Gustavo Marçal Gonçalves da Silva

Cardiorespiratory Fitness in Children and Adolescents

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Assessment, Reference Standards and Associations with Metabolic Risk and Physical Activity

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Academic dissertation submitted pursuing the PhD degree under the Doctoral Course in Physical Activity and Health, organized by the Research Centre in Physical Activity, Health and Leisure (CIAFEL), from the Faculty of Sport – University of Porto, according to the Decree-Law 74 / 2006 of March 24th.

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"Where is the Life we have lost in living? Where is the wisdom we have lost in knowledge? Where is the knowledge we have lost in information?"

– T.S. Eliot, Choruses from "The Rock"

This work is dedicated to my parents, Carlos Alberto and Tânia, and to my partner Luísa. Having you as references and inspiration in my life is a blessing. Thank you for pushing me on the right track …

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ABSTRACT

Primary prevention of Cardiovascular Diseases (CVD) and related disorders starts in the definition of valid tools for targeting subjects at risk and in the delineation of strategies for intervention. Evidences suggest that primary prevention of CVD since paediatric ages should be a priority of public health policies and interventions. Therefore, the assessment of cardiorespiratory fitness (CRF) might be relevant, since CRF has been associated with obesity and other cardio-metabolic risk factors. Thus, the purposes of the present thesis were: to calculate and validate two models to estimate CRF (***Study I***), to calculate and validate CRF reference standards for targeting Portuguese children and adolescents with increased metabolic risk (***Study II***); to analyse the associations between CRF, central adiposity and metabolic risk in youths (***Study III***); and to analyse the associations between the amounts of moderate to vigorous physical activity (MVPA), participation in different contexts of physical activity (PA) and levels of CRF in young people (***Study IV***). A total of 6192 students (3131 girls and 3031 boys) aged 10–18 years participated in the four studies. CRF was assessed by the 20m Shuttle Run Test (SR). Body mass index (BMI), waist circumference (WC), systolic and diastolic blood pressure were assessed with standard procedures. Blood samples were collected to determine concentrations of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides and fasting glucose. Clustered metabolic risk scores were calculated and adjusted for age and sex. Subjects with metabolic risk scores greater than the mean + 1 *SD* were defined as the group at high metabolic risk (HMRS). PA was assessed objectively by accelerometers. Participation in PA contexts was assessed by questionnaire. In the ***Study I***, 114 subjects had their maximal oxygen uptake (VO2max) measured directly during the SR. Multiple Linear Regression (MLR) and Artificial Neural Network (ANN) tests were carried out considering sex, age, height, weight, BMI and SR stages as predictors of VO_2max. Estimations from MLR and ANN were compared with three other previously published equations. In summary, the equation estimated by MLR is more appropriate for Portuguese youths than the equation estimate by ANN or the other three previously published equations (validation coefficient for the MLR model: r=0.84, P<0.001; systematic error=-0.01±5.2, P>0.05; SEE=4.9). In the ***Study II***, normative values for the SR performance were calculated from a population-based study (5559 students) and validated as reference standards for metabolic risk stratification in a validation group (633 subjects). The accuracy of SR laps standards was significant for girls (AUC=0.66; 95% CI=0.58–0.74; P<0.001) and boys (AUC=0.71; 95% CI=0.62–0.79; P<0.001) for identifying subjects at high metabolic risk. The 40^{th} percentile was the best cut-off for SR laps in girls (SENS=0.569; 1-SPEC=0.330) and boys (SENS=0.634; 1-SPEC=0.266). New SR laps reference standards are able to discriminate metabolic risk levels. In the ***Study III***, the associations between CRF, WC and metabolic risk were analysed in a sample of 633 students. With adjustments for age, sex and WC, VO_2max was correlated with MRS (r=-0.095; P<0.05). Participants who were low fit, presented higher levels of MRS (P<0.001) compared to those who were fit, even after adjustment for age, sex and WC. Results suggest that CRF associates with MRS, independently of central adiposity. In the ***Study IV***, the associations between CRF, MVPA and participation in PA contexts were analysed in a sample of 310 youths. The OR for being fit was greater for those who comply with 60min/day in MVPA (OR=2.612; 95%CI=1.614-4.225) in comparison with those who do not. Participation in competitive sports at club levels increased the chances of being fit (OR=13.483; 95%CI=4.560-39.864), independently of MVPA levels. There were positive and significant trends in CRF and objectively measured PA across the levels of engagement in competitive sports (P<0.05). In this analysis, participation in competitive sports, especially at club level, increased the chances to reach healthier levels of CRF and recommended levels of MVPA. In conclusion, the experimental work of the present thesis suggest that a field test such as the SR provides valid estimations of CRF and might be considered an important tool for the stratification of children and adolescents at risk for CVD. CRF is an important marker of cardiovascular health in youths, independently of central adiposity. Participation in competitive sports may be an effective PA context to improve CRF and overall cardiovascular health in young people. Keywords: AEROBIC ENDURANCE, CARDIOVASCULAR RISK FACTORS, METABOLIC SYNDROME, PHYSICAL ACTIVITY, YOUTHS.

RESUMO

A prevenção primária das Doenças Cardiovasculares (DCV) e patologias associadas começa pela definição de ferramentas válidas para identificar sujeitos em risco e no delineamento de estratégias de intervenção. Evidências sugerem que a prevenção primária das DCV em idades pediátricas deve ser prioridade das políticas de saúde pública e de intervenções. Assim, a avaliação da Aptidão Cardiorrespiratória (ACR) é relevante, já que tem sido associada à obesidade e outros fatores de risco das DCV. Desta forma, os objetivos desta tese foram: calcular e validar dois modelos de estimação da ACR (***Estudo I***); calcular e validar padrões de referência para identificar crianças e adolescentes portugueses com elevado risco metabólico (***Estudo II***); analisar associações entre ACR, adiposidade central e fatores de risco metabólico em jovens (***Estudo III***); e analisar associações entre atividades físicas de intensidades moderadas a vigorosas (AFMV), participação em diferentes contextos da atividade física (AF) e níveis de ACR (***Estudo IV***). Um total de 6192 estudantes (3131 raparigas e 3031 rapazes) com idades entre 10 e 18 anos participaram nos estudos. ACR foi estimada a partir do teste do Vaivém (VV). O índice de massa corporal (IMC), o perímetro da cintura (PC), a tensão arterial sistólica e diastólica foram medidos por procedimentos padronizados. Amostras sanguíneas foram recolhidas para determinar os níveis de colesterol total, colesterol de alta densidade, colesterol de baixa densidade, triglicerídeos e glicose em jejum. Valores de risco metabólico foram calculados ajustados à idade e ao sexo. Sujeitos com risco metabólico superior à média + 1*DP* foram definidos como o grupo em risco metabólico elevado (RME). AF foi medida por acelerômetros. A participação em diferentes contextos da AF foi avaliada por questionário. No ***Estudo I***, 114 sujeitos tiveram o seu consumo máximo de oxigênio (VO_2max) medido diretamente durante o VV. Regressão Linear Múltipla (RLM) e Redes Neurais Artificiais (RNA) foram realizadas com sexo, idade, altura, peso, BMI e estágios completos do VV como preditores do VO_2max. As estimativas dos modelos RLM e RNA foram comparadas com estimativas de outras três equações conhecidas na literatura. A equação definida por RLM foi considerada a mais apropriada para jovens portugueses (coeficiente de validação: r=0.84, P<0.001; erro sistemático=-0.01±5.2, P>0.05; EPE=4.9). No ***Estudo II***, valores normativos para o VV foram calculados numa população (5559 estudantes) e testados como padrões de referência para estratificação do risco metabólico num grupo de validação (633 sujeitos). A precisão dos padrões de referências expresso no número de voltas do VV para identificar sujeitos com RME foi significativa para raparigas (AUC=0.66; 95% CI=0.58–0.74; P<0.001) e rapazes (AUC=0.71; 95% CI=0.62–0.79; P<0.001). O percentil 40 foi o melhor ponto de corte para o número de voltas no VV para raparigas (SENS=0.569; 1-SPEC=0.330) e rapazes (SENS=0.634; 1-SPEC=0.266). Os novos valores de referência foram capazes de discriminar diferentes níveis de risco metabólico. No ***Estudo III***, associações entre ACR, PC e risco metabólico foram analisadas numa amostra de 633 estudantes. Com ajustamentos para idade, sexo e PC, VO_2max relacionou-se com o risco metabólico (r=-0.095; P<0.05). Participantes com baixos níveis de ACR apresentam risco metabólico elevado em comparação aos sujeitos com níveis adequados de ACR (P<0.001), mesmo com ajustes para idade, sexo e PC. Estes resultados sugerem que a ACR associa-se com o risco metabólico independentemente da adiposidade central. No ***Estudo IV***, associações entre ACR, AFMV e participação em diferentes contextos da AF foram analisadas numa amostra de 310 jovens. As chances de ter níveis adequados de ACR foram elevadas para os sujeitos que cumprem 60min/dia em AFMV (OR=2.612; 95%CI=1.614-4.225) em comparação com aqueles que não cumprem. A participação desportiva em clubes elevou as chances de apresentar níveis adequados de ACR (OR=4.560-39-864), independentemente dos níveis de AFMV. Houve uma tendência positiva na ACR e na AF com o aumento do nível de exigência das competições desportivas (P<0.05). A participação em desportos competitivos em clubes aumentaram as chances de alcançar valores recomendáveis de ACR e AFMV. Em conclusão, esta tese sugere que o VV oferece estimativas válidas da ACR e pode ser considerado uma importante ferramenta para a estratificação de jovens em risco para as DCV. A ACR é um importante marcador da saúde cardiovascular, independentemente da adiposidade central. A participação em desportos competitivos deve ser um contexto efetivo de realização de AF para aumentar os níveis da ACR e da saúde cardiovascular em geral. Palavras-chave: RESISTÊNCIA AERÓBIA, FATORES DE RISCO CARDIOVASCULAR, SÍNDROME METABÓLICA, ATIVIDADE FÍSICA, JOVENS.

List of Abbreviations

95% CI: 95% confidence interval

95% LOA: 95% limits of agreement

ACR: aptidão cardiorrespiratória

AF: atividade física

AFMV: atividade física moderada a vigorosa

AHA: American Heart Association

ANN: artificial neural network

ANOVA: analysis of variance

AUC: area under the curve

BMI: body mass index

CRF: cardiorespiratory fitness

CVD: cardiovascular diseases

DBP: diastolic blood pressure

DCV: doenças cardiovasculares

DEXA: dual-energy X-ray absorptiometry

DP: desvio padrão

EFF: efficiency

GLM: general linear model

GLU: fasting glucose

HDL-C: high-density lipoprotein cholesterol

HMRS: high metabolic risk score

HOMA: homeostatic model assessment

HR: heart rate

ICC: intra-class correlation coefficient

IMC: índice de massa corporal

LDL-C: low-density lipoprotein cholesterol

LIGPA: light physical activity

LMRS: low metabolic risk score

LMS method: L: Box-Cox power for skewness; M: median; S: coefficient of variation

M: mean

MAP: mean arterial pressure

MLR: multiple linear regression

MODPA: moderate physical activity

MRS: metabolic risk score

MS: metabolic syndrome

MVPA: moderate to vigorous physical activity

OR: odds ratio

PA: physical activity

PACER: progressive aerobic cardiovascular endurance run

PAI: physical activity index

PASW: Predictive Analytic Software

PC: perímetro da cintura

RER: respiratory exchange ratio

RLM: regressão linear múltipla

RME: risco metabólico elevado

RNA: redes neurais artificiais

ROC: receiver-operating characteristic

SBP: systolic blood pressure

SD: standard deviation

SEDPA: sedentary physical activity

SEE: standards error of the estimate

SENS: sensitivity

SPEC: specificity

SPSS: Statistical Package for the Social Sciences

SR: 20m shuttle run test

TC: total cholesterol

TRIG: triglycerides

VIG: vigorous physical activity

VO_2max: consumo máximo de oxigênio / maximal oxygen uptake

VO_2maxANN: maximal oxygen uptake estimated by artificial neural network

VO_2maxBarnett: maximal oxygen uptake estimated by Barnett equation

VO_2maxLéger: maximal oxygen uptake estimated by Léger equation

VO_2maxMLR: maximal oxygen uptake estimated by multiple linear regression

VO_2maxRuiz: maximal oxygen uptake estimated by Ruiz equation

VO_2peak: peak of oxygen uptake

VV: teste do vaivém

WC: waist circumference

WHO: World Health Organization

Z-score: standardized score

GENERAL INTRODUCTION

It is established that cardiovascular diseases (CVD) represents the greatest burden in terms of public health, with massive social and economical implications, both for developed and developing societies. According to the World Health Organization (WHO), 17.3 million people died from CVD in 2008, which represents 30% of all deaths. In Portugal, the mortality due to CVD is approximately 40% of all deaths. The American Heart Association (AHA) suggests that 75%-90% of the CVD epidemic is related to risk factors such as dyslipidaemia, hypertension, diabetes *mellitus* and obesity. These disorders and risk factors are mostly explained by sedentary lifestyles, unhealthy diet, tobacco use and other risk behaviours (Daviglus et al., 2004; Gidding et al., 2006; Kavey et al., 2003; Stamler et al., 1999).

In the In the recent years, CVD risk factors are recognized as a health paediatric concern, since the incidence of risk factors like obesity, hypertension and dyslipidaemias have increased in children and adolescents (Kavey et al., 2003). It is established that there is a tendency of cardiovascular risk factors to cluster in young people (Andersen et al., 2006; Andersen, Wedderkopp, Hansen, Cooper, & Froberg, 2003; Brage et al., 2004; Ribeiro et al., 2004). A well documented metabolic disorder resulting from risk factors clustering is the Metabolic Syndrome (MS), characterized by central obesity, dyslipidaemia, hypertension, glucose intolerance and insulin resistance (Alberti, Zimmet, & Shaw, 2006; IDF, 2007), which affects 30% of young obese and 2.4% of the general paediatric population (Cook, Auinger, Li, & Ford, 2008; Cook, Weitzman, Auinger, Nguyen, & Dietz, 2003). Studies reported a trend in clustered risk factors to track from childhood to adulthood, which is stronger than the trends for a single risk factor (Andersen & Haraldsdottir, 1993; Andersen, Hasselstrøm, Grønfeldt, Hansen, & Karsten, 2004). Furthermore, it is well documented that long-term exposure to cardiovascular risk factors (such as obesity, hypertension, dyslipidaemias, insulin resistance and metabolic syndrome) is associated to increased cardiovascular morbidity and mortality risk (Freedman, Serdula, Srinivasan, & Berenson, 1999; Lauer, Lee, & Clarke, 1988; Li et al., 2003; Mahoney et al., 1996; Nicklas, Von Duvillard, & Berenson, 2002; Srinivasan & Berenson, 1995; Webber, Srinivasan, Wattigney, & Berenson, 1991).

Together with the evidence reporting the occurrence and clustering of cardiovascular risk factors in youths, there are signs that the development of atherosclerosis, the pathology underlying the majority of CVD, starts in childhood, progressing silently until the clinical manifestation (angina and myocardial infarction) and the occurrence of cardiovascular events later in adulthood. Indeed, post-mortem studies demonstrated that advanced atherosclerotic lesions are present in children and adolescents, through autopsy after unexpected death of other causes (Berenson et al., 1998; Berenson et al., 1992). Arterial stiffness, which is considered a marker of the atherosclerotic process and related CVD, has been investigated in children and adolescents. Actually, in young people, increased levels of arterial stiffness were already reported and associated with obesity (Sakuragi et al., 2009; Urbina, Kimball, Khoury, Daniels, & Dolan, 2010) elevated blood pressure (Lydakis et al., 2012), metabolic syndrome (Ferreira et al., 2005; Jae et al., 2010), insulin resistance and other traditional cardiovascular risk factors (Urbina, Gao, Khoury, Martin, & Dolan, 2012).

The body of evidence aforementioned is an indicative that the primary prevention of CVD should begin in childhood. Therefore, the recognition of non-invasive methods for detection and targeting interventions for young people at risk is imperative. The assessment of physical fitness and its measures allow describing the general condition of health (Ortega, Ruiz, Castillo, & Sjöström, 2008). Especially cardiorespiratory fitness (CRF), which is representative of the physiological status and general capacity of cardiovascular and respiratory systems and describes the ability to carry out prolonged exercise (Taylor, Buskirk, & Henschel, 1955). The standard indicator of CRF is the maximal oxygen uptake (VO_2max). Usually, VO_2max is measured directly by ergospirometry in laboratory setting with a progressive exercise test on treadmill or cycle ergometers. Conventionally, VO_2max is described in millilitres consumed in one minute for each unit of body mass [$ml.kg^{-1}.min^{-1}$] or, alternatively in terms of metabolic equivalents [MET (1 MET= 3.5 $ml.kg^{-1}.min^{-1}$)] (Taylor et al., 1955). By analysing the relationship between cardiorespiratory fitness and single cardiovascular risk factors in children and adolescents, evidence from previous investigation indicated that CRF is inversely associated with body mass index (Aires et al., 2008; Aires, Silva, et al., 2010), skinfold thickness (Brage et al., 2004; Ekelund et al., 2007), waist circumference (Ekelund et al., 2007), total cholesterol (Boreham et al., 2001; Eisenmann et al., 2005; Gutin, Yin, Humphries, Bassali, et al., 2005), triglycerides (Brage et al., 2004; Eisenmann et al., 2005; Ruiz et al., 2006), fasting glucose (Eisenmann et al., 2005; Ekelund et al., 2007) and blood pressure (Boreham et al., 2001; Eisenmann et al., 2005; Ekelund et al., 2007). Furthermore, reduced CRF has been more strongly associated with clustered metabolic risk (Andersen et al., 2008; Anderssen et al., 2007; Brage et al., 2004; Ekelund et al., 2007; Lobelo, Pate, Dowda, Liese, & Ruiz, 2009; Ruiz et al., 2006; Ruiz et al., 2007). More recently, reduced CRF was also associated with increased arterial stiffness in young people (Sakuragi et al., 2009). Additionally, longitudinal studies reported that reduced CRF during youth is associated with increased arterial stiffness and metabolic risk later in adulthood (Boreham et al., 2004; Ferreira et al., 2005). Although many findings suggest that CRF is correlated with single and clustered risk factors, few studies analysed whether these relationships are independent of obesity (Andersen et al., 2008; Ekelund et al., 2007). Indeed, obesity seems to be a crucial element to the development of metabolic syndrome (Alberti et al., 2006). Although CRF and obesity are associated, there are evidences supporting the idea that CRF may be considered an independent risk factor for MS and it might be related to metabolic risk through different mechanisms (Andersen et al., 2008; McMurray & Andersen, 2010)

Cardiorespiratory fitness was already reported to be a powerful predictor of cardiovascular morbidity and mortality and all-cause risk of death in adults (Blair et al., 1989). Indeed, CRF is a more powerful predictor of mortality among adults than other established cardiovascular risk factors (Myers et al., 2002). Consequently, for both adults and young people, evidences suggest that CRF is considered a valuable tool for risk stratification and should be inserted in surveillance system for primary prevention of cardiovascular diseases (Ortega et al., 2008).

The definition of reference standard values for cardiovascular risk stratification is an important step for primary prevention of CVD. In adults, the study from Blair et al. (1989) suggests that men and women with CRF values lower than 10 MET (35 $ml.kg^{-1}.min^{-1}$) and 9 MET (31.5 $ml.kg^{-1}.min^{-1}$), respectively, have an increased relative risk of all-cause mortality. On the other hand, a similar approach to define reference values for CRF in children and adolescents is not possible, since the exposure to risk factors was not prolonged enough to trigger cardiovascular events, or even death from CVD, which might just occur later in adulthood. Therefore, the clinical reference of cardiovascular health for establishing reference standards for CRF in young people should be different, and clustered metabolic risk scores have been pointed as a stable marker of cardiovascular health with increased tracking coefficients form childhood to adulthood in comparison with single risk factors (Andersen et al., 2004).

Cardiorespiratory Fitness reference standards for targeting children and adolescents with elevated cardiovascular risk were calculated and validated, taking into consideration the inverse relationship between CRF and the clustering of cardio-metabolic risk factors (Adegboye et al., 2010; Bell, Macek, Rutenfranz, & Saris, 1986; Cureton & Plowman, 2008; Cureton & Warren, 1990; Lobelo et al., 2009; Ruiz et al., 2007; The Cooper Institute, 2007; Welk, Laurson, Eisenmann, & Cureton, 2011). These reference standards were described in units of VO_2max ($ml.kg^{-1}.min^{-1}$) and defined in the basis of different principles: (a) arbitrary, when defined by a group of experts in the research field; (b) statistical, defined from values in the bottom of the distribution for a population, first quintile or first quartiles, for example; and (c) biological, determined from the non-linear relationship between CRF levels and an increased clustered metabolic risk score.

Table 1 shows some reference standards of VO_2max for cardiovascular health risk stratification in children and adolescents. The reference values suggested by the European Group of Pediatric Work Physiology in 1986 were defined arbitrarily by experts (Bell et al., 1986). The cut-off values suggested by FITNESSGRAM™ for each group of age and gender were estimated by regression of reference standards defined for adults (Blair et al., 1989; Cureton & Plowman, 2008). Thus, no clinical validation is recognized for these two mentioned reference standards. For the reference standards suggested in the studies from Ruiz et al. (2007), Lobelo et al. (2009), Adegboye et al. (2010), and Welk et al. (2011), cut point values were estimated by ROC Curve analysis and validated by the differentiation of groups with healthier and deprived levels in a clustered cardio-metabolic risk score. The reference values suggested by these studies were validated on the basis of clinical/biological approaches.

Table 1 – Reference standards (cut-off points) for VO_2max values for cardiovascular risk stratification in children and adolescents

Gender	Age	EGPWP (1986) at risk	FITNESSGRAM (2007) at risk	Ruiz et al. (2007) at risk	Lobelo et al. (2009) at risk	Adegboye et al. (2010) at risk	Welk et al. (2011) at high risk	at risk
Girls	8	-	-	-	-	<37.4	-	-
	9	-	-	<37.0	-	<37.4	-	-
	10	<35.0	<39.0	<37.0	-	<37.4	<37.3	<40.2
	11	<35.0	<38.0	-	-	<37.4	<37.3	<40.2
	12	<35.0	<37.0	-	<36	-	<37.0	<40.1
	13	<35.0	<36.0	-	<36	-	<36.6	<39.7
	14	<35.0	<35.0	-	<36	<33.0	<36.3	<39.4
	15	<35.0	<35.0	-	<36	<33.0	<36.0	<39.1
	16	<35.0	<35.0	-	<35.5	<33.0	<35.8	<38.9
	17	<35.0	<35.0	-	<35.5	<33.0	<35.7	<38.8
	18	<35.0	<35.0	-	<35.5	-	<35.3	<38.6
	19	-	-	-	<35.5	-	-	-
Boys	8	-	-	-	-	<43.6	-	-
	9	-	-	<42.1	-	<43.6	-	-
	10	<40.0	<42.0	<42.1	-	<43.6	<37.3	<40.2
	11	<40.0	<42.0	-	-	<43.6	<37.3	<40.2
	12	<40.0	<42.0	-	<44.1	-	<37.6	<40.3
	13	<40.0	<42.0	-	<44.1	-	<38.6	<41.4
	14	<40.0	<42.0	-	<44.1	<46.0	<39.6	<42.5
	15	<40.0	<42.0	-	<44.1	<46.0	<40.6	<43.6
	16	<40.0	<42.0	-	<40.3	<46.0	<41.1	<44.1
	17	<40.0	<42.0	-	<40.3	<46.0	<41.2	<44.2
	18	<40.0	<42.0	-	<40.3	-	<41.2	<44.3
	19	-	-	-	<40.3	-	-	-

Notes: VO_2max values expressed in $ml.kg^{-1}.min^{-1}$; subjects with values under the above-mentioned values are considered "at risk" of later development of cardiovascular diseases. EGPWP= European Group of Pediatric Work Physiology; FITNESSGRAM™= health related physical fitness test battery suggested by the Cooper Institute.

Although directly measured VO_2max is considered to be the standard indicator of CRF, it is accepted that the methods for assessing this indicator have some limitations. Usually, the objective measurement of VO_2max requires a laboratory with sophisticated equipment, specialized technicians, which may elevate costs for population assessment (Ruiz et al., 2009). Alternatively, several field tests were validated to assess CRF, and several equations were calculated and validated to estimate VO_2max based on the achieved performance in these tests. Field tests such as One-mile Run, 12-minutes Run, 9-minutes Run, 6-minutes Run and the 20-meter Shuttle Run (SR) are several examples of tests included in popular fitness tests batteries like FITNESSGRAM (The Cooper Institute, 2007; The Cooper Institute for Aerobics Research, 1999), EUROFIT (Council of Europe committee for the development of sport, 1988) and PROESP-BR (Gaya & Silva, 2007). The 20m Shuttle Run test (SR), originally designed and validated by Léger and colleagues (Léger, Lambert, Goulet, Rowan, & Dinelle, 1984; Leger & Lambert, 1982; Léger, Mercier, Gadoury, & Lambert, 1988) is one of the most widely used field tests for estimating CRF in youths (Tomkinson, Leger, Olds, & Cazorla, 2003). The SR simulates a progressive workload test until the exhaustion. Summarizing, the test consists in running back and forth between two lines 20 meters apart according to sound signals pacing the running speed, with a gradual increase in the speed for each 1-min stage.

The use of equations to estimate VO_2max from the performance in field tests might be valuable in many ways: it provides known values that are comparable to valid reference standards and it permits the comparisons between studies and populations, even when the subjects were evaluated with different tests and protocols. Especially for the original SR, or other modified versions, several equations were previously calculated for estimating VO_2max (Table 2) from indicators of the individuals' performance achieved in the test (number of completed shuttle/laps, number of completed 1-min stages, time to exhaustion or maximal running speed attained in the test). As it can be observed in Table 2, the equations mainly differ on independent variables assumed as predictors and also in the type of function assumed to express the relationships between the predictors and the outcome (VO_2max). This variety of equations and methodological assumptions accounted in the different studies result from an extensive number of attempts for improving the validation of SR as an indicator of CRF and also to improve the accuracy of the VO_2max estimations in different populations.

It is important to refer one study carried out in Portuguese youths, which aimed to analyse the validation of the SR for the Portuguese population and to assess the agreement between directly measured VO_2max and estimated VO_2max from five different equations in youths aged 13-19 years (Ruiz et al., 2009). The findings of this study were that the equations developed by Léger et al. (1988), Barnett et al. (1993), Matsuzaka et al. (2004), and Ruiz et al. (2008) significantly underestimate the directly measured VO_2max in Portuguese youths. Another important finding was that the equation originally suggested by Léger et al. (1988) presented the worst validation results when compared to the other equations. These findings are of concern, since the equation from Léger et al. (1988) is probably the most used one in studies that appeal to SR for assessing CRF.

It should be recognized that there are no equations universally accepted and valid for all populations, age and gender groups. Thus, it is necessary to know the levels of validation for the equations in each population that is object of research, in order to have proper estimations of VO_2max (Ruiz et al., 2009). It is known that most of the equations for estimating VO_2max fail to provide valid estimations for populations that are different than that one which gives origin to the equation.

Table 2 – Equations for estimating VO_2max from the performance in the 20-m Shuttle Run Test

Authors	Equation to estimate VO_2max expressed in $ml.kg^{-1}.min^{-1}$
Léger et al. (1988)	VO_2max = 31.025 + 3.328*speed - 3.248*age + 0.1536*speed*age
Barnett et al. (1993)	VO_2max = 25.8 - 6.6*sex - 0.2*weight + 3.2*speed VO_2max = 24.2 - 5.0*sex - 0.8*age + 3.4*speed
Matsuzaka et al. (2004)	VO_2max = 25.9 - 2.21*sex - 0.449*age - 0.831*BMI + 4.12*speed VO_2max = 61.1 - 2.20*sex - 0.462*age - 0.862*BMI + 0.192*laps
Mahar et al. (2006)	VO_2max = 47.438 + 0.142*laps + 5.134*sex - 0.197*weight
Ruiz et al. (2008)	VO_2max = (1/(1+EXP(-(1/(1+EXP(-((sex*0.8-0.7)*-1.03329+(age*0.114285714286-1.38571428571)*0.54719+(weight*0.012213740458-0.406870229008)*0.61542+(height*0.0195598978221-2.76356892177)*-0.51381+(stage*0.0842105263158-0.0684210526316)*-0.92239+-0.34242)))*-0.95905+1/(1+EXP(-((sex*0.8-0.7)*-1.19367+(age*0.114285714286-1.38571428571)*-1.54924+(weight*0.012213740458-0.406870229008)*-3.18931+(height*0.0195598978221-2.76356892177)*0.77773+(stage*0.0842105263158-0.0684210526316)*3.31887-0.55696)))*2.19501+1/(1+EXP(-((sex*0.8-0.7)*1.38191+(age*0.114285714286-1.38571428571)*-2.14449+(weight*0.012213740458-0.406870229008)*0.0485+(height*0.0195598978221-2.76356892177)*0.10879+(stage*0.0842105263158-0.0684210526316)*-4.90052+0.53905)))*-2.567+-0.05105)))+0.478945173945)/0.0204587840012
Mahar et al. (2011)	VO_2max = 32.56941 + 0.27297*laps + 3.25225*sex + 0.02961*age VO_2max = 40.34533 + 0.21426*laps – 0.79472*BMI + 4.27293*sex + 0.79444*age VO_2max = 41.76799 + 0.49261*laps - 0.00290*$laps^2$ - 0.61613*BMI + 0.34787*sex*age

Notes: For Léger equation, speed=running speed in the last completed stage. For Barnett equations, speed=maximal running speed, sex=0 for boys and sex=1 for girls. For Matsuzaka equation, speed= maximal running speed (km/h), sex=0 for boys and sex=1 for girls. For Mahar 2006 equation, sex=1 for boys and sex=0 for girls. For Ruiz equation, stage=number of half-completed stage, sex=1 for boys and sex=2 for girls. For Mahar 2011 equation, sex=1 for boys and sex=0 for girls. For all equations, age is included in years, speed in km/h, height cm, body weight kg and BMI in kg/m^2.

Besides the limitations that are related to the external validity of the equations, the distribution of errors may lead to biased interpretations in longitudinal studies or intervention studies. Thus, some representative value of the performance in the field test could be used as reference for risk stratification if its validation for this purpose was tested. Table 3 shows the reference values in terms of SR laps suggested by FITNESSGRAM (The Cooper Institute, 2007; The Cooper Institute for Aerobics Research, 1999).

Table 3 – Reference standards (cut-off points) for completed laps in the 20-m Shuttle Run test for cardiovascular risk stratification in children and adolescents suggested by FITNESSGRAM™

Age	Girls at risk	Boys at risk
10	<7 laps	<23 laps
11	<15 laps	<23 laps
12	<15 laps	<32 laps
13	<23 laps	<41 laps
14	<23 laps	<41 laps
15	<32 laps	<51 laps
16	<32 laps	<61 laps
17	<41 laps	<61 laps
>17	<41 laps	<72 laps

Notes: laps represents the number od completed shuttles for the 20-m Shuttle Run test; subjects with values under the above-mentioned values are considered “at risk” of later development of cardiovascular diseases. FITNESSGRAM™= health related physical fitness test battery suggested by the Cooper Institute (2007).

The FITNESSGRAM™ reference standards (The Cooper Institute, 2007; The Cooper Institute for Aerobics Research, 1999), expressed in SR laps (number of completed shuttles), were estimated by inverting the equation by Léger et al. (1988). Although these reference values were used for many years, the clinical validity of these standards for screening subjects in risk for CVD or exposure to CVD risk factors was never tested. Indeed, it would be advantageous if one or some parameters of the performance in the field test could offer a good measure for risk stratification. A similar rationale was applied when reference standards for overweight and obesity were calculated for body mass index [BMI] (Cole, Bellizzi, Flegal, & Dietz, 2000) and waist circumference [WC] (Taylor, Jones, Williams, & Goulding, 2000). Although it is known that these measurements are not feasible as the gold standard measurements of body composition, it is assumed that both BMI and WC are valid for screening subjects with increased body fatness (Taylor et al., 2000).

Summarizing, studies that presented reference standards clinically valid for VO_2max (Adegboye et al., 2010; Lobelo et al., 2009; Ruiz et al., 2007; Welk et al., 2011) support the idea that might exist a critical value for CRF which is associated with a significant increase in the risk of occurrence and clustering of cardiovascular risk factors in children and adolescents. From the epidemiological point of view, it is important to refer that this association was observed both with directly measured and estimated VO_2max. Therefore, it seems reasonable that CRF may be considered a valuable tool for risk stratification since the early ages.

Not just the stratification of young subjects at risk, but also the identification of strategies for increasing CRF levels and optimize cardiovascular health might be relevant for the prevention of later CVD manifestation. Therefore, it seems logical that regular physical exercise and habitual physical activity should promote several favourable changes in general health. Regarding cardiovascular health, reduced levels of habitual PA have been associated with reduced cardiorespiratory fitness (Dencker et al., 2006), increased overweight and obesity, dyslipidaemias, increased glucose, insulin resistance, elevated triglycerides, hypertension and increased metabolic risk (Andersen et al., 2006; Brage et al., 2004).

Regarding obesity, it makes sense that the total volume of PA has an important role in the regulation of energy balance, i.e. in the equilibrium of energy intake and energy expenditure (Gutin, Yin, Humphries, & Barbeau, 2005). Evidences pointed that not only total PA but also moderate to vigorous PA (MVPA) may be associated with the general cardiovascular health (Andersen et al., 2006; Gutin et al., 2002; Gutin, Yin, Humphries, & Barbeau, 2005).

International recommendations of PA for young people in school ages suggest that children and adolescents should accumulate 60 minutes per day of

MVPA in order to improve physical fitness, cardiovascular, metabolic and bone health and to reduce symptoms of anxiety and depression (Janssen, 2007; Strong et al., 2005; WHO, 2010). It is considered that these PA guidelines are based in solid evidence (Andersen, Riddoch, Kriemler, & Hills, 2011), although, the studies that support these recommendations differ in many methodological aspects such as: studies designs (observational, cross-sectional, longitudinal, experimental, training and/or intervention studies) and assessment of physical activity (self-reported by questionnaires or objectively measured by pedometers or accelerometers). Studies from Brage et al. (2004), Gutin et al. (2005) and Andersen et al. (2006), reported significant associations between total PA, the amount of MVPA and CRF, indicators of obesity and metabolic syndrome. However, the compliance with the recommendation of 60 min per day in MVPA may be attained in different contexts of PA. Active commuting to school, participation in physical education classes, recreational and leisure physical activity, supervised or spontaneous activities, participations in recreational or competitive sports are some of the PA contexts that may contribute to the compliance with PA guidelines. However, little is known about which PA contexts are more effective in promoting the compliance with PA recommendations, increasing CRF and improving cardiovascular health. Some evidence suggest that sports participation is associated with increased levels of physical fitness, lower levels of body fatness, blood pressure and reduced risk of clustering CVD risk factors (Ara et al., 2004; Boreham, Twisk, Savage, Cran, & Strain, 1997). Therefore, it would be expected that children and adolescents engaging in higher levels of competitive sports would reach greater amounts and superior intensities of habitual PA as a result of their participation in exercise training programs. Data from cross-sectional studies indicate that trained young athletes have higher values of VO_2max when compared to their untrained peers (Armstrong, Tomkinson, & Ekelund, 2011; Rowland, Wehnert, & Miller, 2000). Experimental studies based in dose-response analysis of moderate to vigorous physical exercise (>60% of VO_2max) report significant increases in CRF in young people submitted to training programs (Armstrong et al., 2011; Stoedefalke, Armstrong, Kirby, & Welsman, 2000; Tolfrey, Jones, & Campbell, 2004). However, the dose-response relationship between habitual physical activity and CRF is not clearly established in the contexts where regular supervised exercise training within a particular sport is absent (Armstrong et al., 2011). The evidence from cross-sectional analyses report low to moderate correlations (r=0.1 to r=0.4) between indicators of habitual physical activity and CRF (Dencker & Andersen, 2008).

Cardiorespiratory fitness, assessed directly by measuring maximal oxygen uptake, has been proven to be an important marker of cardiovascular health in people of all ages. Evidences suggest that the process leading to cardiovascular diseases starts in childhood and primary prevention should begin since the early ages. Consequently, the systematic assessment of cardiorespiratory fitness with simple measurements, such as field fitness testing, could be considered a paramount extent for surveillance strategies.

Therefore, with public health concerns, especially for the Portuguese youths, the original research carried out in this thesis had the following purposes that were structured in four different studies:

[Study I] To calculate and validate two models to estimate maximal oxygen uptake (VO_2max) in Portuguese youths, aged 10-18 years, using the 20-meter Shuttle Run Test.

[Study I] To calculate and validate reference standards for the 20-m Shuttle Run Test in youths aged 10-18 years for screening children and adolescents with increased metabolic risk.

[Study III] To analyse the associations between cardiorespiratory fitness, central adiposity and metabolic risk in youths.

[Study IV] To analyse the associations between the levels of participation in different contexts of physical activity, levels of moderate to vigorous physical activity and cardiorespiratory fitness.

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Calculation and validation of models for estimating VO_{2max} from the 20-m shuttle run test in children and adolescents

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Objective: The purposes of this study were to calculate and validate two models to estimate maximal oxygen uptake (VO_2max) in Portuguese youths, aged 10-18 years, using a 20-meter shuttle run test (SR). ***Design:*** Subjects (54 girls and 60 boys) were divided into estimation (*n*= 91) and cross-validation (*n*=23) groups, and their VO_{2max} was directly measured by wearing a portable gas analyzer during the SR. The Multiple Linear Regression (MLR) and Artificial Neural Network (ANN) tests were carried out considering sex, age, height, weight, body mass index (BMI) and SR stage as predictors of VO_{2max}. Estimations from MLR and ANN were compared with three other previously published equations. ***Results:*** In summary, the equation estimated by MLR is more appropriate for Portuguese youths than the equation estimate by ANN or the other three previously-published equations (validation coefficient for the MLR model: r=0.84, P<0.001; systematic error=-0.01±5.2, P>0.05; SEE=4.9). ***Conclusion:*** For Portuguese youths, the following equation would be recommended: VO_{2max}= 43.313 + 4.567*sex - 0.560*BMI + 2.785*stage. However, findings from this study also warn researchers that the use of equations to estimate VO_{2max} may not be sensitive enough to detect small changes in individuals' cardiorespiratory fitness in longitudinal observations and intervention studies, according to the dispersions of random error for all equations.

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Key Words: physical fitness; oxygen consumption; youths; multiple linear regression; artificial neural network

INTRODUCTION

There is a good deal of evidence suggesting that cardiorespiratory fitness (CRF) is an important marker of cardiovascular health. In adults, low levels of CRF are considered predictors of mortality as a result of cardiovascular diseases (10, 28, 30). In young people, poor CRF is associated with obesity and features of metabolic syndrome (2, 13, 18, 32, 33).

In terms of measurement, the maximum oxygen uptake (VO_{2max}) is the criterion measure of CRF (38). However, protocols to directly measure VO_{2max} usually require a research laboratory with sophisticated equipment, trained staff and increased costs. Alternatively, several field tests and equations have been developed to estimate VO_{2max}, which are crucial for large sample studies and health surveillance policies.

The 20-meter Shuttle Run Test (SR) created by Léger and colleagues (20) is a widely used field test. The SR, or some modified version, is included in a broad number of physical fitness test batteries. One of the most frequently-used versions is the PACER (Progressive Aerobic Cardiovascular Endurance Run), the standard CRF test for the FITNESSGRAM battery (39).

The equation suggested by Léger et al. (20) to estimate VO_{2max} remains the most frequently used, and it is included by default in the FITNESSGRAM software to estimate VO_{2max} (14). On the other hand, several researchers have developed alternative equations to estimate VO_{2max} for different population groups, including children and adolescents (8, 20, 24, 25, 34), attempting to improve the validity and the accuracy of VO_{2max} estimations.

A common issue that arises in the use of several equations is the external validity of estimations. Usually, when estimation models are employed in a different population than that in which the equation originated, the validity and accuracy of estimations

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decrease (35). These limitations could be related to the variety of methodological options available to estimate and validate equations, including statistical procedures, error analysis and presence/absence of a cross-validation group. In a previous study, the validity and accuracy of five different equations were tested in Portuguese youths (35), including equations estimated by Multiple Linear Regression (8, 20, 25) and Artificial Neural Networks (34). Findings from that study suggested that more research is needed to calculate a valid and precise indicator of CRF, one that is determined from the SR in Portuguese children and adolescents.
Therefore, this study aimed to estimate and validate two different models (MLR and ANN) to predict VO_{2max} in Portuguese youths aged 10-18 years.

MATERIAL AND METHODS

Participants

For this study, a total of 122 healthy young individuals (57 girls and 65 boys) from three schools in the District of Porto, Portugal, volunteered to participate in the study. The study was carried out following the Declaration of Helsinki guidelines for human research. The study's purpose, nature, benefits and risks were explained to participants, parents/guardians and teachers. Informed written consent was obtained from the participants' parents/guardians. The experimental protocol was approved by the Review Committee of the Institutional Scientific Board, as well as by the Foundation of Science and Technology from Portugal.

Anthropometric Measures

Height and weight were measured before testing, with participants wearing shorts and t-shirts only. Height was measured using a Holtain stadiometer (Holtain Ltd., Crymmych, UK) and recorded in centimetres to the nearest millimetre. Weight was measured to the nearest 0.1 kg with a Seca weight scale. Body mass index (BMI) was calculated by the ratio between weight and squared height ($kg.m^{-2}$).

Cardiorespiratory Fitness and Physiological Measurements

Participants performed the SR according to the described by FITNESSGRAM [PACER;(39)]. Briefly, the test consists in running back and forth between two lines 20 meters apart, with running speed determined by audio signals from a pre-recorded music CD. The running speed increases at the end of each one-minute stage. The running speed is 8.0 $km.h^{-1}$ for the first stage, 9.0 $km.h^{-1}$ for the second stage, and thereafter increases by 0.5 $km.h^{-1}$ each minute. The test ends when the subjects twice fail to reach the lines at the time indicated by the audio signals, demonstrating an inability to keep the required pace. All participants were familiar with the test, since the FITNESSGRAM test battery is included in the Portuguese Physical Education curriculum. As a result, most of the students perform the 20m Shuttle Run Test at least three or four times a year. The total number of completed laps was recorded and then transformed into stages. The last completed stage was considering the SR variable and entering it into the equations estimated by this study. All subjects underwent the SR wearing a portable gas analyser (K4*b*2, Cosmed, Rome, Italy) and a heart rate monitor (Polar Electro Oy, Kempele, Finland) to directly measure oxygen consumption (VO_2) and heart rate (HR), respectively. The weight of the Cosmed K4*b*2 was 1.5 kg, including the battery and a specially designed harness. McLaughlin and colleagues (26) reported that it is a valid device when compared with the Douglas bag method. Wearing the portable gas analyser during the 20-m shuttle run test does not significantly alter participants' energy demands (16). Respiratory variables were recorded breath-by-breath, which in turn were averaged over a 10-second period, yielding a "fair" representation of the change in VO_2 during incremental exercise (6). Before each individual test, oxygen and carbon dioxide analysers were calibrated according to the manufacturer's instructions. Directly measured VO_{2max} or VO_{2peak} were the main variables determined using the open-circuit method. Directly measured VO_{2max} was considered when a plateau in the VO_2 curve was detected, defined as an increase in a VO_2 of less than 2 $ml.kg^{-1}.min^{-1}$ with a concomitant increase in speed stage. If a VO_2 plateau was absent (15), the VO_{2peak} was taken and defined as the highest oxygen uptake achieved during the SR at exhaustion (3). For practical reasons, from now on, this paper will refer to the highest VO_2 values achieved in the SR as VO_{2max}. Exhaustion was confirmed when: (1) subjects desired to stop or demonstrated an inability to maintain the required running pace despite strong verbal encouragement; (2) maximal heart rate was greater than 85% of age-predicted maximal heart rate (220-age); (3) the respiratory exchange ratio (RER) was greater than 1.0 at the end of the test; (4) the participants showed symptoms of discomfort and/or signs of high sweating, facial flushing and grimacing (15). Careful control was taken concerning technical and environmental variables that might have had some influence on the results, so that highly reliable metabolic measures could be obtained. The protocol for the SR was carried out in groups of 11 students at time and, for each group of 11 subjects, one student

Table 1. Descriptive values for physical and physiological characteristics and SR performance of study participants.

	Total Sample					Validation Groups	
		All		Girls	Boys	Estimation	Cross-Validation
		(n=114)		(n=54)	(n=60)	(n=91)	(n=23)
Variables	*Minimum*	*Maximum*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*
Age (years)	10.0	18.0	14.5±2.0	14.4±1.9	14.5±2.0	14.5±1.9	14.3±2.3
Height (cm)	141.0	187.0	164.0±9.8	160.6±6.5	*167.1±11.2	164.2±9.6	163.2±10.7
Weight (kg)	34.8	96.0	57.6±10.6	55.8±7.6	*59.2±12.6	58.0±10.1	56.1±12.6
BMI ($kg \cdot m^{-2}$)	16.0	29.9	21.3±2.7	21.6±2.6	21.0±2.8	21.4±2.7	20.8±2.7
SR laps (number of laps completed)	6.0	135.0	47.9±22.3	34.6±12.5	*60.0±22.4	46.9±20.3	52.0±29.0
SR stage (number of stages completed)	0.0	13.0	5.1±2.2	3.9±1.4	*6.3±2.1	5.1±2.0	5.5±2.7
SR maximal speed ($km \cdot h^{-1}$)	8.0	15.0	11.0±1.1	10.3±0.7	*11.6±1.1	11.0±1.0	11.2±1.4
Measured VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	29.2	70.0	48.1±9.5	42.3±7.5	*53.3±8.1	47.8±9.7	49.4±8.9
Measured VO_2max ($ml \cdot min^{-1}$)	1490.5	4649.4	2765.7±737.3	2344.2±420.2	*3145.0±757.3	2766.5±726.0	2762.6±797.2
Maximal Ventilation ($l \cdot min^{-1}$)	63.5	160.7	102.3±19.1	92.1±11.9	*111.5±19.8	102.6±18.9	101.0±20.3
End-exercise RER ($ml \cdot min^{-1}$)	1.0	2.6	1.3±0.3	1.3±0.3	1.3±0.4	1.3±0.3	1.3±0.3
Predicted Maximal HR ($beats \cdot min^{-1}$)	202.0	210.0	205.5±2.0	205.6±1.9	205.5±2.0	205.5±1.9	205.7±2.3
Measured Maximal HR ($beats \cdot min^{-1}$)	162.0	224.0	199.5 ± 9.9	196.7 ± 10.0	**202.0 ± 9.3	198.9 ± 10.3	202.5 ± 7.9

Note: BMI=body mass index; SR= 20-meter shuttle run; VO_{2max}=maximal oxygen uptake; RER=respiratory exchange ratio; HR= heart rate; * P<0.01 for comparisons between sex; **P<0.05 for comparisons between sex.

was randomly selected to run carrying the portable gas analyser, while the others ran wearing a heart rate monitor (Polar Team System). Finally, a total of 122 subjects had run wearing the Cosmed K4*b*2.

Statistical analysis

Two groups of individuals were separated to estimate and cross-validate the equations. Approximately 80% of participants were randomly assigned in the estimation group. The remaining 20% were defined as the cross-validation group. Descriptive statistics (expressed as mean ± *SD*) were determined to provide anthropometric and physiological characteristics of the participants. Independent t-tests were performed for comparisons between sex and validation subgroups. Two new equations were calculated using the Multiple Linear Regression (MLR) and Artificial Neural Network (ANN) estimation models. Sex, age, weight, height, body mass index (BMI) and the last stage completed (stage) were set up as predictors or inputs (independent) variables. The outcome or the output (dependent) variable was the VO_{2max}, measured directly in the SR by means of the portable gas analyser (measured VO_{2max}). All variables were expressed in their original units, i.e., sex (0=girls and 1=boys), age (years), weight (kg), height (cm), BMI ($kg.m^{-2}$), speed ($km.h^{-1}$) and measured VO_{2max} ($ml.kg^{-1}.min^{-1}$). Before running estimation procedures, Pearson correlation coefficients (r) were examined to observe the relationship between measured VO_{2max} and predictors. The MLR model was constructed using the stepwise method. The VO_{2max} estimated by the MLR model was named VO_{2max}MLR. The ANN model was built using the multilayered perceptron method. Predictors and outcome variables were normalized to a 0-1 interval as an ANN step in order to facilitate the model's learning. The resulting multilayered perceptron model consists of the following ANN architecture: 6 inputs (predictors), 4 hidden units (such as a latent dimension for another multivariate analysis) and 1 output (dependent). The best mathematical resolution for the ANN models tested was the logistic activation functions between input variables and hidden units and between hidden units and output. More recently, ANN models have been explored in the context of medicine, health, physical activity and sports sciences (9, 19, 21, 31, 36, 37). For more information about the related techniques used in this study, see Ruiz et al. (34). The VO_{2max} estimated by the ANN model was defined as VO_2maxANN. The normal distribution of the residuals was tested for both the MLR and ANN models. To verify whether the estimation models calculated by this study were better than previously published equations, three other equations were selected for comparison: Léger's

Table 2. Newly developed equations to estimate VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$) from the SR.

Method	*Equation and Inputs*
MLR	$VO_2maxMLR$= 43.313 + 4.567*sex - 0.560*BMI + 2.785*stage
ANN	$VO_2maxANN$= (1/(1+EXP(-((1/(1+EXP(-(+((stage)/11)*-5.309 + (sex)*-1.968+((age-10)/8)*4.394 + ((height-141)/46)*1.881 + ((weight-37)/59)*3.078 + ((BMI-16.23)/13.68)*4.429 - 4.302))))*-1.782 + (1/(1+EXP(-(+((stage)/11)*1.790 + (sex)*2.253 + ((age-10)/8)*1.770 + ((height-141)/46)*-1.060 + ((weight-37)/59)*4.978 + ((BMI-16.23)/13.68)*-3.610-2.705))))*9.988 + (1/(1+EXP(-(+((stage)/11)*5.528 + (sex)*-6.357 + ((age-10)/8)*-1.068 + ((height-141)/46)*0.663 + ((weight-37)/59)*1.333 + ((BMI-16.23)/13.68)*0.825-1.608))))*6.384 + (1/(1+EXP(-(+((stage)/11)*8.144 + (sex)*-0.724 + ((age-10)/8)*-0.329 + ((height-141)/46)*6.170 + ((weight-37)/59)*-0.573 + ((BMI-16.23)/13.68)*0.373-4.679))))*-4.278 -3.886))))*39.83 + 29.17

Inputs: sex (0=girls; 1=boys); age (years); height (cm); weight (kg), BMI ($kg \cdot m^{-2}$) and stage (number of stages completed); MLR=multiple linear regression (stepwise method); ANN=artificial neural network (multilayered perceptron method); BMI= body mass index; equations are expressed with this shape for easy use with an Excel spreadsheet or SPSS syntaxes.

equation (20) [VO_{2max}Léger], Barnett's equation (8) [VO_{2max}Barnett], and Ruiz's equation (34) [VO_{2max}Ruiz]. The Léger's equation was chosen because it was the original equation created with the SR. Findings from a previous study (35) suggest that Barnett and Ruiz equations had better agreement results with measured VO_2max. The equation selected from Barnett et al. (8) used sex, age and speed as predictors. All five equations were tested for validity and error analyses as recommended (7, 17). Simple linear regression was used to calculate the validity correlation (correlation between the criterion measure and the estimation – measured and estimated VO_{2max}) and the standard error of the estimate (SEE). A paired-sample T-test was used to examine the mean differences (systematic error) between measured and estimated VO_{2max} (7). The agreement between measured and estimated $VO2_{max}$ was observed with the Bland-Altman method (11, 12) for VO_{2max}MLR and VO_{2max}ANN. All analyses were completed with SPSS 19.0 (SPSS Inc., Chicago, United States), with a significance level of 0.05.

RESULTS

After the determination of exhaustion parameters, those subjects who did not meet the exhaustion criteria (end-exercise RER superior to 1.0) were excluded from analyses. Thus, all analyses were carried out with a final sample of 114 subjects (54 girls and 60 boys), separated in the estimation (n=91) and cross-validation (n=23) groups. A VO_{2max} plateau was detected in 87.7% of participants. Table 1 shows descriptive statistics for participants' physical characteristics and SR performance. Significant statistical differences were found between sex for height, weight, SR laps, SR stage, SR speed, measured VO_{2max}, maximal ventilation and measured maximal HR. No differences were found between estimation and cross-validation groups (P>0.05).

Sex, age, height, weight, BMI and stage were considered to be predictors for measured VO_{2max}. Correlation analyses indicated significant Pearson-r coefficients between measured VO_{2max} and age (r=0.26; P<0.001), height (r=0.24; P<0.001), BMI (r=-0.34; P<0.001) and stage (r=0.77; P<0.001). When groups were split by sex, the variables age (r=0.26; P<0.001), weight (r=-0.35; P<0.05) and stage (r=0.68; P<0.001) were correlated with measured VO_{2max} in girls and age (r=0.36; P<0.01), BMI (r=-0.38; P<0.01) and stage (r=0.65; P<0.001) were correlated with measured VO_{2max} in boys. Table 2 provides equations estimated for the multiple linear regression and the artificial neural network methods. The residuals for both equations were normally distributed (non-significant Kolmogorov-Smirnov test; P>0.05).

To test the validity and the accuracy of the estimated VO_{2max} (Table 3), the SEE, the validity correlation, the systematic error and the 95% limits of agreement (LOA) were observed for the two newly estimated equations (MLR and ANN) and for the other previous published equations (Léger, Barnett and Ruiz). SEE ranged between 4.9 (estimated MLR equation for the cross-validation group) and 7.1 $ml.kg^{-1}.min^{-1}$ (Léger's equation for the total sample). Validation coefficients (correlation between estimated and measured VO_{2max}) were significant for all equations (0.86>r>0.67; P<0.001). No mean difference (systematic error; P>0.05) was detected in the comparison between measured and estimated VO_{2max} for the newly developed equations (MLR and ANN). However, a significant underestimation of measured VO_{2max} (P<0.001) was found for Léger and Ruiz equations in the total sample and in the cross-validation group.

Table 3. Descriptive values for estimated VO_2max and validation parameters.

Equation	*Estimated VO_2max (Mean ± SD)*	*Mean Difference (95% LOA)*	*r*	*SEE*
Total Sample (n=114)				
MLR	48.1 ± 8.1	0.0 (-11.2; 11.2)	0.80**	5.7
ANN	47.8 ± 8.5	-0.3 (-10.0; 9.4)	0.86**	5.0
Léger	45.2 ± 5.9	-2.9 (-16.8; 11.0)*	0.67**	7.1
Barnett	47.5 ± 5.4	-0.6 (-14.0; 12.9)	0.71**	6.8
Ruiz	44.1 ± 9.6	-4.0 (-16.8; 8.8)*	0.77**	6.2
Cross-validation (n=23)				
MLR	49.3 ± 9.4	-0.1 (-10.2; 10.0)	0.84**	4.9
ANN	47.9 ± 8.8	-1.5 (-12.8; 9.8)	0.79**	5.6
Léger	46.6 ± 7.5	-2.7 (-10.6; 7.3)*	0.82**	5.2
Barnett	48.5 ± 6.5	-0.9 (-11.4; 9.6)	0.80**	5.4
Ruiz	45.2 ± 10.7	-4.2 (-17.0; 8.6)*	0.80**	5.5

Note: MLR=multiple linear regression model; ANN=artificial neural network model; VO_2max=maximal oxygen uptake; Mean Difference = VO_{2max} estimated by the equation - VO_{2max} measured directly (expressed in $ml \cdot kg^{-1} \cdot min^{-1}$); LOA=limits of agreement (mean difference ± 1.96*SD*); *r* = Pearson correlations between estimated and measured VO_2max (validation coefficient); SEE = standard error of the estimate expressed in $ml \cdot kg^{-1} \cdot min^{-1}$; **P*<0.001 for comparison between estimated and measured VO_{2max}; ***P*<0.001 for correlations between estimated and measured VO_{2max}.

By analysing the 95%LOA, it would be expected that errors of estimation by these five equations would lie between –17.0 (for Ruiz's equation) and +12.9 $ml.kg^{-1}.min^{-1}$ (for Barnett's equation). The Bland-Altman plots were explored in the cross-validation group for the two newly estimated equations (MLR and ANN), as represented in Figure 1. For the observed plots, the systematic error and the dispersion of random error (95% LOA) were reduced for VO_{2max}MLR in comparison to VO_{2max}ANN.

DISCUSSION

In the present study, two new models to predict the maximal oxygen uptake (VO_{2max} – the standard measure for cardiorespiratory fitness) were estimated and validated for Portuguese children and adolescents, based on their performance in the 20-meter shuttle run test. Also, the validity of three other previously-published equations [Léger's, Barnett's and Ruiz's equations (8, 20, 34)] was verified for Portuguese youths. In summary, the new equation estimated from the multiple linear regression could be considered the most satisfactory to estimate VO_2max for Portuguese children and adolescents. The strengths of the study lie in the strategy to estimate and validate the two new equations, based on several assumptions: (a) the objective and direct measure of oxygen uptake while children and adolescents performed the SR; (b) the employment of two different statistical methods for estimating equations; (c) the analysis of data on three group levels (total sample, estimation group and cross-validation group); (d) the analysis of equation accuracies in a cross-validation group; and (e) the use of robust statistics for validation analysis (7, 11, 12, 17).

The findings of the current study are of interest and are timely, since recent studies (1, 23, 33) have reported that reference-based standards for cardiorespiratory fitness expressed in units of VO_{2max} ($ml.kg^{-1}.min^{-1}$) are valid for targeting young people at risk for metabolic syndrome and cardiovascular diseases. Also, the findings are opportune because there are a variety of equations for the prediction of VO_{2max} that are based in SR performance, and there is no agreement in the literature concerning the appropriate equation for different populations. Moreover, the SR is widely used in Portugal. The FITNESSGRAM battery is the standard set of physical fitness tests implemented by the Portuguese Physical Education Curriculum.

The SR is a logically valid test, since its pacing simulates the criterion test to which it is most often compared: a maximal graded exercise test (14). In the present study, the validation model used by Ruiz and colleagues (34, 35), where subjects ran during the SR wearing a portable gas analyser, was used as an alternative to the usual laboratory treadmill reference test. In this case, the SR was assumed to be a maximally graded test, since physiological variables were measured directly and exhaustion criteria were satisfactorily achieved. Metabolic variables (VO_2 and VCO_2) were measured breath-by-breath and averaged across 10-second periods, which allowed a trustworthy depiction of the metabolism response to incremental effort. This sampling interval is more precise than 30 or 60 seconds periods to determine the VO_2 plateau and/or the VO_{2max}. On the other hand, raw breath-by-breath acquisition is subject to variability due to fluctuations in breathing frequency and tidal volume (6). This approach yields a more sensitive method than the backward VO_{2max} extrapolation technique used by Léger et al. (20). The backward extrapolation is

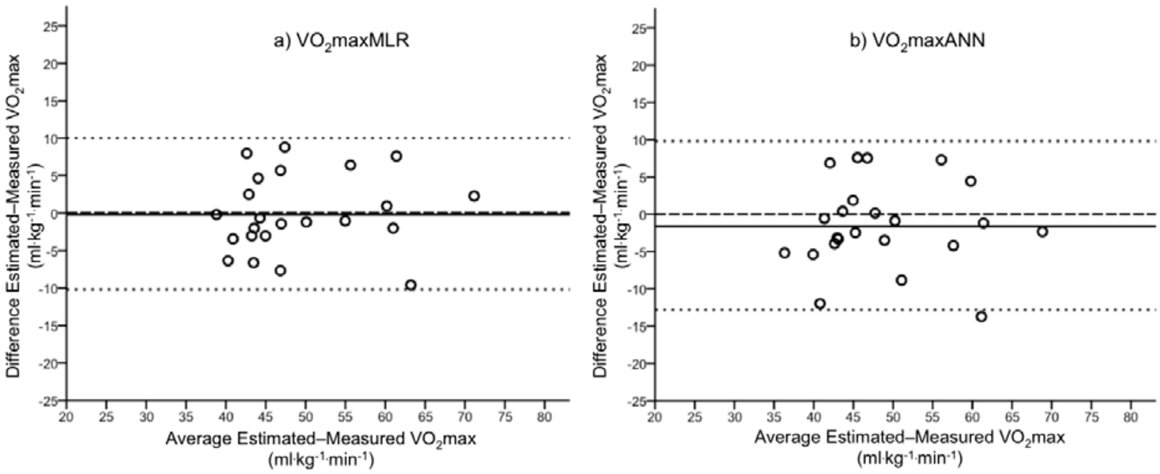

Figure 1. Bland-Altman plots for directly measured VO_{2max} and estimated VO_{2max}. Figure 1a and 1b respectively represents results for MLR and ANN equations in Bland-Altman plots. Solid lines represent mean differences (systematic error) between estimated and measured VO_{2max}. Upper and lower broken lines represent 95% limits of agreement (mean difference±1.96 standard deviations of differences). Centered broken lines represent the reference line for 0 (zero) mean differences.

considered an outdated method (16, 34, 35), since it does not allow the detection of the VO_2 plateau, and provides only an estimation of actual VO_2. The use of new techniques, such as a field measure using a portable gas analyser, has enhanced the understanding of metabolic kinetics during maximal effort measurement, or any other habitual daily task.

A possible discrepancy factor for the comparison of equations is based on the fact that other studies determine VO_{2max} values as directly measured from treadmill-based protocols, using this as the reference (standard) measure (8, 16, 24, 25). The exercise-mode differences in protocol to determine the reference measure of VO_{2max} seem to indicate some discrepancy in energy demands. Metsios et al. (27) and Flouris et al. (16) suggested that VO_{2max} for shuttle running appears to be higher than for treadmill running, but is still strongly correlated (r=0.96). This can be attributed to differences in exercise mode and technique, since shuttle runs incorporate pivot and turning movements characterizing another muscular and, consequently, metabolic involvement (16, 27). Previous studies (8, 22, 34, 35) showed that Léger's equation (20) has an acceptable but not exceptionally strong concurrent validity, although Léger's equation (20) is still used by the FITNESSGRAM software (14). Therefore, some effort should be made to improve VO_2max prediction. Those efforts should consider methodological/statistical alternatives, as well as the selection/inclusion of CRF predictors.

The selection of variables assumed to be VO_{2max} predictors were based on gender differences detected in height, weight, SR laps, SR stages, SR speed, measured VO_{2max}, maximal ventilation and maximal heart rate. Also, by exploring the relationship between the predictors and the outcome, age, height, weight, BMI and stage were found to be correlated with measured VO_{2max}, which is in accordance with previous research (8, 24, 25, 34). Furthermore, it is expected that the inclusion of sex, age and anthropometrical measures would improve the percentage of the VO_2max variance explained by prediction models (8, 24, 25, 34). Despite this, age, height and weight, for example, are indicators of growth and maturation, expressing changes in body size and composition, which can influence the expression of VO_{2max} (4, 5, 29).

Regarding the analysis of errors and validation coefficients, no significant systematic error was detected for the models estimated from the Portuguese population (MLR and ANN). However, a significant underestimation of VO_2max was found for the Léger and Ruiz equations. The results for validation correlations are in accordance with previous studies, varying between 0.79 and 0.86 for MLR and ANN. For Barnett et al. (8), correlations ranged between 0.82 and 0.85 for Hong Kong Chinese students. Matsuzaka et al. (25) reported correlations of 0.76 and 0.75 for Japanese children and adolescents. Mahar et al. (24) showed correlations between 0.65 and 0.67 for North American youths. Ruiz et al. (34) reported a correlation of 0.96 between measured and estimated $VO2_{max}$. However, it should be highlighted that higher validation coefficients were described for the estimation samples. Actually, few studies tested the estimated equations in a cross-validation group (24, 34). When the accuracy of a certain equation is tested in a cross-validation group, generally, validation coefficients drop, even when estimation and cross-validation groups are similar. This was observed when the developed ANN model was employed in the cross-validation group and when the previous published equations were tested in the total sample of the present study, corroborating with findings from a previous study (35). The only exception for this behaviour was observed in the present findings for the developed MLR equation, which obtained improved validation values in the cross-validation group.

In the search for an estimation model that better predicts VO_{2max} for Portuguese youths, MLR and ANN were calculated. While MLR models are common and easier to understand, ANN models are more complex and characterized by a "black box" nature (34). By this complexity, it would be expected that the explained variance and the SEE for ANN models would be more satisfactory. Indeed, this is only for the estimation group. When ANN models are utilized in a different group of individuals, like the cross-validation sample, validation parameters are more favourable to the use of the MLR model, as suggested by these results. Also, the stepwise method used in the MLR model reduces the presence of some co-linearity between predictors, which is not an option for ANN models. Based on the mathematical law of parsimony, since the more complex model achieves no substantial improvement, it is more appropriate to choose the simplest one. When comparing the estimations between MLR and ANN models, no statistical differences were detected.

The findings from the present study should be considered together with some limitations. The equations estimated by this study were calculated and validated for the Portuguese population. Further research should be done to explore the accuracy of these equations in other populations. Moreover, it should be considered that other variables could be included and analysed as potential predictors. The maturation status, for example, could eventually contribute to the understanding of the variation of $VO2_{max}$, as well as the predictors, over the growth and development process. Eventually, physical activity levels could also be included in the analyses. Unfortunately, the lack of this information does not allow exploration of whether the estimation models would behave in a different way if maturation status and physical activity levels were accounted for. The results also point to a substantial dispersion of random error (95%LOA), existing in all equations. Researchers should consider the possibility of biased results when the analysis requires more sensitive differential data, as required in longitudinal and intervention studies, for example, where SR assessed CRF. If that was the case, maybe a raw result from the SR, such as the number of completed laps or stages, could be more sensitive for detecting small but significant changes in CRF. However, for prospective studies, the clinical value of reference standards for of VO_2max standards for the discrimination of children SR laps is not known. On the other hand, the accuracy and adolescents at high and low cardio-metabolic risk had already been described (1, 23, 33). Also, FITNESSGRAM Reference Standards for the number of laps were arbitrarily determined by inverting the equation from Léger for VO_{2max} reference standards (14). From the perspective of future research, it would be useful to verify whether the raw value of the SR performance could determine a cut-off that could indicate subjects with a poor cardio-metabolic profile.

In conclusion, the model estimated from the multiple linear regression in the current study would be recommended for estimating the maximal oxygen uptake in Portuguese children and adolescents, as suggested by analyses of systematic error, validation coefficients and the standard error of the estimates, especially in the cross-validation group. However, users should be aware that the equations to estimate VO_{2max} could not be sensitive enough to detect small changes in individuals' CRF in longitudinal observations and intervention studies, according to the dispersions of random error.

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Normative and criterion-related standards for shuttle run performance in youth

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Normative and Criterion-Related Standards for Shuttle Run Performance in Youth

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The purpose of this study was to calculate and validate reference standards for the 20-m shuttle run test (SR) in youths aged 10–18 years. Reference standards based on the number of completed SR laps were calculated by LMS method in a reference group of 5559 students. Cut-off values for SR laps were determined and tested by ROC curve analysis in a validation group (633 students), from which waist circumference, HDL-cholesterol, triglycerides, fasting glucose and mean arterial pressure were assessed to calculate a metabolic risk score, later dichotomized in low and high metabolic risk (HMRS). The accuracy of SR laps standards was significant for girls (AUC = 0.66; 95% CI = 0.58–0.74; $p < .001$) and boys (AUC = 0.71; 95% CI = 0.62–0.79; $p < .001$) for identifying subjects at HMRS. The 40th percentile was the best cut-off for SR laps in girls (SENS = 0.569; 1-SPEC = 0.330) and boys (SENS = 0.634; 1-SPEC = 0.266). New SR laps reference standards are able to discriminate metabolic risk levels, and may provide a valuable tool for early prevention of cardiovascular risk factors.

It is well established that lower cardiorespiratory fitness (CRF) relates to poor cardio-metabolic conditions, being also a predictor of mortality and morbidity by cardiovascular diseases (CVD) and diabetes mellitus in adults (7,39). In youth, CRF might have a health protective effect since it has been inversely associated with body fat (4), features of the metabolic syndrome (8), and arterial compliance (27). Therefore, CRF evaluation is paramount to identify subjects at risk or monitoring adverse health outcomes (28–30).

The best indicator for CRF is the maximal oxygen uptake ($VO_{2max;}$ 34). By establishing VO_{2max} values to discriminate young people at high and low of cardio-metabolic risk, CRF reference standards were validated (1,23,30). However, the methods for direct assessment of VO_{2max} usually require a laboratory with sophisticated equipment, trained staff and increased costs (33). Alternatively, VO_{2max} can be estimated from the performance achieved in laboratory or field tests by using validated equations (17,20,21,31). On the other hand, those equations showed bias

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and increased random error (33), especially for subjects in extreme (low) performance values, potentially leading to misclassification of the CRF and metabolic risk levels. Thus, when using submaximal or field tests it would be interesting that the raw data of the performance objectively measured (i.e., power output, stage, number of shuttles or achieved distance) could be used as a reference standard to classify the CRF levels and to identify the individual's clinical metabolic risk status.

The 20-m Shuttle Run Test (SR) created by Léger et al. (21) is a widely used field test. The SR, or some adapted version of it, is part of several fitness tests batteries, like FITNESSGRAM (35). However, reference standards for CRF expressed in SR laps developed by FITNESSGRAM were calculated by regressing VO_{2max} values associated to the risk of CVD or all-cause mortality in adults (7,13). Thus, the clinical validity of SR laps reference standards for discriminating youths with poor cardio-metabolic profiles is not known. Therefore, the purpose of the current study was to calculate population-based reference standards for SR laps in children and adolescents, and validate their clinical value in the screening of subjects with high metabolic risk.

Methods

Subjects

A total of 6192 students (3131 girls and 3031 boys) aged 10–18 years from two districts from the North of Portugal participated in this study. Participants were further divided into two groups, reference group [5559 students (2789 girls and 2770 boys)] and validation group [633 students (372 girls and 261 boys)], to calculate and validate new CRF reference standards. The study was carried out following the guidelines for Human research from the Declaration of Helsinki. The study's purpose, nature, benefits, risks and discomforts were explained to participants, parents/guardians and teachers before running the study. Informed written consent was obtained from all participants' parents/guardians. The institutional research review board approved the study.

Anthropometric Measurements

Body mass and height were measured following standard procedures (24). Body height was measured to the nearest mm in bare or stocking feet with adolescents' standing upright against a stadiometer (Holtain Ltd.—Crymmych, Pembrokeshire, UK). Weight was measured to the nearest 0.10kg, with students lightly dressed, using an electronic weight scale (Tanita Inner Scan BC 532; Tanita Corporation—Tokyo, Japan). Body mass index (BMI) was calculated from the ratio of body weight (kg)/body height (m^2). Waist circumference (WC) was measured to the nearest mm with a metallic tape at the superior border of the iliac crest, according to a previously described protocol (36).

Cardiovascular Disease Risk Factors

Systolic (SBP) and diastolic (DBP) blood pressures were measured using the Colin Press Mate Non-Invasive Blood Pressure Monitor (model BP 8800p; Colin Medical Instruments Corporation—San Antonio, TX, USA). A trained technician took measurements. Two measurements were taken from children in seating position after five and ten minutes rest. The mean of these two measurements was used for further data analysis. Mean arterial pressure (MAP) was calculated as: $MAP = [(SBP\text{-}DBP)/3]+DBP$.

After 12–14 hr of fasting, capillary whole blood samples were collected from the earlobe using a 35-µl lithium heparin-coated capillary tube and immediately assayed using the Cholestech LDX Analyzer (Cholestech Corporation—Hayward, CA, USA) for determination of serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TRIG) and fasting glucose (GLU) levels. Low-density lipoprotein cholesterol (LDL-C) was estimated by the equation: $LDL\text{-}C = TC—HDL\text{-}C—(0.20 \times TRIG)$ (15).

A continuous metabolic risk score (MRS) was calculated from WC, HDL-C, TRIG, GLU and MAP. For each variable, standardized scores (Z-scores) were calculated according to age and sex. HDL-C Z-score was multiplied by -1 to indicate higher metabolic risk with increasing value. MRS was calculated as the average of the five standardized variables. A lower MRS was indicative of a better overall metabolic profile. MRS was later dichotomized, whereas values above mean plus 1 standard deviation for MRS were classified at high metabolic risk (HMRS). Values under this cut-off were considered at low metabolic risk (LMRS).

Cardiorespiratory Fitness

Participants performed the SR according to protocol describe by FITNESSGRAM [the adapted version of SR: the Progressive Aerobic Cardiovascular Endurance Run—PACER] (35). Briefly, the test consists in running back and forth between two lines 20 m apart, with running speed determined by audio signals from a prerecorded music CD. The running speed increases in the end of each one-minute stage. The running speed is 8.0 km·h^{-1} for the first stage, 9.0 km·h^{-1} for the second stage and thereafter increases by 0.5 km·h^{-1} each minute. The test ends when subjects fails twice the audio signals to reach the lines, demonstrating inability to keep the required pace. All participants were familiar with the test, since the FITNESSGRAM test battery is included in the Portuguese Physical Education curriculum. Total number of completed shuttles (SR laps) was recorded and considered as the main representative parameter of CRF level.

Data Analysis

Descriptive data are presented as mean and standard deviation (M± *SD*) and One-Way ANOVA was used to identify differences between groups.

Normative data were calculated for the reference group using the LMS method in a similar approach to that used for establishing nutritional standards for BMI (10). The LMS method basically calculates smoothed developmental curves for parameters M (median), S (coefficient of variation) and L (Box-Cox power for skewness), for a common age units X-axis. Using penalized likelihood, each one of L, M and S curves can be fitted by polynomials as cubic splines by nonlinear regression. Having these three parameters in each age point, it is possible to calculate the number of SR laps equivalent to any percentile (9,11,12). Based on these previously described procedures, normative data for SR laps by age and sex were calculated for the following percentiles: 10th, 20th, 30th, 40th, 50th, 60th, 70th, 80th and 90th. Subjects from the validation group were classified from 0 to 9 according to the corresponding percentile in SR laps (SRrank) for age and sex references.

To determine which SR laps decile could be the best cut-off value to discriminate subjects at HMRS and LMRS, ROC curve analysis was done considering SRrank, the test variable, and HMRS/LMRS, the state variable. The area under curves (AUC) and its respective 95% confidence interval (95% CI) were observed indicating the overall ability of using SR laps deciles for targeting HMRS. For each decile, sensitivity (SENS), specificity (SPEC), 1-SPEC and efficiency (EFF) were observed to examine the accuracy of the cut-off value as a diagnostic parameter. SENS is the proportion of subjects at HMRS with less SR laps than the decile used to discriminate HMRS/LMRS (true-positives). SPEC represents the proportion of LMRS with SR laps equal or greater than the decile used to discriminate HMRS/LMRS (true-negatives), and 1-SPEC is the proportion of LMRS smaller than the decile used to discriminate HMRS/LMRS (false-positives). EFF, calculated as EFF=(SENS+SPEC)/2, represents the overall performance of a particular cut-off value and higher values indicates higher EFF for the cut-off. The SR decile for each sex and age group with the highest EFF was established as the best cut-off.

Furthermore, logistic regression was used to describe the risk of being at HMRS for those subjects classified as low fit (under the best cut-off value) in comparison with those classified as fit (equal or above the cut-off). Results are shown in odds ratio (OR) and respective 95% confidence intervals.

All analyses were completed with LMS Chart Maker Light, version 2.3, Microsoft Excel 2007 and PASW version 18.0, with a significance level of 0.05.

Results

Characteristics of subjects separated by sex and group are presented in Table 1. Differences were found between reference and validation groups for height, weight, BMI and WC, with higher values for the validation group in girls. For boys, validation group had higher values for height and SR laps. Differences between sexes were found in height, weight, WC and SR laps in the reference group, with higher values for boys. In the validation group, boys had higher values than girls for height, weight, BMI and SR laps.

Validation group was classified for SR laps according to deciles calculated in the reference group. In the validation group 85.3% of subjects were classified as being in LMRS, whereas 14.7% in HMRS. ROC curve analyses indicate significant accuracy for SR laps deciles in the discrimination of subjects in LMRS and HMRS. The accuracy was significant for both girls (AUC = 0.66; 95% CI = 0.58–0.74; $p < .001$) and boys (AUC = 0.71; 95% CI = 0.62–0.79; $p < .001$).

Table 2 shows the performances of different percentiles as cut-off points for SR laps for targeting HMRS. The overall rates of true-positives (SENS), true-negatives (SPEC) and false-positives (1-SPEC) were greater to the 40th percentile of SR laps, comparatively to the others, and consequently leading to a higher efficiency in both sexes. Thus, SR laps corresponding to the 40th percentile are considered the best cut-off to discriminate subjects at HMRS/LMRS. Table 3 shows the corresponding reference values (40th percentile) determined by this study and the reference values for SR laps suggested by FITNESSGRAM (35).

Table 1 Characteristics of Participants According to Sample and Sex Group

Variables	Reference Group		Validation Group	
	Girls (*n* = 2789)	Boys (*n* = 2770)	Girls (*n* = 372)	Boys (*n* = 261)
	M± *SD*	M± *SD*	M± *SD*	M± *SD*
Age (yr)	14.4 ± 2.4	14.3 ± 2.3	14.4 ± 2.0	14.2 ± 2.0
Height (cm)	157.6 ± 8.9 [b, c]	163.2 ± 13.3 [b, d]	159.2 ± 7.5 [b]	166.3 ± 11.2
Weight (kg)	53.6 ± 11.6 [b, d]	57.8 ± 15.4	56.2 ± 11.4 [b]	59.5 ± 13.2
BMI (kg/m^2)	21.4 ± 3.6 [a, c]	21.4 ± 3.8	22.0 ± 3.7 [b]	21.3 ± 3.2
Waist Circumference (cm)	73.3 ± 9.5 [b, d]	75.0 ± 10.5	76.4 ± 9.8	75.1 ± 8.7
20-m Shuttle Run (laps)	29.8 ± 13.4 [b]	51.3 ± 23.9 [a, c]	29.9 ± 11.8 [b]	55.1 ± 24.4

[a] $P < 0.05$ for differences between sex within reference and validation groups;

[b] $P < 0.001$ for differences between sex within reference and validation groups;

[c] $P < 0.05$ for differences between reference and validation groups within sexes;

[d] $P < 0.001$ for differences between reference and validation groups within sexes.

Table 2 Performance of Different Percentiles for SR Laps as Cut-Off Points for CRF Reference

Sex	Percentiles								
Parameter	10th	20th	30th	40th	50th	60th	70th	80th	90th
Girls									
Sensitivity	0.173	0.308	0.462	0.596	0.654	0.712	0.827	0.923	0.981
Specificity	0.941	0.857	0.785	0.670	0.589	0.455	0.318	0.206	0.100
1-Specificity	0.059	0.143	0.215	0.330	0.411	0.545	0.682	0.794	0.900
Efficiency	0.557	0.582	0.623	0.633	0.621	0.583	0.572	0.564	0.540
Boys									
Sensitivity	0.122	0.366	0.488	0.634	0.659	0.732	0.829	0.878	0.976
Specificity	0.969	0.886	0.799	0.734	0.655	0.550	0.437	0.275	0.153
1-Specificity	0.031	0.114	0.201	0.266	0.345	0.450	0.563	0.725	0.847
Efficiency	0.546	0.626	0.643	0.684	0.657	0.641	0.633	0.577	0.564

Table 3 CRF Reference Standards Expressed in SR Laps

	New Reference Standards [a]						FITNESSGRAM Reference Standards [b]					
	SR laps		Stage [c]		VO_{2max} [d]		SR laps		Stage [c]		VO_{2max} [d]	
Age	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls	Boys
10	18	25	3	4	43,9	46,3	18	25	1	3	39,1	43,9
11	20	26	3	4	42,1	44,6	20	26	2	3	39,6	42,1
12	23	31	3	4	40,3	42,9	23	31	2	4	37,8	42,9
13	24	37	4	5	41,1	43,8	24	37	3	5	38,5	43,8
14	24	44	4	6	39,4	44,8	24	44	3	5	36,7	42,1
15	26	50	4	6	37,7	43,3	26	50	4	6	37,7	43,3
16	27	57	4	7	36,0	44,6	27	57	4	7	36,0	44,6
17	28	60	4	7	34,3	43,1	28	60	5	7	40,2	43,1
18	28	62	4	8	32,6	44,6	28	62	5	8	38,6	44,6

[a] Reference standards suggested by the current study;

[b] FITNESSGRAM reference standards suggested by The Cooper Institute for Aerobics Research (2004);

[c] SR stage corresponding to the number of SR laps;

[d] Estimated VO_{2max} from SR laps using the Léger equation (21) [VO_{2max} = 31,025 + 3,238 * maximal running speed—3,248 * age + 0,1536 * maximal running speed * age].

By applying the SR laps reference standards (40th percentile in Table 3) in the validation group, subjects were classified as low fit (girls: 36.5%; boys: 32.5%) and fit (girls: 73.5%; boys: 67.5%). In the comparison between low fit and fit girls (Table 4), differences were found in weight, BMI, WC, MRS and SR laps. In boys, differences were detected in BMI, WC, TC, TRIG, MRS and SR laps. In general, low fit subjects showed greater values for cardio-metabolic risk factors and poorer CRF in comparison with those classified as fit. Logistic regression indicates that low fit girls were 3.02 times (95% CI = 1.65–5.51; $p < .001$) more likely to be at HMRS than fit girls. Low fit boys were 4.73 times (95% CI = 2.34–9.54; $p < .001$) more likely to be at HRMS than those who were fit.

In a subanalysis, participants from validation group were classified according to the clinical definition of metabolic syndrome (2,18), instead of classifying subjects according to the HMRS reference (MRS > mean + 1 standard deviation). Thus, 2.7% of girls and 3.1% of boys would be diagnosed as MS positive cases. The accuracy of SR laps reference standards determined by this study for targeting MS cases is described by the following rates: SENS = 0.900, SPEC = 0.649, 1-SPEC = 0.351 and EFF = 0.775 for girls; and SENS = 1.000, SPEC = 0.696, 1-SPEC = 0.304 and EFF = 0.848 for boys. By applying the FITNESSGRAM reference standards, SENS = 0.800, SPEC = 0.583, 1-SPEC = 0.417 and EFF = 0.691 for girls; and SENS = 1.000, SPEC = 0.688, 1-SPEC = 0.312 and EFF = 0.844 for boys. These results indicate that the accuracy is higher for the newly suggested standards in comparison with FITNESSGRAM standards for targeting MS positive cases, especially for girls.

Discussion

The relevance and strengths of the current study are to provide new standards for cardiorespiratory fitness assessed by the number of laps in the SR, and the more robust and consistent methodological approach for its determination. In the current study, which was conducted in a relative large reference population, well-established and recommended methodological approaches were followed to estimate normative data and criterion-referenced standards. These approaches were previously employed for other health outcomes, such as nutritional status (10) and cardiovascular disease biomarkers (37). Different CRF deciles calculated from the reference group were tested in a validation group as recommended (38), relating CRF values to the incidence of HMRS and seeking a threshold beyond risk escalates (22,37). The option for the deciles calculation from the reference group using the LMS method is justified by the attempt to avoid abnormal oscillations on the variation of mean, standard deviation and skewness through time (age), which is common in developmental curves originated by cross-sectional data (9,12). Since deciles for SR laps were calculated as a function of age for each sex group, it was thought that the LMS method would be a prudent pathway to estimate the normative values for the new standard. The parsimony of the LMS method for estimating developmental norms was already observed in the description of standards for anthropometric parameters (11) and blood pressure (19), but not in the context of physical fitness. For the definition of critical values of SR laps to discriminate low and high metabolic risk, ROC curve analyses were carried out as recommended (37). ROC curves give information about the general ability of a parameter as a marker of health and to certify the selection of a certain value as a critical threshold by analyzing also the levels of misclassification (1,37).

Previously, recommended CRF standards were determined arbitrarily by experts (6) or derived from adults VO_{2max} standards (7,13,14). More recently, three studies have examined the clinical validation of VO_{2max} reference values in youth and reported cut-offs very similar to those suggested by FITNESSGRAM, 37.0 and 42.1 ml/kg/min for girls and boys, respectively (1,23,30). Those values were obtained from the indirectly estimated VO_{2max} by using performance-based equations. Indeed, values suggested by Ruiz et al. (30) and Adegboye, et al. (1) were established from the maximal power output attained in a maximal cycle-ergometer test (17,20), while those proposed by Lobelo et al. (23) were extrapolated from the heart hate values obtained in a submaximal treadmill exercise test (3,26). Although the use of indirectly estimated VO_{2max} values facilitates results comparisons between studies, this widespread procedure might have some limitations. The presence of heteroscedasticity, bias and increased random error, enhances the possibility of misclassification of CRF levels (low fit vs. fit), due to the over or underestimation of VO_{2max} (33). These limitations could lead to a reduction in the sensitivity of the estimated VO_{2max} to monitor small changes in CRF (5,33). On the other hand, a raw indicator of CRF, i.e., SR laps, can be used advantageously by overcoming the above-mentioned limitations. Indeed, the results of this study seems to support the assumption that performance-based indicators, such as SR laps, are of value as valid reference standards of CRF and in the discrimination of subjects in LMRS or HMRS.

While the SR standards determined in the current study were calculated and validate, FITNESSGRAM standards for SR laps were established by inverting the Léger equation (21) and calculating the number of SR laps for a corresponding VO_{2max} reference value, which highlight the novelty of our results. The comparison between the standards of the current study and those used by FITNESSGRAM (Table 3) indicates some differences in the cut-off values. For girls, it seems that our standards are more demanding until the age of 14 and less demanding from 15 to 18 years of age. For boys, our standards are more demanding for 10 and 11 years and less demanding from 12 to 18 years of age. The discrepancy is more prominent in girls since the differences in SR laps represent variations of one minute stage for 10, 17 and 18 years of age. From the metabolic point of view, those differences are considerable in terms of energy demand, leading to a variation from -5 to +3 ml/kg/min of oxygen uptake, if values were converted by Léger equation, like in the FITNESSGRAM. Consequently, these divergences could lead to misclassifications in low fit/fit categories.

Although this was not the main purpose of the current study, a subanalysis was conduced to verify whether our reference standards and those suggested by FITNESSGRAM were accurate to discriminate positive cases of MS. Higher rates of true-positive (SENS) and false-positive cases (1-SPEC) were found for the standards determined by this study in comparison with those suggested by FITNESSGRAM. Differences in accuracy were more substantial for girls, where a 10% difference was found in sensibility, which is a considerable difference from a clinical point of view.

Together with previous results and despite methodological differences (1,23,30), current findings support the existence of a hypothetical CRF level linked to a more favorable cardio-metabolic profile. In the comparison between low fit and fit groups (Table 4), differences with statistical significance were observed for the metabolic risk score. However, by observing other characteristics and risk factors, differences were found in BMI, WC and SR for boys and girls, in weight for girls and TC and TRI for boys only. As mentioned in previous studies (23,30), differences between low fit and fit subjects are more notorious in features of overweight and body fat than to others cardio-metabolic risk factors. These results are

Table 4 Characteristics of Validation Group According to Sex and CRF Levels

	Girls		*Boys*	
	Low Fit (*n* = 136)	Fit (*n* = 236)	Low Fit (*n* = 85)	Fit (*n* = 176)
	M± *SD*	M± *SD*	M± *SD*	M± *SD*
Age (yr)	14.3 ± 2.1	14.5 ± 1.9	13.9 ± 2.1	14.3 ± 1.9
Height (cm)	158.3 ± 7.9	159.7 ± 7.2	164.5 ± 12.2	167.1 ± 10.7
Weight (kg)	59.3 ± 14.1 [a]	54.4 ± 9.1	61.0 ± 16.5	58.8 ± 11.4
BMI (kg/m^2)	23.5 ± 4.6 [b]	21.2 ± 2.7	22.2 ± 4.3 [b]	20.8 ± 2.4
Waist Circumference (cm)	80.4 ± 11.7 [b]	74.1 ± 7.6	78.5 ± 10.4 [b]	73.5 ± 7.2
Total Cholesterol (mg/dl)	149.1 ± 23.9	147.0 ± 25.4	147.2 ± 27.9 [a]	139.2 ± 24.6
HDL-Cholesterol (mg/dl)	47.1 ± 12.6	47.0 ± 10.8	43.0 ± 11.2	43.1 ± 11.8
LDL-Cholesterol (mg/dl)	91.0 ± 22.3	89.8 ± 23.3	93.6 ± 25.0	87.5 ± 23.9
Triglycerides (mg/dl)	63.4 ± 21.5	61.8 ± 19.6	59.5 ± 22.1 [b]	51.9 ± 12.4
Fasting Glucose (mg/dl)	84.0 ± 6.9	84.4 ± 6.7	87.4 ± 7.4	87.0 ± 6.7
Systolic Blood Pressure (mm Hg)	118.7 ± 13.9	117.2 ± 12.5	122.4 ± 14.4	121.6 ± 13.2
Diastolic Blood Pressure (mm Hg)	64.4 ± 9.5 [a]	62.1 ± 9.4	60.6 ± 9.7	61.7 ± 9.2
Mean Arterial Pressure (mm Hg)	82.5 ± 9.9	80.5 ± 9.5	81.2 ± 10.3	81.7 ± 9.5
Metabolic Risk Score (z-score) [c]	0.1 ± 0.5 [b]	-0.1 ± 0.4	0.2 ± 0.6 [b]	-0.1 ± 0.5
20-m Shuttle Run (laps)	18.6 ± 5.0 [b]	36.4 ± 9.5	30.9 ± 12.6 [b]	66.8 ± 19.5

[a] $P < 0.05$ for differences between low fit and high fit subjects within sexes in validation group;

[b] $P < 0.001$ for differences between low fit and high fit subjects within sexes in validation group;

[c] Metabolic Risk Score expressed in z-scores adjusted for age and sex.

in agreement with previous findings showing that WC is the strongest marker of metabolic syndrome (25).

The prevalence of low fit students (35%) in the validation group is preoccupying, and similar to those found in other populations (23,30). In addition, low CRF levels are associated with increased risk for HMRS (23,30). Therefore, there is enough evidence to promote intervention programs to improve physical activity levels to enhance CRF and metabolic profiles. Although CRF variation is broadly explained by genetic factors (40), there is a theoretical framework with substantial evidence pointing that physical activity and regular exercise also play an important role in the regulation of CRF levels (16,32), features of metabolic syndrome (8) and obesity (16).

The findings from the current study should be considered together with some limitations. The sample of participants was selected by convenience and the distributions on reference and validation groups were determined by students/parents' acceptance for blood analysis, which is crucial for the description of cardio-metabolic risk factors. Moreover, these findings just offer a valid indication of the current metabolic condition, being impossible to confirm future development of metabolic syndrome or cardiovascular diseases.

In terms of public health, schools have a crucial position in health surveillance policies. It guarantees the access to a majority of children and adolescents and configures a powerful setting for primary prevention for several diseases. Concerning the cardiovascular health, the procedures to access some risk factors are invasive and not suitable for screening subjects at risk among large populations. However, performance in the 20-m shuttle run test seems to be a valid tool for targeting children and adolescents at risk for obesity and metabolic syndrome. Current findings encourage the assessment of physical fitness in schools as a key component for health monitoring systems. Physical fitness field tests like the 20-m shuttle run are suitable for school environments. It allows evaluating a large number of subjects with little equipment and reduced costs in an optimized time. In addition, schools can provide opportunities to improve healthier behaviors like physical activity and adequate diet, so that obesity and cardiovascular health could be controlled.

Conclusion

The new criterion-referenced standards for SR laps are valid and seem to discriminate children and adolescents with high metabolic risk from those with healthier cardio-metabolic profiles. In addition, this study supports the clinical significance of having a high CRF from early ages. In practice, the SR together with the new reference standards constitute valid tools for monitoring cardiovascular health in youths and for targeting groups for future intervention.

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Table 3 – CRF reference standards expressed in SR laps (RECTIFIED FROM ORIGINAL)

	New Reference Standards [a]						FITNESSGRAM Reference Standards [b]					
	SR laps		Stage [c]		VO_2max [d]		SR laps		Stage [c]		VO_2max [d]	
Age	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**	**Boys**
10	18	25	3	4	43,9	46,3	7	23	1	3	39,1	43,9
11	20	26	3	4	42,1	44,6	15	23	2	3	39,6	42,1
12	23	31	3	4	40,3	42,9	15	32	2	4	37,8	42,9
13	24	37	4	5	41,1	43,8	23	41	3	5	38,5	43,8
14	24	44	4	6	39,4	44,8	23	41	3	5	36,7	42,1
15	26	50	4	6	37,7	43,3	32	51	4	6	37,7	43,3
16	27	57	4	7	36,0	44,6	32	61	4	7	36,0	44,6
17	28	60	4	7	34,3	43,1	41	61	5	7	40,2	43,1
18	28	62	4	8	32,6	44,6	41	72	5	8	38,6	44,6

[a] Reference standards suggested by the present study;

[b] FITNESSGRAM reference standards suggested by The Cooper Institute for Aerobics Research (2004);

[c] SR stage corresponding to the number of SR laps;

[d] Estimated VO_2max from SR laps using the Léger equation (21) [VO_2max= 31,025 + 3,238*maximal running speed – 3,248*age + 0,1536*maximal running speed*age].

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Silva, G., Aires, L., Martins, C., Mota, J., Oliveira, J., Ribeiro, J.C.

Cardiorespiratory fitness associates with metabolic risk independently of central adiposity

Cardiorespiratory fitness associates with metabolic risk independently of central adiposity

Gustavo Silva, Luísa Aires, Clarice Martins, Jorge Mota, José Oliveira and José Carlos Ribeiro

Abstract

This study aimed to analyze the associations between cardiorespiratory fitness (CRF), waist circumference (WC) and metabolic risk in children and adolescents. Participants were 633 subjects (58.7% girls) aged 10-18 years. Metabolic risk score (MRS) was calculated from HDL-cholesterol, triglycerides, fasting glucose and mean arterial pressure. MRS was dichotomized into low and high metabolic risk (HMRS). Maximal oxygen uptake (VO_2max) was estimated from the 20m Shuttle Run Test. The first quartile of CRF was set as the low fit group. The fourth quartile of WC was defined as high central adiposity. With adjustments for age, sex and WC, VO_2max was correlated with MRS (r=-0.095; P<0.05). WC was correlated with MRS (r=0.150; P<0.001) after adjustments for age, sex and VO_2max. Participants who were low fit, presented higher levels of MRS (P<0.001) compared to those who were fit, even after adjustment for age, sex and WC. In comparison with subjects who were fit with normal central adiposity, an increased risk for being at HMRS was found for participants who were low fit with high central adiposity (OR=2.934; 95%CI= 1.690-5.092) and for those who were low fit with normal central adiposity (OR=2.234; 95%CI=1.116-4.279). Results suggest that CRF associates with MRS, independently of central adiposity.

Keywords: Aerobic Capacity; Waist Circumference; Obesity; Metabolic Syndrome; Cardiovascular Risk Factors; Youths.

Introduction

Cardiorespiratory fitness (CRF) is recognized as a great marker of cardiovascular health. In adults, lower levels of CRF are associated with an increased risk of all-cause and CVD mortality [5, 17]. In youths, reduced CRF is associated with obesity and features of metabolic syndrome [3, 7], a condition of clustered cardiovascular risk factors. Indeed, the definitions of reference standards for CRF were established from the risk of mortality in adults [5, 17]. However, the same strategy to define cardiovascular risk for young people is unreasonable, since the time of exposure to cardiovascular risk factors was reduced and insufficient to trigger cardiovascular events. Alternatively, a clustered metabolic risk score has been used as a reference of cardiovascular health in youths for the determination of CRF standards [1, 16, 20, 21, 26]. On the other hand, obesity is a key characteristic of metabolic syndrome and it has been included in the metabolic risk score calculated for the validation of CRF reference standards in youths.

Current definitions of metabolic syndrome [2, 15] have included waist circumference (WC) as an indicator of central adiposity, i.e. intra-abdominal and visceral fat, which regulates some physiological mechanisms underlying the progress of insulin resistance, dyslipidemia, and atherogenesis [2]. Consequently, it would be informative to understand whether central adiposity mediates the relationship between CRF and other cardio-metabolic features in children and adolescents.

Therefore, the purpose of the present study was to analyze the associations between CRF, abdominal fatness and metabolic risk factors.

Methods

Subjects

A total of 633 students (372 girls and 261 boys) aged 10-18 years from two districts from the North of Portugal participated in this study. The experimental protocol was approved by the Review Committee of the Scientific Board of the Faculty of Sport of the University of Porto as well as by the Foundation of Science and Technology and performed in accordance with the ethical standards required by the International Journal of Sports Medicine [14].

Anthropometric Measurements

Height was measured to the nearest mm using a Holtain stadiometer. Body mass was measured to the nearest 0.10kg, with students lightly dressed, using an electronic weight scale (Tanita Inner Scan BC 532). Body mass index (BMI) was calculated from the ratio of body weight (kg)/body height (m^2). Waist circumference (WC) was measured to the nearest mm with a metallic tape at the superior border of the iliac crest, according to a previously described protocol [25].

Cardiovascular Disease Risk Factors

Systolic (SBP) and diastolic (DBP) blood pressures were measured using the Colin Press Mate Non-Invasive Blood Pressure Monitor (model BP 8800p). Two measurements were taken from children in seating position after five and ten minutes rest. The mean of these two measurements was used for further data analysis. Mean arterial pressure (MAP) was calculated as: *MAP = [(SBP-DBP)/3]+DBP*.

After 12-14 hours of fasting, capillary whole blood samples were collected from the earlobe using a 35-µl lithium heparin-coated capillary tube and immediately assayed using the Cholestech LDX® Analyzer for determination of serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TRIG) and fasting glucose (GLU) levels. Low-density lipoprotein cholesterol (LDL-C) was estimated by the equation [12]: *LDL-C = TC – HDL-C – (0.20 x TRIG)*.

A continuous metabolic risk score (MRS) was calculated from standardized scores (Z-scores) for HDL-C, TRIG, GLU and MAP. For each variable included in MRS, Z-scores were calculated according to age and sex. HDL-C Z-score was multiplied by -1 to indicate higher metabolic risk with increasing value. MRS was calculated as the average of the four standardized variables. A lower MRS was indicative of a better overall metabolic profile. MRS was later dichotomized, whereas values above mean plus 1 standard deviation for MRS were classified at high metabolic risk (HMRS). Values under this cut-off were considered at low metabolic risk (LMRS).

Cardiorespiratory Fitness

Participants performed the 20m Shuttle Run Test according to the protocol suggested by FITNESSGRAM [24]. All participants were familiar with the test, since the FITNESSGRAM test battery is included in the physical education curriculum in a National Program. Total number of completed shuttles (SR laps) was recorded and converted in completed stages (speed level). The maximal oxygen uptake (VO_2max) was estimated with the following equation [22]: $VO_2max = 43.313 + 4.567*sex - 0.560*BMI + 2.785*stages$.

Statistics

Descriptive data are presented as mean and standard deviation (Mean ± *SD*) and One-Way ANOVA was used to identify differences between gender and CRF groups. Partial correlation analysis was used to analyse the relationships between WC, VO_2max and metabolic risk factors, with adjustments for confounders (age, sex and WC). Standardized Z-scores adjusted for age and sex were calculated for WC and CRF (VO_2max). Quartiles for the adjusted z-scores of WC and VO_2max were computed. The fourth quartile of WC was established as “high central adiposity”, while the remaining quartiles were grouped as “normal central adiposity”. The first quartile of VO_2max was established as “low fit”, while the remaining quartiles were set as “fit”. General Linear Model (Analysis of Covariance) was used to analyse differences between CRF groups with adjustments for age, sex and WC. Logistic regression was used to describe the risk of being at the group with the high metabolic risk (HMRS) according to groups of CRF and central adiposity. Results for logistic regressions are shown as odds ratio (OR) and respective 95% confidence intervals. All analyses were completed with SPSS 19.0 (SPSS Inc., Chicago, United States), with a significance level of 0.05.

Results

Descriptive characteristics of participants are shown in Table 1. In the comparisons between genders, boys were taller, heavier and presented greater values of GLU, SBP and CRF, while girls had higher values of BMI, TC, HDL-C, TRIG and DBP.

Table 1 – Participant's characteristics according to sex for anthropometric measures, cardio-metabolic risk factors and cardiorespiratory fitness

Variables	Girls (*n*=372)	Boys (*n*=261)
Age (years)	14.4±2.0	14.2±2.0
Height (cm)	159.2±7.5	166.3±11.2 [b]
Weight (kg)	56.2±11.4	59.5±13.2 [a]
BMI (kg/m^2)	22.0±3.7 [a]	21.3±3.2
Waist Circumference (cm)	76.4±9.8	75.1±8.7
Total Cholesterol (mg/dl)	147.7±24.9 [a]	141.8±26.0
HDL-Cholesterol (mg/dl)	47.0±11.5 [b]	43.1±11.6
LDL-Cholesterol (mg/dl)	90.2±22.9	89.5±24.4
Triglycerides (mg/dl)	62.4±20.3 [b]	54.4±16.6
Fasting Glucose (mg/dl)	84.3±6.7	87.1±6.9 [b]
Systolic Blood Pressure (mm Hg)	117.7±13.0	121.9±13.6 [b]
Diastolic Blood Pressure (mm Hg)	63.0±9.5 [a]	61.3±9.4
Mean Arterial Pressure (mm Hg)	81.2±9.7	81.5±9.8
Metabolic Risk Score (z-score) [1]	0.0±0.5	0.0±0.6
20m Shuttle Run (laps)	29.9±11.8	55.1±24.4 [b]
VO2max (ml.kg^{-1}.min^{-1})	40.1±4.6	52.2±7.0 [b]

Notes: Descriptive values are Mean ± *SD*; [a] $P<0.05$; [b] $P<0.001$; [1] Metabolic Risk Score calculated from the average of age-sex adjusted z-scores from HDL-cholesterol, triglycerides, fasting glucose and mean arterial pressure.

Table 2 shows partial correlations for the associations of CRF, WC and metabolic risk factors. With adjustments for age and sex, VO_2max was correlated with WC, TRIG, SBP, DBP, MAP and MRS, while WC was associated with HDL-C, TRI, SBP, DBP, MAP and MRS. After adjustments for age, sex and WC, VO_2max remained associated with TC, TRIG and MRS. When correlations were controlled for age, sex and VO_2max, WC was associated with TC, HDL-C, GLU, SBP, DBP, MAP and MRS.

Table 2 – Partial correlations between cardiorespiratory fitness, waist circumference and cardio-metabolic risk factors

Variables	VO_2max	WC	VO_2max	WC
Control Variables:	Age and Sex	Age and Sex	Age, Sex and WC	Age, Sex and VO_2max
Waist Circumference	-0.499 [b]	-	-	-
Total Cholesterol	-0.060	-0.056	-0.102 [a]	-0.100 [a]
HDL-Cholesterol	0.024	-0.109 [a]	-0.035	-0.112 [a]
LDL-Cholesterol	-0.037	-0.014	-0.050	-0.037
Triglycerides	-0.159 [b]	0.120 [a]	-0.115 [a]	0.047
Fasting Glucose	-0.017	0.083 [a]	0.029	0.086 [a]
Systolic Blood Pressure	-0.099 [a]	0.183 [b]	-0.009	0.155 [b]
Diastolic Blood Pressure	-0.115 [a]	0.121 [b]	-0.064	0.074
Mean Arterial Pressure	-0.120 [a]	0.162 [b]	-0.046	0.119
Metabolic Risk Score [1]	-0.191 [b]	0.223 [b]	-0.095 [a]	0.150 [b]

Notes: [a] $P<0.05$; [b] $P<0.001$; [1] Metabolic Risk Score calculated from the average of age-sex adjusted z-scores from HDL-cholesterol, triglycerides, fasting glucose and mean arterial pressure.

Table 3 shows the comparison between CRF levels for metabolic risk factors. In the unadjusted comparison, there was a trend in the direction of the fit group for healthier levels of WC, HDL-C, TRI, SBP, DBP, MAP, MRS and CRF. After adjustments for age and sex, the same trends in differences were observed. In the analysis where comparisons were adjusted for age, sex and WC, differences remained significant for TRI, MRS and CRF.

Table 3 – Differences cardio-metabolic risk factors across CRF levels with adjusted ANCOVA

Variables	Low Fit (n=158)	Fit (n=475)	*F* without adjustments	*F* adjusted for age and sex	*F* adjusted for age, sex and WC
Waist Circumference (cm)	83.7±10.5	73.2±7.3	193.928 [b]	212.803 [b]	-
Total Cholesterol (mg/dl)	144.7±23.2	145.5±26.2	0.139	0.134	0.159
HDL-Cholesterol (mg/dl)	43.6±11.4	46.0±11.7	5.415 [a]	5.039 [a]	1.009
LDL-Cholesterol (mg/dl)	90.7±21.4	89.7±24.2	0.232	0.174	0.474
Triglycerides (mg/dl)	64.1±22.0	57.4±18.0	14.318 [b]	16.644 [b]	8.711 [a]
Fasting Glucose (mg/dl)	85.7±7.2	85.3±6.9	0.295	0.207	0.471
Systolic Blood Pressure (mm Hg)	122.1±14.0	118.6±13.1	8.227 [a]	10.322 [a]	1.080
Diastolic Blood Pressure (mm Hg)	63.9±9.8	61.8±9.3	5.823 [a]	7.396 [a]	1.874
Mean Arterial Pressure (mm Hg)	83.3±10.0	80.7±9.6	8.318 [a]	10.513 [a]	1.893
Metabolic Risk Score (z-score) [1]	0.2±0.6	-0.1±0.5	24.680 [b]	24.622 [b]	5.999 [a]
20m Shuttle Run (laps)	23.6±11.7	45.9±21.7	151.254 [b]	324.018 [b]	270.346 [b]
VO2max (ml.kg-1.min-1)	38.4±5.9	47.4±7.7	181.970 [b]	689.494 [b]	414.154 [b]

Notes: Descriptive values are Mean ± *SD*; [a] $P<0.05$; [b] $P<0.001$; [1] Metabolic Risk Score calculated from the average of age-sex adjusted z-scores from HDL-cholesterol, triglycerides, fasting glucoses and mean arterial pressure.

Table 4 presents the logistic regression models for estimating the odds ratios (OR) for being at high metabolic risk (HMRS). In the first model, the OR for being at HMRS was greater for low fit subjects in comparison with their fit counterparts. In the second model, subjects with high central adiposity presented an increased OR for being at HMRS, in comparison with subjects with normal central adiposity. For the third model, CRF and WC levels were included together in the same analysis as independent variables and it was observed that being low fit increased the risk for being at HMRS, independently of central adiposity levels. In the fourth and final logistic regression analysis, CRF and WC levels were combined and grouped into four categories. In comparisons with participants who were fit with normal central adiposity (reference category), the risk for being at HMRS was increased in low fit subjects with high or normal central adiposity. The OR was not significant increased for those who were fit with high central adiposity in comparison with the reference category.

Table 4 – Logistic regression models to estimate the odds ratios for being at high metabolic risk [*]

Models	Independent Variables and Levels	OR (95% CI)
Model 1	*CRF Levels*	
	Ref.: Fit	
	Low Fit	2.437 (1.551-3.829)[b]
Model 2	*WC Levels*	
	Ref.: Normal Central Adiposity	
	High Central Adiposity	2.030 (1.283-3.210)[a]
Model 3	*CRF Levels*	
	Ref.: Fit	
	Low Fit	2.061 (1.237-3.433)[a]
	WC Levels	
	Ref.: Normal Central Adiposity	
	High Central Adiposity	1.456 (0.864-2.451)
Model 4	*Combined CRF/WC Levels*	
	Ref.: Fit with Normal Central Adiposity	
	Low Fit with High Central Adiposity	2.934 (1.690-5.092)[b]
	Low Fit with Normal Central Adiposity	2.234 (1.166-4.279)[a]
	Fit with High Central Adiposity	1.607 (0.785-3.292)

Notes: [*] High metabolic risk: values greater than mean plus 1 standard deviation for metabolic risk score (calculated from the average of age and sex adjusted z-scores from HDL-cholesterol, triglycerides, fasting glucoses and mean arterial pressure); [a] *P*<0.05; [b] *P*<0.001;

Discussion

Main findings from the present study showed that CRF and central adiposity are independently associated with single or clustered cardiovascular risk factors in children and adolescents. Also, differences between CRF levels in a metabolic risk score are independent of central adiposity levels. Although both low CRF and higher central adiposity increased the risk of being at high metabolic risk, it seems that being fit may attenuate this risk for those youths with elevated central adiposity.

These results highlight the relevance of CRF as a marker of metabolic and cardiovascular health in youths. Results from previous research indicated that CRF is inversely associated with TC [6, 10, 13], TRIG [7, 10, 18], GLU [10, 11], blood pressure [6, 10, 11], WC [11] and clustered metabolic risk [3, 4, 7, 11, 16, 18, 20, 21]. In the present study, after adjustments for age and sex, CRF was associated with WC, TRIG, blood pressure and MRS. When analyses were controlled for age, sex and WC, CRF was still associated with MRS, although the relationship was attenuated. Similar trends were reported in previous investigations. Ekelund et al. [11] also described that CRF and clustered metabolic risk were associated and the correlation was attenuated when the analysis was adjusted for WC as a confounder. Andersen et al. [3] analyzed the odds ratios for being at high metabolic risk over the quartiles of CRF and concluded that the lower quartiles of fitness had an increased risk even after adjustments for fatness. In the comparison between two fitness groups in the present study, there were differences in most of the cardiovascular risk factors with healthier levels for the fit group. After adjustments for WC, differences in TRIG and MRS remained significant, supporting the notion that the differences between fitness levels were independent of central adiposity. Indeed, when the risk for being at high metabolic risk was estimated, the low fit group showed an increased odds ratio in comparison with the fit group and this association was also independent of central adiposity levels. Finally, when fitness and central adiposity levels were combined, the risk for having an increased metabolic score was greater for low fit subjects with normal or high central adiposity. It should be highlighted that there was a meaningful reduction in the risk of being at high metabolic risk for subjects who had high central adiposity and were fit, in comparison with those who were low fit and had high central adiposity too. These associations suggest that being “fat but fit” might reduce the consequences of obesity over some markers of cardiovascular health, which is consistent with previous findings [2, 8-10, 15].

Findings from the present study should be pondered with the consideration of some limitations. Maximal oxygen uptake, the standard measure of cardiorespiratory fitness, was estimated from the performance in a field test (20m Shuttle Run). However, previous evidences suggest that these estimations are valid and accurate indicators of aerobic capacity [19, 22]. Additionally, it should be pointed that the strength of the relationships described in the present results should be taken with regardless to the measures of exposures and outcomes. The lack of a standard measurement for central fat distribution was also a limitation, given high costs and restricted use in large-scale studies. On the other hand, waist circumference seems to provide simple and yet effective measures of central adiposity in children and adolescents, as an alternative to dual-energy X-ray absorptiometry (DEXA) and others standard measures of body composition [23]. Although the current international standards for the definition of metabolic syndrome in youths [2, 15] take into account waist circumference, HDL-C, triglycerides, fasting glucose and blood pressure, the absence of fasting insulin and homeostatic model assessment (HOMA) did not allowed inferences regarding the dependent and independent associations between cardiorespiratory fitness, abdominal obesity and insulin resistance metabolism. Finally, inferences in the direction of causal effects were limited due to the cross-sectional nature of the data.

Concluding, evidences from the present analysis support the idea that CRF is a powerful independent indicator of metabolic and cardiovascular health in young ages. In terms of primary prevention of cardiovascular diseases, the assessment of CRF is an important tool for targeting candidates for future intervention. Furthermore, data from the present study suggests that interventions should promote physical activity with sufficient intensities to induce changes in CRF with the scope of reducing metabolic risk instead of focusing only in changes in body composition and weight loss. Current results suggest that even overweight children could benefit of healthier metabolic conditions after improvements in CRF.

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Associations between the contexts of practice of physical activity, levels of moderate to vigorous physical activity and cardiorespiratory fitness in children and adolescents

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Gustavo Silva, Lars Bo Andersen, Luísa Aires, Jorge Mota, José Oliveira and José Carlos Ribeiro

Abstract

The purpose of this study was to analyse the associations between the levels of participation in different contexts of physical activity (PA), moderate to vigorous physical activity (MVPA) and cardiorespiratory fitness (CRF) levels. The study comprised 310 subjects (183 girls and 127 boys) aged 11-18 years. Participation levels in PA contexts were assessed by questionnaire and habitual PA was measured objectively with accelerometers. Twenty meters Shuttle Run Test was used to estimate CRF. Logistic regression analyses were carried out with CRF as the outcome. The OR for being fit was greater for those who comply with 60min/day in MVPA (OR=2.612; 95%CI=1.614-4.225) in comparison with those who not. Participation in competitive sports at club levels increased the chances of being fit (OR=13.483; 95%CI=4.560-39.864), independently of MVPA levels. There were positive and significant trends in CRF and objectively measured PA across the levels of engagement in competitive sports (P<0.05). Concluding, participation in competitive sports at club level is more effective than other contexts of PA to reach healthier levels of CRF and recommend levels of MVPA.

Keywords: accelerometers, questionnaires, sports participation, aerobic capacity, youth.

Introduction

Cardiorespiratory fitness (CRF) has been considered an independent and additive marker of cardiovascular health. In adults, low CRF is associated with increased risk of cardiovascular morbidity, and all cause and cardiovascular mortality (Blair et al., 1989; Wei, Gibbons, Kampert, Nichaman, & Blair, 2000). In youth, the evidence from epidemiological studies suggest that CRF is associated with the clustering of CVD risk factors (Anderssen et al., 2007), which tends to track from adolescence through adulthood (Andersen, Hasselstrøm, Grønfeldt, Hansen, & Karsten, 2004). CRF might have a health protective effect since it has been inversely associated with body fat (Andersen et al., 2008), features of the metabolic syndrome (Andersen et al., 2008; Brage et al., 2004) and arterial stiffness (Reed et al., 2005). More recently, it was suggested that CRF could be considered an independent risk factor for metabolic disorders in youths (Andersen et al., 2008). Therefore, CRF evaluation is paramount to identify subjects at risk or monitoring adverse health outcomes (Ruiz et al., 2006; Ruiz, Ortega, Meusel, & Sjöström, 2007; J.R. Ruiz et al., 2007).

There is a plausible framework suggesting that physical activity (PA) and exercise of moderate to vigorous intensities could promote favourable changes in CRF and cardiovascular risk factors. Higher levels of moderate to vigorous physical activity (MVPA) are associated with improved CRF (Gutin, Yin, Humphries, & Barbeau, 2005), reduced fatness (Gutin et al., 2002), favourable lipid profile (Tolfrey, Jones, & Campbell, 2004) and reduced metabolic risk (Andersen et al., 2006; Brage et al., 2004). Although PA and CRF are correlated, they are independently associated with metabolic risk factors (Andersen et al., 2008; Ekelund et al., 2007), which means that they may affect cardiovascular health through different ways.

Physical activity is usually described in relation to intensity, duration and frequency, which together constitute the volume of activity (Caspersen, Powell, & Christenson, 1985; Corder, Ekelund, Steele, Wareham, & Brage, 2008). In these terms and based on the compilation of previous evidences (Janssen, 2007; Strong et al., 2005), the World Health Organization recommend that school aged people should accumulate at least 60 minutes in MVPA daily (WHO, 2010). The accomplishment of PA guidelines could be ensured in different contexts. However, data is still restricted about how youngsters' PA varies across types and contexts and how these relationships extend for other parameters of health. A better understanding of these variables might contribute to enhance the effectiveness of interventions and public health policies (Koorts et al., 2011). Therefore, the purpose of this study was to analyse the associations between the engagement in different contexts of PA, levels of MVPA and CRF levels.

Methods

Participants

Anthropometric measures, cardiorespiratory fitness and physical activity were assessed in 397 girls and 292 boys from public schools in the District of Porto, Portugal. From this group, 310 subjects (183 girls and 127 boys aged 11-18 years old) with complete data and valid accelerometers measurements constituted the final sample of the study. Excluded participants (379 individuals) had no valid data for objectively measured physical activity by accelerometers. In the comparison between subjects with complete and incomplete data, no differences (P>0.05) were found for age, height, weight, BMI, SR laps, VO_2max and PAI. The proportion of girls (46.1%) and boys (43.5%) with complete data was similar (χ^2=0.460; P=0.497). The study was carried out following the Declaration of Helsinki guidelines for human research. The study's purpose, nature, benefits and risks were explained to participants, parents/guardians and teachers. Informed written consent was obtained from the participants' parents/guardians. The study protocol was approved by the Review Committee of the Scientific Board of the

Faculty of Sport of the University of Porto as well as by the Foundation of Science and Technology from Portugal.

Anthropometric Measurements

Body mass and height were measured following standard procedures (Lohman, Roche, & Martorell, 1988). Body height was measured to the nearest mm in bare or stocking feet with adolescents' standing upright against a stadiometer (Holtain Ltd. – Crymmych, Pembrokeshire, UK). Weight was measured to the nearest 0.1 kg, with scholars lightly dressed, using an electronic weight scale (Tanita Inner Scan BC 532; Tanita Corporation – Tokyo, Japan). Body mass index (BMI) was calculated from the ratio of body weight (kg)/body height (m^2).

Cardiorespiratory Fitness

Participants performed the 20m Shuttle Run Test according to the FITNESSGRAM™ protocol (The Cooper Institute for Aerobics Research, 2004). All participants were familiar with the test, since the FITNESSGRAM™ test battery is included in the physical education curriculum in a National Program. Total number of completed shuttles (SR laps) was recorded. Based on the performance achieved in shuttle-run test, maximal oxygen uptake (VO_2max), which is considered as the main representative parameter of CRF level, was estimated according the following equation (Silva et al., 2012): VO_2max= 43.313 + 4.567*sex - 0.560*BMI + 2.785*stages. FITNESSGRAM™ reference standards for VO_2max (Welk, Laurson, Eisenmann, & Cureton, 2011) were used to classify participants as low fit (below health fitness zone) and fit (health fitness zone).

Context of Practice of Physical Activity

The physical activity contexts of practice were assessed by a questionnaire (Ledent, Cloes, & Piéron, 1997), which have been previously determined to have good reliability with strong intra-class correlation coefficients (ICC: 0.92–0.96) (Mota & Esculcas, 2002; Raitakari et al., 1994). The questionnaire consists of five questions, each one with four answer choices (four-point ordinal scale): Question 1 - Do you take part in organized sport outside school? Never (1); Less than once a week (2); At least once a week (3); Almost every day (4); Question 2 - Do you take part in non-organized sport outside school? Never (1); Less than once a week (2); At least once a week (3); Almost every day (4); Question 3 - How many times per week do you take part in sport or PA for at least 20 minutes outside school? Never (1); Less than once a week (2); Between once a week and once a month (3); Twice or more times a week (4); Question 4 - How many hours per week do you usually take part in physical activity outside school, which causes you to get out of breath or sweat? Never (1); 30 min to 1 hour (2); 2 to 3 hours (3); 4 hours or more (4); Question 5 - Do you take part in a competitive sport? Never (1); No, but I participated (2); Yes, at school (3); Yes, in a club (4). A physical activity index (PAI) was obtained according to the total sum of the points (maximum 20) with increasing ranks from the sedentary to physically active levels.

Objectively Measured Physical Activity

PA was objectively assessed by accelerometers (GTM1, Actigraph, Florida) during 7 consecutive days and data was recorded in 15 seconds sampling periods (epochs). The standard software PROPERO (developed by the Centre of Research in Childhood Health of the University of Southern Denmark, 2011) was used to reduce the raw activity data from the accelerometers into daily physical activity. Time periods with at least 10 consecutive minutes of zero counts recorded were excluded from analysis assuming that the monitor was not worn. A minimum recording of 10-hours/day was the criteria to accept daily PA data as valid. Individual's data were only accepted for analysis if at least two-week days and one weekend day were successfully assessed. For each outcome from accelerometer-reduced data, values were adjusted for the average wearing time (734.3±59.8 min/day) and for differences between weekdays and weekend days. The main outcomes of reduced data were: total physical activity [total PA (counts/min/day)], time in sedentary physical activity [SEDPA (min/day)], light physical activity [LIGPA (min/day)], moderate physical activity [MODPA (min/day)], vigorous physical activity [VIGPA (min/day)] and moderate to vigorous physical activity [MVPA (min/day)]. To determine the time spent in PA of different intensities, the following counts intervals were considered: 0-499 for SEDPA, 500-1999 for LIGPA, 2000-3999 for MODPA, ≥4000 for VIGPA (Ekelund et al., 2007) and ≥ 2000 for MVPA (Andersen et al., 2006). An average of 60 minutes/day spent in MVPA for accepted valid days was set as an operational definition of compliance with PA guidelines (Janssen, 2007; Strong et al., 2005). Accordingly, subjects with an average of less than 60min/day of MVPA were classified as low active, and those with 60min/day or more of MVPA were considered active.

Statistics

For the descriptive analysis, data from continuous variables were expressed as mean and standard deviation (M±SD). Age, height, SR laps, VO_2max, PAI and SEDPA were normally distributed. Total PA, LIGPA, MODPA, VIGPA and MVPA were positively skewed and thus transformed to their natural logarithm for comparisons tests. Independent T-test was employed to test mean differences between gender and CRF levels. Nominal and ordinal variables were described as absolute and relative frequencies. Chi-square statistic was used to test for frequencies differences between sexes and CRF groups across the levels of engagement in each PA context. Pearson correlation coefficients (r) were used to examine how age was correlated with VO_2max, total PA, MVPA and PAI. Logistic regression was employed with CRF levels (0=low fit and 1=fit) as the dependent variable and PA contexts and MVPA levels set as independent variables. For each independent variable, the unadjusted (crude) association with the outcome was explored. Finally, a multiple logistic regression (adjusted analysis) was carried out with all independent variables and potential confounders (age, sex and age*sex interaction) included in the model. Stepwise method with backward selection was employed retaining the independent variables with P value <0.05 in the final model. The same approach preceded assuming MVPA levels (0=low active and 1=active) as the outcome and PA contexts as predictors. General Linear Models (GLM) adjusted for age and sex were conducted to test trends and differences across the levels of participation in competitive sports. Analyses were carried out with the definitive sample of 310 participants with complete data. All analyses were carried out in SPSS 19.0 with significance level set at 0.05.

Results

Participant's characteristics (*n*=310) separated by sex, cardiorespiratory fitness and physical activity levels are presented in Table 1. Compared to girls, boys were significantly taller and heavier. Moreover, boys and those classified as fit, compared to girls and those classified as low fit, showed higher values for CRF, PAI, total PA, time spent in MODPA, VIGPA, and MVPA. Girls, and the low fit participants, had higher values of time spent in SEDPA. BMI was higher for low fit participants. Participants classified as active, compared to the low active group, showed higher values in height, weight, SR laps, VO_2max, PAI, total physical activity, LIGPA, MODPA, VIGPA and MVPA. Time spent in SEDPA was higher for low active subjects.

Table 1 – Descriptive data according to sex, cardiorespiratory fitness and physical activity levels

	Sex		CRF Levels		PA levels	
Variables	Girls (*n*=183)	Boys (*n*=127)	Low Fit (*n*=149)	Fit (*n*=161)	Low Active (*n*=198)	Active (*n*=112)
Age (years)	14.5 ± 1.6	14.5 ± 1.6	14.3 ± 1.7	14.7 ± 1.5	14.5 ± 1.6	14.5 ± 1.7
Height (cm)	160.7 ± 5.7	169.9 ± 9.5^{b}	161.1 ± 6.7	167.6 ± 9.2^{b}	163.6 ± 8.0	166.0 ± 9.8^{a}
Weight (kg)*	56.3 ± 10.6	65.2 ± 12.8^{b}	59.4 ± 13.1	60.4 ± 11.5	58.6 ± 11.7	62.3 ± 13.0^{a}
Body Mass Index (kg/m^2)*	21.7 ± 3.6	22.5 ± 3.5	22.8 ± 4.0	21.4 ± 2.9^{a}	21.8 ± 3.6	22.5 ± 3.5
20m Shuttle Run (laps)	33.5 ± 12.1	58.0 ± 22.0^{b}	28.0 ± 8.4	58.0 ± 18.1^{b}	40.3 ± 19.0	49.4 ± 22.5^{b}
VO_2max (ml/kg/min)	41.4 ± 4.6	52.5 ± 6.8^{b}	39.6 ± 3.6	51.9 ± 5.7^{b}	44.7 ± 7.3	48.2 ± 8.2^{b}
Physical Activity Index	11.1 ± 3.7	14.4 ± 3.2^{b}	10.8 ± 3.6	13.9 ± 3.5^{b}	11.7 ± 3.8	13.7 ± 3.7^{b}
Total PA (counts/min/day)*	419.7 ± 121.0	524.0 ± 169.7^{b}	419.5 ± 123.8	502.2 ± 164.2^{b}	375.8 ± 78.1	615.7 ± 127.0^{b}
Time in Sedentary PA (min/day)	578.5 ± 40.2^{b}	557.6 ± 45.8	578.0 ± 41.1^{a}	562.5 ± 45.0	590.3 ± 31.7^{b}	534.0 ± 38.8
Time in Light PA (min/day)*	108.0 ± 29.0	112.0 ± 28.3	108.8 ± 28.8	110.4 ± 28.8	105.1 ± 26.2	117.7 ± 31.3^{a}
Time in Moderate PA (min/day)*	38.8 ± 17.0	47.5 ± 20.4^{b}	38.6 ± 17.3	45.8 ± 19.8^{b}	31.9 ± 10.2	60.8 ± 16.6^{b}
Time in Vigorous PA (min/day)*	9.0 ± 9.2	17.2 ± 14.3^{b}	8.9 ± 9.1	15.6 ± 13.8^{b}	7.0 ± 5.9	21.9 ± 14.6^{b}
Time in MVPA (min/day)*	47.8 ± 21.5	64.8 ± 28.9^{b}	47.5 ± 21.6	61.5 ± 28.1^{b}	38.9 ± 11.9	82.7 ± 20.3^{b}

Notes: Values are Mean ± *SD*; ^{a} $P<0.05$, ^{b} $P<0.001$ for group comparisons; * natural log transformed variables were used for comparisons but untransformed data are shown.

Correlation analyses indicated that age was not correlated with VO_2max (r=0.081; P=0. 276), total PA (r=-0.089; P=0.232), MVPA (r=-0.007; P=0.925) or PAI (r=-0.080; p=0.281) in girls. In boys, age was correlated with VO_2max (r=0.440; P<0.001), but not with total PA (r=0.039; P=0.667), MVPA (r=0.071; P=0.430) and PAI (r=-0.001; P=0.988).

The proportions of participants classified as fit were different for girls and boys (χ^2=113.273; P<0.001). 26.8% of girls and 88.2% of boys were classified as fit according to FITNESSGRAM™ standards for CRF. There were also differences between the proportions of girls (24.6%) and boys (52.8%) that meet a daily average of 60 minutes spent in MVPA (χ^2=25.774; P<0.001). Moreover, 65.2% of active participants were classified as fit, while 55.6% of low active participants were low fit (χ^2=12.320; P<0.001). In Table 2, the proportion of participants in each PA context level is described according to sex, CRF and PA levels. In the lower levels of engagement in the PA contexts, the proportions were higher for girls, low fit and low active participants. For higher scores in PA contexts, the proportions were increased for boys, and for those who are fit or active.

Table 3 describes logistic regression models for estimating odds ratios OR for being fit and for being active, according to engagement levels in PA contexts. Crude analyses indicated a significant trend for the association between age and CRF (β=0.177; 95%CI=0.035-0.319; P<0.001; OR=1.194; 95%CI=1.035–1.376). In comparison with girls, boys presented an increased odds ratio (OR) for being fit (OR=20.419; 95%CI=10.870-38.356). The interaction age*sex (boys) was also associated with CRF levels (OR=1.246; 95%CI=1.190-1.304). In the unadjusted analysis (crude), each independent variable presented some level with an increased OR for being fit, in comparison with reference categories. To estimate the OR for being fit, all PA contexts were included in the adjusted model (multiple logistic regression) together with age, sex, age*sex interaction and MVPA levels. After seven steps of backward selection, only the level of engagement in competitive sports, adjusted for sex and age*sex interaction, remained associated with the chances of being fit.

Table 2 – Frequencies in categories of physical activity contexts according to sex, cardiorespiratory fitness levels and physical activity levels

	Sex		CRF Levels		MVPA levels	
PA Contexts and Engagement Levels	Girls (*n*=183)	Boys (*n*=127)	Low Fit (*n*=149)	Fit (*n*=161)	Low Active (*n*=198)	Active (*n*=112)
Question 1 - Do you take part in organized sport outside school?						
Never	92 (78.6%)	25 (21.4%)	79 (67.5%)	38 (32.5%)	91 (77.8%)	26 (22.2%)
Less than once a week	9 (50.0%)	9 (50.0%)	7 (38.9%)	11 (61.1%)	8 (44.4%)	10 (55.6%)
At least once a week	60 (57.7%)	44 (42.3%)	50 (48.1%)	54 (51.9%)	64 (61.5%)	40 (38.5%)
Almost every day	22 (31.0%)	49 (69.0%)	13 (18.3%)	58 (81.7%)	35 (49.3%)	36 (50.7%)
	$\chi^2 = 42.363^{b}$		$\chi^2 = 43.532^{b}$		$\chi^2 = 19.531^{b}$	
Question 2 - Do you take part in non-organized sport outside school?						
Never	82 (73.9%)	29 (26.1%)	72 (64.9%)	39 (35.1%)	82 (73.9%)	29 (26.1%)
Less than once a week	21 (56.8%)	16 (43.2%)	14 (37.8%)	23 (62.2%)	20 (54.1%)	17 (45.9%)
At least once a week	63 (52.9%)	56 (47.1%)	50 (42.0%)	69 (58.0%)	74 (62.2%)	45 (37.8%)
Almost every day	17 (39.5%)	26 (60.5%)	13 (30.2%)	30 (69.8%)	22 (51.2%)	21 (48.8%)
	$\chi^2 = 18.774^{b}$		$\chi^2 = 21.322^{b}$		$\chi^2 = 9.514^{a}$	
Question 3 - How many times per week do you take part in sport or PA for at least 20 minutes outside school?						
Never	41 (87.2%)	6 (12.8%)	34 (72.3%)	13 (27.7%)	34 (72.3%)	13 (27.7%)
Less than once a week	29 (78.4%)	8 (21.6%)	23 (62.2%)	14 (37.8%)	27 (73.0%)	10 (27.0%)
Between once a week and once a month	44 (66.7%)	22 (33.3%)	34 (51.5%)	32 (48.5%)	48 (72.7%)	18 (27.3%)
Twice or more times a week	69 (43.1%)	91 (56.9%)	58 (36.3%)	102 (63.8%)	89 (55.6%)	71 (44.4%)
	$\chi^2 = 39.514^{b}$		$\chi^2 = 23.303^{b}$		$\chi^2 = 9.747^{a}$	
Question 4 - How many hours per week do you usually take part in physical activity outside school, which causes you to get out of breath or sweat?						
Never	58 (84.1%)	11 (15.9%)	48 (69.6%)	21 (30.4%)	52 (75.4%)	17 (24.6%)
30 min to 1 hour	82 (65.1%)	44 (34.9%)	60 (47.6%)	66 (52.4%)	83 (65.9%)	43 (34.1%)
2 to 3 hours	39 (41.5%)	55 (58.5%)	37 (39.4%)	57 (60.6%)	51 (54.3%)	43 (45.7%)
4 hours or more	4 (19.0%)	17 (81.0%)	4 (19.0%)	17 (81.0%)	12 (57.1%)	9 (42.9%)
	$\chi^2 = 45.618^{b}$		$\chi^2 = 22.723^{b}$		$\chi^2 = 8.346^{a}$	
Question 5 - Do you take part in a competitive sport?						
Never	50 (74.6%)	17 (25.4%)	48 (71.6%)	19 (28.4%)	55 (82.1%)	12 (17.9%)
No, but I participated	68 (59.6%)	46 (40.4%)	54 (47.4%)	60 (52.6%)	73 (64.0%)	41 (36.0%)
Yes, at school	44 (66.7%)	22 (33.3%)	38 (57.6%)	28 (42.4%)	40 (60.6%)	26 (39.4%)
Yes, in a club	21 (33.3%)	42 (66.7%)	9 (14.3%)	54 (85.7%)	30 (47.6%)	33 (52.4%)
	$\chi^2 = 25.550^{b}$		$\chi^2 = 46.131^{b}$		$\chi^2 = 17.154^{b}$	

Notes: Values are counts (percentages); [a] $P<0.05$, [b] $P<0.001$ for group comparisons.

Table 3 – Logistic regression models estimating the odds ratios for being fit and for meeting PA guidelines (being active)

	Dependent Variable: CRF levels (0=Low Fit; 1=Fit)		Dependent Variable: MVPA levels (0=Low Active; 1=Active)	
	Crude Analysis	Multiple Logistic Regression (Method: Backward Stepwise)	Crude Analysis	Multiple Logistic Regression (Method: Backward Stepwise)
Independent Variables	OR (95%CI)	OR (95%CI) *	OR (95%CI)	OR (95%CI) **
Question 1 - Do you take part in organized sport outside school? [1]				
Less than once a week	3.267 (1.174-9.092)[a]		4.375 (1.567-12.215)[a]	
At least once a week	2.245 (1.301-3.875)[a]		2.187 (1.215-3.939)[a]	
Almost every day	9.275 (4.537-18.963)[b]		3.600 (1.903-6.810)[b]	
Question 2 - Do you take part in non-organized sport outside school? [1]				
Less than once a week	3.033 (1.404-6.552)[a]		2.403 (1.110-5.206)[a]	
At least once a week	2.548 (1.495-4.342)[a]		1.719 (0.980-3.018)	
Almost every day	4.260 (1.995-9.097)[b]		2.699 (1.297-5.616)[a]	
Question 3 - How many times per week do you take part in sport or PA for at least 20 minutes outside school? [1]				
Less than once a week	1.592 (0.633-4.002)		0.969 (0.368-2.547)	
Between once a week and once a month	2.462 (1.105-5.483)[a]		0.981 (0.424-2.267)	
Twice or more times a week	4.599 (2.248-9.410)[b]		2.086 (1.025-4.249)[a]	
Question 4 - How many hours per week do you usually take part in physical activity outside school, which causes you to get out of breath or sweat? [1]				
30 min to 1 hour	2.514 (1.352-4.677)[a]		1.585 (0.819-3.066)	
2 to 3 hours	3.521 (1.822-6.805)[b]		2.579 (1.305-5.099)[a]	
4 hours or more	9.714 (2.915-32.377)[b]		2.294 (0.825-6.382)	
Question 5 - Do you take part in a competitive sport? [1]				
No, but I participated	2.807 (1.471-5.356)[a]	3.126 (1.308-7.468)[a]	2.574 (1.238-5.354)[a]	2.267 (1.069-4.809)[a]
Yes, at school	1.861 (0.905-3.830)	2.248 (0.862-5.867)	2.979 (1.344-6.604)[a]	2.878 (1.271-6.515)[a]
Yes, in a club	15.158 (6.267-36.664)[b]	13.483 (4.560-39.864)[b]	5.042 (2.273-11.181)[b]	3.451 (1.504-7.916)[a]
Compliance with PA Guidelines [2]				
60min/day or more in MVPA	2.612 (1.614-4.225)[b]		-	-

Notes: [1] Reference Category=Never; [2] Reference Category=less than 60min/day in MVPA; [a] P<0.05, [b] P<0.001; * Model Adjusted for sex and age*sex interaction; ** Model Adjusted for sex.

When the compliance with an average of 60min/day of MVPA was established as the outcome, no trend was detected for the relationship between age and MVPA levels (β=0.082; 95%CI=-0.132-0.158; *P*=0.857; OR=1.194; 95%CI=0.877–1.171). The OR for being active was higher in boys (OR=3.424; 95%CI=2.110 – 5.558) when compared to girls. The interaction age*sex (boys) was also associated with MVPA levels (OR=1.086; 95%CI=1.050-1.122). In the unadjusted analysis, most of PA context had some level of engagement associated with an increased OR for being active, except for the participation in sport or PA for at least 20 minutes outside school (question 3). Multiple logistic regression, after seven steps of backward selection, showed that taking part in competitive sports (question 5) increases the OR for being active, with adjustment for sex.

Table 4 presents descriptive values for physical characteristics, CRF and PA according to the participation in competitive sports. GLM with adjustments for age and sex indicated significant trends for SR laps, VO_2max, PAI, total PA, SEDPA, MODPA, VIGA and MVPA. With exception of SEDPA, which was higher for those who never took part in competitive sports, greater values of CRF and PA were found for those who participate in competitive sports in a club.

Table 4 – Descriptive data and General Linear Model testing for trend across levels of engagement in competitive sports (question 5)

	Do you take part in competitive sport?				
Dependent Variables	Never (n=67)	No, but I participated (n=114)	Yes, at school (n=66)	Yes, in a club (n=63)	F for trend
Height (cm)	162.4 ± 8.4	165.2 ± 8.6	162.3 ± 7.9	167.9 ± 9.0	1.728
Weight (kg)*	58.6 ± 10.8	59.5 ± 12.7	59.9 ± 14.8	62.3 ± 10.1	0.288
Body Mass Index (kg/m2)*	22.2 ± 3.2	21.7 ± 3.4	22.6 ± 4.7	22.0 ± 2.6	1.326
20m Shuttle Run (laps)	33.2 ± 14.8	41.0 ± 17.1	40.2 ± 19.6	62.8 ± 21.4	26.046 [b]
VO2max (ml/kg/min)	42.1 ± 6.2	45.6 ± 6.4	44.2 ± 8.2	52.5 ± 7.5	17.284 [b]
Physical Activity Index	8.9 ± 2.9	11.5 ± 2.8	13.2 ± 3.2	17.1 ± 1.9	89.022 [b]
Total PA (counts/min/day)*	399.1 ± 112.9	454.7 ± 130.1	471.1 ± 138.3	534.9 ± 200.8	4.785 [a]
Time in Sedentary PA (min/day)	582.1 ± 37.0	573.9 ± 43.6	564.8 ± 42.2	555.3 ± 48.3	2.841 [a]
Time in Light PA (min/day)*	108.4 ± 24.2	105.6 ± 30.3	114.7 ± 29.4	113.0 ± 28.9	1.725
Time in Moderate PA (min/day)*	36.3 ± 18.6	42.7 ± 17.9	42.7 ± 17.8	47.8 ± 20.8	2.970 [a]
Time in Vigorous PA (min/day)*	7.5 ± 6.5	12.2 ± 10.0	12.0 ± 11.6	18.3 ± 17.8	3.784 [a]
Time in MVPA (min/day)*	43.8 ± 21.6	54.9 ± 22.3	54.7 ± 24.3	66.1 ± 33.5	4.515 [a]

Notes: Values are Mean ± *SD*; [a] $P<0.05$ and [b] $P<0.001$ for General Linear Model testing the trend across levels of engagement in competitive sports with adjustments for sex and age; * natural log transformed variables were used for comparisons but untransformed data are shown.

Discussion

Present findings showed that there are associations between compliance with PA guidelines of 60min/day of MVPA and being fit, involvement in competitive sports in a club and being fit, and participating in competitive sports and comply with PA recommendations. Also, in comparisons with girls, boys are much more likely for being fit and for complying with PA guidelines. Stepwise logistic regressions including the different PA questions suggested that being involved in competitive sports in a club may be more important to ensure healthier CRF levels than complying with 60min/day in MVPA defined from accelerometer measurements.

Evidence from previous studies suggests that sports participation is associated with enhanced fitness, reduced body fat, low blood pressure, and reduced risk for the clustering of CVD risk factors in youths (Ara et al., 2004; Boreham, Twisk, Savage, Cran, & Strain, 1997). It could be expected that young people committed to higher levels of competitive sports would be involved in greater volumes of PA resulting in training processes. Data from cross-sectional studies indicated that trained young athletes have higher values of VO_2peak in comparison with their untrained peers (Armstrong, Tomkinson, & Ekelund, 2011; Rowland, Wehnert, & Miller, 2000). Furthermore, data from experimental exercise-training studies reported significant improvements in youths' CRF (Armstrong et al., 2011; Stoedefalke, Armstrong, Kirby, & Welsman, 2000; Tolfrey et al., 2004). However, although there is plentiful data in the literature supporting that exercise-training programs improve CRF, there appears to be no meaningful dose-response relationship between habitual daily PA and maximal oxygen uptake (Armstrong et al., 2011). Cross-sectional studies with accelerometer measured PA and measured or estimated VO_2peak reported low-to-moderate correlations (r values from 0.1 to 0.4) (Dencker & Andersen, 2008). On the other hand, data from the present study indicated that children and adolescents who take part in competitive sports at club level spend more time in MVPA and have higher VO_2max (Table 4).

Findings from the present study add new evidence in the area of health-related PA and fitness. It seems that not just the study of intensity, frequency, duration and volume in which PA is carried out, but also the understanding of the context in which PA takes place may contribute to develop strategies for improving youths' PA and health. When PA contexts were analysed separately, results from the present study showed that healthier levels of CRF were associated with greater levels of engagement in organized sport outside the school, non-organized sport outside the school, PA for more than 20min outside the school, breath taking or sweating PA outside the school and competitive sports. When PA contexts were taken together in a stepwise analysis, and adjusting for physical activity levels assessed by accelerometers, participation in competitive sports becomes an independent factor to increase the odds to have healthier levels of CRF. Therefore, regular training for competitive sports might be a strategy to empower health-related PA and to enhance CRF, thereby improving cardio-protection.

Consistent with data previously reported (Machado Rodrigues et al., 2010; J.R. Ruiz et al., 2007; Trost et al., 2002; Welk et al., 2011), in the present study boys tended to be more physically active and have greater CRF levels than girls, although the odds of boys for being fit seems to be somewhat unique in comparison with girls. It is known that boys are more engaged in sports and intensive (moderate to vigorous) physical activities, while girls are more likely to be involved in lighter activities and leisure PA (Mota & Esculcas, 2002). Moreover, it is alarming that girls scored lower levels of engagement for all the five PA contexts analysed in the present study. Sex per se is considered a biological determinant of PA, which can also be affected by psychological, social and environmental factors (Seabra, Mendonca, Thomis, Anjos, & Maia, 2008; Uijtdewilligen et al., 2011). However, it is not clear how these determinants interact with sex in order to influence overall PA and the participation in PA in distinguished contexts.

Collectively, these findings suggest that girls are in greater risk for being insufficient active, which might contribute to reduced fitness levels. However, to the best of our knowledge, this is the first study using the new FITNESSGRAM^{TM} reference standards (Welk et al., 2011), which makes difficult comparisons with other populations. On the other hand, up to the present date it was not possible to ascertain if the new FITNESSGRAM^{TM} reference standards (Welk et al., 2011) are too much demanding for girls, and therefore, overestimating the proportion of those classified as unfit. Nevertheless, girls seem to be a target group for specific PA interventions.

Physical activity guidelines (Janssen, 2007; Strong et al., 2005; WHO, 2010) suggest that children and young people should accumulate 60 min/day of moderate to vigorous physical activity in order to improve physical fitness, bone health, cardiovascular and metabolic health biomarkers and to reduce symptoms of anxiety and depression. It is accepted that these recommendations are based on solid evidence (Andersen, Riddoch, Kriemler, & Hills, 2011), although the differences in study designs and methods for assessing PA and CVD risk factors have to be considered. Some considerations should be addressed when compliance with PA guidelines assessed with accelerometers is the object of analysis. The options that researchers take when reducing the data (number of days, minimum daily wearing time and intensities cut-offs) and whether the compliance with 60min/day in MVPA is analysed daily or on the average of accepted days strongly affect the prevalence of subjects complying with recommendations (Beets, Bornstein, Dowda, & Pate, 2011). In our analyses, we assumed an operational definition of an adjusted average of 60min/ day for accepted days as the reference for reaching recommended PA levels. In fact, some of the evidence in which the guidelines are based performed their analyses in terms of average values (Andersen et al., 2006). Indeed, more research is needed to resolve issues with intensity cut points and interpretation of PA guidelines (Beets et al., 2011).

Findings from the present study should also be taken into consideration with some limitations. Although the results clearly suggest associations between the levels of CRF and different features of PA and engagement in sport, the cross-sectional design does not allow elucidating a dose-response effect between these PA domains and cardiovascular health in young people. On the other hand, a positive point of the current data was the combination of the assessment of physical activity by questionnaire and accelerometers. While questionnaires can provide information about the context, type, structure and organization of physical activity and sports which young people are involved in, accelerometers allow directly assessment of intensity, frequency, duration and volume of physical activity (Corder et al., 2008; Ekelund, Tomkinson, & Armstrong, 2011). Together with CRF data, this approach for assessing PA allowed to analyse the settings that really seemed to be prognostic determinants of the former health markers. Thus, it seemed that the participation in competitive sports is more effective than other contexts of PA to reach healthier levels of CRF, and compliance with PA recommendations. Other organized or structured physical activity contexts, may contribute to achieve healthier PA and CRF levels, like for instance Physical Education classes or leisure-time physical activities. However, because in these contexts there are additional educational and social purposes, they are not so effective to generate the appropriate dose of MVPA to reach health benefits.

In conclusion, there was an independent association between the participation in competitive sports, compliance of MVPA guidelines and cardiorespiratory fitness levels. Current results indicate that being active and/or involved in competitive sports increases the chances of having high cardiorespiratory fitness. Our findings suggest a pathway for promoting healthier levels of cardiorespiratory fitness through the engagement in competitive sports, increasing the chances of compliance with PA guidelines.

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OVERALL DISCUSSION

Main findings from the original research carried out in this thesis support the idea that the performance in a field test, the 20-meter Shuttle Run is a valid marker of the cardiorespiratory fitness (**Study I**) and a suitable tool for the stratification of cardio-metabolic risk in children and adolescents (**Study II**). Furthermore, this measurement of CRF might be an important indicator of increased metabolic risk, independently of central adiposity levels (**Study III**). Additionally, assuming CRF as an independent risk factor for cardiovascular diseases, the competitive sports might be a relevant context to achieve higher levels of moderate to vigorous physical activity and to improve cardiovascular health profile (**Study IV**).

All the research comprised in this thesis was carried out with the perspective that CRF is a powerful marker of cardiovascular health since the early ages, even when assessed by a field test. In Portugal, an adapted version of the original SR (PACER – Progressive Aerobic Cardiovascular Endurance Run) is widely used, since the evaluation of physical fitness with the FITNESGRAM^{TM} battery (The Cooper Institute, 2007; The Cooper Institute for Aerobics Research, 1999) is a standard procedure in the Portuguese Physical Education curricula. Additionally, SR have been used exhaustively by Portuguese and other international researchers for the assessment of CRF (Aires, Andersen, et al., 2010; Aires et al., 2011; Guerra, Ribeiro, Costa, Duarte, & Mota, 2002; Martins, Silva, Santos, Ribeiro, & Mota, 2008; Mota et al., 2002; Nielsen & Andersen, 2003; Reed, Warburton, Macdonald, Naylor, & McKay, 2008; Sakuragi et al., 2009). In a previous study accomplished from our research group (Ruiz et al., 2009), we analysed the criterion-validity of SR and five different equations for the estimation of VO_2max in Portuguese youths. Main findings from this previous study suggest that validity was moderate for the five equations analysed, from Léger et al. (1988), Barnett et al. (1993) [two equations], Matsuzaka et al. (2004) and Ruiz et al. (2008). For the correlations with measured VO_2max, coefficients ranged from 0.59 (Léger equation) to 0.76 (Ruiz equation). Furthermore, the standard error of estimates varied from 5.3 (Barnett equation a) to 6.5 $ml.kg^{-1}.min^{-1}$ (Léger equation) and the systematic error (the mean difference between estimated and measured VO_2max) ranged from 1.3 (Barnett equation b) to 5.5 $ml.kg^{-1}.min^{-1}$ (Léger equation). With the exception for the Barnett equation b, the other four equations significantly underestimate the directly measured VO_2max. A worrying result from this study was that the Léger equation, the most cited and used, provides the less valid estimation of VO_2max. We have considered that the attempt to estimate new equations adjusted for the Portuguese youth population would be valuable, due to the validation and errors observed in previous studies. Indeed, with the **Study I** (Silva, Oliveira, et al., 2012), we improved the validity of SR as a tool for assessing CRF in Portuguese youth. In a cross validation group, we described a decreased standard error of the estimate of VO_2max (4.9 $ml.kg^{-1}.min^{-1}$), a reduced systematic error (-0.1 $ml.kg^{-1}.min^{-1}$) and an increased validation coefficient (r=0.84; P<0.001) for the equation calculated by linear regression. However, some advantages and disadvantages in using equations to estimate VO_2max should be elucidated. If the interest were to compare results between populations assessed by different CRF tests, the expression of VO_2max (in $ml{\cdot}kg^{-1}{\cdot}min^{-1}$) estimated by an appropriate equation in each population would satisfy this limitation. On the other hand, the substantial dispersion of random error existing in equations could lead to biased results when the analysis require more sensitive within-individual differential data, like in longitudinal analyses and intervention studies. If that was the case, may be a raw indicator of SR performance, such as the number of laps or stages completed in the test, could be more sensitive to detect small but significant changes in CRF. Indeed, the performance in the SR seemed to be *per se* a good indicator of CRF with increased correlations with directly measured VO_2max, r=0.75 (P<0.001) for the number of laps and r=0.77 (P<0.001) for completed stages [**Study I** (Silva, Oliveira, et al., 2012)]. Additionally, the use of equations that were originally determined in other populations might lead to non-negligible errors of assessment and, by consequence, misclassification in risk stratification.

Recently, several studies suggested that there might exist an ideal level of cardiorespiratory fitness that could be associated with reduced cardio-metabolic risk in children and adolescents (Adegboye et al., 2010; Lobelo et al., 2009; Ruiz et al., 2007; Welk et al., 2011). These studies assumed VO_2max expressed in $ml.kg^{-1}.min^{-1}$ as the parameter of CRF, and a metabolic risk score, calculated from the clustering of obesity and other traditional cardiovascular risk factors, as a reference of cardiovascular health. On the basis of these previous studies and having into consideration the findings from the **Study I**, we assumed that the performance in SR *per se* could also be considered an important measurement for the cardiovascular risk stratification in Portuguese youths. With this perspective, we carried out the **Study II** (Silva, Aires, Mota, Oliveira, & Ribeiro, 2012) to estimate reference standards for the performance in the SR that were associated with an increased metabolic risk. Figure 1 shows the comparisons between the original reference standards proposed by FITNESSGRAM (The Cooper Institute, 2007; The Cooper Institute for Aerobics Research, 1999) and the cut-off values calculated in **Study II** (Silva, Aires, et al., 2012). For boys (Figure 1a), the latest standards are more demanding for the ages 10, 11, and 14 years, and less demanding for the ages 12, 13, 15, 16, 17 and 18 years, when compared with previous FITNESSGRAM standards. For girls (Figure 1b), the standards suggested by **Study II** are harder to accomplish by the subjects from 10 to 14 years, and less demanding than FITNESSGRAM standards for those aged between 15 to 18 years. Indeed, the performance to identify young subjects with increased risk for metabolic syndrome was superior for the reference values suggested by **Study II** in comparison with the original FITNESSGRAM reference standards for SR laps, especially in girls. In practice, the differences ranged from -10 to 3 laps for boys and from -13 to 11 laps for girls, which correspond to differences between -5 to +3 $ml.kg^{-1}.min^{-1}$ if these values in SR laps were converted to VO_2max by the Léger equation.

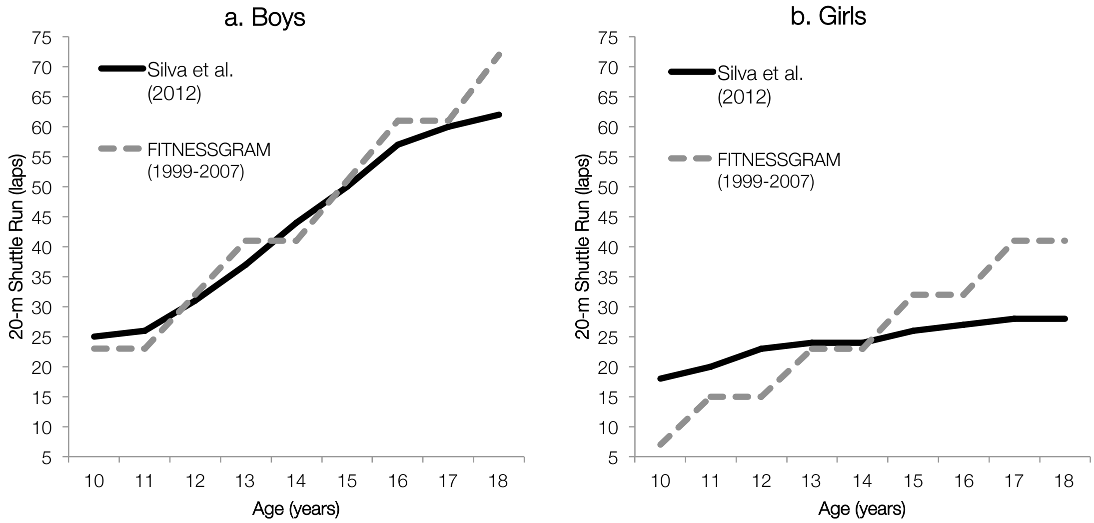

Figure 1 – Reference standards for 20-m shuttle run (number of completed laps) for boys (Figure 1a) and girls (Figure 1b). Straight lines represent the reference standards suggested by Silva et al. (2012). Dashed lines represent reference standards proposed by FITNESSGRAM (1999-2007).

Taking into consideration the performance of previous reference standards suggested for VO_2max values in ml.kg^{-1}.min^{-1} (Adegboye et al., 2010; Lobelo et al., 2009; Ruiz et al., 2007; Welk et al., 2011), the results for risk stratification presented in the **Study II** are completely acceptable. The area under the curve (AUC) for our reference standards was 0.710 (95% CI=0.620-0.790; P<0.001) for boys and 0.660 (95% CI=0.580-0.740; P< 0.001) for girls' cut-off values. The efficiency, the best trade-off ratio for true-positive and true-negative cases, for the latest reference standards suggested for SR laps was 0.684 in boys and 0.633 in girls. These findings are similar to those reported for reference standards based on VO_2max values (Adegboye et al., 2010; Lobelo et al., 2009; Ruiz et al., 2007; Welk et al., 2011). These results support the idea that reference standards based in SR laps are so effective for cardio-metabolic risk stratification in youths as much as VO_2max reference values, which highlights the practical relevance for the findings from **Study II**. Additionally, 90% of girls and 100% of boys that were classified as being positive cases of metabolic syndrome according to the IDF definition (IDF, 2007) were low fit. These results highlight the relevance of the new reference values calculated from the performance in SR, as excellent indicators for stratification of youths with increased metabolic risk in the school setting. In comparison with previous FITNESSGRAM™ reference standards, our cut-off values were 10% more effective to identify girls that were classified as positive cases of metabolic syndrome, and no difference were found among boys. Taking into consideration some of the aforementioned limitations regarding the use of equations to estimate VO_2max, it might be advantageous to use SR laps as the reference standard to identify children and adolescents with increased metabolic risk. This procedure might reduce the chances of misclassification of subjects accounted by errors in the estimation of VO_2max.

In the **Study III**, we analysed the associations between CRF, central adiposity and clustered metabolic risk factors. For this study, we assumed CRF expressed in VO_2max values ($ml.kg^{-1}.min^{-1}$) estimated from SR with the equation previously validated for Portuguese youths (**Study I**). CRF was classified according to age and sex adjusted quartiles. A metabolic risk score was calculated from four traditional metabolic risk factors (excluding the adiposity component): HDL-cholesterol (HDL-C), triglycerides (TRIG), glucose (GLU) and mean arterial pressure (MAP). In the main analysis, there was a significant trend for decreasing metabolic risk with the increasing of CRF levels. When waist circumference (WC) was included in the analysis as a covariate, the trend was attenuated but remained significant. When we analysed the combined associations between CRF/WC levels and metabolic risk, our results suggested that children and adolescents with increased central adiposity might decrease the odds ratio for an increased metabolic risk if their CRF was increased. These results support the rationale that CRF might have a significant effect over metabolic risk, independently of central adiposity in Portuguese youths. These findings are in accordance with the associations, trends and relationships described in previous studies (Andersen et al., 2008; Eisenmann, Welk, Ihmels, & Dollman, 2007; Eisenmann, Welk, Wickel, & Blair, 2007; Ekelund et al., 2007). For instance, Andersen et al. (2008) demonstrated that the association between CRF and metabolic risk score had approximately the same strength as with fatness, and similar results were obtained with skinfold thickness and waist circumference.

Interestingly, similar findings were described in studies with different protocols and tests to assess CRF and adiposity. Despite differences in methodological and statistical approaches, collectively, these findings support with strong evidence the thought that CRF is a powerful and independent marker of cardiovascular health since childhood and adolescence. Although the clinical manifestations of CVD rarely occur in youths, it is preoccupying that cardiovascular and metabolic risk factors tend to cluster and to track from adolescence to adulthood, and this is strongly related to physical fitness (Andersen et al., 2004). In adults, CRF is the strongest predictor of mortality among normal subjects and those with CVD (Blair et al., 1989; Myers et al., 2002). Therefore, the maintenance of increased CRF levels over individuals' growth and development might have some cardioprotective effect even when exposed to single or clustered risk factors. Together, these evidences reinforce the notion that low CRF should warrant as much attention as others major cardiovascular risk factors in health surveillance policies.

In the sequence of the previous studies, we assumed CRF as an independent risk factor for the development of CVD since the early ages. Thus, we thought that it would be relevant not just to develop strategies of assessment and risk stratification, but also to identify some evidence to support future intervention, in a more involved perspective of primary prevention of CVD. Assuming estimated CRF levels as the outcome, we aimed to analyse whether levels of moderate to vigorous physical activity and the participation in different contexts of physical activity were associated with healthier levels of CRF (Study IV). Although the strong evidence supporting the recommendations of 60 min per day of moderate to vigorous physical activity for subjects in school ages (Andersen et al., 2011), our results suggest that the participation in competitive sports might have an independent association with increased CRF levels. These results are in accordance with previous data showing that participation in organized sports is associated with decreased levels of traditional cardiovascular risk factors (Ara et al., 2004; Boreham et al., 1997). Cross-sectional analysis and exercise-training studies suggest that training, which is an important component of the participation in competitive sports, lead to significant improvements in CRF and general cardiovascular health (Armstrong et al., 2011; Rowland et al., 2000; Stoedefalke et al., 2000; Tolfrey et al., 2004). A multilevel longitudinal analysis that we carried out in a previous study (Aires et al., 2012) showed that the participation in competitive sports over 4 years, especially in the club level, was associated with increased CRF over time. On the other hand, associations between objectively measured physical activity and CRF have been reported to range from low to moderate (Dencker & Andersen, 2008). Our results (Study IV), pointed that there was an independent association between the participation in competitive sports, compliance of MVPA guidelines and cardiorespiratory fitness levels, which might suggest a pathway for promoting healthier levels of cardiorespiratory fitness through the engagement in competitive sports, increasing the chances of compliance with PA guidelines.

Finally, it should be underlined that findings from the present thesis were taken in a perspective of a school-based population of Portuguese youths. These findings highlight the relevance of school in terms of public health. It seems that health surveillance policies should focus their efforts for primary prevention of cardiovascular diseases at school, once school is the perfect setting to access the majority of the youngsters' population. Indeed, it is worth including the evaluation of some health parameters in the schools routine, once indicators of health-related physical fitness are easy to operate by teachers and health professionals. The procedures to access most of traditional risk factors are invasive and not suitable for screening subjects at risk among large populations. Indeed, some procedures such as collection of blood samples are uncomfortable for most of the children. On the other hand, the assessment of cardiorespiratory fitness, especially through the 20-meter Shuttle Run Test, might be considered a valuable and simple tool for targeting children and adolescents in risk of later development of CVD and other metabolic related disorders. Additionally, schools offer a favourable environment to intervene over obesity, low fitness and sedentary lifestyles. Therefore, it is increasingly important to recognize the role of schools in public health strategies and politics, to improve general health and reduce the burden of CVD.

CONCLUSIONS

This thesis adds new findings to the body of evidences suggesting that cardiorespiratory fitness should be considered an essential independent risk factor for cardiovascular diseases since paediatric ages. Moreover, compiling the results from the investigations originated in the present thesis, the following conclusions should be outlined:

- Equations estimated from Portuguese youths provide valid estimates of maximal oxygen uptake (VO_2max), the standard measure of cardiorespiratory fitness. The model calculated from the linear regression should be recommended to estimate VO_2max from the 20-meter Shuttle Run Test in future studies carried out with Portuguese children and adolescents.
- Reference standards based on the performance in the 20-meter Shuttle Run Test expressed in laps that were calculated from the Portuguese youth population provide a valuable tool for screening children and adolescents with increased metabolic risk.
- Low cardiorespiratory fitness estimated from the 20-meter shuttle run test is associated with increased metabolic risk, independently of central adiposity, in children and adolescents.
- In Portuguese youths, participation in competitive sports is associated with the compliance of recommended levels of moderate to vigorous physical activity and with increased cardiorespiratory fitness. Therefore, participation in competitive sports should be promoted since the early ages in order to improve cardiovascular health.

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