitted work.

The remaining authors have no conflicts of interest relevant to this article to disclose.

ACKNOWLEDGEMENTS

Funding: This work was supported by St Olav's Hospital and The Norwegian University of Science and Technology [grant number #9527], Sports Medicine Australia Research Foundation, and The Wesley and St Andrew's Research Institute [grant number #2014-01].

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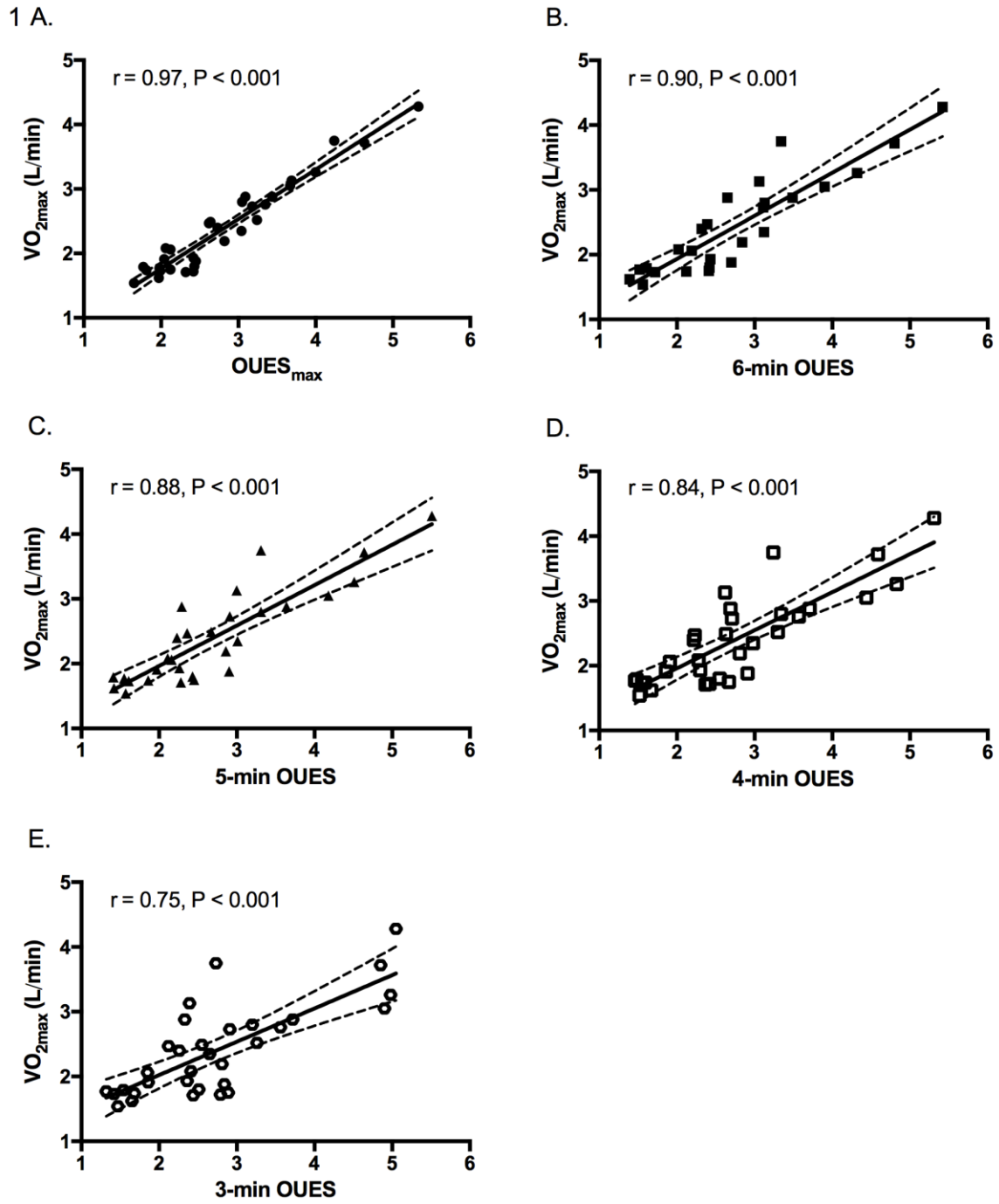

Figure 1. Linear relationship between $\dot{V}O_{2max}$ (L/min) and **A.** $OUES_{max}$, **B**. 6-min OUES, **C.** 5-min OUES, **D.** 4-min OUES, E. 3-min OUES at baseline. Dotted lines represent 95% confidence intervals. Error bars show standard deviation from the mean. No units for OUES.

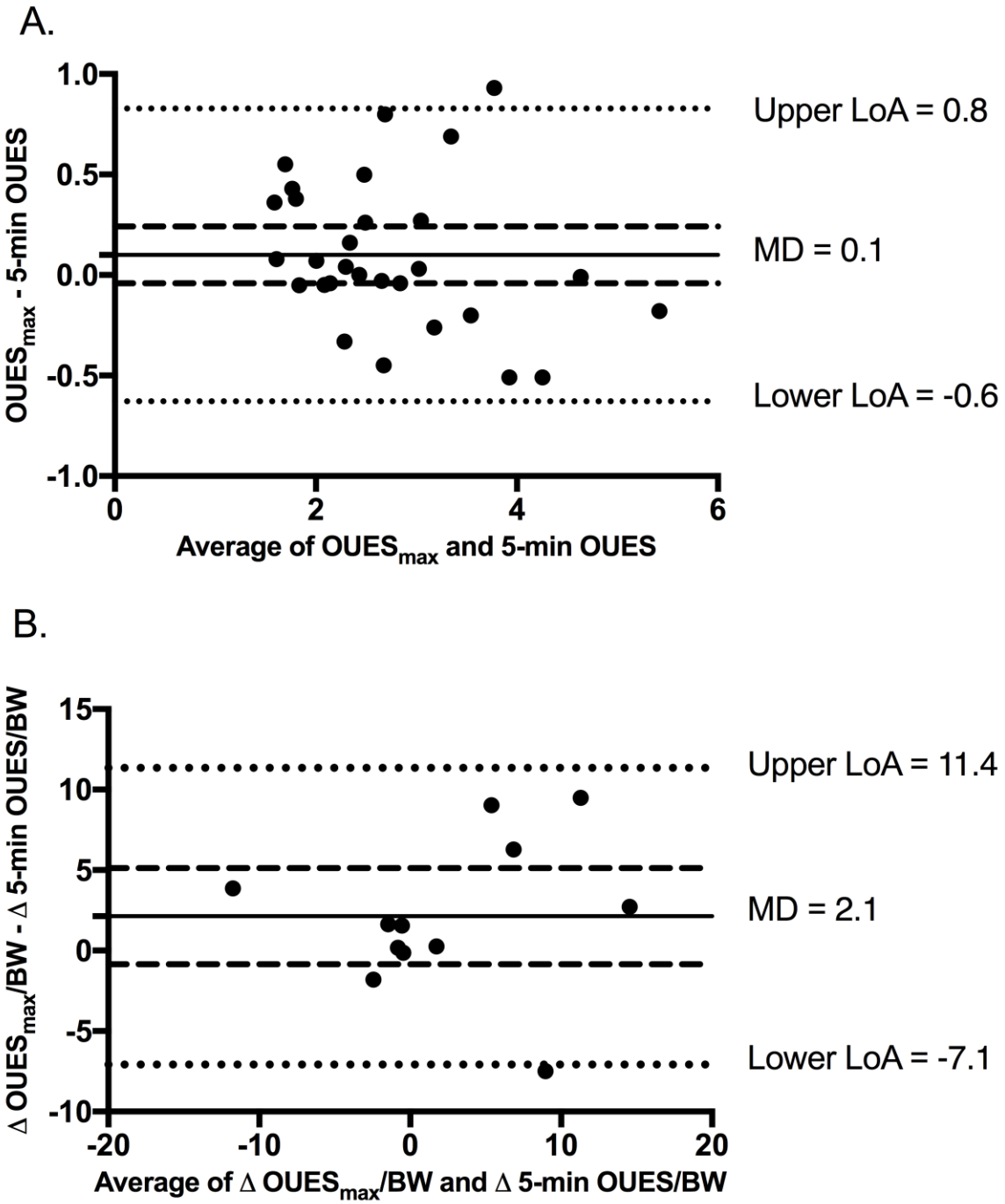

Figure 2. A. Bland-Altman plot comparing absolute $OUES_{max}$ (reference) with absolute 5-min OUES. **B**. Bland-Altman plot comparing change in relative $OUES_{max}$ (reference) with change in relative 5-min OUES. The plots show the mean difference between the two measures (solid line), 95 % confidence intervals of the mean difference (dashed line) and the upper and lower limits of agreement (dotted line). MD = mean difference; LoA = limits of agreement.

Table 1. Baseline clinical characteristics and cardiopulmonary exercise test responses in 1) participants who achieved $\dot{V}O_{2peak}$ at baseline, 2) participants who achieved $\dot{V}O_{2max}$ at baseline, and 3) participants who also achieved $\dot{V}O_{2max}$ after the 12-week intervention.

	$\dot{V}O_{2peak}$ at baseline (n = 60)	$\dot{V}O_{2max}$ at baseline (n = 32)	$\dot{V}O_{2max}$ pre and post intervention (n = 13)
Age, y	11.3 ± 2.3	12.8 ± 1.9*	13.5 ± 1.9
Sex, F (%)	56	56	62
Tanner stage of puberty	2 (1 – 3)	3 (2 – 4)	3 (2.5 – 4.5)
Height, cm	155.5 ± 11.8	160.0 ± 12.6	163.1 ± 12.1
Height z-score	1.15 ± 1.06	0.78 ± 0.96	0.70 ± 0.91
Weight, kg	72.2 ± 19.2	77.4 ± 18.5	80.7 ± 19.1
Weight z-score	2.37 ± 0.54	2.21 ± 0.51	2.13 ± 0.51
Body mass index, kg/m^2	29.4 ± 4.6	29.7 ± 3.9	29.9 ± 3.8
Body mass index z-score	2.16 ± 0.31	2.08 ± 0.28	2.02 ± 0.25
Body fat, %	43.6 ± 6.3	45.2 ± 6.2	43.4 ± 6.6
Rest			
Heart rate, bpm	88 ± 16	77 ± 9*	73 ± 12
Systolic blood pressure, mm Hg	113 ± 8	115 ± 8	113 ± 9
Diastolic blood pressure, mm Hg	63 ± 9	65 ± 6	65 ± 7
Maximal exercise			
Heart rate, bpm	192 ± 12	194 ± 10	193 ± 10
$\dot{V}O_{2max/peak}$#, L/min	2.28 ± 0.61	2.40 ± 0.71	2.48 ± 0.70
$\dot{V}O_{2max/peak}$#, mL/kg/min	31.9 ± 5.5	31.2 ± 5.5	31.2 ± 5.6
Minute ventilation L/min	76.1 ± 23.1	87.4 ± 20.3*	95.0 ± 17.1
Respiratory exchange ratio	1.01 ± 0.11	1.14 ± 0.07**	1.09 ± 0.10
Exercise time, min	7.94 ± 1.68	7.71 ± 1.89	7.83 ± 1.74

	$\dot{V}O_{2peak}$ at baseline (n = 60)	$\dot{V}O_{2max}$ at baseline (n = 32)	$\dot{V}O_{2max}$ pre and post intervention (n = 13)
Max speed, km/h	6.5 (5.5 – 7.0)	7.8 (6.5 – 12.0)*	7.5 (6.8 – 8.0)
Max gradient, %	12.0 (12.0 –14.0)	10.0 (8.0 –12.0)*	11.0 (10.0 –12.0)
$OUES_{max}$	2.93 ± 0.69	2.82 ± 0.89	2.91 ± 0.90
$OUES_{max}$ /BW	41.3 ± 7.8	36.6 ± 7.0*	36.3 ± 7.0
Submaximal exercise			
6-min OUES	2.79 ± 0.84	2.77 ± 1.01	3.08 ± 0.92
6-min OUES/BW	39.5 ± 7.9	36.3 ± 7.6	37.8 ± 6.9
% of $\dot{V}O_{2max/peak}^{\#}$ at 6-min OUES	75 ± 14	77 ± 14	77 ± 15
5-min OUES	2.77 ± 0.88	2.73 ± 1.03	2.95 ± 0.97
5-min OUES/BW	38.6 ± 8.5	35.4 ± 8.6	37.4 ± 7.7
% of $\dot{V}O_{2max/peak}^{\#}$ at 5-min OUES	65 ± 13	65 ± 15	64 ± 14
4-min OUES	2.71 ± 0.90	2.75 ± 1.00	2.95 ± 0.94
4-min OUES/BW	37.7 ± 9.0	35.5 ± 9.0	36.8 ± 7.9
% of $\dot{V}O_{2max/peak}^{\#}$ at 4-min OUES	52 ± 11	55 ± 15	54 ± 14
3-min OUES	2.68 ± 0.90	2.73 ± 1.04	2.89 ± 0.90
3-min OUES/BW	37.4 ± 9.4	35.4 ± 10.3	36.1 ± 7.6
% of $\dot{V}O_{2max/peak}^{\#}$ at 3-min OUES	39 ± 8	42 ± 12	40 ± 10

Data presented as mean ± SD, or median (IQR). * $P < 0.05$; ** $P < 0.001$; P values signify between-group differences for participants who achieved $\dot{V}O_{2peak}$ at baseline compared to participants who achieved $\dot{V}O_{2max}$ at baseline.

$\dot{V}O_{2max}$, maximal oxygen uptake; $\dot{V}O_{2peak}$, peak oxygen uptake; OUES, oxygen uptake efficiency slope; $OUES_{max}$, maximal OUES; OUES/BW, oxygen uptake efficiency slope normalised to body weight. $^{\#}$ $\dot{V}O_{2peak}$ for n = 60

Table 2. Univariate and multivariate linear regression to examine the predictive value of OUES markers for $\dot{V}O_{2max}$ at baseline (n = 32) and change in $\dot{V}O_{2max}$ (n = 13). Model 1 accounts for Tanner **stage of puberty** and sex at baseline, while Model 2 accounts for change in percent body fat.

Variable	r value	β	Standard Error	P value
Unadjusted Model (n = 32)				
$\dot{V}O_{2max}$				
$OUES_{max}$	0.97	0.769	0.038	<0.001
6-min OUES	0.90	0.668	0.066	<0.001
5-min OUES	0.88	0.626	0.067	<0.001
4-min OUES	0.84	0.589	0.070	<0.001
3-min OUES	0.75	0.569	0.092	<0.001
Variable	**R^2 value**	**β**	**Standard Error**	**P value**
Model 1 adjusted for Tanner stage of puberty and sex (n = 32)				
$\dot{V}O_{2max}$				
$OUES_{max}$	0.93	0.771	0.052	<0.001
6-min OUES	0.83	0.611	0.082	<0.001
5-min OUES	0.80	0.567	0.079	<0.001
4-min OUES	0.74	0.512	0.084	<0.001
3-min OUES	0.63	0.430	0.108	<0.001
Variable	**r value**	**β**	**Standard Error**	**P value**
Unadjusted Model (n = 13)				
Δ $\dot{V}O_{2max}$/BW				
Δ $OUES_{max}$/BW	0.82	0.590	0.126	0.001
Δ 6-min OUES/BW	0.82	0.528	0.132	0.004

Δ 5-min OUES/BW	0.76	0.602	0.163	0.004
Δ 4-min OUES/BW	0.74	0.588	0.162	0.004
Δ 3-min OUES/BW	0.44	0.310	0.192	0.134

Variable	**R^2 value**	**β**	**Standard Error**	**P value**
Model 2 adjusted for change in percent body fat (n = 13)				
Δ $\dot{V}O_{2max}$/BW				
Δ $OUES_{max}$/BW	0.83	0.599	0.118	0.005
Δ 6-min OUES/BW	0.74	0.523	0.148	0.036
Δ 5-min OUES/BW	0.63	0.636	0.218	0.049
Δ 4-min OUES/BW	0.61	0.708	0.254	0.058
Δ 3-min OUES/BW	0.25	0.333	0.323	0.428

$\dot{V}O_{2max}$, maximal oxygen uptake; $OUES_{max}$, maximal OUES; OUES, oxygen uptake efficiency slope; OUES/BW; oxygen uptake efficiency slope normalised to body weight.

Table 3. Protocol for 5-min OUES measurement and calculation procedures from a maximal cardiopulmonary exercise test.

Participant evaluation	• If the participant is willing to attempt a maximal effort, then follow the full exercise protocol below. • If the participant is non-cooperative, showing signs of anxiety or has behavioural difficulties, then follow the exercise protocol outlined but terminate the test at 5-minutes (5-min submaximal protocol). • A 4-minute warm up should be performed; total exercise test time for the 5-min submaximal protocol will be 9 minutes.
Exercise protocol	• Treadmill, ramp protocol consisting of 1-minute stages. • Warm up: 4 minutes, 4 km/hr • Start of protocol: 4 km/hr, 2 % gradient • Increase in gradient (2 %) each minute up to 12% (< 12 years) or up to 16 % (≥ 12 years) • Increase in speed (1km/h) each minute until volitional exhaustion
Gas analysis system	Continuous breath-by-breath
Post-processing epoch length	5-min OUES should be calculated using 10-second epochs
OUES calculation	1. Plot log$\dot{V}$E (L/min) against $\dot{V}O_2$ (L/min) for each 10 second epoch from the start of the test to 5 minutes (warm up should be excluded). 2. Apply a linear trendline to produce the following equation: $\dot{V}O_2 = a$ (log$\dot{V}$E) + b where OUES is defined by *a*.
Interpretation	• If $\dot{V}O_{2max}$ is reached, this should be used as the gold-standard measure of CRF. • If $\dot{V}O_{2max}$ is not attained or if a 5-min submaximal protocol was performed, 5-min OUES should be calculated and used as the measure of CRF.

Supplementary Table 1. Changes in anthropometric and cardiopulmonary exercise test responses in participants who reached $\dot{V}O_{2max}$ pre- and post-intervention (n=13).

Variable	
Height, cm	1.3 ± 0.9*
Weight, kg	0.5 ± 4.1
BMI, kg/m^2	-0.3 ±1.6
Body fat, %	-1.5 ± 3.0
Maximal exercise	
HR, bpm	-0.8 ± 4.9
Absolute oxygen uptake, $L \cdot min^{-1}$	0.09 ± 0.49
Relative oxygen uptake, $mL \cdot kg^{-1} \cdot min^{-1}$	2.85 ± 5.49
Minute ventilation ($\dot{V}E$) $L \cdot min^{-1}$	6.5 ± 15.2
Respiratory exchange ratio	-0.02 ± 0.12
Exercise time, min	-0.32 ± 1.34
Max OUES	0.27 ± 0.64
Max OUES/BW	3.4 ± 7.6
Submaximal exercise	
6-min OUES	0.25 ± 0.71
6-min OUES/BW	3.6 ± 8.5
% of $\dot{V}O_{2max}$ at 6-min OUES	0 (-7-4)
5-min OUES	0.09 ± 0.55
5-min OUES/BW	1.6 ± 7.2
% of $\dot{V}O_{2max}$ at 5-min OUES	1.7 (-5-11)
4-min OUES	0.07 ± 0.51
4-min OUES/BW	1.6 ± 6.9
% of $\dot{V}O_{2max}$ at 4-min OUES	0 (-4-7)
3-min OUES	0.11 ± 0.57
3-min OUES/BW	2.40 ± 7.78
% of $\dot{V}O_{2max}$ at 3-min OUES	0 (-3-5)

Data are presented as mean ± SD or median (IQR). F, female; HR, heart rate; BP, blood pressure; OUES, oxygen uptake efficiency slope; OUES/BW, oxygen uptake efficiency slope normalised to body weight. *p<0.001 for within-group t-test.

Supplementary Table 2. Bootstrap of coefficients for linear regression analysis derived from participants who achieved $\dot{V}O_{2max}$ at baseline (n = 32).

	β	Standard Error	P-value	95% Confidence Interval	
				Lower	Upper
Constant	0.682	0.159	0.001	0.321	0.959
5-min OUES	0.614	0.064	0.001	0.466	0.708
Tanner Puberty Stage	0.019	0.061	0.729	-0.081	0.158

OUES, oxygen uptake efficiency slope