TITLE PAGE

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Linking cardiorespiratory fitness classification criteria to early subclinical atherosclerosis in children.

Running title:

Recommended cardiorespiratory fitness for vascular health

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Abbreviations

BMI	body mass index
CRF	cardiorespiratory fitness
DXA	dual-energy radiographic absorptiometry
IMT	intima-media thickness

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ABSTRACT

Background: It is unclear if cardiorespiratory fitness (CRF) can be used as a screening tool for premature changes in carotid intima-media thickness (cIMT) in paediatric populations.

Aim: The purpose of this cross-sectional study was three-fold: 1) to determine if CRF can be used to screen increased cIMT; 2) to determine an optimal CRF cut-off to predict increased cIMT; 3) to evaluate its ability to predict increased cIMT among children in comparison with existent CRF cut-offs.

Methods: cIMT was assessed with high-resolution ultrasonography and CRF was determined using a maximal cycle test. Receiver operating characteristic (ROC) analyses were conducted in boys (n=211) and girls (n=202) aged 11–12 years-old to define the optimal sex-specific CRF cut-off to classify increased cIMT (≥P75). Logistic regression was used to examine the association between the CRF cut-offs with the risk of having an increased cIMT.

Results: The optimal CRF cut-offs to predict increased cIMT were 45.81 and 34.46 ml.kg^{-1}.min^{-1} for boys and girls, respectively. The odds-ratios (OR) for having increased cIMT among children who were unfit was up to 2.8 times the odds among those who were fit (95%CI: 1.40-5.53). Considering the existent CRF cut-offs, only those suggested by Adegboye et al. 2011 and Boddy et al. 2012 were significant in predicting increased cIMT.

Conclusion: CRF cut-offs (boys: ≤45.8; girls: ≤34.5 ml.kg^{-1}.min^{-1}) are associated with thickening of the arterial wall in 11-12 years-old children. Low CRF is an important cardiovascular risk factor in children and our data highlight the importance of obtaining an adequate CRF.

Key Words

Aerobic evaluation; intima-media thickness; common carotid artery; recommended values; paediatric; risk

INTRODUCTION

There are many simple anthropometric and physiological measures that may predict the onset and progression of cardiovascular and metabolic disease. Body mass index (BMI) and waist circumference are accepted measures for this purpose and are associated with carotid intima-media thickness (cIMT) in children (Lamotte et al. 2010), an intermediate phenotype for early atherosclerosis (Ferreira et al. 2002; Pahkala et al. 2013) and a solid predictor of future vascular events (Lorenz et al. 2007). Although an inverse association between cardiorespiratory fitness (CRF) and metabolic risk in children has been shown (Andersen et al. 2008; Anderssen et al. 2007), and adolescent levels of CRF were inversely and independently associated with premature changes in cIMT in adults (Ferreira et al. 2002), the use of CRF as a screening tool for early subclinical atherosclerosis is not established in paediatric populations. This is potentially important because by assessing CRF, simple risk stratification can be performed in the school setting, and a strategy for detecting individuals at risk in public health settings for potential further investigation can then be created (Adegboye et al. 2011).

The purpose of this study was three-fold: 1) to determine if CRF can be used to screen increased cIMT in children; 2) to determine an optimal CRF cut-off to predict increased cIMT in children; and 2) to evaluate its ability to predict increased cIMT among children in comparison with existent CRF cut-offs for cardiometabolic health.

METHODS

Study population

Participants were 413 children (202 girls) aged 11 to 12 years-old, enrolled in 2012 from 6 schools of Portugal. This cross-sectional study was approved by the ethics committee of the Faculty of Human Kinetics - University of Lisbon, Portugal. Children provided assent for their participation and informed consent was obtained from their parents or legal tutors. The study population was sequentially studied without specific exclusion criteria, hence the investigation did not specifically target children who were overweight/obese, or of any particular fitness level.

Anthropometrics

Height and sitting height were measured to the nearest 0.1 cm and body mass was measured to the nearest 0.1 kg on a scale with an attached stadiometer (model 770, Seca, Deutschland), wearing minimal clothing and no shoes. Leg length was calculated by subtracting sitting height from height. Body mass index (BMI) was calculated and categorized according to the established criteria (Cole and Lobstein 2012).

Dual-energy X-ray absorptiometry (DXA)

Total-body scans were performed by DXA and analysed using an extended analysis program for body composition (Hologic Explorer-W, fan-beam densitometer, software QDR 12.4, USA) to determine total body fat and trunk fat. The coefficients of variation for repeated measurements in our laboratory for total and regional DXA measurements are reported elsewhere (Santos et al. 2014).

Maturity

Maturity offset, that is, time before or after peak height velocity, was predicted with the equation of Mirwald et al. 2002 using the following variables: leg length, sitting height, age, weight, and height.

Cardiorespiratory Fitness

CRF was indirectly determined by a cycle test with progressively increasing workload using an electronically braked cycle ergometer (Monark 828 E Ergomedic; Monark, Sweden). Initial and incremental workloads were 20 W for children weighing <30 kg and 25 W for children ≥30 kg (Klasson-Heggebø et al. 2006). The workload was increased every 3-min until the peak effort of the participants was reached. Heart rate was recorded continuously (Polar Electro Oy, Finland) throughout the test. Criteria defined for a peak effort were heart rate >185 bpm or the subjective judgment by the observer that the participant could no longer continue, even after encouragement. Peak power output and peak oxygen consumption ($ml.min^{-1}$) were calculated according to the formulas by Hansen et al. 1989. Peak oxygen consumption was normalized by weight ($ml.kg^{-1}.min^{-1}$) and termed CRF from here on. The test has been previously validated against direct measurement of peak oxygen consumption (Klasson-Heggebø et al. 2006).

Hemodynamics

The heart rate at rest, brachial systolic and diastolic blood pressure were measured after 10 min with the participants in the supine position using an automated oscillometric cuff (HEM-907-E, Omron, Japan). Two measurements were obtained and if these values deviated by >5 mmHg, a third measurement was performed. The average of the closest 2 values was used. The pulse pressure was calculated for adjustment purposes since pulse pressure was positively correlated with mean cIMT (Stabouli et al. 2012).

Intima-Media Thickness

cIMT was defined as the distance between the leading edge of the lumen–intima interface to the leading edge of the media–adventitia interface of the far wall of the right carotid artery using an ultrasound scanner equipped with a linear 13 MHz probe (MyLab One, Esaote, Italy) and implemented with a previously validated radiofrequency-based tracking of arterial wall that allows a real-time determination of common carotid far-wall thickness (QIMT®) with high spatial and temporal resolution (Hoeks et al. 1997). cIMT was automatically measured, and distension curves were acquired within a segment of the carotid artery about 1 cm before the flow divider, where the operator places the region of interest. The coefficients of variation for repeated measurements in our laboratory for carotid IMT and diameter are reported elsewhere (Melo et al. 2014).

Statistical analyses

Data are presented as means and standard deviation unless stated otherwise. All variables were checked for normality. Receiver operating characteristic (ROC) analysis were used to determine the efficacy of CRF for correctly identifying children with increased IMT for each sex group. Early subclinical atherosclerosis was defined as cIMT≥75th percentile (P75) for age, and sex (Howard et al. 1993) as defined by Doyon et al. 2013 in a study aimed to establish a cIMT international reference data set for the childhood and adolescence period. The decision for the optimal cut-off was the cut-off value with the highest accuracy that maximized the sum of the sensitivity and specificity. Participants from this study were then classified as fit or unfit using the generated cut-offs and those listed in Table 1. Student's t-test was used to assess the statistical significance of the difference in cIMT and other physiologically relevant variables among CRF groups. Differences were tested controlling for other covariates (e.g. age and maturity offset) using the Bonferroni adjustment. Chi-square tests were performed for the comparison of proportions expressed as a percentage.

Odds-ratio (OR) with 95% confidence intervals (95%CIs) for having increased cIMT were calculated via multiple logistic regression entering sex and age as confounding variables. The statistical analyses were computed and analysed using the SPSS Statistics 19.0 and MedCalc 10.1.2.0.

RESULTS

The descriptive characteristics of the participants are displayed in Table 2. Boys had lower BMI, total body fat, trunk fat, maturity offset, chronotropic responses, CRF and diastolic blood pressure than girls ($p<0.05$). No significant differences were found for cIMT ($p>0.05$). Overall prevalence of overweight and obesity in this study was 28.8% (boys: 24.6%; girls: 33.1%).

The potential of CRF to discriminate increased cIMT was higher than would be expected by chance. The diagnostic accuracy of the ROC generated thresholds was 0.60 (95% CI 0.53-0.66; p=0.07) in boys and 0.55 (95% CI 0.48-0.62; p=0.43) in girls. Sex dependent ROC curves analyses with generated and existent cut points, plus sensitivity and specificity values are shown in Table 3. In both boys and girls, the highest sensitivity was found with the criteria by Boddy et al. 2012 whereas the highest specificity was found with the criteria by Bell et al. 1986 in boys and by the generated criteria in girls.

Figure 1 displays the prevalence of children who miss the recommended CRF according to the cut-offs generated in this study and those from Table 1. Significant differences were found between the prevalence of unfit boys (Bell et al. 1986, The Cooper Institute for Aerobics Research 1994 and Ruiz et al. 2007) and girls (The Cooper Institute for Aerobics Research 1994, Ruiz et al. 2007, Adegboye et al. 2011, Boddy et al. 2012) from the cut-offs generated in the present study and those determined by the cut-offs in Table 1 ($p<0.05$). The highest prevalence of unfit boys and girls resulted from the cut-offs by Boddy et al. 2012 whereas the lowest prevalence was set by the cut-offs of The Cooper Institute for Aerobics Research 1994 in boys and those of the present study in girls.

Unfit children had higher BMI, total body fat, trunk fat, systolic blood pressure, diastolic blood pressure, diameter and lower CRF ($p<0.05$) (Table 4). Participants classed as unfit by the generated

CRF cut-offs in this study and those by Bell et al. 1986, Lobelo et al. 2009, Adegboye et al. 2011 and Boddy et al. 2012 had also significantly increased cIMT independently of pulse pressure in comparison to those classed as fit.

The OR for having increased cIMT among children who were unfit was up to 2.8 times the odds among those who were fit (Table 5). In addition to the CRF cut-offs generated in the present study, only those suggested by Adegboye et al. 2011 and Boddy et al. 2012 were significant in predicting increased cIMT.

DISCUSSION

The results of the present study suggest that children failing to meet CRF standards have significantly increased cIMT compared with those who meet them. To our knowledge, this is the first study to show that low CRF in children is associated with a higher risk of increased cIMT while simultaneously strengthening the clinical validity of CRF thresholds for children in relation to their cardiovascular risk profile. Although there are no universally accepted recommendations for health-related levels of fitness in children, using the thresholds in Table 1, 64.40±10.66% of boys and 61.11±13.27 of girls in the present study apparently have healthy CRF. These prevalence rates are lower than those reported for children in Australia (boys: 71.00%; girls: 77.00%) (Catley and Tomkinson 2013), USA (boys: 71.00%; girls: 69.00%) (Lobelo et al. 2009) and England (boys: 82.50%; girls: 84.15%) (Sandercock et al. 2012), and are in line with the prevalence rates for children from 10 European countries (boys: 61.00%; girls: 57.00%) (Ortega et al. 2011). The percentage of children failing to meet the required standards of CRF is of concern as associations exist between adolescent CRF and total body fat (Eisenmann et al. 2005), serum lipids (Boreham et al. 2002), subclinical atherosclerosis (Ferreira et al. 2002), large artery stiffness (Ferreira et al. 2003), and blood pressure (Hasselstrøm et al. 2002), later in adulthood. Using cross-sectional designs, the present study and others (Anderssen et al. 2007; Melo et al. 2014; Ruiz et al. 2006) have also shown differences in physiologically relevant variables of body composition, hemodynamics and cIMT, according to CRF level in children. Several studies failed to establish CRF and physical activity as predictors of vascular structure in children and adolescents (Morrison et al. 2010; Ried-Larsen et al. 2013; Trigona et al. 2010). Conversely, others have shown an inverse association between physical activity and aortic IMT (Pahkala et al. 2011) and a favourable effect of CRF on aortic IMT but not cIMT in adolescents (Pahkala et al. 2013). Interventional studies have also provided mixed results on the ability of exercise training to reverse subclinical atherosclerosis in children (Meyer et al. 2006; 2011; Woo et al. 2004) so further longitudinal and/or intervention studies are needed to examine the impact of having low CRF in childhood on the odds of having atherosclerosis later in life.

In terms of risk, children aged 9–10 years-old who met the FITNESSGRAM standards were 2-3 times (boys: 2.42; girls: 3.09) more likely to have a lower cardiovascular disease risk score when compared with those who did not meet them (Ruiz et al. 2007). Likewise, Lobelo et al. 2009 found that boys aged 12-15 years-old who met the CRF standards were 5.17 times more likely to have a low cardiovascular disease risk when compared with those who did not meet them, although statistical significance was not found for females. The results from our study extend these associations to children with early subclinical manifestations of cardiovascular pathology with important implications not only for understanding pathophysiological mechanisms, but also for public health policies. The risk of having increased cIMT among children who were unfit was up to 2.8 times [1.82 (Boddy et al. 2012) and 1.92 (Adegboye et al. 2011)] the odds among those who were fit and the standards were significant in predicting increased cIMT.

The larger cIMT by 0.04 mm in the unfit group compared with the fit group in this study may result in a higher risk of cardiovascular disease later in life. Schools may play an important role in identifying children with low CRF via standardized tests promoting positive fitness-enhancing behaviours (Lobelo et al. 2009). Valuable CRF standards in the prediction of increased cIMT in children may be provided by Bell et al. 1986, Lobelo et al. 2009, Adegboye et al. 2011 and Boddy et al. 2012 and those from the present study.

The prevalence of children in this study below the early subclinical atherosclerosis threshold set by Doyon et al. 2013 is remarkably low. According to the author’s international reference data set for children's cIMT, the P75 of 11-12 years old boys and girls varies between 0.405 mm and 0.413 mm. In our study, the P75 varies between 0.55-0.56 mm in boys and 0.53-0.55 mm in girls. These variances may partly be explained by differences in the site of measure and analysis technic.

The area under the curve values are similar (Adegboye et al. 2011; Lobelo et al. 2009) or slightly lower than those of other studies (Boddy et al. 2012; Ruiz et al. 2008) aimed to define optimal cut-offs for CRF and to evaluate its ability to predict clustering of cardiovascular risk factors among children and adolescents. Unsurprisingly, cut-offs proposed by the previous works were relatively similar to those generated in this study in boys (Adegboye et al. 2011; Boddy et al. 2012; Lobelo et al. 2009) and girls (Bell et al. 1986; Lobelo et al. 2009). Resemblances among the cut-offs truly support the existence of a theoretical health-related standard value for CRF in children linked to a more favourable cardiovascular disease risk profile, even when accessed by a subclinical measure of atherosclerosis such as cIMT.

LIMITATIONS

This study did not have the ambition to create a standard classification system for CRF. Rather, the findings presented were intended to serve as a hypothetical basis and stimulus to consider international healthy ranges of CRF based on cIMT in a paediatric perspective. These cut-off values ought to be evaluated in a wider separate test group in whom outcomes could be verified independently, thereby allowing the definition of groups with and without increased cIMT.

CRF was indirectly determined by a cycle test with progressively increasing workload using an electronically braked cycle ergometer. A recognized limitation on the use of cycle ergometers in Portuguese children is that they are not used to cycling (Andersen et al. 2008; Anderssen et al. 2007). Therefore, the cycle ergometer test used may be less suitable for Portuguese children, and this might explain their lower CRF level and peak power output (Adegboye et al. 2011; Andersen et al. 2008). However, we did use a maximal test requiring a peak effort, which is a better indicator of CRF than submaximal tests (Rowland et al. 1993). Some children did not reach the maximal heart rate threshold, but these children did not have a different cIMT. Thus this was unlikely to affect our findings. Although the method by Hansen et al. 1989 was designed to provide gross peak oxygen consumption values, a high correlation was also demonstrated between the directly measured value and the calculated value relative to body weight (r=0.84-0.96), indicating that this method of predicting peak oxygen consumption could be used as an accurate and valid method of establishing CRF levels when scaled to body weight.

The sex adjusted areas under the curve for the generated cut-offs were not significant, mostly likely due to sample size as a strong trend towards significance was found in boys, an important step conferring utility of the prediction. Still, the observed area under the curve and the sensitivity, especially in girls, was low which may indicate a low discriminatory ability. However, sensitivity and specificity of a test may have different consequences depending on the clinical and public health settings of interest. In view of the benefits that regular aerobic exercise has in children, increasing CRF level (Ortega et al. 2008) and reducing cardiovascular disease risk (Ruiz et al. 2009), false-positive cases (failing to identify children as high risk who really are at high risk) are of greater concern than false-negative cases (increasing CRF level of low-risk children) (Adegboye et al. 2011).

CONCLUSION

CRF was associated with early subclinical atherosclerosis in 11-12 years-old children. In addition, results suggest a theoretical CRF cut-off (boys: ≤45.8; girls: ≤34.5 ml.kg^{-1}.min^{-1}) to predict increased cIMT in children. Low CRF is an important predictor of a potent cardiovascular risk factor in children and our data highlight the importance of obtaining an adequate CRF to refer unfit children onto intervention programs/services.

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REFERENCES

Adegboye, A.R., Anderssen, S.A., Froberg, K., Sardinha, L.B., Heitmann, B.L., Steene-Johannessen, J., Kolle, E., and Andersen, L.B. 2011. Recommended aerobic fitness level for metabolic health in children and adolescents: a study of diagnostic accuracy. Br J Sports Med. **45**(9): 722-728. doi:10.1136/bjsm.2009.068346

Andersen, L.B., Sardinha, L.B., Froberg, K., Riddoch, C.J., Page, A.S., and Anderssen, S.A. 2008. Fitness, fatness and clustering of cardiovascular risk factors in children from Denmark, Estonia and Portugal: the European Youth Heart Study. Int J Pediatr Obes. **3 Suppl 1**58-66. doi:790629714 [pii] 10.1080/17477160801896366

Anderssen, S.A., Cooper, A.R., Riddoch, C., Sardinha, L.B., Harro, M., Brage, S., and Andersen, L.B. 2007. Low cardiorespiratory fitness is a strong predictor for clustering of cardiovascular disease risk factors in children independent of country, age and sex. Eur J Cardiovasc Prev Rehabil. **14**526-531. Available from:

Bell, R.D., Macek, M., Rutenfranz, J., and Saris, W. 1986. Health indicators and risk factors of cardiovascular diseases during childhood and adolescence. Champaign, Illinois, USA: Human Kinetics.

Boddy, L.M., Thomas, N.E., Fairclough, S.J., Tolfrey, K., Brophy, S., Rees, A., Knox, G., Baker, J.S., and Stratton, G. 2012. ROC generated thresholds for field-assessed aerobic fitness related to body size and cardiometabolic risk in schoolchildren. PLoS One. **7**(9): e45755. doi:10.1371/journal.pone.0045755

Boreham, C., Twisk, J., Neville, C., Savage, M., Murray, L., and Gallagher, A. 2002. Associations between physical fitness and activity patterns during adolescence and cardiovascular risk factors in young adulthood: the Northern Ireland Young Hearts Project. Int J Sports Med. **23 Suppl 1**S22-26. doi:10.1055/s-2002-28457

Catley, M.J. and Tomkinson, G.R. 2013. Normative health-related fitness values for children: analysis of 85347 test results on 9-17-year-old Australians since 1985. Br J Sports Med. **47**(2): 98-108. doi:10.1136/bjsports-2011-090218

Cole, T.J. and Lobstein, T. 2012. Extended international (IOTF) body mass index cut-offs for thinness, overweight and obesity. Pediatr Obes. **7**(4): 284-294. doi:10.1111/j.2047-6310.2012.00064.x

Doyon, A., Kracht, D., Bayazit, A.K., Deveci, M., Duzova, A., Krmar, R.T., Litwin, M., Niemirska, A., Oguz, B., Schmidt, B.M., Sözeri, B., Querfeld, U., Melk, A., Schaefer, F., Wühl, E., and Consortium, C.S. 2013. Carotid artery intima-media thickness and distensibility in children and adolescents: reference values and role of body dimensions. Hypertension. **62**(3): 550-556. doi:10.1161/HYPERTENSIONAHA.113.01297

Eisenmann, J.C., Wickel, E.E., Welk, G.J., and Blair, S.N. 2005. Relationship between adolescent fitness and fatness and cardiovascular disease risk factors in adulthood: the Aerobics Center Longitudinal Study (ACLS). Am Heart J. **149**(1): 46-53. doi:10.1016/j.ahj.2004.07.016

Ferreira, I., Twisk, J.W., Stehouwer, C.D., van Mechelen, W., and Kemper, H.C. 2003. Longitudinal changes in .VO2max: associations with carotid IMT and arterial stiffness. Med Sci Sports Exerc. **35**(10): 1670-1678. doi:10.1249/01.MSS.0000089247.37563.4B

Ferreira, I., Twisk, J.W., Van Mechelen, W., Kemper, H.C., Stehouwer, C.D., and Study, A.G.a.H.L. 2002. Current and adolescent levels of cardiopulmonary fitness are related to large artery properties at age 36: the Amsterdam Growth and Health Longitudinal Study. Eur J Clin Invest. **32**(10): 723-731. doi:1066 [pii]

Hansen, H.S., Froberg, K., Nielsen, J.R., and Hyldebrandt, N. 1989. A new approach to assessing maximal aerobic power in children: the Odense School Child Study. Eur J Appl Physiol Occup Physiol. **58**(6): 618-624. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2731531

Hasselstrøm, H., Hansen, S.E., Froberg, K., and Andersen, L.B. 2002. Physical fitness and physical activity during adolescence as predictors of cardiovascular disease risk in young adulthood. Danish Youth and Sports Study. An eight-year follow-up study. Int J Sports Med. **23 Suppl 1**S27-31. doi:10.1055/s-2002-28458

Hoeks, A.P., Willekes, C., Boutouyrie, P., Brands, P.J., Willigers, J.M., and Reneman, R.S. 1997. Automated detection of local artery wall thickness based on M-line signal processing. Ultrasound Med Biol. **23**(7): 1017-1023. doi:S0301562997001191 [pii]

Howard, G., Sharrett, A.R., Heiss, G., Evans, G.W., Chambless, L.E., Riley, W.A., and Burke, G.L. 1993. Carotid artery intimal-medial thickness distribution in general populations as evaluated by B-mode ultrasound. ARIC Investigators. Stroke. **24**(9): 1297-1304. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8362421

Klasson-Heggebø, L., Andersen, L.B., Wennlöf, A.H., Sardinha, L.B., Harro, M., Froberg, K., and Anderssen, S.A. 2006. Graded associations between cardiorespiratory fitness, fatness, and blood pressure in children and adolescents. Br J Sports Med. **40**(1): 25-29; discussion 25-29. doi:40/1/25 [pii]

10.1136/bjsm.2004.016113

Lamotte, C., Iliescu, C., Libersa, C., and Gottrand, F. 2010. Increased intima-media thickness of the carotid artery in childhood: a systematic review of observational studies. Eur J Pediatr. doi:10.1007/s00431-010-1328-y

Lobelo, F., Pate, R.R., Dowda, M., Liese, A.D., and Ruiz, J.R. 2009. Validity of cardiorespiratory fitness criterion-referenced standards for adolescents. Med Sci Sports Exerc. 4**1(**6): 1222-1229. doi:10.1249/MSS.0b013e318195d491

Lorenz, M.W., Markus, H.S., Bots, M.L., Rosvall, M., and Sitzer, M. 2007. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. Circulation. 1**15(**4): 459-467. doi:CIRCULATIONAHA.106.628875 [pii] 10.1161/CIRCULATIONAHA.106.628875

Melo, X., Santa-Clara, H., Pimenta, N.M., Martins, S.S., Minderico, C.S., Fernhall, B., and Sardinha, L.B. 2014. Intima-Media Thickness in 11-13 Years-Old Children: Variation Attributed to Sedentary Behavior, Physical Activity, Cardiorespiratory Fitness and Waist Circumference. J Phys Act Health. doi:10.1123/jpah.2013-0501

Meyer, A.A., Kundt, G., Lenschow, U., Schuff-Werner, P., and Kienast, W. 2006. Improvement of early vascular changes and cardiovascular risk factors in obese children after a six-month exercise program. J Am Coll Cardiol. 4**8(**9): 1865-1870. doi:S0735-1097(06)01963-2 [pii] 10.1016/j.jacc.2006.07.035

Mirwald, R.L., Baxter-Jones, A.D., Bailey, D.A., and Beunen, G.P. 2002. An assessment of maturity from anthropometric measurements. Med Sci Sports Exerc. 3**4(**4): 689-694. Available from: http://www.ncbi.nlm.nih.gov/pubmed/11932580

Morrison, K.M., Dyal, L., Conner, W., Helden, E., Newkirk, L., Yusuf, S., and Lonn, E. 2010. Cardiovascular risk factors and non-invasive assessment of subclinical atherosclerosis in youth. Atherosclerosis. 2**08(**2): 501-505. doi:S0021-9150(09)00598-X [pii] 10.1016/j.atherosclerosis.2009.07.034

Ortega, F.B., Ruiz, J.R., Castillo, M.J., and Sjöström, M. 2008. Physical fitness in childhood and adolescence: a powerful marker of health. Int J Obes (Lond). 3**2(**1): 1-11. doi:10.1038/sj.ijo.0803774

Ortega, F.B., Artero, E.G., Ruiz, J.R., España-Romero, V., Jiménez-Pavón, D., Vicente-Rodriguez, G., Moreno, L.A., Manios, Y., Béghin, L., Ottevaere, C., Ciarapica, D., Sarri, K., Dietrich, S., Blair, S.N., Kersting, M., Molnar, D., González-Gross, M., Gutiérrez, A., Sjöström, M., Castillo, M.J., and study, H. 2011. Physical fitness levels among European adolescents: the HELENA study. Br J Sports Med. 4**5(**1): 20-29. doi:10.1136/bjsm.2009.062679

Pahkala, K., Heinonen, O.J., Simell, O., Viikari, J.S., Ronnemaa, T., Niinikoski, H., and Raitakari, O.T. 2011. Association of physical activity with vascular endothelial function and intima-media thickness. Circulation. 1**24(**18): 1956-1963. doi:CIRCULATIONAHA.111.043851 [pii] 10.1161/CIRCULATIONAHA.111.043851

Pahkala, K., Laitinen, T.T., Heinonen, O.J., Viikari, J.S., Rönnemaa, T., Niinikoski, H., Helajärvi, H., Juonala, M., Simell, O., and Raitakari, O.T. 2013. Association of Fitness With Vascular Intima-Media Thickness and Elasticity in Adolescence. Pediatrics. doi:10.1542/peds.2013-0041

Ried-Larsen, M., Grøntved, A., Froberg, K., Ekelund, U., and Andersen, L.B. 2013. Physical activity intensity and subclinical atherosclerosis in Danish adolescents: The European Youth Heart Study. Scand J Med Sci Sports. doi:10.1111/sms.12046

Rowland, T.W., Rambusch, J.M., Staab, J.S., Unnithan, V.B., and Siconolfi, S.F. 1993. Accuracy of physical working capacity (PWC170) in estimating aerobic fitness in children. J Sports Med Phys Fitness. 33(2): 184-188. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8412055

Ruiz, J.R., Rizzo, N.S., Hurtig-Wennlöf, A., Ortega, F.B., Wärnberg, J., and Sjöström, M. 2006. Relations of total physical activity and intensity to fitness and fatness in children: the European Youth Heart Study. Am J Clin Nutr. 84(2): 299-303. doi:84/2/299 [pii]

Ruiz, J.R., Ortega, F.B., Rizzo, N.S., Villa, I., Hurtig-Wennlöf, A., Oja, L., and Sjöström, M. 2007. High cardiovascular fitness is associated with low metabolic risk score in children: the European Youth Heart Study. Pediatr Res. 61(3): 350-355. doi:10.1203/pdr.0b013e318030d1bd

Ruiz, J.R., Sui, X., Lobelo, F., Morrow, J.R., Jackson, A.W., Sjöström, M., and Blair, S.N. 2008. Association between muscular strength and mortality in men: prospective cohort study. BMJ. 337a439. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18595904

Ruiz, J.R., Castro-Piñero, J., Artero, E.G., Ortega, F.B., Sjöström, M., Suni, J., and Castillo, M.J. 2009. Predictive validity of health-related fitness in youth: a systematic review. Br J Sports Med. 43(12): 909-923. doi:10.1136/bjsm.2008.056499

Sandercock, G., Voss, C., Cohen, D., Taylor, M., and Stasinopoulos, D.M. 2012. Centile curves and normative values for the twenty metre shuttle-run test in English schoolchildren. J Sports Sci. 30(7): 679-687. doi:10.1080/02640414.2012.660185

Santos, D.A., Dawson, J.A., Matias, C.N., Rocha, P.M., Minderico, C.S., Allison, D.B., Sardinha, L.B., and Silva, A.M. 2014. Reference values for body composition and anthropometric measurements in athletes. PLoS One. 9(5): e97846. doi:10.1371/journal.pone.0097846

Seeger, J.P., Thijssen, D.H., Noordam, K., Cranen, M.E., Hopman, M.T., and Nijhuis-van der Sanden, M.W. 2011. Exercise training improves physical fitness and vascular function in children with type 1 diabetes. Diabetes Obes Metab. 13(4): 382-384. doi:10.1111/j.1463-1326.2011.01361.x

Stabouli, S., Kotsis, V., Karagianni, C., Zakopoulos, N., and Konstantopoulos, A. 2012. Blood pressure and carotid artery intima-media thickness in children and adolescents: the role of obesity. Hellenic J Cardiol. 53(1): 41-47. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22275742

The Cooper Institute for Aerobics Research 1994. FITNESSGRAM - the Prudential FITNESSGRAM test administration manual. Dallas, Texas, USA.

Trigona, B., Aggoun, Y., Maggio, A., Martin, X.E., Marchand, L.M., Beghetti, M., and Farpour-Lambert, N.J. 2010. Preclinical noninvasive markers of atherosclerosis in children and adolescents with type 1 diabetes are influenced by physical activity. J Pediatr. 157(4): 533-539. doi:S0022-3476(10)00323-9 [pii] 10.1016/j.jpeds.2010.04.023

Woo, K.S., Chook, P., Yu, C.W., Sung, R.Y., Qiao, M., Leung, S.S., Lam, C.W., Metreweli, C., and Celermajer, D.S. 2004. Effects of diet and exercise on obesity-related vascular dysfunction in children. Circulation. 109(16): 1981-1986. doi:10.1161/01.CIR.0000126599.47470.BE 01.CIR.0000126599.47470.BE [pii]

TABLES

Table 1: Health-related cut-offs for CRF in children and adolescents

Author	Sex	Sample (*n*)	Age (years)	Cut-offs ($ml.Kg.^{-1}min^{-1}$)	Method
Bell et al. 1986	Boys	---	Adolescents	40.0	Expert judgement
	Girls	---	Adolescents	35.0	
The Cooper Institute for Aerobics Research 1994[a]	Boys	NHANES data	11	37.3	Criterion referenced standards
			12	37.6	
	Girls		11	37.3	
			12	37.0	
Ruiz et al. 2007	Boys	429	9-10	42.1	ROC analysis
	Girls	444		37.0	
Lobelo et al. 2009	Boys	677	12-15	44.1	ROC analysis
	Girls	570		40.3	
Adegboye et al. 2011	Boys	1219	9	43.6	ROC analysis
	Girls	1181		37.4	
Boddy et al. 2012	Boys	8382	9-11	46.6	ROC analysis
	Girls	8237		41.9	

[a] Updated data released in 2010 (http://cooperinstitute.org/healthyfitnesszone)

Table 2: Characteristics of the study group and sex comparisons.

		Boys			Girls			All		
n		211			202			413		
Age	(years)	11.37	±	0.48	11.33	±	0.47	11.35	±	0.48
Weight	(kg)	43.65	±	10.55	45.93	±	9.51*	44.76	±	10.11
Height	(cm)	150.30	±	7.39	152.00	±	7.01*	151.13	±	7.25
BMI	($kg.m^{-2}$)	19.15	±	3.48	19.77	±	3.34	19.46	±	3.43
Total body fat	(kg)	11.20	±	6.12	14.13	±	5.73*	12.72	±	6.09
Trunk fat	(kg)	4.05	±	2.86	5.47	±	2.79*	4.80	±	2.91
Maturity offset	(years)	-1.82	±	0.58	-0.03	±	0.55*	-0.95	±	1.06
Heart rate at rest	(bpm)	87.68	±	14.02	90.75	±	14.90*	89.18	±	14.52
Heart rate at peak effort	(bpm)	193.50	±	11.49	196.25	±	10.26*	194.85	±	10.98
Peak power output	($W.kg^{-1}$)	2.88	±	0.64	2.47	±	0.48*	2.68	±	0.60
CRF	($ml.kg^{-1}.min^{-1}$)	44.84	±	9.02	38.87	±	7.03*	42.41	±	8.48
Systolic blood pressure	(mmHg)	110.32	±	9.64	111.38	±	11.44	110.84	±	10.56
Diastolic blood pressure	(mmHg)	60.34	±	7.30	62.83	±	8.86*	61.56	±	8.09
Diameter of the carotid artery	(mm)	6.41	±	0.47	6.11	±	0.45*	6.26	±	0.48
cIMT	(mm)	0.50	±	0.08	0.49	±	0.08	0.50	±	0.08
cIMT<P75[b]	(%)	13.0			15.2			14.0		

Abbreviations: BMI: body mass index; CRF: Cardiorespiratory fitness; cIMT: intima-media thickness of the common carotid artery; P75[b]: 75^{th} percentile by Doyon et al. 2013; * Significant differences between boys and girls ($p<0.05$)

Table 3: Receiver operating characteristic curves analysis with cIMT≥P75[b] as classification variable in boys and girls according to different cut-offs*

	Boys			Girls		
	CRF Cut-offs	Sensitivity	Specificity	CRF Cut-offs	Sensitivity	Specificity
Bell et al. 1986	40.0	30.2	81.3	35.0	25.0	76.9
The Cooper Institute for Aerobics Research 1994[a]	37.3	22.9	81.2	37.3	36.9	69.2
	37.6	24.0	81.2	37.0	35.2	69.2
Ruiz et al. 2007	42.1	36.3	78.1	37.0	36.9	69.2
Lobelo et al. 2009	44.1	45.8	75.0	40.3	53.4	53.9
Adegboye et al. 2011	43.6	45.8	78.1	37.4	36.9	65.4
Boddy et al. 2012	46.6	57.0	65.6	41.9	63.6	42.3
Present Study	45.8	52.0	75.0	34.5	24.4	88.5

Abbreviations: CRF: cardiorespiratory fitness ($ml.kg^{-1}.min^{-1}$)

* Coordinates of the ROC curve for approximate criterion values (0.1); [a] Updated data released in 2010 (http://cooperinstitute.org/healthyfitnesszone); P75[b]: 75th percentile by Doyon et al. 2013

Table 4: Physiologically relevant variables according to CRF level

		Boys		Girls		All	
		Fit	Unfit	Fit	Unfit	Fit	Unfit
n		111	100	157	45	268	145
BMI	($kg.m^{-2}$)	17.21 ± 1.68	21.31 ± 3.70	18.75 ± 2.57	23.33 ± 3.23*	18.11 ± 2.36	21.94 ± 3.70*
Total body fat	(%)	20.77 ± 4.38	29.14 ± 7.46*	27.89 ± 5.67	36.71 ± 6.13*	25.17 ± 6.25	31.72 ± 7.88*
Trunk fat	(%)	16.22 ± 3.40	25.50 ± 8.60*	23.69 ± 6.83	33.99 ± 7.42*	20.83 ± 6.93	28.40 ± 9.13*
Heart rate at rest	(bpm)	87.91 ± 13.34	87.44 ± 14.81	90.57 ± 15.00	91.37 ± 14.80	89.47 ± 14.35	88.66 ± 14.86
CRF	($ml.kg^{-1}.min^{-1}$)	51.66 ± 5.13	37.27 ± 5.79*	42.46 ± 5.55	30.81 ± 2.95*	46.27 ± 7.03	35.26 ± 5.89*
Systolic blood pressure	(mmHg)	108.27 ± 8.73	112.60 ± 10.13*	109.19 ± 10.17	119.02 ± 12.42*	108.81 ± 9.59	114.59 ± 11.25*
Diastolic blood pressure	(mmHg)	58.90 ± 7.00	61.94 ± 7.30*	62.08 ± 8.19	65.44 ± 9.87*	60.76 ± 7.87	63.03 ± 8.31*
Diameter of carotid artery	(mm)	6.32 ± 0.48	6.51 ± 0.44*	6.07 ± 0.46	6.24 ± 0.38*	6.17 ± 0.48	6.42 ± 0.44*
cIMT	(mm)	0.49 ± 0.08	0.51 ± 0.08*	0.48 ± 0.08	0.52 ± 0.08*	0.48 ± 0.08	0.52 ± 0.08*
cIMT<P75^b	(%)	21.6	8.0*	14.6	6.7	17.5	7.6*

Overall, no significant differences were found for age and maturity between groups of CRF level.

Abbreviations: BMI: body mass index; CRF: Cardiorespiratory fitness; cIMT: intima-media thickness of the common carotid artery; * Significant differences between fit and unfit children ($p<0.05$); P75^b: 75th percentile by Doyon et al. 2013

Table 5: Logistic regression analyses on associations between CRF cut-offs for children and increased cIMT.

Authors	OR (sex adjusted)	Lower 95%CI	Higher 95% CI
Bell et al. 1986	1.60	0.80	3.21
The Cooper Institute for Aerobics Research 1994[a]	1.50	0.81	2.76
Ruiz et al. 2007	1.58	0.84	2.95
Lobelo et al. 2009	1.79	0.95	3.37
Adegboye et al. 2011	1.92	1.03	3.58
Boddy et al. 2012	1.82	1.04	3.20
Present study	2.78	1.40	5.53

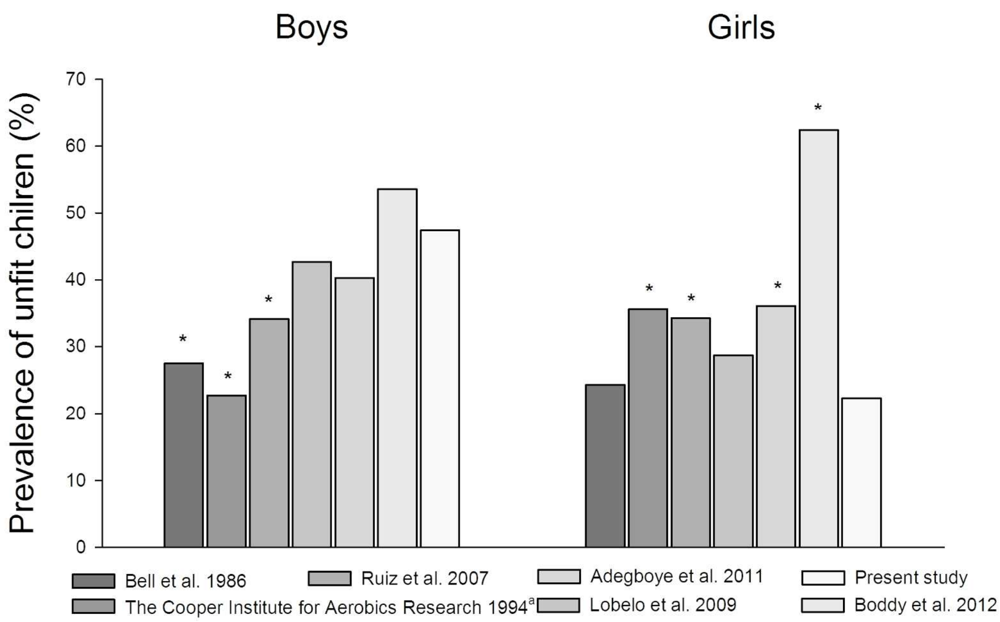
[a]Updated data released in 2010 (http://cooperinstitute.org/healthyfitnesszone).

FIGURES

Figure 1: Prevalence of children attaining the recommended CRF according to the different cut-offs from Table 1 and the generated CRF standards from the present study.

[a] Updated data released in (2010)

* Prevalence is significantly different from that of the present study using the generated cut-offs ($p<0.05$)



Prevalence of children attaining the recommended CRF according to the different cut-offs from Table 1 and the generated CRF standards from the present study.
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