Peak oxygen uptake cut-points to identify children at increased cardiometabolic risk - The PANIC Study

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Abstract

We aimed to develop cut-points for directly measured peak oxygen uptake ($\dot{V}O_{2peak}$) to identify boys and girls at increased cardiometabolic risk using different scaling methods to control for body size and composition. Altogether 352 children (186 boys, 166 girls) aged 9–11 years were included in the analyses. We measured $\dot{V}O_{2peak}$ directly during a maximal cycle ergometer exercise test and lean body mass (LM) by bioelectrical impedance. We computed a sex- and age-specific cardiometabolic risk score (CRS) by summing important cardiometabolic risk factors and defined increased cardiometabolic risk as >1 standard deviation above the mean of CRS. Receiver operating characteristics curves were used to detect $\dot{V}O_{2peak}$ cut-points for increased cardiometabolic risk. Boys with $\dot{V}O_{2peak}$ <45.8 mL·kg body mass $(BM)^{-1}\cdot min^{-1}$ (95% confidence interval [CI] = 45.1 to 54.6, area under the curve [AUC] = 0.86, p<0.001) and <63.2 $mL\cdot kg\ LM^{-1}\cdot min^{-1}$ (95% CI = 52.4 to 67.5, AUC = 0.65, p=0.006) had an increased CRS. Girls with $\dot{V}O_{2peak}$ <44.1 $mL\cdot kg\ BM^{-1}\cdot min^{-1}$ (95% CI = 44.0 to 58.6, AUC = 0.67, p=0.013) had an increased CRS. $\dot{V}O_{2peak}$ scaled by $BM^{-0.49}$ and $LM^{-0.77}$ derived from log-linear allometric modelling poorly predicted increased cardiometabolic risk in boys and girls. In conclusion, directly measured $\dot{V}O_{2peak}$ <45.8 $mL\cdot kg\ BM^{-1}\cdot min^{-1}$ among boys and <44.1 $mL\cdot kg\ BM^{-1}\cdot min^{-1}$ among girls were cut-points to identify those at increased cardiometabolic risk. Appropriately controlling for body size and composition reduced the ability of cardiorespiratory fitness to identify children at increased cardiometabolic risk. Keywords: Aerobic fitness, Metabolic health, Adiposity, Children, Allometric scaling, Maximal exercise

1 INTRODUCTION

An increased cardiometabolic risk in childhood has been associated with an elevated risk of metabolic syndrome and type 2 diabetes, increased arterial stiffness, endothelial dysfunction, and preclinical carotid atherosclerosis in adulthood.[1–4] Therefore, the early identification of children with increased cardiometabolic risk is important to prevent cardiometabolic diseases in adulthood. Decreased cardiorespiratory fitness (CRF), independent of the levels of physical activity, has been considered a strong determinant of increased cardiometabolic risk in children and adolescents.[5–9] However, only a few studies have investigated cut-points for CRF to identify children at increased cardiometabolic risk.[10–17]

Previous studies have suggested that peak oxygen uptake ($\dot{V}O_{2peak}$) lower than 44.0 mL·kg body mass (BM)$^{-1}$·min^{-1} in boys and lower than 39.5 mL·kg BM^{-1}·min^{-1} in girls are indicative of increased cardiometabolic risk.[10–12,17] Furthermore, the most recent FITNESSGRAM guidelines suggest that boys with $\dot{V}O_{2peak}$ 37.3 to 41.2 mL·kg BM^{-1}·min^{-1} and girls with $\dot{V}O_{2peak}$ 35.3 to 37.3 mL·kg BM^{-1}·min^{-1} depending on their age have an increased risk of metabolic syndrome.[13,18] However, these studies have measured workload or heart rate during a submaximal treadmill or cycle ergometer exercise test, a stage reached during a 20-meter shuttle run test, or other types of exercise tests and converted these measures of performance into an estimate of $\dot{V}O_{2peak}$[10–13,15,17] instead of measuring $\dot{V}O_{2peak}$ directly during a maximal exercise test continued until exhaustion. Estimated $\dot{V}O_{2peak}$ obtained from these types of exercise tests is problematic in that it has, at best, 50% agreement with directly measured $\dot{V}O_{2peak}$.[19] Furthermore, $\dot{V}O_{2peak}$ thresholds obtained from these studies are based on $\dot{V}O_{2peak}$ divided by BM that is confounded by body fat content and may invalidate $\dot{V}O_{2peak}$ as a measure of CRF in children with increased body mass and particularly adiposity.[10–13,17,20–22]

Allometric scaling of $\dot{V}O_{2peak}$ by measures of body size and composition using log-linear regression can partly overcome the problems related to scaling of $\dot{V}O_{2peak}$ by BM using the ratio standard method.[23] Nonetheless, allometrically scaled $\dot{V}O_{2peak}$ for lean body mass (LM) is regarded superior to allometrically scaled $\dot{V}O_{2peak}$ for BM in order to account for variance in body fat content in the expression of $\dot{V}O_{2peak}$ among children and adolescents.[24–29] Thus, allometrically scaled $\dot{V}O_{2peak}$ by LM has been recommended as the best approach in expressing $\dot{V}O_{2peak}$ among children and adolescents.[29] However, there are few studies on the associations of CRF with cardiometabolic risk having appropriately controlled for body size and composition using the allometric methods or the ratio standard methods.[22] Using these approaches has attenuated the associations of CRF with cardiometabolic risk,[22] suggesting that CRF expressed in these manners has inferior predictive power compared to CRF scaled by BM.

The aim of this study was to provide cut-points for $\dot{V}O_{2peak}$ measured directly during a maximal cycle ergometer exercise test among boys and girls to identify those who are at increased cardiometabolic risk. We used different methods for scaling $\dot{V}O_{2peak}$ to control for body size and composition.

2 METHODS

2.1 Study design and study population

The Physical Activity and Nutrition in Children (PANIC) Study is an ongoing physical activity and dietary intervention study (ClinicalTrials.gov NCT01803776) in a population sample of primary school children living in the city of Kuopio, Finland. Altogether 736 children 6–9 years of age who had been registered for the first grade in one of the 16 public schools of the city of Kuopio were invited for baseline examinations in 2007–2009.

Altogether 512 children (248 girls, 264 boys), who accounted for 70% of those invited, participated in the baseline examinations in 2007–2009. The participants did not differ in sex

distribution, age, or body mass index standard deviation score (BMI-SDS) from all children who started the first grade in the city of Kuopio in 2007–2009 based on data from the standard school health examinations performed for all Finnish children before the first grade.[30] The present analyses are based on the 2-year follow-up data. We had complete data on variables needed in the analyses for 352 children (186 boys, 166 girls) 9–11 years of age. Of these children, 99.1% are Caucasians.

The PANIC Study protocol was approved by the Research Ethics Committee of the Hospital District of Northern Savo. A written informed consent was acquired from the parent or caregiver of each child and every child provided assent to participation.

2.2 Assessment of cardiorespiratory fitness

We assessed CRF by a maximal exercise test using the Ergoselect 200 K® electromagnetic cycle ergometer coupled with a paediatric saddle module (Ergoline, Bitz, Germany), as explained in detail earlier.[30] The children and their parents and caregivers were informed about the exercise test in the invitation letter. A research nurse and a research physician gave the children instructions on how to perform the exercise test. The children were familiarised with the exercise test protocol two years earlier during baseline examination. They were also allowed to practice cycling with the ergometer and using the paediatric mask 10 minutes before the exercise test. The exercise test protocol, supervised by the research physician and assisted by the research nurse, included a 2.5-minute anticipatory period with the child sitting on the ergometer; a 3-minute warm-up period with a workload of five watts; a 1-minute steady-state period with a workload of 20 watts; an exercise period with an increase in the workload of one watt per six seconds until exhaustion, and a 4-minute recovery period with a workload of five watts.

The children were asked to keep the cadence stable and within 70–80 revolutions per minute. The children were verbally encouraged to exercise until voluntary exhaustion. Heart rate was measured continuously throughout the exercise test using a 12-lead electrocardiogram (ECG) registered by the Cardiosoft® V6.5 Diagnostic System (GE Healthcare Medical Systems, Freiburg, Germany). The exercise test was considered maximal if the peak heart rate was at least 185 beats per minute and the respiratory exchange ratio was at least 1.0.[31] However, the research physician also adjudged the exercise test maximal among 21 (6%) children with a peak heart rate of 179-184 beats per minute, because the cadence dropped below 65 revolutions per minute although the children still had the motivation to continue and the reason for terminating the test suggested a maximal effort had been provided.[30] The peak workload was defined as the workload at the end of the exercise test.

The respiratory gas analysis was performed during the exercise test from the beginning of the 2.5-minute anticipatory period before the exercise test to the end of the 4-minute recovery period after the exercise test using the Oxycon Pro® respiratory gas analyzer (Jaeger, Hoechberg, Germany) and the Hans-Rudolph® paediatric mask (Shawnee, Kansas, USA). $\dot{V}O_{2peak}$ was measured using the breath-by-breath method and was averaged over consecutive 15-second periods. $\dot{V}O_{2peak}$ and the respiratory exchange ratio were defined as the highest 15-seconds average values recorded during the last minute of the exercise test.[30]

2.3 Assessment of cardiometabolic risk factors

Cardiometabolic risk factors were assessed in the morning for two children, first of them arriving at 08:00 and second at 09:15. The research nurse measured body height, body weight, and waist circumference using standard protocols.[32] Body mass index (BMI) was calculated by dividing body weight with body height squared and BMI-SDS using national references.[33] The prevalence of overweight and obesity was defined using age- and sex-specific cut-points.[33]

Total body fat mass, body fat percentage (BF%), and LM were measured twice; the children having fasted for 12 hours, voided the bladder; and standing in light underwear, using the InBody® 720 bioelectrical impedance device (Biospace, Seoul, South Korea). We have found a good agreement between BF% and LM measured with bioelectrical impedance and those derived from dual-energy X-ray absorptiometry.[34] The research nurse measured blood pressure manually from the right arm by a calibrated Heine 130 Gamma G7® aneroid sphygmomanometer (Heine Optotechnik, Herrsching, Germany). The measurement protocol included a 5-minute rest and thereafter three measurements in a sitting position at 2-minute intervals. The mean of all three values was used as the systolic and diastolic blood pressure. The research nurse took venous blood samples using a standard protocol after a 12-hour fast and the children having seated for 10 minutes. The assessment of serum insulin and plasma glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides, have been explained in detail earlier.[35]

2.4 Assessment of puberty

The research physician assessed pubertal status using a 5-stage scale described by Tanner.[36,37] Boys were defined having entered clinical puberty if their testicular volume assessed by an orchidometer was ≥4 mL (Tanner stage ≥2).[37] Girls were defined having entered clinical puberty if their breast development had started (Tanner stage ≥2).[36]

2.5 Calculation of cardiometabolic risk score

We calculated a continuous cardiometabolic risk score using population-specific and age- and sex-standardized Z-scores for waist circumference, insulin, glucose, HDL cholesterol, triglycerides, and the average of systolic and diastolic blood pressure by a formula: waist circumference + insulin + glucose - HDL cholesterol + triglycerides + the average of systolic and diastolic blood pressure.[38] We defined elevated cardiometabolic risk as ≥1 standard

deviation above the mean of the cardiometabolic risk score in the present study population. The rationale for using this approach is the existing evidence on the ability of a continuous cardiometabolic risk score in children to predict cardiometabolic diseases in adulthood.[39] The clustering of risk factors may also provide a more sensitive and clinically more relevant evaluation of increased cardiometabolic risk than using individual risk factors,[40,41] and may compensate for day-to-day fluctuations observed in the levels of individual risk factors.[42]

2.6 Allometric scaling of peak oxygen uptake

Allometric scaling of $\dot{V}O_{2peak}$ was performed by the log-linear regression model[43] with sex and BM or LM as independent variables and $\dot{V}O_{2peak}$ as a dependent variable. The scaling exponent (b) was identified in the allometric equation $\dot{V}O_{2peak} = Y/X^b$, where X is the anthropometric scaling variable (BM or LM). $\dot{V}O_{2peak}$ and X were log transformed and least squares regression with the equation ln ($\dot{V}O_{2peak}$) = lnY / b ln(X) was used to obtain the scaling exponent b. We found that the scaling exponent b for BM was 0.49 (95% confidence interval [CI] = 0.43 to 0.55) and that for LM was 0.77 (95% CI = 0.70 to 0.85). To test if the slopes of the association of $\dot{V}O_{2peak}$ with BM and LM were similar in boys and girls, we added the interaction term to the model. The interactions of sex with BM ($p = 0.362$) and LM ($p = 0.932$) were not statistically significant. These allometric models were able to remove the associations of $\dot{V}O_{2peak}$ with BM ($r = -0.072$, $p = 0.328$) and LM ($r = 0.022$, $p = 0.939$), indicating their validity in scaling CRF.

2.7 Statistical methods

Student's t-test, Mann–Whitney U test, and Chi-square test were used to compare basic characteristics between boys and girls. The associations of measures of CRF with the cardiometabolic risk score were studied using linear regression analyses. The associations were adjusted for age and study group and additionally for puberty. Receiver operating

characteristics (ROC) curves were used to investigate $\dot{V}O_{2peak}$ cut-points associated with increased cardiometabolic risk. We decided not to provide cut-points for allometrically scaled $\dot{V}O_{2peak}$ because they would depend on the scaling exponent b that is specific to our study population. Moreover, a recent review has highlighted that no general scaling exponent b is available.[29] The area under the curve (AUC) was used as a measure of the effectiveness of the predictor variable to identify correctly children having a cardiometabolic risk score ≥ +1 SD (sensitivity) and to identify correctly children having a cardiometabolic risk score <1 SD (specificity). An AUC of 1.0 represents the ability to identify perfectly children having a cardiometabolic risk score ≥ +1 SD from other children, whereas an AUC of 0.5 indicates no greater predictive ability than chance. We also compared the cardiometabolic risk score in the categories of $\dot{V}O_{2peak}$ scaled by BM and LM among boys and girls combined using analysis of covariance with Sidak correction adjusted for age and the study group. In these analyses, we used sex-specific categories of $\dot{V}O_{2peak}$ scaled by BM and LM according to the distributions of these variables [very low (<2.5 %), low (2.5–15.9 %), medium (16–83.9 %), high (84–97.5 %), very high (>97.5 %)] as defined in our earlier study in the same paediatric population.[30] We combined children in the two highest categories because of the small number of children in the highest category to increase statistical power in these analyses. Student's t-tests, Mann–Whitney U tests, Chi-square tests, and linear regression analyses were conducted using the SPSS statistics software, version 23.0 (IBM Corp, Armonk, NY, USA). ROC curve analyses were performed using the MedCalc® statistical software, version 16.1 (MedCalc® Software bvba, Ostend, Belgium). Our power calculation provided a sample size of 352 children. In this calculation, we determined the effect size that corresponds to the power of 0.8 at 0.05 alpha to test differences in the associations of CRF within sex-specific categories and cardiometabolic risk. Cohen's *d* effect size for F-test ($d = 0.177$) was derived using the G*Power statistical

software, version 3.1.9.2. Cohen's *d* effect size for F-test is interpreted as small for $d = 0.1$, medium for $d = 0.25$, and large for $d = 0.4$.

3 RESULTS

3.1 Characteristics of children

Boys had more LM, less fat mass, a lower BF%, a higher waist circumference, lower insulin, higher glucose, higher HDL cholesterol, and higher $\dot{V}O_{2peak}$ scaled by BM and LM compared to girls (Table 1).

3.2 Associations of peak oxygen uptake with cardiometabolic risk

Absolute $\dot{V}O_{2peak}$ expressed in $mL \cdot min^{-1}$ was directly associated with cardiometabolic risk in boys and girls adjusted for age and the study group (Table 2). $\dot{V}O_{2peak}$ scaled by BM and LM were inversely related to cardiometabolic risk in boys and girls after these adjustments. Allometrically scaled $\dot{V}O_{2peak}$ expressed in $mL \cdot kg\ BM^{-0.49} \cdot min^{-1}$ and $mL \cdot kg\ LM^{-0.77} \cdot min^{-1}$ were also inversely associated with cardiometabolic risk in boys. Moreover, allometrically scaled $\dot{V}O_{2peak}$ expressed in $mL \cdot kg\ LM^{-0.77} \cdot min^{-1}$, but not allometrically scaled $\dot{V}O_{2peak}$ expressed in $mL \cdot kg\ BM^{-0.49} \cdot min^{-1}$, was associated with cardiometabolic risk among girls. These relationships in boys and girls were unaltered after additional adjustment for puberty (data not shown).

3.3 Peak oxygen uptake in identifying children with increased cardiometabolic risk

In boys, $\dot{V}O_{2peak}$ less than 45.8 $mL \cdot kg\ BM^{-1} \cdot min^{-1}$ and $\dot{V}O_{2peak}$ less than 63.2 $mL \cdot kg\ LM^{-1} \cdot min^{-1}$ were associated with increased cardiometabolic risk. Allometrically scaled $\dot{V}O_{2peak}$ expressed in $mL \cdot kg\ BM^{-0.49} \cdot min^{-1}$ and in $mL \cdot kg\ LM^{-0.77} \cdot min^{-1}$ also differentiated boys with increased cardiometabolic risk (Table 3).

In girls, $\dot{V}O_{2peak}$ less than 44.1 mL·kg BM^{-1}·min^{-1} was associated with increased cardiometabolic risk. However, neither $\dot{V}O_{2peak}$ scaled by LM nor allometrically scaled $\dot{V}O_{2peak}$ for BM or LM was able to differentiate girls with increased cardiometabolic risk.

3.4 Cardiometabolic risk among children in categories of peak oxygen uptake

Cardiometabolic risk decreased in a dose-dependent manner with increasing categories of $\dot{V}O_{2peak}$ expressed in mL·kg BM^{-1}·min^{-1} among children ($p<0.001$ for linear trend) (Figure 1). Moreover, children in the highest category of $\dot{V}O_{2peak}$ expressed in mL·kg LM^{-1}·min^{-1} also had a lower cardiometabolic risk than children in other categories of $\dot{V}O_{2peak}$ (Figure 1).

DISCUSSION

We found a strong inverse association between directly measured $\dot{V}O_{2peak}$ scaled by BM and cardiometabolic risk among boys and girls. $\dot{V}O_{2peak}$ less than 45.8 mL·kg BM^{-1}·min^{-1} in boys and 44.1 mL·kg BM^{-1}·min^{-1} in girls was associated with increased cardiometabolic risk with moderate sensitivity and specificity. However, the inverse associations of $\dot{V}O_{2peak}$ scaled by LM or $\dot{V}O_{2peak}$ scaled by $BM^{-0.49}$ and $LM^{-0.77}$ derived from log-linear allometric modelling with cardiometabolic risk were markedly weaker. $\dot{V}O_{2peak}$ less than 63.2 mL·kg LM^{-1}·min^{-1} was linked to increased cardiometabolic risk in boys, although sensitivity was poor. Furthermore, $\dot{V}O_{2peak}$ scaled by allometric methods was able to differentiate boys at increased cardiometabolic risk with adequate sensitivity but poor specificity. Neither $\dot{V}O_{2peak}$ scaled by LM nor allometrically scaled $\dot{V}O_{2peak}$ for BM or LM was able to differentiate girls with increased cardiometabolic risk.

Our results are in agreement with previous findings that CRF measured in exercise test laboratories or using field tests and scaled by BM using the ratio standard method had a strong inverse association with cardiometabolic risk in children.[10–12] However, the inverse relationship between CRF scaled by BM and cardiometabolic risk is partly confounded by

adiposity, because CRF divided by BM is a measure of both CRF and body fat content. We observed that using $\dot{V}O_{2peak}$ scaled by LM or allometric scaling of $\dot{V}O_{2peak}$ for BM or LM instead of $\dot{V}O_{2peak}$ scaled by BM attenuated the magnitude of the inverse association between CRF and cardiometabolic risk by 50-75%. These results are in consonance with the observation that estimated $\dot{V}O_{2peak}$ scaled by BM provided spurious associations with cardiometabolic risk among children and the view that $\dot{V}O_{2peak}$ scaled allometrically or by fat free mass would be a better measure to estimate the magnitude of the association between CRF and cardiometabolic risk.[22] Notwithstanding, the measures of CRF scaled by LM may also be influenced by adiposity because individuals with higher fat mass also have higher LM.[44] In the present cross-sectional study, adjusting for puberty had no effect on the relationships between $\dot{V}O_{2peak}$ and cardiometabolic risk in boys or girls. Therefore, longitudinal studies are needed to clarify the role of CRF in cardiometabolic health during growth and maturation. It is also important to note that CRF is strongly influenced by genetic factors[45] and some genetic variants have been reported to modify the relationship between CRF and cardiometabolic risk factors, such as adiposity, insulin resistance, and elevated blood pressure.[45] In our study, genetics may play a role in the inverse association between CRF controlled for body fat and cardiometabolic risk.[46]

The cut-point for $\dot{V}O_{2peak}$ of 45.8 mL·kg BM^{-1}·min^{-1} to identify boys aged 9-11 years at increased cardiometabolic risk in our study corresponds well with the previously reported cut-point for $\dot{V}O_{2peak}$ of 43.6 mL·kg BM^{-1}·min^{-1} among boys aged 8-11 years.[10] However, the cut-point for $\dot{V}O_{2peak}$ of 44.1 mL·kg BM^{-1}·min^{-1} to identify girls aged 9-11 years at increased cardiometabolic risk in our study was notably higher than the cut-point for $\dot{V}O_{2peak}$ of 37.0 mL·kg BM^{-1}·min^{-1} observed among girls aged 9-10 years in a previous study.[15] The reason for a higher threshold for $\dot{V}O_{2peak}$ among girls in our study than in the earlier study may be that Finnish girls aged 9-11 years are more fit than girls of the same age in other paediatric populations. However, Welk and co-workers[13] found that the cut-point for estimated $\dot{V}O_{2peak}$

to identify children aged 10-11 years at increased cardiometabolic risk was 40.2 mL·kg BM^{-1}·min^{-1} in both sexes. The diversity in cut-points may also be due to the different age ranges of children because most of the earlier studies have reported pooled data of various age groups.[10,11,14] There is some evidence that the cut-point for $\dot{V}O_{2peak}$ decreases with increasing age in girls, whereas it remains relatively stable in boys.[31] One explanation for this sex difference may be that body fat content increases more in girls than in boys during maturation that introduces more confounding by adiposity in the measurement of CRF in girls.[47] Another reason for the array of cut-points for $\dot{V}O_{2peak}$ scaled by BM may be that the prevalence of overweight has varied among the study populations.[13,23] Moreover, different assessments of CRF and cardiometabolic risk factors and different definitions of increased risk may explain the incongruence in CRF cut-points among these studies.[10–13,17] In consonance with earlier studies,[10,12,15] the prevalence of increased cardiometabolic risk defined by ≥ +1 SD of the cardiometabolic risk score in our study was 25% among boys and 34% in girls.

We found moderate sensitivity and specificity of the measures of CRF in predicting increased cardiometabolic risk which corresponds to those of previous studies.[10–12,15] Sensitivity for $\dot{V}O_{2peak}$ scaled by BM was 75% in boys and 69% in girls. This sex difference may be due to a stronger inverse association between CRF and cardiometabolic risk in boys than in girls.[13] Nonetheless, the false positive rate would be too high for screening children with increased cardiometabolic risk using $\dot{V}O_{2peak}$ scaled by BM. There is a trade-off between false-positive and false-negative rates. Classification accuracy may lead to the problem of fictitious interpretation when applying cut-points with a high false positive rate than those with a high false negative rate. Improving CRF may decrease cardiometabolic risk, however, a large number of false positive cases would result in failure to correctly identify children at increased risk, in contrast to recommending increased physical activity to improve CRF in false negative cases.[10] It is important that children are not subject of the social stigma associated with being

276 erroneously classified as being at increased cardiometabolic risk. In addition, it is better to err
277 on the side of caution so that children who truly are at increased risk are not deprived of health
278 care.[10]

279 Loftin and co-workers suggested that $\dot{V}O_{2peak}$ should be allometrically scaled for LM due to
280 the involvement of skeletal muscle in locomotion.[29] We found that $\dot{V}O_{2peak}$ scaled by LM had
281 worse ability to differentiate boys with increased cardiometabolic risk than $\dot{V}O_{2peak}$ scaled by
282 BM. Neither $\dot{V}O_{2peak}$ scaled allometrically nor $\dot{V}O_{2peak}$ scaled by LM could differentiate girls
283 with increased cardiometabolic risk. Appropriately scaled CRF resulted in a poor prediction of
284 cardiometabolic risk; whereas, CRF scaled by BM using the ratio standard method, was a better
285 predictor of cardiometabolic risk. The reason for this is that CRF scaled by BM combines the
286 information from these two measures such that both decreased CRF and increased weight
287 and/or body fat content are associated with increased cardiometabolic risk. Proposing age-
288 specific cut-points for CRF scaled by BM using the ratio standard method offers clear
289 diagnostic utility in identifying children at increased cardiometabolic risk, which tracks well
290 from childhood into adulthood.[39] There was a linear decrease in cardiometabolic risk with
291 increasing categories of $\dot{V}O_{2peak}$ scaled by BM. Similarly, children in the highest category of
292 $\dot{V}O_{2peak}$ scaled by LM had reduced cardiometabolic risk compared to other children. However,
293 the differences in cardiometabolic risk across the categories of $\dot{V}O_{2peak}$ scaled by LM were
294 markedly smaller than those of $\dot{V}O_{2peak}$ scaled by BM.

295 The strengths of this study include a large population sample of children 9-11 years of age, the
296 direct assessment of $\dot{V}O_{2peak}$, and the use of $\dot{V}O_{2peak}$ scaled allometrically for BM and LM. Our
297 study provides a robust threshold for $\dot{V}O_{2peak}$ scaled by BM in these children aged 9-11 years,
298 however, we cannot extrapolate our findings to other age groups. Our study participants were
299 Caucasian children, so the cut-points may not be generalised to children of different ethnic
300 groups. A limitation of the study is its cross-sectional design that does not allow us to arrive at

a conclusion regarding the causality of the association between CRF and cardiometabolic risk. Therefore, longitudinal studies are warranted in order to investigate whether a decrease in adiposity-independent measures of CRF is associated with an increase in cardiometabolic risk over time among children and adolescents. In addition, it would be important to provide evidence for the effects of growth and maturation on the cut-points for CRF using different methods to scale CRF for body size and composition.

In conclusion, we found that directly measured $\dot{V}O_{2peak}$ less than 45.8 mL·kg BM^{-1}·min^{-1} in boys 9-11 years of age and less than 44.1 mL·kg BM^{-1}·min^{-1} in girls 9-11 years of age was associated with increased cardiometabolic risk with moderate sensitivity and specificity. The association of CRF scaled by BM with cardiometabolic risk was markedly weaker than that of CRF scaled by LM because scaling by LM reduced the dependence of the measure of CRF on adiposity. Appropriately controlling for body size and composition reduced the ability of CRF to identify boys and girls at increased cardiometabolic risk.

5 PERSPECTIVES

Cardiometabolic risk tracks from childhood into adulthood and the early identification of individuals at increased risk is essential in developing public health actions targeted at preventing cardiometabolic diseases. Our results showed that CRF scaled by BM, which is partly confounded by adiposity, had a strong inverse association with cardiometabolic risk among children. Appropriately controlling for body size and composition markedly attenuated the predictive ability of CRF. The strong inverse association between CRF scaled by BM and cardiometabolic risk suggests that CRF scaled by BM can be used in screening children at increased cardiometabolic risk. However, children may be erroneously classified as being at increased risk, which may subject them to social stigma. Hence, there should be cautious

interpretation and utilization of CRF thresholds so that children who truly are at increased cardiometabolic risk are not deprived of appropriate intervention.

A markedly weakened relationship between CRF and increased cardiometabolic risk when adiposity was appropriately controlled for raises the question of whether there is an aetiological link between CRF and cardiometabolic health in children. Hence, longitudinal research is needed to establish whether decreased CRF, using appropriate scaling methods to control for body size and composition, increases cardiometabolic risk among children.

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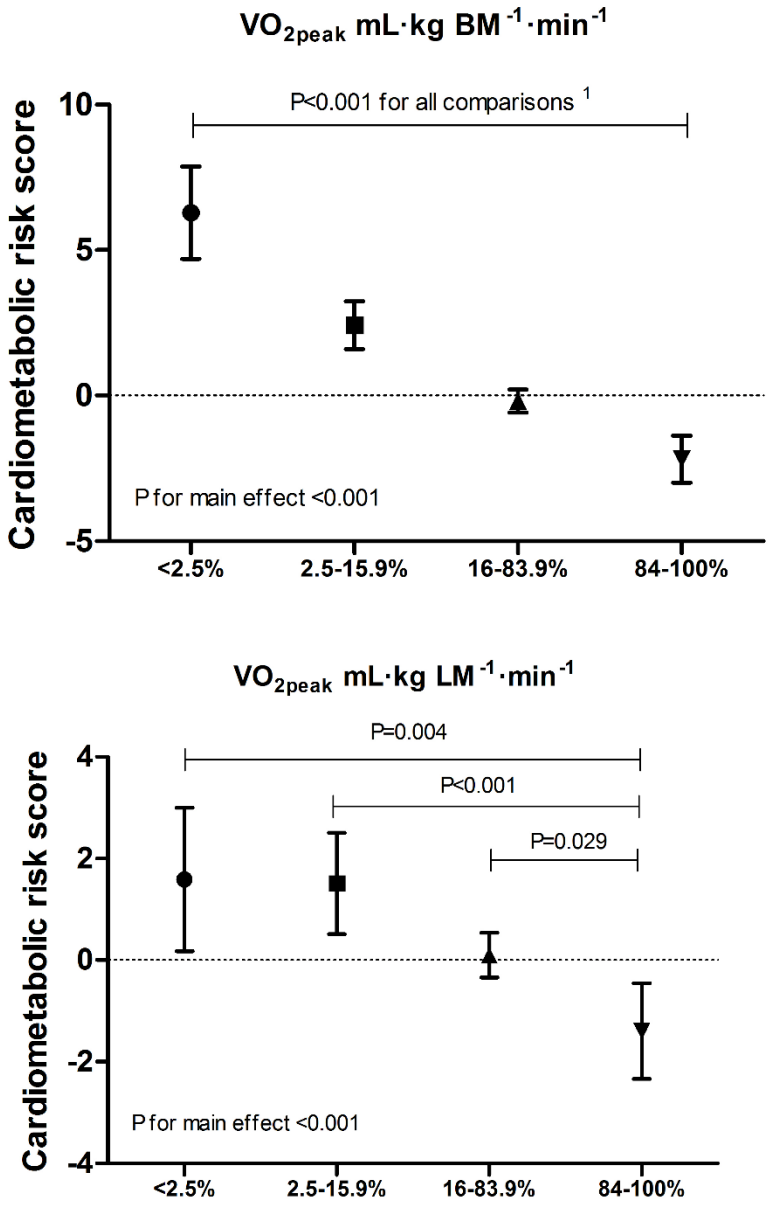

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509 Figure 1: Differences in cardiometabolic risk score according to sex-specific $\dot{V}O_{2peak}$
510 distribution using analysis of covariance (ANCOVA) with Sidak correction, adjusted for age
511 and study group

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515 **Table 1.** Characteristics of 352 children

	Boys (n=186)	Girls (n=166)	P for difference
Age (y)	9.8 (0.5)	9.8 (0.4)	0.696
Body height (cm)	141.5 (6.0)	140.4 (6.6)	0.127
Body weight (kg)	34.3 (9.7)*	33.1 (9.6)*	0.248
Clinical Puberty (%)†	15.1	37.7	<0.001
Overweight and obesity (%)	19.9	18.7	0.773
Waist circumference (cm)	61.7 (9.1)*	59.4 (9.5)*	0.012
Body fat mass (kg)	5.5 (5.6)*	6.1 (5.8)*	0.009
Body fat percentage (%)	15.9 (11.1)*	18.3 (11.0)*	<0.001
Lean body mass (kg)	27.0 (3.6)	25.3 (3.5)	<0.001
Serum insulin (pmol/L)	4.9 (3.9)*	6.1 (4.4)*	<0.001
Plasma glucose (mmol/L)	5.0 (0.3)*	4.9 (0.4)*	<0.001
Plasma HDL cholesterol (mmol/L)	1.7 (0.3)	1.6 (0.3)	0.040
Plasma triglycerides (mmol/L)	0.6 (0.3)*	0.5 (0.3)*	0.916
Systolic Blood Pressure (mm Hg)	100.0 (8.0)	101.0 (7.0)	0.795
Diastolic Blood Pressure (mm Hg)	61.2 (7.5)	61.4 (7.7)	0.830
Peak heart rate (beats/min)	198.8 (8.7)	200.1 (8.6)	0.167
Peak Respiratory exchange ratio	1.1 (0.1)	1.1 (0.1)	<0.001
$\dot{V}O_{2peak}$ (mL·kg BM^{-1}·min^{-1})	52.0 (7.0)	46.3 (6.9)	<0.001
$\dot{V}O_{2peak}$ (mL·kg LM^{-1}·min^{-1})	66.7 (6.5)	61.9 (6.5)	<0.001
$\dot{V}O_{2peak}$ (mL·kg $BM^{-0.49}$·min^{-1})	314.0 (37.0)	277.0 (33.0)	<0.001
$\dot{V}O_{2peak}$ (mL·kg $LM^{-0.77}$·min^{-1})	224.0 (21.0)	207.0 (21.0)	<0.001

Cardiometabolic risk score	0.18 (3.6)	0.13 (3.5)	0.878

The values are means (standard deviations) from the Student's t-test for normally distributed variables, medians (interquartile ranges) from the Mann–Whitney U test for variables with skewed distributions*, or percentages from the Chi-square test for categorical variables.

BM, body mass; HDL, high density lipoprotein; LM, lean body mass; $\dot{V}O_{2peak}$; peak oxygen uptake.

†Boys were defined having entered clinical puberty if their testicular volume assessed by an orchidometer was ≥4 mL (Tanner stage ≥2).[37] Girls were defined having entered clinical puberty if their breast development had started (Tanner stage ≥2).[36]

Table 2. Associations of peak oxygen uptake with cardiometabolic risk score in boys and girls

	Boys (n=186)		Girls (n=166)	
	β	*p*	*β*	*p*
$\dot{V}O_{2peak}$ (mL·min^{-1})	0.229	0.002	0.356	<0.001
$\dot{V}O_{2peak}$ (mL·kg BM^{-1}·min^{-1})	-0.577	<0.001	-0.484	<0.001
$\dot{V}O_{2peak}$ (mL·kg LM^{-1}·min^{-1})	-0.252	0.001	-0.245	0.001
$\dot{V}O_{2peak}$ (mL·kg BM$^{-0.49}$·min^{-1})	-0.261	<0.001	-0.123	0.127
$\dot{V}O_{2peak}$ (mL·kg LM$^{-0.77}$·min^{-1})	-0.185	0.012	-0.166	0.036

The values are standardised regression coefficients and *p*-values from linear regression models adjusted for age and the study group.

546 **Table 3.** Receiver operating characteristics curve analyses to predict increased cardiometabolic risk in boys and girls.

	Boys (n=186)						Girls (n=166)					
	Cut-points	Sensitivity	Specificity	AUC	95% CI	*p*-value	Cut-points	Sensitivity	Specificity	AUC	95% CI	*p*-value
$\dot{V}O_{2peak}$ (mL·kg BM^{-1}·min^{-1})	< 45.8	75.0	85.4	0.86	0.80 - 0.90	< 0.001	44.1	69.6	69.2	0.69	0.59 - 0.74	0.013
$\dot{V}O_{2peak}$ (mL·kg LM^{-1}·min^{-1})	< 63.2	50.0	76.6	0.65	0.58 - 0.72	0.006	*			0.57	0.49 - 0.65	0.332
$\dot{V}O_{2peak}$ (mL·kg $BM^{-0.49}$·min^{-1})	**	78.6	55.0	0.66	0.59 - 0.73	0.027	*			0.50	0.42 - 0.58	0.977
$\dot{V}O_{2peak}$ (mL·kg $LM^{-0.77}$·min^{-1})	**	75.0	46.8	0.61	0.54 - 0.68	0.047	*			0.53	0.45 - 0.61	0.633

547 AUC, area under the curve; CI, confidence interval;

548 *Scaled $\dot{V}O_{2peak}$ could not differentiate girls with cardiometabolic risk.

549 **Cut-points were not provided because they would depend on the scaling exponent that are specific to our study population.