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Cardiorespiratory fitness and the development of cardiovascular risk factors in children and adolescents

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Clarice Maria de Lucena Martins

To my parents and brothers

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ABSTRACT

The aim of the present study was to analyze the cardiorespiratory fitness (CRF) behavior throughout the years and its role as a predictor of cardiovascular disease (CVD) risk factors in children and adolescents from Oporto – Portugal. The present thesis is structured in four papers that compound the main part of the study.

Paper I analyzed trends in CVD risk factors and CRF in five years. Two cross sectional studies were performed including 138 subjects in 1998 and 110 in 2003. The data showed a significant year effect ($p=0.000$) for CRF in boys and girls and stability for the other CVD risk factors.

Paper II analyzed different categories of CRF and obesity and the relation with CVD risk factors in youth. The study was carried-out in 2006 with 392 children and adolescents aged 10-16 years-old of both genders. The fit-obese and fit-nonobese groups presented significant differences in waist circumference (WC), triglycerides, sum of skinfolds and LDL cholesterol.

The III paper investigated the relationship between CVD risk factors, CRF and three different indicators of fatness, and if these relationships are independent by each other. In 2006, 491 children and adolescents aged 10-16 years-old of both genders were evaluated. Fitness was associated with clustering risk factors. Belonging to the unfit category increased the risk of having high metabolic risk score - MRS ($\beta=.158$; $p<0.05$). Obesity indicators presented significant relationship with the MRS ($\beta=.033$, .010, and .014 for body mass index, waist circumference and percentage of fat respectively).

The paper IV analyzed the 5-year longitudinal relationship between CRF and CVD risk factors in 153 children and adolescents from 1998 to 2003. For each of the CVD risk factors (BMI, TC, SBP and DBP) two models were analyzed. The first model was only adjusted for time, while the second model was further adjusted for gender and age. In both models, a significant main effect was found for BMI ($p\geq0.05$).

The studies highlighted that: 1. a significant marked low CRF level over time in adolescents of both genders was observed; 2. regardless fatness, participants with higher CRF levels presented lower prevalence of CVD risk factors; 3. both fitness and fatness are associated with clustered risk factors by different pathways; 4. low levels of CRF are associated with higher levels of BMI over time. As a result, even at young ages, the beneficial impact of increasing levels of CRF would be of great clinical relevance.

RESUMO

O objectivo do presente estudo foi analisar o comportamento da aptidão cardiorrespiratória (ACR) ao longo dos anos e o seu papel como um predictor de factores de risco de doenças cardiovasculares (DCV) em crianças e adolescentes do Porto - Portugal. A presente tese está estruturado em quatro artigos que compõem o estudo.

O artigo I analisou as tendências comportamentais dos factores de risco cardiovascular e da ACR em cinco anos. Dois estudos transversais incluindo 138 indivíduos em 1998 e 110 em 2003 foram realizados. Os dados indicaram um efeito significativo dos cinco anos de diferença entre as duas avaliações para a ACR em meninos e meninas p = 0,000) e de estabilidade para os demais factores de risco de DCV.

O artigo II objectivou analisar diferentes categorias de ACR e de obesidade e suas relações com os factores de risco cardiovascular em crianças e adolescentes. O estudo foi realizado em 2006, com 392 sujeitos com idades compreendidas entre os 10-16 anos, de ambos os sexos. Os indivíduos pertencentes aos grupos aptos-obesos e aptos-não obesos apresentaram diferenças significativas na circunferência da cintura (CC), triglicérides, soma das dobras cutâneas e colesterol LDL.

O artigo III investigou a relação entre os factores de risco para DCV, a ACR e três diferentes indicadores de adiposidade, além de investigar se estas relações são independentes umas das outras. Par tal, em 2006, 491 crianças e adolescentes com idades entre os 10-16 anos e de ambos os sexos foram avaliadas. Verificou-se que a ACR está associada à agregação de factores de risco de DCV. Pertencer à categoria baixa ACR aumentou a probabilidade de ter um score de risco metabólico (SRM) elevado (β =. 158, p <0,05). Os indicadores de obesidade apresentaram relação significativa com o SRM (β =.033, .010, .014 para o índice de massa corporal, circunferência da cintura e percentual de gordura, respectivamente).

O artigo IV analisou a relação longitudinal entre ACR e factores de risco cardiovascular em 153 crianças e adolescentes, de 1998 a 2003. Para cada um dos factores de risco de DCV (IMC, CT, PAS e PAD) foram analisados dois modelos. O primeiro modelo foi ajustado apenas para o tempo, enquanto o segundo modelo foi ajustado para o sexo e a idade. Em ambos os modelos foi encontrado um efeito significativo da ACR sobre o IMC ($p \geq 0,05$).

O trabalho aqui apresentado destacou: 1. uma significativa redução dos níveis de ACR ao longo do tempo em adolescentes de ambos os sexos; 2. independente da adiposidade, sujeitos com níveis mais elevados de ACR apresentaram menor prevalência de factores de risco para DCV; 3. tanto a ACR quanto a obesidade estão associados à agregação de factores de risco, através de diferentes vias; 4. baixos níveis de ACR estão associados a níveis mais elevados de IMC ao longo do tempo. Como resultado, mesmo em idades jovens, o impacto positivo do aumento dos níveis de ACR seria de grande relevância clínica.

RÉSUMÉ

L´objectif de cette étude était d'analyser le comportement cardio-fitness (CFR) au fil des anées et de comprendre son rôle comme un facteur prédictif des risques de maladies cardiovasculaires (MCV) chez les enfants et adolescents dans la ville de Porto - Portugal. Cette thèse est structurée en quatre articles scientifiques qui constituent la partie principale de l'étude.

L´artilce I a analysé les tendances des facteurs de risque des MCV et de CRF. Deux études transversales ont été effectuées avec 138 sujets en 1998 et 110 en 2003. Les données ont révélé un effet de l´âge significatif (p = 0,000) pour le CRF dans les garçons et les filles et une stabilité pour les autres facteurs de risque de MCV.

L´artilce II a analysé les différentes catégories du CRF et de l'état d´engraissement et la relation avec les facteurs de risque des MCV chez les jeunes. L'étude a été effectuée en 2006 avec 392 enfants et adolescents âgés de 10-16 ans comprenant les deux sexes. Le fit-obèses et fit-nonobèses groupes ont présenté des resultats avec différences significatives pour le tour de taille, dans les niveaux des triglycérides, dans la somme des plis cutanés et dans les niveaux du cholestérol LDL.

L´article III s´agit d´une recherche sur les relations entre les facteurs de risque des maladies cardiovasculaires, le CRF et trois indicateurs de l'état d'engraissement, et si ces relations sont indépendantes entre eux. En 2006, 491 enfants et adolescents âgés de 10-16 ans comprenant les deux sexes ont été évalués. La variable fitness était associé avec l´agrégation des facteurs de risque. Les niveaux les plus élevés du score de risque metabolique (SRM)ont été enregistrés dans les sujets appartenant à la categorie non-fitness - SRM (β =. 158, p <0,05). Les indicateurs d´obésité ont démontré une étroite relation avec les SRM (β =. 033, .010, et ,014 pour l'indice de masse corporelle, le tour de taille et le pourcentage de matières grasses, respectivement).

Le quatriéme article a analysé la relation longitudinal entre le CRF et les facteurs de risque de MCV dans 153 enfants et adolescents entre 1998 et 2003. Pour chacun des facteurs de risque de maladie cardiovasculaire (IMC, TC, SBP et DBP), deux modèles ont été analysés. Le premier modèle été ajusté en fonction des temps, le deuxième modèle a été ajusté pour le sexe et l'âge. Dans les deux modèles, un effet principal a été trouvée pour l'IMC (p ≥ 0,05).

Les études ont mis en évidence que: 1.un faible niveau du CRF chez les adolescents des deux sexes a été observé au cours du temps; 2. malgré l´état d'engraissement, les participants avec des niveaux plus élevés du CRF ont presenté une faible prévalence des facteurs de risque de MCV, 3. À la fois le fitness et l´état d'engraissement sont associés à l´agrégation des facteurs de risque, toutefois, par voies différentes; 4. des faibles niveaux du CRF sont associés à des niveaux plus élevés d'IMC. En conséquence, même à un jeune âge, les effets bénéfiques d'une augmentation des niveaux du CRF serait d'une grande valeur clinique.

LIST OF ABBREVIATIONS

ANOVA	Analysis Of VAriance
BP	Blood Pressure
BMI	Body Mass Index
CI	Confidence Interval
CRF	CardioRespiratory Fitness
CVD	Cardiovascular Diseases
DBP	Diastolic Blood Pressure
GLUC	GLUCose
HDL	High Density Lipoproteins
HOMA	HOmeostasis Model Assessment
LDL	Low Density Lipoproteins
MRS	Metabolic Risk Score
MS	Metabolic Syndrome
NHANES	National Health and Nutrition Examination Survey
SBP	Systolic Blood Pressure
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
TC	Total Cholesterol
TEM	Total Erros of the Mean
TRIG	TRIGlycerides
US	United States
USA	United States of America
VO_2máx	Maximal oxygen consumption
WC	Waist Circumference
WHO	World Health Organization
%FAT	Percentage of Fat Mass

LIST OF TABLES

LIST OF PUBLICATIONS

1. Martins C, Silva F, Santos MP, Ribeiro JC, Mota J. Trends of cardiovascular risk factors clustering over time. A study in two cohorts of Portuguese adolescents. PES, 20:74-83. 2008

2. Martins C, Gaya AR, Silva F, Mota, J. Cardiorespiratory fitness, fatness and cardiovascular diseases risk factors in children and adolescents from Porto. (Submitted in November, 2008)

3. Martins C, Andersen LB, Aires L, Mota J. Association between fitness, fatness, different indicators of fitness, and clustered cardiovascular diseases risk factors in portuguese children and adolescents. (Submitted in December, 2008)

4. Martins C, Santos R, Gaya AR, Twisk J, Ribeiro JC, Mota J. Cardiorespiratory fitness predicts later body mass index, but not others cardiovascular risk factors from childhood to adolescence. Am J Hum Biol. 21 (1): 121-122. 2008.

1. Introduction

1. Introduction

The increasing levels of inactivity or deaths caused by chronic diseases, especially those caused by the cardiovascular system have lead to efforts in order to minimize the epidemic profile of nowadays society. Those efforts have been focused on the detection and prevention of risk factors associated to cardiovascular diseases (CVD), namely obesity, sedentary lifestyle, smoking habits, diabetes mellitus, arterial hypertension, high lipid profile, and heredity (Fisberg et al., 2001; Gus et al., 2002).

Although a large proportion of clinical manifestations associated to CVD appear in adulthood, in the 1930s researchers started questioning if the CVD risk factors appear only in adulthood or since childhood. Nowadays we have concrete evidences that CVD predisposal factors could appear (Frelut, 2003), cluster (Andersen et al., 2003) and have increasing prevalence in children.

In adult population, the CVD have been responsible for a large proportion of deaths all over the world (Smith et al., 2004). It is supposed that some CVD risk factors, like hypertension, diabetes or hypercholesterolemia could be influenced by cardiorespiratory fitness (CRF) (Laaksonen, 2002; Carnethon et al., 2003).

Some authors investigated the relationship between CVD risk factors and CRF in adulthood and found an inverse relationship between clustered CVD risk factors, inflammatory factors, and physical activity, with subsequent alterations of the CRF. High levels of CRF are also associated with low levels of Metabolic Syndrome (MS) (Kullo et al., 2002; Laaka et al., 2003) and with clustered risk factors. There are some evidences that low levels of CRF are an independent predictor of MS or its precursors (Benson et al., 2006; LaMonte & Blair, 2006;

Lobelo et al.; 2007) This fact suggests that effective modifications in CRF could attenuate CVD risk factors prevalence (Laaksonen et al., 2002; Carnethon et al., 2005; Simmons et al., 2008).

While in adults it is well established that increased CRF levels have a protective effect against CVD risk factors (Church et al., 2001), in youth the important role of CRF in attenuating the CVD risk factors development is controversial. Several studies reported that low CRF at childhood and adolescence is a predictor of CVD risk factors such as abnormal lipids profile (Andersen et al., 2004; Twisk et al., 2002), hypertension (Carnethon et al., 2003; Hasselstrom et al., 2002) and overall or central adiposity (Byrd-Wiliams et al., 2008; Psarra et al., 2005) later in life. However, there are other studies showing that in young populations, the inverse correlation between CRF and CVD risk factors is adulthood is weak (Ruiz et al., 2009)

There is a conflicting and poor consistent position in the literature about the mediation of the relationship between CRF and CVD risk factors. Some authors considered that obesity, and also CRF (Eisenmann et al., 2007a); Ruiz et al., 2009) should be taken into account when analyzing the CVD risk factors in pediatric population. For example, Nielsen and Andersen (2003) evaluated a sample of 13.557 adolescents and observed that both CRF and body mass index (BMI) represent important and independent predictors of blood pressure. In a children and adolescents Estonian and Sweden sample, the CRF was inversely related with significant Homeostasis Model Assessment (HOMA) and insulin levels variation in children with high levels of obesity and waist circumference (Ruiz et al., 2007). In a British cross-sectional study, Stratton and others (2007) observed that children aged between 9 to 11-years-old tended to increase their BMI, while CRF presented an inverse behavior. Even when considering children with normal BMI according to health parameters, CRF levels are also decreasing, independently of BMI. In parallel, when analyzing 1362 Greek children and adolescents, Nassis and others (2005) observed that high CRF levels could reduce obesity prevalence in children. Reinforcing this idea, Ortega and others (2005) consider that high CRF levels are synonymous of a cardiovascular healthy future.

Although long-term follow-ups are rare (Matton et al., 2007), some longitudinal or cross-sectional studies showed the health benefits of CRF, as well as some evidences that the levels of CRF in children and adolescents have declined. However, the results are not consistent and there is a lack of information on Portuguese children.

Thus, if the levels of CRF in children are decreasing, and considering its important role in attenuating the prevalence of CVD risk factors in adulthood, it is important to know how the CRF behaves throughout the years in Portuguese youth and how it influences the prevalence of other risk factors.

Understanding whether CRF in children and adolescents could predict/mediate a better health profile has an important value. So, we do not verify which individuals are in or out health parameters of CRF, but how the CRF behaves throughout the years and how low levels of CRF could independently influence the appearance and development of CVD risk factors in children and adolescents.

Taking into account the abovementioned scientific context concerning: 1. the increasing levels of CVD risk factors in pediatric ages; 2. the decreasing levels of CRF in youth; 3. the important responsibility of CRF and obesity in the development of CVD risk factors in children and adolescents, and 4. the conflicting position of CRF in the development of CVD risk factors in youngsters, the main purpose of this thesis was to:

"Analyze the CRF behavior throughout the years and its role as a predictor of CVD risk factors in children and adolescents from Oporto – Portugal".

Regarding it, the present thesis is structured to answer distinct questions that support the specific aims of each of the four papers that compound the thesis:

1. How do the CRF and the CVD risk factors behave in young ages throughout the years?

PAPER I:

"Trends of cardiovascular risk factors clustering over time. A study in two cohorts of Portuguese adolescents"

2. Do youngsters with low levels of CRF have a better metabolic profile regardless of whether they are obese or not?

PAPER II:

"Cardiorespiratory fitness, fatness and cardiovascular diseases risk factors in children and adolescents from Porto"

3. Is there a relationship between CRF, CVD risk factors, and different indicators of obesity? If so, is this relation independent or mediated by other factors?

PAPER III:

"Association between fitness, different indicators of fatness, and clustered cardiovascular diseases risk factors in Portuguese children and adolescents"

4. Does CRF influence the prevalence of other CVD risk factors throughout the years?

PAPER IV:

"Cardiovascular fitness predicts later body mass index, but not other cardiovascular risk factors from childhood to adolescence"

2. Theoretical Background

Researches have evidenced that when studying the CVD´ etiology, it is necessary to study not only one but a combination of manifestations that potentiates its appearance and development. Those manifestations are called risk factors. Relevant studies like the *Framingham Study* (Massachusetts, USA), the *Tecumseh Study* (Michigan, USA*),* and others, have established the concept of CVD risk factor like a mean to forecast morbidity situations related to the CVD (Kannel, 1971). The risk factors have an individual harmful action that is aggravated when they occur together for a particular subject (Genest and Cohn, 1995). Clustering risk factors is known as the coexistence of several risk factors in a same subject (Twisk, 2000) and it is associated to cardiovascular events in adults. This fact reinforces the idea of considering the association of all the risk factors as a higher clinical relevance (Andersen et al., 2003).

The CVD has a variable etiology, and could be associated to modifiable or non-modifiable risk factors. The non-modifiable risk factors represent those of hereditary character (age, sex, family history), while the modifiable ones include obesity, sedentary lifestyle, smoking habits, stress, and others (Twisk et al., 2001), and constitute the focus of prevention programs.

From these modifiable risk factors, the obesity or the overweight, defined as abnormal or excessive fat accumulation that presents a risk to health (WHO, 2000), gained more attention in the current scenario.

The genesis of obesity is considered of extreme importance once this disorder is associated with a high risk for diabetes mellitus and CVD (Pi-Sunyer, 1991). Throughout history, Men associated the genesis of obesity to genetic factors like hormonal imbalance caused by failure into one or more endocrine glands. Physiological studies indicated that the obesity etiology is associated to a combination of several factors. In the youth population, the etiology of obesity is also related to multiple risk factors (Skinner et al., 2004).

Firstly identified in developed countries´ populations, with high economic power, obesity has become a disease of epidemic proportions and nowadays is considered a major public health problem. These are alarming data, especially when it shows that the number of obese people is increasing especially among children and adolescents (Yoshinaga, 2004), even becoming an epidemic problem (Homer, 2009).There are evidences suggesting that the obese adult population tends to a further increase in the near future (Silventoinen et al., 2004).

Recently, this disease has gained the status of the most common pediatric disease, not only in technologically developed countries but also in countries under development (Burniat et al., 2002; Ebbeling et al., 2002). In the last few decades there is a growing of young and obese European population (Rolland-Cachera et al., 2002;; Luciano et al., 2003; Agneta et al., 2003), and Portugal is not an exception to the rule.

When mentioning the increasing prevalence of obesity, it is also important to emphasize that obesity is an increased factor for situations such as insulin resistance, diabetes, cancer, biliary disorders, sleep apnea, arteriosclerosis and consequently CVD (Aronne & Segal, 2002).

It is evident that the etiology of obesity begins at young ages. It is also evident that obesity is related to an increased risk for CVD. Thus, despite most of the cardiovascular events occur in the 5th decade of life, there are some concrete evidences that the CVD precursors, like obesity, have their genesis during childhood and adolescence (McGill et al., 2000). Berenson and others

(1998), in a study realized with children, identified similar lesions in children's aorta like those observed in adults. These discoveries emphasized the fact that the arteriosclerosis has its genesis in childhood, and could be a pediatric disease that evolves through the years.

Several studies that deal with obesity have shown that excess body fat in children and adolescents has direct adverse effects on the cardiovascular system, similarly to those effects occurred in adults (Reilly et al., 2003).

Other studies indicate that there is correlation between obesity and risk for CVD (Pituelli Suaréz et al., 2008). Some of these studies indicate that children and adolescents with higher proportions of body fat have higher blood pressure levels (Zwiauer et al., 1994), cholesterol, triglycerides, and glucose than those non-obese (Grilo, 1994). Moreover, an exploratory factor analysis indicated that obesity is strongly correlated with CVD risk clustering in adolescents (Goodman et al., 2005).

Though the CVD occur in later life, the risk factors for its development appear in children and adolescents. Furthermore, not only one risk factor isolated like obesity for example, but its clustering has been identified in children and adolescents (Andersen et al., 2003).

The assessment of body composition in children is an important method of early identification (Teixeira et al., 2001) to prevent the CVD´s development. However, the variety of methods and procedures used like skinfolds or waist circumference (WC), for example, have led to widely divergent estimates (Lohman, 1992), according to region, race, age, among other factors.

Considering the obesity increasing prevalence and its associated disorders, many efforts have been done in order to reduce its increasing prevalence and consequently the related CVD risk factors. The American Heart Association indicated the CRF as a key component of the physical activity performed to improve health (Morris & Froelicher, 1993). In the last two decades, the US Preventive Task Force and the International Federation of Sports Medicine reinforced this idea (Blair et al., 1996).

CRF is an attribute, component of physical fitness (Riddoch & Boreham, 1996), reflected in the overall capacity of the cardiovascular and respiratory systems to carry out prolonged exercise (Taylor et al., 1955) and is a physiologic trait (Eisenmann, 2007).

Numerous health benefits of CRF in adults have been extensively documented (Kesaniemi et al., 2001). Several studies emphasized that moderate to high levels of CRF and physical activity are associated with reduced risk of CVD (Carnethon et al., 2005), MS (LaMonte, et al., 2005), diabetes type II (Bassuk & Manson, 2005), among other causes of mortality in adults. Nowadays there are increasing data suggesting that high levels of CRF provide indicators able to diagnose illness or death, especially those caused by the cardiovascular system (LaMonte & Blair, 2006).

Regarding that, in adults a strong inverse relationship between CRF and the prevalence of risk factors for CVD is established (Rana et al., 2006).

In children, however, this relationship is conflicting (Musa et al., 2002; Thomas et al., 2003). In general, the studies examined the association between CRF and clustering risk factors and found that there is an inverse association between fitness levels and metabolic risk profile (Anderssen et al., 2007; DuBose et al., 2007 ; Ekelund et al., 2007 ; Hurting-WennlÖf et al., 2007). Ruiz et al., (2009) indicated that higher levels of CRF reduce the risk of developing MS.

CRF levels are also inversely associated to obesity indicators, such as WC and skinfolds (Klasson-HeggebØ et al., 2006). There are studies indicating that high CRF may reduce the hazards of obesity in children (Nassis et al., 2005). However, a meta-analysis study indicated that there are inconclusive evidences that changes in CRF are associated with changes in weight gain (Ruiz et al., 2009)

Despite the abovementioned, when low levels of CRF are associated to overweight and obesity in youth population, this superposition of risks is determinant to the development of other CVD risk factors in children and adolescents (Gutin et al., 2005; Ruiz et al, 2006a; Ruiz et al, 2006b; MØller et al., 2007).

A Spanish study examined the association between CRF with blood lipids and a composite index of blood lipids and fasting glycaemia in adolescent, with possible interactions with weight status and observed that CRF was related to the composite index of blood lipids and glycaemia in both overweight and non-overweight adolescents. However, in further analysis, for the same levels of CRF, this composite index was significantly higher in overweight adolescents (Mesa et al., 2006).

So, added to the idea of CRF being related to the reduction of risk factors during childhood, more recent studies even suggest that this relationship is mediated by body fat (Einsenmann et al., 2007a; Einsenmann et al., 2007b). Rizzo et al.(2007), in a study with children and adolescents aged 9 and 15-years-old, observed that CRF is inversely related to metabolic risk, and body fat has a pivotal role in this relationship. However, it is difficult to determine whether adiposity confound, mediate or modify the relationship.

Whether fit children and adolescents have less CVD risk factors than their unfit peers, even being obese, remains controversial but CRF could be partially responsible for deleterious consequences of CVD risk factors in youth (Katzmarzyk et al., 2005)

Several cross sectional and longitudinal divergent studies in this field tried to elucidate the relationship between CRF, CVD risk factors and obesity. It is assumed that CRF in children and adolescents is a powerful marker of adult healthier profile and that it tracks from childhood over adolescence into adulthood (Biddle et al., 2004; Ruiz et al., 2009). Hence, understanding the secular changes in CRF plays a crucial role in preventive strategies against CVD.

Secular trends data for CRF are rather scarce and the time period between comparisons is in general not as extensive as desirable. Recent studies showed results from data that evaluated CRF in youth population along the years. In general, those results show that levels of CRF in children and adolescents is declining tremendously. A recent study that highlighted the aerobic performance of Australian and New Zealand children and adolescent showed a marked decline in CRF in recent decades (Tomkinson & Olds, 2007). When evaluating Finish children and adolescents' CRF from 1976 and 2001, Huotari and others (2009) observed the same tendency for boys and girls aged 13-to 18-years-old. A secular trend study with Flemish subjects revealed decreased values for CRF, added to increasing values for weight, BMI and skinfolds (Matton et al., 2007).

However, there is no consensus regarding the tendency above cited. In a Danish study, boys between the mid-1980s and late-1990s demonstrated a decline in CRF levels, while in girls, no overall difference was found during the same period (Wedderkopp et al., 2004). In a study published 3 years later, analyzing data from the late-1990s and early-2000s, an inverse result was found. A significant decline in CRF was observed for Danish girls, but not for boys (MØller et al., 2007). Similarly, in children from the United States, it was observed a decline in maximal aerobic power for girls, but for boys, stability in these levels was found (Malina, 2007). In a study with American adolescents, Eisenmann and others (2002) verified stability in absolute and relative peak VO_2 among boys and girls. The girls, particularly those 15-year-old age and older, had a decreased peak VO_2 by approximately 20%. It was concluded that CRF has not decreased in USA, except in adolescent girls over the past few decades.

In several longitudinal analysis, the eventual relationship between CRF and CVD risk factors in children and adolescents were studied and it was concluded that low levels of CRF in youth ages could predict CVD risk factors, for example abnormal lipid profile (Hasselstrom et al., 2002; Twisk et al., 2002, Andersen et al., 2004), total or central obesity (Boreham et al., 2002; Psarra et al., 2005; Einssenmann et al., 2005), and hypertension (Carnethon et al., 2003) later in life.

Despite Einsenmann and other (2005) having verified a significant relation between adolescents CRF and adult body fat, a lack of association was observed between adolescent CRF and adult cholesterol, BP, and glucose.

Byrd-Williams and others (2008) have shown that in overweight Hispanic boys, a great CRF level at baseline is protective against adiposity increasing. In girls no changes were observed. Boreham and coleagues (2002) have analyzed if there is a relationship between CVD risk factor profile in young adulthood and antecedent physical activity and physical fitness (Shuttle Run Test, physical activity and sports participation by a self-report recall questionnaire) at 12 and 15-years old subjects. It was observed that the promotion of physical fitness during adolescence may reduce exposure to other risk factors lasting into early adulthood.

The intrinsic longitudinal and cross-sectional studies´ adversities are evident. Research developed by Ribeiro et al. (2003), and others, showed the CVD risk factors prevalence in children and adolescents from Porto. However, in specialized literature we have a poor knowledge about its indicators´ behaviour over the years, and if there is any other factor that could influence this relationship. If we look at CRF as an easy indirect measure variable, evaluating this parameter is of fundamental importance given that with this information it is possible to provide primary prevention and minimize the number of deaths caused by CVD in Portuguese population. When analyzing the position of CRF as a cardiovascular health indicator, it is possible to verify the greater or lesser individuals´ predisposition to chronic degenerative symptoms. Also, longitudinal studies developed from childhood to adolescence have the potential of analyzing the changes that maturational alterations could promote in CVD risk factors and mapping strategies for further CVD detections (Janz et al., 2002).

Thus, supposing that levels of CRF are decreasing in children and adolescents, levels of obesity are increasing, and those variables have a pivotal role in the development of CVD risk factors, the knowledge in these topics is of fundamental importance. So, the main point in this subject is the divergent results presented in the literature. On the one hand there are some divergences showing that CRF is decreasing along the years and a convergent idea of the inverse association between CRF levels and prevalence of CVD risk factors in children and adolescents as on the other, data are divergent in concluding the role of CRF in this association. It remains unclear if CRF has an independent function in the relationship or if this relationship is mediated by other indicator, such as obesity.

3. Methodology

STUDY DESIGN

The studies presented in this thesis were carried out as part of two longitudinal research project conducted in Porto (Portugal) area, looking at the prevalence of CVD risk factors and levels of physical fitness in children and adolescents of both genders.

The first project corresponds to a 5-year follow-up study that started in 1998 and finished in 2003 and evaluated children and adolescents aged 8-15 years-old.

During this period, 30 schools were selected and stratified (17 primary schools and 13 high schools) from all Porto's districts in a way that at least one school represented each district. Children and adolescents were chosen at random from the 3rd till the 9th school grade, according to general school system rules. From this project, papers I and IV were elaborated.

The second project started in 2006/2007 and will finish five years later. In this thesis, only data from the cross-sectional analysis collected in 2006/2007 is presented. The sample comprised children and adolescents aged 10-16 years-old of both genders were evaluated from 2 schools of Porto district, Portugal. Subjects were chosen at random from the 5th till the 12th school grade, according to general school system rules as above cited. From this project, papers II and III were constructed.

Considering that not all the sample carried out two evaluations, or not all performed all measurements of the variables under study, it was chosen to best explain the methods in each of the specific studies. The following topics summarize each of the evaluations that were done.

The basic characteristics of the participant and the examined variables in each of the four studies are presented in table 1.

Table 1. Basic characteristics of the studies

Study	Year	Population	Sample	Age	Variables
I	1998 2003	1998 – 529 2003 - 350	248 (138 in 1998 and 110 in 2003)	14-15	BMI, BP, CRF, TC
II	2006/2007	1165	392 (173 boys and 219 girls)	10 to 16	BMI, BP, CRF, TC, LDL/HDL, TRIG, GLUC
III	2006/2007	1165	491 (223 boys and 268 girls)	10 to 16	BMI, BP, CRF, TC, LDL/HDL, TRIG, GLUC
IV	1998 2003	1998 – 529 2003 - 350	153 (66 in 1998 and 87 in 2003)	1998 – 8/10 2003 - 13/15	BMI, BP, CRF, TC

BMI = body mass index; BP = blood pressure; CRF = cardiorespiratory fitness; TC = total cholesterol; LDL/HDL = low density lipoprotein / high density lipoprotein; TRIG = triglycerides; GLUC = glucose.

SAMPLING PROCEDURES

Daily Evaluation protocol

Subjects were identified through his/her code number and code of the school. Fasting blood samples were taken followed by BP measurements. The children were then given breakfast followed by the determination of their maturational stage. Finally the shuttle-run test was performed. The variables were measured between 8:00 and 11:00am.

Blood sampling

In papers I and IV, capillary blood samples of participants were taken from the earlobe after at least 12 hours fasting in order to obtain values of plasmatic TC. The blood samples were drawn in capillary tubes (33 μl, Selzer) coated with lithium heparin and immediately assayed using Reflotron Analyser (Boehringer Mannheim, Indianapolis, IN) in the first moment of the project.

In papers II and III, other blood variables, such as LHD and HDL cholesterols, triglycerides and glucose were determined. Given that, the blood samples were drawn in capillary tubes (33 μl, Selzer) coated with lithium heparin and immediately assayed using Colestech LDX® Analyser. The sample was applied into a Cholestech LDX® cassette and the analyser separates the plasma and the blood cells. Cassettes were stored in the refrigerator after reception. The Cholestech LDX® analyser has been proven to provide good agreement with laboratory measures for population-based screaming for cardiovascular risks factors (Shemesh et al., 2006).

The mean of two measurements was considered for statistical procedures.

Blood pressure

Blood pressure (BP) was measured using the Dinamap adult/pediatric and neonatal vital signs monitors, model BP8800. Measurements were taken by a trained technical and with all children sitting after at least 5min rest. Two measurements were taken after five and ten minutes rest. The mean of these two measurements was used for further data analysis. If the two measurements differed by 2mmHg or more the protocol was repeated (two new measurements, which could not exceed 2mmHg). This procedure was used in a previous study in similar characteristics population and it was observed a mean intra-tester Total Erros of the Mean (TEM) of 1.2% (Duarte et al., 2000)

Anthropometric Measures and Body Composition

Anthropometric methods were used to measure body weight and body height. Body height was measured to the nearest mm in bare or stocking feet with the adolescent standing upright against a Holtain Stadiometer. Weight was measured to the nearest 0.10kg, lightly dressed and after having breakfast, using an electronic weight scale (Seca 708 portable digital beam scale). BMI was calculated from the ratio of body weight (kg) / body height (m^2).

To evaluate the waist circumference (WC), the National Health and Nutrition Examination Survey – NHANES (1996) protocol was used. A bony landmark is first located and marked. The subject stands and the examiner, positioned at the right of the subject, palpates the upper hip bone to locate the right iliac crest. Just above the uppermost lateral border of the right iliac crest, a horizontal mark is drawn, and then crossed with a vertical mark on the midaxillary line. The measuring tape is placed in a horizontal plane around the abdomen at the level of this marked point on the right side of the trunk. The plane of the tape is parallel to the floor and the tape is snug, but does not compress the skin. The measurement is made at a normal minimal respiration

Body fat was determined by tricipital and subscapular skinfolds, according to Heyward (1991). Each skinfold was measured twice and in a successive way, in the right side of the body. However if in these two measurements there was a difference above 5% a third measure was performed. The final result consisted of the mean of the two or three measurements for each skinfold. An Harpender caliper with a constant pressure of 10 g/mm^2 was used and all measurements were completed by the same observer. The percentage of fat (%FAT) was estimated from skinfolds measurements, according to Slaughter et al. (1988) equations.

Maturational Stage

Regarding the maturational stage, the adolescents were inquired separately during physical examination. Each subject self-assessed his/her stages of secondary sex characteristics. Stage of breast in females and pubic hair in males was evaluated according to the criteria of Tanner (1962*).* Previous study showed a correlation of 0.73 between ratings on two occasions (three day interval) in a sub-sample of 50 selected subjects. Concordance between self-assessments of sexual maturity status and physician assessment ranged from 63% for girls and 89% for boys (Mota et al., 2002). In this study all adolescents were in stages 4 and 5 according to Tanner's criteria.

Cardiorespiratory Fitness (CRF)

In papers I and IV, CRF was predicted by maximal multistage 20m shuttle-run test according to procedures described from Fitnessgram (1994). The FITNESSGRAM was selected because of its easy of administration to large numbers of subjects, and in addition its choice of reliable and valid health-related physical fitness measures (Cooper Institute for Aerobics Research, 1999). The Shuttle Run Test predicted maximal aerobic capacity and after converting scores, a predicted maximal oxygen uptake (VO_2max) was obtained. Furthermore, the 20 meter-shuttle run test showed good correlation with VO_2max (r=0.80) suggesting that could be used as a measure of aerobic fitness in children (Ahmaidi et al., 1992*).*

Regarding that VO_2max expressed per unit body mass ($ml.kg^{-1}.min^{-1}$) has been criticized (Armstrong & Welsman, 1997), in paper II and III the CRF was expressed per number of completed laps achieved in the Shutlle Run Test. There are several studies that assessed CRF fitness by the number of completed laps achieved in Shuttle-Run Test (Ruiz et al., 2009).

Statistical analysis

Different methods were used according to the aims of each specific study. The table below (table 2) synthesized the type of analysis, the covariates inserted and the electronic package program used for each of the studies.

Table 2. Statistic Treatment required in each of the specific studies

Study	Analysis	Adjustement	Program
I	Descriptive statistics Independent t-test Qui-square Univariate Analysis of Variance - GLM	Gender Time point	SPSS 13.0
II	Descriptive statistics Independent t-test Qui-Square Test ANOVA – Oneway	Gender Age	SPSS 14.0
III	Descriptive statistics Multiple linear regression Univariate Analysis of Variance - GLM	Gender Age	SPSS 15.0
IV	Descriptive statistics Paired t-test Multilevel analysis	Gender Time point Age	MLwiN 1.1 SPSS 14.0

4. Papers

Study I

Pediatric Exercise Science, 2008, 20, 74-83

Trends of Cardiovascular Risk Factors Clustering Over Time: A Study in Two Cohorts of Portuguese Adolescents

Clarice Martins, Francisco Silva, Maria Paula Santos, José Carlos Ribeiro, and Jorge Mota

This study analyzes trends in CVD risk factors and aerobic performance. Two cross-sectional studies were performed including 138 (58 boys and 80 girls) in 1998 and 110 (44 boys and 66 girls) in 2003 adolescents. Although in cardiorespiratory fitness (CRF) boys performed better than girls, they had lower body mass index (BMI) and total cholesterol (TC) than girls. The data also showed a significant year effect ($p = .000$) for CRF in both boys and girls. The sex–age group interaction was not significant ($p > .05$). This cross-sectional study revealed a marked low CRF level over time in both boys and girls.

Although clinical manifestations of cardiovascular diseases (CVD) appear late in adulthood, there are studies suggesting that some risk factors like obesity (3), hypertension (21), hypercholesterolemia (11), inactivity, or low physical fitness can already be detected in childhood (14,19).

There is a multiplicative effect of the biological risk factors when they occur together for a particular subject (15), which might have higher clinical relevance (2). Furthermore, CVD risk factor clustering have been identified in children and adolescents (2,19).

The important role of physical fitness in preventing CVD risk factors and the inverse relationship between physical fitness levels and the prevalence of CVD risk factors has been suggested (6,16,17). In addition, low levels of physical activity and CRF are both associated with higher risk of all-cause and disease-specific mortality (33).

In the last 20 years, the number of studies on CVD risk factors in the pediatric population has increased significantly, mainly focusing on epidemiologic, etiologic, and prevention data. In Portugal, there are few data concerning epidemiologic transition of the different CVD risk factors in youth population. Indeed, prevalence modifications in CVD risk factors within the same age group at different times remain unclear in the youth population. Because prevalence of CVD risk factors is a serious problem, we hypothesized that after 5 years, youngsters of the same ages tend to have a higher prevalence of CVD risk factors, and this could be reflected in its clustering.

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Therefore, the aim of the current study was to investigate trends in CVD risk factors and aerobic performance in a sample of Portuguese adolescents between 1998 and 2003 using a cross-sectional approach.

Participants and Methods

Design and Sample

This study was carried out as part of a longitudinal research project conducted in the Porto (Portugal) area, looking at the prevalence of CVD risk factors and levels of physical fitness in children and adolescents of both sexes ages 8–15 years. The longitudinal study is a 5-year follow-up study (1998–2003), comprising 30 schools (i.e., 17 primary schools and 13 high schools). The schools were selected and stratified from all Porto districts so that at least one school represented each district. Children and adolescents were chosen at random from the 3rd through 9th school grade, according to general school system rules and as previously described (27). A total of 529 children in 1998 and 350 in 2003 have fully completed all the protocols. For this study, the sample comprised two cohorts including 138 adolescents (58 boys and 80 girls) in 1998 and 110 (44 boys and 66 girls) in 2003 age 14–15 years. All adolescents, parents, and schools approved the study protocol, and all parents signed an informed consent. Students were apparently healthy and free of medical treatment. In both cohorts, all measures were carried out by the same group (physical education teachers, medical doctor). This study was approved by the National Foundation of Science.

Daily Evaluation Protocol

In relation to the daily measurement protocol, children were first identified through their code number and code of the school. Second, blood samples were taken followed by blood pressure measurements. The children were then given breakfast followed by the determination of their maturational stage. Finally the shuttle-run test was performed. The variables were measured between 8:00 and 11:00 a.m.

Blood Sampling

Capillary blood samples of participants were taken from the earlobe after at least 12 hr of fasting to obtain values of plasmatic total cholesterol (TC). The blood samples were drawn in capillary tubes (33 µl, Selzer) coated with lithium heparin and immediately assayed using a Reflotron Analyser (Boehringer Mannheim, Indianapolis, IN). The mean of two measurements was considered for statistical procedures. Risk for hypercholesterolemia was determined by National Cholesterol Educational Program cut-off points for children and adolescents (23). Therefore, adolescents were classified as normal, with risk, or with high risk for hypercholesterolemia.

Blood Pressure

Blood pressure (BP) was measured using the Dinamap adult/pediatric and neonatal vital signs monitors, model BP8800. Measurements were taken by a trained technician and with all children sitting after at least 5 min of rest. Two measurements were taken after 5 and 10 min of rest. The mean of these two measurements was used for statistical analysis. If the two measurements differed by 2 mmHg or more, the protocol was repeated (two new measurements, which could not exceed 2 mmHg). This detailed process has been described elsewhere (12). Elevated (at risk) levels of systolic (SBP) and diastolic (DBP) blood pressure were defined according to age and sex-adjusted 4th quartile of SBP or DBP.

Anthropometric Measures

Anthropometric methods were used to measure body weight and body height. Body height was measured to the nearest millimeter in bare or stocking feet with the adolescent standing upright against a Holtain stadiometer. Weight was measured to the nearest 0.10 kg, lightly dressed and after having breakfast, using an electronic weight scale (Seca 708 portable digital beam scale). Body mass index (BMI) was calculated from the ratio of body weight (kg) to body height (m^2). For purposes of this study, participants were classified in obese, overweight, or normal weight, according to internationally accepted BMI cut-off points (8).

Maturational Stage

Regarding the maturational stage, the adolescents were reviewed separately during physical examination. Participants self-assessed their stages of secondary sex characteristics. Breast stage in females and pubic hair in males were evaluated according to the criteria of Tanner (32). Previous study showed a correlation of 0.73 between ratings on two occasions (3-day interval) in a subsample of 50 selected subjects (22). Concordance between self-assessments of sexual maturity status and physician assessment ranged from 63% for girls to 89% for boys (22). In this study all adolescents were in Stages 4 and 5 according to Tanner's criteria.

Cardiorespiratory Fitness (CRF)

Cardiorespiratory fitness was predicted by maximal multistage 20-m shuttle-run test according to procedures described from FITNESSGRAM (13). FITNESSGRAM was selected because of its ease of administration to large numbers of subjects and its choice of reliable and valid health-related physical fitness measures (9). The shuttle-run test predicted maximal aerobic capacity, and, after converting scores, a predicted maximal oxygen uptake (VO_2max) was obtained. There are several studies that applied the shuttle-run test to estimate VO_2max in children (1). Furthermore, the 20-m shuttle-run test showed good correlation with VO_2max (r = .80), suggesting that could be used as a measure of aerobic fitness in children (35). Analysis was conducted including the percentage of students meeting the adopted age-adjusted criterion-referenced health standards (Health Fitness Zone) for individual test items in the FITNESSGRAM test battery (13). Children were then classified according to the age and sex-specific cut-off points of FITNESSGRAM criteria, as belonging to the healthy zone, under, or above the healthy zone, respectively.

Statistical Analysis

Statistical analysis was performed using SPSS (Windows, version 13.0). Mean and standard deviation was used to characterize the sample. The comparisons between cohorts were done by independent *t* test for anthropometric and maturational variables as well as the chi-square test for proportions in CVD risk factors and clustering of risk factors. General linear models with Bonferroni's adjustments for multiple comparisons were used to examine the differences in the CVD risk factors, adjusting for sex and time point. Level of significance was set up at $p \leq .05$.

Results

Table 1 shows descriptive statistics (mean ± *SD*) of anthropometric and maturational variables for boys and girls, respectively. Regardless, the time-point-considered boys were significantly taller and heavier than girls. No differences were found for maturational stage. Within gender, only girls showed a statistically significant difference in body mass between the two time points.

Table 2 shows a significant main effect for sex in CRF, BMI, and TC. Whereas in CRF boys performed better than girls, they had lower BMI and TC than girls. The data also showed a significant year effect ($p = .000$) for CRF as both boys and girls showed lower CRF values in the second time point. No other statistically significant differences were found. The sex–age group interaction was not significant ($p > .05$). The data also showed that regardless of gender, the percentage of adolescents at risk for CRF (under health zone) was higher in second cohort. No other statistically significant difference was reported.

Table 1 Sample Characteristics

	1998	2003	*p*
	X (*SD*)	*X* (*SD*)	
Boys			
Age (years)	14.8 (0.8)	14.6 (0.6)	.37
Weight (kg)	56.80 (11.60)	58.35 (13.13)	.53
Height (cm)	165.17 (7.96)	164.80 (7.71)	.81
Maturational stage	4.66 (.48)	4.61 (.49)	.67
	X (*SD*)	*X* (*SD*)	
Girls			
Age (years)	14.5 (0.5)	14.6 (0.6)	.57
Weight (kg)	53.65 (7.94)	57.73 (11.40)	.01*
Height (cm)	158.10 (5.35)	160.07 (6.76)	.06
Maturational stage	4.61 (.49)	4.76 (.43)	.06

*$p \leq .05$.

Table 2 Mean (*X*) and Standard Deviation (*SD*) of Risk Factors in 1998 and 2003 for Boys and Girls

	Boys (N = 102)		Girls (N = 146)							
	1998 (N = 58)	2003 (N = 44)	1998 (N = 80)	2003 (N = 66)	Year		Sex		Year × Sex	
CVD risk factors	X (SD)	X (SD)	X (SD)	X (SD)	F	p	F	p	F	p
BMI (kg/m^2)	20.70 (3.28)	21.36 (3.84)	21.44 (2.90)	22.52 (4.23)	3.555	0.06	4.267	0.04*	.211	0.65
SBP (mmHg)	125.41 (8.52)	122.65 (12.07)	121.52 (9.58)	120.49 (10.77)	4.166	0.06	3.129	0.08	1.264	0.26
DBP (mmHg)	64.19 (8.41)	62.78 (8.29)	64.91 (8.60)	64.05 (8.30)	1.803	0.18	1.352	0.25	.252	0.62
TC (mg/dL)	145.83 (21.31)	141.68 (23.15)	163.87 (28.17)	161.47 (29.25)	1.202	0.27	31.215	0.001*	.080	0.78
VO_2max (ml/kg/m)	51.36 (4.48)	47.19 (5.75)	44.98 (4.60)	38.88 (5.19)	61.768	0.000*	132.667	0.000*	2.939	0.09

*$p \leq .05$.

Discussion

This study analyzes trends in CVD risk factors and aerobic performance in a sample of Portuguese adolescents between 1998 and 2003. Because the onset of chronic disease risk factors lies in early childhood, it is of great importance to examine the risk trends in order that effective preventive strategies targeting those at risk start as early as possible.

Our findings showed that regardless of gender, there were no statistical significant differences in the two cohorts for TC, SBP, SDP, and BMI mean values, which might suggest some stability in those variables over time. Only CRF showed a time-point effect. Indeed, despite the cross-sectional design, our findings revealed that adolescents' CRF levels were significantly lower (6.1% in girls and 4.2% in boys) at the second time point. Thus, this outcome should be highlighted because our data also showed that the percentage of adolescents at risk of CRF (under health zone) was significantly higher ($p \leq .05$) at the second time point in both boys and girls. Our findings agree with data showing that, in adolescents, CRF has been decreasing over time (24). Furthermore, a meta-analysis analyzed 55 reports of CRF performance in youth and observed a fast secular decline in the 20-m shuttle-run test performance over the last 20 years (32). Overall, the percentage of adolescents at risk for CRF are at the same magnitude as Irish adolescents age 12–15 years, in which 23–31% presented low CRF (5), and data from a sample of English adolescents showed that boys more often reach the CRF health zones criterion than girls (29). Because there is a strong relationship between CRF and physical activity (6) and higher levels of fitness are related to sports participation and other physically demanding leisure activities (25), it might be that the lower CRF levels over time could be an indicator that levels of physical activity have been decreasing in this population.

Adolescence has been described as a period in which a drastic reduction in levels of physical activity is seen (30). This might justify the high percentage of girls at risk for CRF that was observed in our study and described in a cross-sectional study of Portuguese youth (28). In addition, it might represent a serious problem in the relationship of fitness and obesity. Despite that, we did not find statistically significant differences between the two time points in BMI either when expressed as continuous or categorical (normal weight and overweight). Moreover, it should be noted that the percentage of overweight boys (6%) and girls (10%) was higher in the second cohort. Thus, our findings showed a lower CRF performance at the second time point, which was concurrent with higher level of BMI. Because usually overweight and obese boys and girls showed lower CRF than did normal-weight peers (25), with an emphasis in girls since childhood (21), our data claim a particular attention in strategies that might counteract this tendency.

Although no statistically significant differences were found, CVD risk factors tend to cluster in a higher proportion in 2003 than 1998 in both genders. Whereas previous studies have shown that risk factors tend to cluster even in pediatric years (2) and that there is a relationship between the number of risk factors and the severity of symptoms (4), it is difficult to compare our results with those reported in the literature. One reason is related to the different risk factors considered in the definition of risk factor clustering. Table 3 shows CVD risk factors according to international cutoff points. It was observed to be a significant main effect in CRF for both genders.

Table 3 Percentage of CVD Risk Factors According to Time Point in Boys and Girls

	Boys			Girls		
CVD risk factors	1998	2003	*p*	1998	2003	*p*
TC						
normal	91.4	84.1	.357	62.5	63.6	.740
risk	6.9	13.6		26.3	28.8	
high risk	1.7	0.0		11.3	7.6	
SBP						
no risk	72.4	75	.769	82.5	72.7	.206
risk	27.6	25		17.5	25.8	
DBP						
no risk	77.6	81.8	.601	68.8	78.8	.126
risk	22.4	18.2		31.3	19.7	
VO_2max						
under	0.0	13.6	.004*	3.8	31.8	.000*
health	60.3	61.4		31.3	47	
above	39.7	20.5		65	19.7	
BMI						
normal	81	75	.463	81.3	71.2	.153
overweight	19	25		18.8	28.8	
Cluster						
0 or 1	89.7	88.4	.870	88.8	86.2	.662
2 or more	10.3	11.6		11.2	13.8	

*$p \leq .05$ (chi-square test).

Although no statistically significant differences have been found, it seems, however, that a higher percentage of CVD risk factors clustering is due to a lower CRF performance at the second time point. This is a worthy outcome because a cluster of biological risk factors track over time in children and young adults (17,33). In addition, Raitakari et al. (26), in a cross-sectional study of atherosclerosis precursors in children and young adults, showed that 25% of the participants remained in last tertile after 6 years of follow-up. Furthermore, low CRF is an independent risk factor for CVD (7) and is correlated with visceral adipose tissue in young boys and girls (35). So, although we did not find statistically significant differences, our results might have some importance from a preventive point of view because they potentially pointed out some future health negative implications.

Because clustering of risk factors showed an increased risk for morbidity and mortality (35), our data highlights the need for identification of CVD risk factor clustering early during childhood and adolescence. This might lead to prevention strategies and the development of policies and public health programs in these age groups. Under special caution should be those identified as at risk for low CRF in schools because that identification in clinical settings is difficult (25).

The strength of this study is that it examined the trends within populations of same age groups. This potentially gives additional information to data collected in other population surveys and allows a better understanding of targeting of the preventive strategies to being implemented.

Nevertheless, some limitations should be recognized. First, the sample size was limited, which prevented generalizations. Therefore, a Portuguese youth representative sample is needed to create parameters to develop preventive programs. Second, the cross-sectional design does not allow assigning causality and prevents causal inferences from being drawn. Third, the indirect CRF measures are a potential weakness of this study, but the use of direct measures has been largely described with good reliability in the literature. Furthermore, the easy administration of the shuttle-run test and its common use in large-scale studies makes it as a valuable tool for studying CRF in youth.

Conclusion

This cross-sectional study revealed a markedly low CRF level over time in both boys and girls. This outcome, as well as the higher percentage of obese adolescents and a higher risk factor clustering between the two time points, might have some health negative implications in the future.

Acknowledgments

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Study II

CARDIORESPIRATORY FITNESS, FATNESS AND CARDIOVASCULAR DISEASES RISK FACTORS IN CHILDREN AND ADOLESCENTS FROM PORTO

ABSTRACT

The present study analyzed different categories of CRF and obesity and the relation with CVD risk factors in youth. We hypothesized that youngsters with low levels of CRF have higher values of CVD risk factors, regardless they are obese or not. This study was carried-out as a part of a longitudinal research project conducted at Porto and Braga districts, Portugal, with children and adolescents aged 10-16 years-old of both genders. A total of 392 children have participated in the study (173 boys and 219 girls). To analyze the dependence between student's CRF and levels of obesity (non-overweight and overweight/obese), a Qui-Square Test was used. For the purpose of this study, a new variable with four groups was created: non-overweight + unfit (37.4%), non-overweight + fit (35%), overweight/obese + unfit (11%) and overweight/obese + fit (10%). An ANOVA – Oneway was used to compare the differences according to fitness and fatness groups. Level of significance was set up at $p \le 0.05$. The main finding of this study was that regardless fatness, participants with higher CRF levels presented lower prevalence of CVD risk factors.

1. INTRODUCTION:

In adults, obesity is a strong risk factor for type 2 diabetes and cardiovascular diseases (CVD) [1]. There is strong evidence that in men and women, physical activity and cardiorespiratory fitness (CRF) may protect from the adverse effects of obesity on health [2]. Results from diverse studies suggest that having a moderate to high CRF is associated with lower risk for health outcomes [3;4] such as cardiovascular diseases [5] and all-cause mortality [6].

In youth, it is evident the worldwide epidemic obesity [7]. Observational studies have shown that childhood obesity is associated with a metabolic risk profile [8;9] and a sedentary lifestyle is suggested to be implicated in this trend [10].

Results from several studies showed that there is an inverse correlation between obesity and levels of CRF in children and adolescents [11; 12; 13;14] and it is strongly evident that low levels of CRF and overweight are related to CVD risk factors [9; 11].

Whether fit children and adolescents have less CVD risk factors than their unfit peers, even being obese, remains controversial and CRF could be partially responsible for deleterious consequences of CVD risk factors in youth[15]. The present study analyzed different categories of CRF and obesity and the relation with CVD risk factors in youth. We hypothesized that youngsters with low levels of CRF have higher values of CVD risk factors, regardless they are obese or not.

2. PARTICIPANTS AND METHODS:

Design and Sample

This study was carried-out as a part of a longitudinal research project looking to the prevalence of CVD risk factors and levels of physical fitness in children and adolescents aged 10-16 years-old of both genders. This study was conducted at Porto and Braga districts, Portugal. Children and adolescents were chosen at random from the 3rd till the 12th school grade, according to general school system rules and previously described [16]. A total of 392 children have participated in the study (173 boys and 219 girls). Parents and schools approved the study protocol and all parents signed an informed consent. Students were apparently healthy and free of medical treatment. All measures were carried out by a specialized group (Physical Education teachers, medical doctor).

Daily Evaluation protocol

Subjects were firstly identified through his/her code number and code of the school. Secondly blood samples were taken followed by blood pressure measurements. The children were then given breakfast followed by the determination of their maturational stage. Finally the shuttle-run test was performed. The variables were measured between 8:00 and 11:00am.

Blood sampling

Capillary blood samples of participants were taken from the earlobe after at least 12 hours fasting in order to obtain values of plasmatic total cholesterol (TC), high density lipoprotein cholesterol (HDL), fasting glucose (GLUC) and triglycerides (TRIG). The blood samples were drawn in capillary tubes (33 μl, Selzer) coated with lithium heparin and immediately assayed using Colestech LDX Analyser.

Blood pressure

Blood pressure (BP) was measured using the Dinamap adult/pediatric and neonatal vital signs monitors, model BP8800. Measurements were taken by a trained technician and with all children sitting after at least 5min rest. Two measurements were taken after five and ten minutes rest. The mean of these two measurements was used for statistical analysis. If the two measurements differed by 2mmHg or more the protocol was repeated (two new measurements, which could not exceed 2 mmHg). Detailed process has been described elsewhere [16].

Anthropometric Measures

Body height was measured to the nearest mm in bare or stocking feet with the adolescent standing upright against a Holtain Stadiometer. Weight was measured to the nearest 0.10kg, lightly dressed and after having breakfast, using an electronic weight scale (Seca 708 portable digital beam scale). Body mass index (BMI) was calculated from the ratio of body weight (kg) / body height (m^2). For purposes of this study, participants were classified in overweight or normal weight, according to internationally accepted BMI cut-off points [17].

Maturational Stage

Regarding the maturational stage, the adolescents were inquired separately during physical examination. Each subject self-assessed his/her stages of secondary sex characteristics. Stage of breast in females and pubic hair in males was evaluated according to the criteria of Tanner [18]. Previous study showed a correlation of 0.73 between ratings on two occasions (three day interval) in a sub-sample of 50 selected subjects and concordance between self-assessments of sexual maturity status and physician assessment ranged from 63% for girls and 89% for boys [19].

Cardiorespiratory Fitness (CRF)

CRF was predicted by maximal multistage 20m shuttle-run test according to procedures described from [20]. The FITNESSGRAM was selected because of its easy of administration to large numbers of subjects, and in addition its choice of reliable and valid health-related physical fitness measures [21]. The Shuttle Run Test predicted maximal aerobic capacity and after converting scores, a predicted maximal oxygen uptake (VO_2max) was obtained. There are several studies which applied the shuttle run test to estimate VO_2max in children [22].

Furthermore, the 20 meter-shuttle run test showed good correlation with directly measured VO_2max (r=0.80) suggesting that could be used as a measure of aerobic fitness in children [23]. Analysis was conducted including the percentage of students meeting the adopted age-adjusted criterion referenced health standards (Health Fitness Zone) for individual test items in the Fitnessgram test battery [20]. Children were then classified according to the age and sex-specific cut-off points of Fitnessgram criteria, as belonging to a healthy zone or under a healthy zone.

Statistical analysis

Descriptive statistics were used in order to characterize the sample. In childhood there is not a clinical criterion for the metabolic syndrome (MS). They differ in detail and inclusion criteria [24], and none of the cut-off points apply specifically to children [25]. In these sense, a specific metabolic score was computed. The values presented for glucose, triglycerides, HDL-C/TC, LDL, HDL, TC, and systolic and diastolic blood pressure consist in a computed standardized value by age, gender and maturational stage for each of the variables as follows: standardized value = (value – mean)/ standard deviation. Similar procedures were described elsewhere [26]. Concerning that there were variables differing between genders, an Independent Sample t-Test was used in order to compare those means.

To analyze the dependence between student's CRF and levels of obesity (non-overweight and overweight/obese), a Qui-Square Test was used. For the purpose of this study, a new variable with four groups was created: non-overweight + unfit (37.4%), non-overweight + fit (35%), overweight/obese + unfit (11%) and overweight/obese + fit (10%). An ANOVA – Oneway was used to compare the differences according to fitness and fatness groups.

Analysis was performed with the statistical software package SPSS 14.0 for Windows and level of significance was set up at $p \leq 0.05$.

RESULTS:

Because the aim of this study was to investigate differences between fit-fat groups, and not age or gender differences per se, only main effects of obesity and fitness in CVD risk factors are discussed. Participants´ anthropometric and physical characteristics are presented in table 1. In all 392 children 22.45% were overweight and obese. BMI was significantly lower and laps completed in the Shuttle-run Test were significantly higher ($p \leq 0.05$) in fit children. Weight and height did not differ between groups.

Table 1: Anthropometric and physical characteristics of the subjects

Variables	Non-overweight[a]		Overweight/obese[a]	
	Unfit	Fit	Unfit	Fit
n	157	147	46	42
Age	14.47 (1.80)	13.60 (2.05)	13.85 (1.94)	12.14 (2.04)
Weight	51.38 (8.44)	51.53 (10.84)	66.75 (9.30)	62.60 (12.59)
Height	159.92 (7.80)	161.80 (11.83)	159.97 (8.54)	161.53 (11.35)
BMI	20.23 .(223)	19.44 (1.93)*	26.00 (2.25)	23.73 (1.93)*
CRF (Laps completed)	27.53 (9.60)	53.95 (21.03)*	21.04 (9.63)	42.95 (20.35)*

[a] Values are means ± s.d.

* $p \leq 0.05$ between fit and unfit within the same BMI category.

Table 2 presents sample characteristics by gender for each of the variables. Statistically significant differences were observed for triglycerides, HDL and TC cholesterol, glucose and laps in the Shuttle Run Test. Boys presented statistically significant lower values for triglycerides, TC and HDL cholesterols than girls and significant higher values of glucose and completed laps.

Table 2: Sample characteristics [b]

CVD Risk Factors	GENDER	$\overline{X}$	SD	t	Sig. (2-tailed)	95% Confidence Interval of the Difference	
						Lower	Upper
Triglycerides	female	59,25	19,156	3,327	,001*	2,475	9,623
	male	53,20	17,515				
TC / HDL	female	4,117	4,3319	-,077	,939	-,877	,811
	male	4,150	4,3611				
TC	female	150,53	25,318	-2,631	,009*	-11,597	-1,679
	male	143,90	26,013				
HDL	female	45,92	10,861	-2,363	.019*	-4,748	-,435
	male	43,32	11,497				
Glucose	female	83,90	7,092	-2,772	,006*	-3,358	-,571
	male	85,87	7,337				
LDL	female	92,7672	24,34674	1,197	,232	-1,823	7,496
	male	89,9308	23,86099				
BP	female	141,3942	16,02733	-,625	,532	-4,353	2,253
	male	142,4444	17,81220				
Shuttle Run Test	female	28,32	10,789	-12,760	,000*	-26,185	-19,193
	male	51,00	23,353				

[a] Independent Sample t-test
* p≤0.05 between gender

Table 3 shows the descriptive characteristics of each fitness-fatness group. The sample was divided in four groups: group 1= Non-overweight + unfit; group 2 = Non-overweight + fit; group 3 = Overweight/obese + unfit; and group 4 = Overweight/obese + fit.

As expected and showed in table 3, waist circumference and sum of skinfolds were significantly different between the non-overweight (1 and 2) and the overweight/obese (3 and 4) groups (p≤0.001). Groups 1 and 2 have no significant differences between them. Groups 3 and 4 presented significant different WC values. The unfit group (group 3) presented higher WC values than the fit one (group 4).

Concerning the two fit groups, there were significant differences in WC, triglycerides, sum of skinfolds and LDL cholesterol.

Table 3: F values showing differences in CDV risk factors by fit-fat groups

CVD Risk Factors Z-scores	Groups	N	$\overline{X}$	SD	95% CI Lower	95% CI Upper	F	Sig.
WC	1	156	-,235	,770	-,357	-,113		
	2	147	-,365	,703	-,480	-,251	68,790	,000*
	3	45	1,302	1,039	,989	1,614		
	4	42	,790	,893	,511	1,068		
	Total	390	,003	,982	-,094	,101		
TC	1	157	,102	,959	-,048	,253		
	2	147	,0421	,918	-,107	,192	3,026	,029*
	3	46	-,349	1,007	-,648	-,049		
	4	42	-,132	,987	-,440	,175		
	Total	392	,001	,960	-,093	,097		
HDL	1	157	-,051	,969	-,203	,101		
	2	147	-,125	,801	-,256	,005	4,626	,003*
	3	46	,445	1,314	,055	,835		
	4	42	,182	1,125	-,168	,533		
	Total	392	,004	,990	-,093	,102		
Trigl	1	157	-,062	,888	-,202	,077		
	2	147	-,173	,638	-,278	-,069	6,222	,000*
	3	46	,343	1,372	-,063	,751		
	4	42	,396	1,461	-,058	,852		
	Total	392	-,007	,975	-,104	,089		
TC/HDL	1	157	-,016	1,009	-,175	,142		
	2	147	-,049	,986	-,210	,110	,551	,648
	3	46	,153	,732	-,063	,371		
	4	42	-,044	,896	-,323	,234		
	Total	392	-,011	,958	-,107	,083		
Glucose	1	157	-,027	,982	-,182	,127		
	2	147	-,028	1,013	-,194	,136	,674	,568
	3	46	,152	,910	-,117	,422		
	4	42	,124	,882	-,150	,399		
	Total	392	,009	,975	-,087	,106		
BP	1	157	,483	2,331	,115	,850		
	2	147	,410	2,222	,048	,772	1,069	,362
	3	46	-,098	2,004	-,693	,497		
	4	42	,063	2,125	-,599	,725		
	Total	392	,342	2,233	,120	,564		
∑Skinfolds	1	390	-,016	1,745	-,833	-,321		
	2	157	-,107	,918	-,515	-,081	35,451	,000*
	3	147	-,040	1,006	1,014	2,326		
	4	46	,349	,977	,890	1,606		
	Total	390	-,016	1,745	-,1901	,1574		
LDL	1	157	-.360	.748	-.478	-.242		
	2	147	-.323	.722	-.441	-.205		
	3	46	1.342	.735	1.123	1.560	96.010	.000*
	4	42	.944	.736	.714	1.173		
	Total	392	-.006	.967	-.102	.089		

[b] Anova – Oneway, all variables were adjusted for age, sex and maturational stage
* p≤0.05

Table 4 resumes all the significant comparisons between groups.

Table 4: Multiple Comparison between groups

CVD Risk Factors		Fit x fatness groups			
		1	2	3	4
Waist circumference	1		-	*	*
	2	-		*	*
	3	*	*		*
	4	*	*	*	
		1	2	3	4
Total cholesterol	1		-	*	-
	2	-		-	-
	3	*	-		-
	4	-	-	-	
		1	2	3	4
HDL Cholesterol	1		-	*	-
	2	-		*	-
	3	*	*		-
	4	-	-	-	
		1	2	3	4
Triglycerides	1		-	-	*
	2	-		*	*
	3	-	*		-
	4	*	-	-	
		1	2	3	4
∑ skinfolds	1		-	*	-
	2	-		*	*
	3	*	*		-
	4	*	*	-	
		1	2	3	4
LDL Cholesterol	1		-	*	*
	2	-		*	*
	3	*	*		-
	4	*	*	-	

* $p \leq 0.05$

DISCUSSION:

The aim of this study was to analyze different categories of CRF with regard to obesity status and its relationship with CVD risk factors in youth. The independent contribution of BMI and fitness to CVD has been unclear. Since the onset of CVD risk factors as well as obesity might lie in youth[27] it is of great importance to examine the associated risks in order that effective preventive strategies targeting those at risk start as early as possible.

The main finding of this study was that regardless obesity level the fit participants showed a better lipid profile (LDL cholesterol) than their unfit counterparts. This was also true with regard to WC, which highlight the fact that being fit may reduce some of the negative health implications of obesity. Further, our study showed that those that are unfit and are obese showed significantly worse lipid profile (TC; HDL; LDL) and anthropometric variables (WC and ∑ skinfolds) than their unfit but normal-weight peers, which, in turn, pointed out that most of the health benefits of leanness are limited to fit youngsters.

Although a lack of consensus concerning to the use of different test procedures and the appropriate use of cut-points to establish fitness performance related to health associated to the existing controversy whether fit youth have less CVD risk factors than their unfit peers, even being obese, expresses a difficulty of comparison and interpretation of the results, our data showed similar results as found in adults. Indeed it was shown that self-reported PA and functional capacity were more important than weight status for CV risk stratification in women, suggesting that that the CV risks of obesity may be explained in part by the adverse effects of low fitness [28]. Other study in men showed that unfit, lean men also had a higher risk of all-cause and CVD mortality than men who were fit and obese [29].

With regard to adolescents our data are consistent with some data in the literature, which hypothesizes that higher levels of CRF are associated with a better lipid profile (or CVD) even in obese. In a study with 4072 European children and adolescents, it was observed a curvilinear graded relation between CRF, WC, sum of skinfolds and blood pressure [30]. In the Québec Family Study, 610 children and adolescents were evaluated and it was observed that 11 to 30% of the variance in the risk profile was explained by physical fitness, including CRF [31]. A recent study shows that low levels of childhood physical activity and CRF are associated with the presence of the MS in adolescent [32]. When examining the relationship among fatness and CRF on indices of insulin resistance and sensitivity in children, it was observed that CRF attenuates the differences in insulin sensitivity within BMI categories, which reinforce the important role of fitness even among obese children [33]. Also, when evaluating levels of obesity in children it was observed that central and total obesity were lower in overweight and obese children with high level of CRF [2].

Rizzo et al. [26] suggest that because of the strong inverse correlation between CRF and fatness, low CRF could be, in part, the onset of some adverse consequences attributed to fatness. All those studies corroborate in some sense with our findings and highlight the important need of increasing the levels of CRF in children and adolescents in order to minimize the prevalence of CVD risk factors in this population.

Conclusion

The main finding of this study was that regardless fatness, participants with higher CRF levels presented lower prevalence of CVD risk factors.

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Study III

ASSOCIATION BETWEEN FITNESS, DIFFERENT INDICATORS OF FATNESS, AND CLUSTERED CARDIOVASCULAR DISEASES RISK FACTORS IN PORTUGUESE CHILDREN AND ADOLESCENTS

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ABSTRACT

Introduction: Although an inverse association between obesity and levels of CRF has been suggested, there is little evidence showing an interaction between CRF and fatness in relation to CVD risk factors. Abdominal fat and low

CRF may both increase the risk of clustered CVD risk. It may therefore be of value to describe the independent association of these traits in relation to clustering of CVD risk factors. Aim: (1) to investigate the relationship between CVD risk factors, CRF and three different indicators of fatness, and (2) investigate if these relationships are independent by each other. Methods: This study was carried-out at Porto, Portugal, with children and adolescents aged 10-16 years-old of both genders (491 children, 223 boys and 268 girls). Standardized metabolic risk scores (MRS) were computed for six CVD risk factors. Multiple linear regression and Univariate Analysis of Variance – GLM were used and level of significance was set up at $p \leq 0.05$ using SPSS 15.0. Results: Fitness was associated with clustering risk factors. Fit youngsters presented a better profile for each of risk factors analyzed isolated. Belonging to the unfit category increased the risk of having high MRS (β=.158; $p<0.05$) but when models were adjusted for each of the fatness indicators, the relationship between fitness and MRS disappeared, and obesity indicators presented significant relationship with the MRS (β=.033, .010, and .014 for body mass index, waist circumference and percentage of fat respectively). Conclusion: Both fitness and fatness are associated with clustered risk factors by different pathways.

INTRODUCTION

Clustering of CVD risk factors is known as the co-existence of several risk factors in the same subject (1). There is a multiplicative effect of the biological risk factors when they occur together for a particular subject (2), which may have higher clinical relevance. Furthermore, CVD risk factors clustering have been identified in children and adolescents (3).

CRF is a direct marker of physiological condition, reflecting the capacity of the cardiovascular and respiratory systems to provide oxygen during a continuous physical activity, carrying out prolonged exercises (4). Recent studies have shown that not only obesity (5) or physical activity (6) but also cardiorespiratory fitness (CRF) should be studied when analyzing the prevalence of CVD risk factors in youth population. Indeed, while some observational studies have shown that childhood obesity is associated with a higher metabolic risk profile

(7), results from other surveys such as the Quebec Family Study indicated that in different BMI categories, higher levels of CRF were associated with a better CVD risk profile (8). Further, in Danish children it was suggested a strong relationship between activity and metabolic risk in children with low cardiorespiratory fitness (9).

Although an inverse association between obesity and levels of CRF has been suggested (10), there is little evidence showing an interaction between CRF and fatness in relation to CVD risk factors (6). Moreover, there is some evidence that youngsters with a high CRF profile have a healthier cardiovascular profile not only during adolescence but also in later life (11), and this evidence seems to be independent of body weight (12). Therefore, based on this scarce data about the inter-relationship between CRF and adiposity, some studies are attempting to determine the contribution of each variable on CVD risk factors by controlling for the simultaneous effect of the other variable (13).

Abdominal fat and low CRF may both increase the risk of clustered CVD risk. It may therefore be of value to describe the independent association of these traits in relation to clustering of CVD risk factors.

Therefore, the aim of the present study was 1. to investigate the relationship between cardiovascular risk factors, CRF and three different indicators of fatness (BMI; %Fat and wais circumference), and 2. investigate if these relationships are independent by each other.

2. SUBJECTS AND METHODS:

Design and Sample

This study was carried-out as a part of an observational research project looking at the prevalence of CVD risk factors and levels of physical fitness in children and adolescents aged 10-16 years-old of both genders. The study was conducted at Porto district, Portugal. Children and adolescents were chosen at random from the 4th till the 12th school grade, according to general school system rules, which has previously been described (14).

From a total of 516 students that agreed to participate in the study, a total of 491 children did all measurements (223 boys, 45,4% and 268 girls, 54,6%). Children, parents and schools approved the study protocol.The nature, benefits, and risks of the study were explained to the volunteers, and parents written informed consent was obtained before the study, consistent with the Helsinki Declaration. The evaluation methods and procedures were approved by the Scientific Board of the Faculty of Sports of the University of Porto.

All measures were carried out by a specialized group (Physical Education teachers, medical doctor).

Daily Evaluation protocol

Subjects were identified through his/her code number and code of the school. Fasting blood samples were taken followed by blood pressure measurements. The children were then given breakfast followed by the determination of their maturational stage. Finally the shuttle-run test was performed. The variables were measured between 8:00 and 11:00am.

Blood sampling

Capillary blood samples of participants were taken from the right earlobe after at least 12 hours fasting in order to obtain values of plasmatic total cholesterol (TC), high density lipoprotein cholesterol (HDL) and fasting glucose (GLUC). The blood samples were drawn in capillary tubes (33 □l, Selzer) coated with lithium heparin and immediately assayed using Colestech LDX® Analyser.

The sample was applied into a Cholestech LDX® cassette and the analyser separates the plasma and the blood cells. Cassettes were stored in the refrigerator after reception. The Cholestech LDX® analyser has been proven to provide good agreement with laboratory measures for population-based screaming for cardiovascular risks factors (15).

Blood pressure

Blood pressure (BP) was measured using the Dinamap adult/pediatric and neonatal vital signs monitors, model BP8800. Measurements were taken by a trained technician and with all children sitting after at least 5min rest. Two measurements were taken after five and ten minutes rest. The mean of these two measurements was used for statistical analysis. If the two measurements differed by 2mmHg or more the protocol was repeated (two new measurements until the difference did not exceed 2 mmHg).

Anthropometric Measures and Body Fat

Body height was measured to the nearest mm in bare or stocking feet with the adolescent standing upright against a Holtain Stadiometer. Weight was measured to the nearest 0.1 kg, lightly dressed and after having breakfast, using an electronic weight scale (Seca 708 portable digital beam scale). Body mass index (BMI) was calculated from the ratio of body weight (kg) / body height (m2).

To evaluate the waist circumference (WC), the NHANES (16) protocol was used. A bony landmark is first located and marked. The subject stands and the examiner, positioned at the right of the subject, palpates the upper hip bone to locate the right iliac crest. Just above the uppermost lateral border of the right iliac crest, a horizontal mark is drawn, then crossed with a vertical mark on the midaxillary line. The measuring tape is placed in a horizontal plane around the abdomen at the level of this marked point on the right side of the trunk. The plane of the tape is parallel to the floor and the tape is snug, but does not compress the skin. The measurement is made at a normal minimal respiration

Body fat was determined by tricipital and subscapular skinfolds, according to Heyward (17). Each skinfold was measured twice and in a successive way, in the right side of the body. However if in these two measurements there was a difference above 5% a third measure was performed. The final result consisted of the mean of the two or three measurements for each skinfold. An Harpender caliper with a constant pressure of 10 g/mm2 was used and all measurements were completed by the same observer. The percentage of fat (%fat) was estimated from skinfolds measurements, according to Slaughter et al. (18) equations.

Maturational Stage

Regarding the maturational stage, the adolescents were inquired separately during physical examination. Each subject self-assessed his/her stages of secondary sex characteristics. Stage of breast development in females and pubic hair in males was evaluated according to the criteria of Tanner (19). Previous study showed a correlation of 0.73 between ratings on two occasions (three day interval) in a sub-sample of 50 selected subjects and concordance between self-assessments of sexual maturity status and physician assessment ranged from 63% for girls and 89% for boys (20).

Cardiorespiratory Fitness (CRF)

CRF was predicted by maximal multistage 20m shuttle-run test according to procedures described by Legér et al (21). The FITNESSGRAM test battery (22) which comprehends several physical fitness tests was selected because of its easy of administration to large numbers of subjects, and in addition its choice of reliable and valid health-related physical fitness measures (23). From the tests that compound the FITNESSGRAM, only The Shuttle Run Test, which predicts maximal aerobic capacity according to the number of completed laps, was obtained. Furthermore, the 20 meter Shuttle Run Test showed good correlation with directly measured VO2max (r=0.80) suggesting that it could be used as a measure of aerobic fitness in children (24). Nevertheless, VO2max expressed per unit body mass (ml.kg-1.min-1) has been criticized (25). Therefore, the CRF was expressed per number of completed laps achieved in the Shutlle Run Test. There are several studies that assessed cardiorespiratory fitness by the number of completed laps in Shuttle-Run Test (26).

Children were then categorized in fit or unfit according to adopted age-adjusted criterion referenced health standards (Health Fitness Zone) for individual CRF test item in the Fitnessgram test battery, as belonging to a healthy zone (fit) or under a healthy zone (unfit).

Statistical analysis

Descriptive statistics were used in order to characterize the sample. Given that fact, there is not a clinical criteria for the metabolic syndrome and in the literature different definitions differ in detail and inclusion criteria (27), none of its cutoff points apply specifically to children (28).

There are two reasons for not using cut off points when we constructed the composite score: 1) no consensus about the level of the cut off points, and 2) it reduces information to use cut offs instead of continuous scores.

Standardized metabolic risk scores (MRS) were computed for each risk factor. The following variables were included in the MRS: glucose, HDL-C, LDL-C, TC, blood pressure (systolic and diastolic), and triglycerides. Each of these variables was standardized as follows: standardized value = (value – mean)/ standard deviation. The HDL-C standardized values were multiplied by -1 to confer higher risk with increasing value for the purpose of calculating the MRS, which was obtained as the mean of the 6 standardized scores. This approach has been used before in youth population (29). Multiple linear regression analysis was used in order to investigate the relationships between fatness, fitness and the MRS. Four independent variables (WC, %FAT, BMI, and CRF) and two dependent variables (MRS and CRF) were performed in separate models.

The Univariate Analysis of Variance – GLM was used to determine if different levels of fitness (Unfit and Fit) were related with clustered metabolic disorders, independent of fatness indicators (Zscore of BMI, WC and % fat). Four different models were analyzed. In the first model, the influence of CRF in the MRS, without obesity indicators was analyzed. The three subsequent models indicate the influence of both CRF and obesity indicators in the MRS. For each model, two analyses were done. In the first one, a crude metabolic risk score was constructed and the analysis was adjusted for sex and maturational status. As there was no interaction between the terms, age and sex specific standardized metabolic risk scores (MRS) were computed and all subjects analyzed together. Analysis was performed with the statistical software package SPSS 15.0 for Windows and level of significance was set up at $p \leq 0.05$.

3. RESULTS

Table 1 shows descriptive statistics (mean±SD) of all variables, separately for fit and unfit subjects. It was observed that in general, unfit subjects tend to have higher mean values of metabolic risk factors, especially the lipid profile. Total cholesterol, HDL cholesterol and triglycerides levels different between groups. Unfit children presented higher values for glucose and LDL cholesterol as well, though this tendency was not significant.

Regarding the adiposity indicators, fit subjects presented significant lower values for BMI, waist circumference and percentage of fat, when compared to unfit.

Table 1: Descriptive statistics. Differences between fit and unfit groups

	Fit		Unfit			
Variables	N	Mean (SD)	N	Mean (SD)	t	p
Age	206	13.48 (.13)	208	14.42 (.14)	4.824	NS
Weight	206	54.09 (12.0)	208	55.00 (10.7)	.802	NS
Height	206	161.33 (11.5)	208	159.36 (8.1)	-2.009	.045
BMI	206	20.55 (2.8)	208	21.53 (3.3)	3.294	.001
Completed laps	189	51.51 (21.3)	208	26.17 (9.9)	-14.923	.000
TC	206	145.13 (25.7)	208	150.61 (25.8)	2.164	.031
HDL	206	46.00 (11.1)	208	43.69 (11.0)	2.110	.035
LDl	206	90.85 (23.8)	208	92.54 (24.2)	.714	NS
Trigl	206	52.94 (15.0)	208	60.40 (21.1)	4.143	.000
Glucose	206	84.17 (7.3)	208	85.41 (7.1)	-1.753	NS
BP	206	141.25 (17.2)	208	142.74 (15.7)	.922	NS
WC	206	73.29 (8.0)	208	75.84 (8.8)	3.073	.002
% fat	205	19.90 (6.3)	207	26.52 (8.2)	9.228	.000
MRS	206	-.035 (.46)	208	.029 (.48)	1.390	NS

Metabolic Risk Score (TC, HDL, LDL, Triglycerides, Glucose, BP)

Relationships between the MRS, the three indicators of fatness and fitness, and the relationship between fitness and fatness indicators are shown in table 2. All the variables were expressed as z-score values. In this analysis, significant relationships between the MRS, fat indicators and fitness were observed. The strongest association was observed when MRS and fitness were related to percentage of fat (.172 and for -.372 respectively).

Table 2: Relationships between the MRS, fitness and fatness

Variables	MRS			Fitness		
	F	Sig	CI (95%)	F	Sig	CI (95%)
WC	.142	.004	(-1.344; -. 429)	-.195	.000	(-12.793; 18.689)
%FAT	.172	.000	(-.909; -.237)	-.372	.000	(-18.206; 3.535)
BMI	.163	.000	(-1.195; -. 404)	-.162	.001	(-19.938; 7.518)
Fitness	-.130	.005	(-1.456; - .379)	-	-	-

Multiple linear regressions adjusted for sex and maturational status
* p≤0.05

In table 3, four different models of predicting metabolic disorders were analyzed by an univariate linear regression model. The main outcome was the MRS and the independent variables were fitness, BMI, WC and % fat. The first model shows fitness as predictor of MRS and indicates that the differences between

the fit and the unfit group was B=.158. The other three models represent the same idea, with further adjustment for BMI, WC and %fat, respectively.
The results indicate that after adjustment for fat, the relationship between fitness and MRS disappears for all the three adiposity indicators.

Table 3: Fitness as predictor of clustered risk factors. All models adjusted for sex and maturational status, with further adjustments for obesity indicators

Models		B	p	CI (95%)
Model 1	Unfit	.158	.025	(,020 ; ,296)
Model 2	Unfit	.132	.058	(-,004 ; ,268)
	BMI	.033	.000	(,017 ; ,040)
Model 3	Unfit	.123	.079	(-,014 ; ,261)
	WC	.010	.001	(,004 ; ,016)
Model 4	Unfit	.094	.189	(-,047 ; ,235)
	% Fat	.014	.000	(,007 ; ,020)

Dependent Variable: Metabolic risk score; Fit health zone is the reference category
Model 1 = fitness; Model 2 = fitness and BMI; Model 3 = fitness and WC; Model 4 = fitness and %fat
* p≤0.05

Discussion:

This study analyzes whether the association between fitness and CVD is independent of fatness.
Since the onset of chronic disease risk factors lies in early childhood, it is of great importance to examine the potential risk in order to make effective preventive strategies targeting those at risk as early as possible. It is important to point out that interventions targeting fitness may change fitness rapidly while interventions targeting fatness may take longer time before major effects are seen.
The main outcome of this study was that both fitness and fatness were associated with clustering risk factors in children and adolescents.
Although our data showed that our fit youngsters presented a better data with regard each of CVD risk factors analyzed isolated (table 1). The results of the regression analysis (table 3) showed that belonging to the unfit category increased the risk of having high MRS (B=.158; $p<0.05$). However, when models were adjusted for each of the indicators of adiposity (BMI, %FAT; WC), the relationship between being fit and MRS disappeared. All the three fat

indicators were associated with MRS, after adjustments for gender, age and maturation. Thus, our findings agree with similar studies showing that higher levels of CRF are inversely associated with healthier CVD profile in children and adolescents when CVD risk factors were computed as a clustered metabolic risk score (6, 30).

We observed a statistically significant association between CRF and MRS. Though, after adjustment for adiposity, this association disappeared. This observation could let to the interpretation of fitness not being important to this relationship between fitness, fatness and MRS. However, this may not be true. The interpretation depends on how the causal chain is. If fatness is an intermediate link between low fitness and CVD risk then the fitness would disappear after adjustment for fatness, but low fitness would still be the cause.

Overweight and obesity are associated with an increased risk of CVD risk factors early in life. Our data seems to confirm that adiposity, regardless the indicator used, is a strong predictor of MRS. Reinforcing this idea, Eisenmann et al. (5) presented some evidences for considering not only obesity, but both adiposity indicator and CRF when interpreting CVD risk factors in the young population. Some data pointed out that moderate to higher levels of CRF have been associated with lower abdominal adiposity, suggesting that a mechanism might exist by which CRF attenuates the health risk of obesity (31). The association between fitness and MRS changed when adjusted for fatness could support the hypothesis that MRS is not entirely mediated by fitness or fatness, but only part of this association is mediated by a singular factor.

In addition, obesity has been shown to be strongly associated with insulin resistance (32) and other CVD risk factors. When evaluating the relation between CRF and insulin sensitivity in U.S youth, Imperatore et al. (33) observed that in boys, higher CRF was associated with high insulin sensitivity, independent of BMI. In girls this association disappeared after controlling for BMI. Given that insulin resistance could be a predictor of obesity and cardiovascular risk factors (34), the findings of these studies could explain some of our results and suggest that maybe the insulin resistance could be the mediator link between CRF, obesity and clustered risk factors. If we assume that fatness is the main mediator of the relationship between clustered risk factors and CRF (35) we do not take in consideration the fact that insulin resistance could be the link behind this relation. Further, insulin sensitivity is mainly related to the muscle tissue, because a great portion of the carbohydrate is stored or burned in the muscle (36). One-leg training models have shown that insulin sensitivity is very local. Glucose uptake can be doubled in the trained leg compared to the untrained leg, and this is independent of fatness (the legs share the rest of the body) (37).

All those studies corroborate in some sense with our findings and highlight the important need of increasing the levels of CRF in children and adolescents in order to minimize the prevalence of CVD risk factors in this population. Nevertheless, some limitations should be pointed-out. Firstly, the small sample size might explain some of our lack of association. Secondly, CRF was assessed indirectly. Indeed, there are many concerns regarding the use of running tests as an indicator of CRF in young children. Performance in the growing years can be compromised in many children due to their relative immaturity from a biomechanical and energy efficiency perspective (38), as well as their motivation, especially in girls (39). However, the easy administration of shuttle-run test and its common use in large scale studies makes it a valuable tool for studying CRF in youth. Furthermore, this study could benefit from additional collected data, such as combined behavioural variables and social background characteristics, which could enhance the outcomes.

Conclusion

In this study it was observed significant relationships between clustered CVD risk factors, fatness indicators, especially percentage of fat, and fitness. After adjustment for fatness, the relationship between fitness and clustered risk factors disappears for all the three adiposity indicators. In conclusion, both fitness and fatness are associated with clustered risk factors by different pathways.

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Study IV

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Short Report

Cardiorespiratory Fitness Predicts Later Body Mass Index, but Not Other Cardiovascular Risk Factors from Childhood to Adolescence

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ABSTRACT We analyzed the 5-year longitudinal relationship between cardiorespiratory fitness (CRF) and cardiovascular diseases (CVD) risk factors in children. A total of 153 students (66 boys and 87 girls) were evaluated in 1998 and 2003. Multilevel modeling was used to determine the effect of CRF across time (Model 1, adjusted for time and Model 2—Model 1 with further adjustment for gender and age). In both models, a significant main effect was found for body mass index (BMI) ($P \leq 0.05$). Data showed that in children, lower levels of CRF are associated with higher levels of BMI over a 5-year follow-up period. Am. J. Hum. Biol. 21:121–123, 2009.

In the past few decades maximal and submaximal aerobic capacity have been measured in order to evaluate athletic performance. Recently, cardiorespiratory fitness (CRF) has become an important measuring variable in clinical health settings. It has been suggested that numerous risk factors for cardiovascular diseases (CVD) could be influenced by CRF (Laaksonen et al., 2002). Results from youth crosssectional and longitudinal studies pointed-out CRF as a key CVD health marker in youth (Lobelo and Ruiz, 2007). A recent study about the importance of CRF and physical activity (PA) in the development of metabolic disorders and CVD risk factors showed that CRF is independently associated with individual and clustered metabolic risk factors in children (Ekelund et al., 2007). There are also some evidences that CRF levels tracked from childhood and adolescence into adulthood (Hassestrom et al., 2002).

Longitudinal studies from childhood to adolescence have the potential of defining maturational changes in CVD risk factors and may provide insight into the prediction of future CVD (Janz et al., 2002). Despite that, the longitudinal data analyses of such relationships in youth are controversial and limited evidences exist. Therefore, the aim of this study was to analyze the 5-year longitudinal relationship between CRF and some CVD risk factors [blood pressure (BP), cholesterol and body mass index (BMI)] in children and adolescents of both genders.

METHODS

Design and sample

This study was based on a longitudinal research project aiming to analyze the prevalence of CVD risk factors and levels of CRF in children aged 8–9 years of both genders, in Porto, Portugal, and described elsewhere (Martins et al., 2008). For this study, data from a 5 year follow-up (1998–2003) was taken. A total of 153 children have fully completed all the protocol in the period of September–November 1998 (time 0) and 2003 (time 1). Parents and schools approved the study protocol, and students were healthy and free of medical treatment.

Blood sampling

Capillary blood samples of participants were taken from the earlobe after at least 12 h of fasting in order to obtain values of plasmatic total cholesterol (TC). The blood samples were drawn in capillary tubes (33 μl, Selzer) coated with lithium heparin and immediately assayed using Reflotron Analyzer (Boehringer Mannheim, Indianapolis, IN). The mean of two measurements was used for statistical analysis.

Blood pressure

BP was measured using the Dinamap adult/pediatric and neonatal vital signs monitors, model BP8800. Measurements were taken by a trained technician and with all children sitting after at least 5 min rest. Two measurements were taken after 5 and 10 min rest. The mean of the two measurements was used for statistical analyses. If the two measurements differed by 2 mm Hg or more the protocol was repeated (two new measurements).

Anthropometric measures

Body height was measured to the nearest mm in bare or stocking feet with the subject standing upright against a Holtain Stadiometer. Weight was measured to the nearest 0.10 kg, lightly dressed and after having breakfast, using an electronic weight scale (Seca 708 portable digital beam scale). BMI was calculated from the ratio of body weight (kg)/body height (m^2).

Maturational stage

Participants' self-assessed their maturational stages, according to Tanner's criteria (Tanner, 1962). A previous study showed a correlation of 0.73 between ratings on two occasions (3 day intervals) in a subsample of 50 selected subjects and a concordance between self-assessments of sexual maturity status and physician assessment ranged

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TABLE 1. Mean and standard deviation for the different variables measured in 1998 and 2003

	Boys (N – 66)		Girls (N – 87)	
Variables	1998	2003	1998	2003
Age (years)	9.12 (0.87)	14.56 (1.04)*	9.09 (0.90)	14.29 (0.93)*
Weight (kg)	34.61 (9.25)	59.21 (14.07)*	33.90 (8.10)	57.16 (11.12)*
Height (cm)	134.43 (7.33)	164.83 (8.50)*	133.90 (7.51)	160.43 (6.47)*
BMI	18.89 (3.57)	21.67 (4.00)*	19.69 (3.11)	22.18 (4.00)*
TC (mg/dl)	161.80 (30.00)	143.16 (23.09)*	170.41 (26.57)	160.30 (29.08)*
SBP (mm Hg)	114.93 (10.39)	120.37 (12.44)*	117.15 (9.03)	119.73 (10.19)*
DBP (mm Hg)	57.82 (10.31)	61.79 (9.60)*	59.58 (9.53)	63.87 (8.14)*
VO_2max (ml/kg/min)	49.63 (4.43)	46.09 (5.90)*	48.10 (2.76)	39.62 (5.22)*

*$P \leq 0.05$; paired sample t-test

TABLE 2. Unstandardized regression coefficients (b) and 95% confidence intervals (95% CI) for each CVD risk factor

		BMI[b] (95% CI)	TC[b] (95% CI)	SBP[b] (95% CI)	DBP[b] (95% CI)
VO_2 max	Model 1[a]	−0.14 (−0.20; −0.09)*	−0.46 (−1.02;0.95)	−0.04 (−0.18;0.25)	−0.13 (−0.33;0.06)
	Model 2[b]	−0.15 (−0.21; −0.09)*	−0.18 (−0.77;0.41)	0.04 (−0.18;0.27)	−0.02 (−0.22;0.19)

BMI, body mass index; TC, total cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.
[a]Model 1, adjusted for time.
[b]Model 2, further adjusted for gender and age.
*$P \leq 0.05$.

from 63% for girls and 89% for boys (Mota et al., 2002). At the baseline, all children were in Stages 1 and 2 (prepuberty) of maturation, and at time 1, all adolescents were classified in Stages 4 and 5 (postpuberty), according to Tanner's criteria.

Cardio respiratory fitness

CRF was predicted by maximal multistage 20 m shuttle-run test according Fitnessgram test battery (FITNESSGRAM, 1994). The Shuttle Run Test predicted maximal aerobic capacity and after converting scores, a predicted maximal oxygen uptake (VO_2max) was obtained. The Shuttle Run Test showed a good correlation with directly measured VO_2max (r = 0.80) suggesting that it could be used as a measure of aerobic fitness in children (Vincent et al., 1999). Analysis was conducted including the adopted age-adjusted criterion referenced health standards (Fitness Zone) for individual test items in the Fitnessgram.

Statistical analysis

Descriptive statistics were used in order to characterize the sample, and paired t-tests were used to analyze the change over time in each of the characteristics. Multilevel analysis was used to determine the effect of CRF (VO_2max) on the CVD risk factors over time. For the multilevel analysis, a two level structure seemed to be appropriate (i.e. observations clustered within individuals). For each of the CVD risk factors considered (BMI, TC, SBP, and DBP) two models were analyzed. The first model was only adjusted for time, whereas the second model was further adjusted for gender and age. If a significant relationship was found, a third step that consisted in analyzing the interaction with genders was investigated. Multilevel analyses were performed with MLwiN (version 1.1), whereas the other analyses were performed with SPSS (version 14). The significance level was 5%.

RESULTS

Table 1 shows descriptive statistics (mean ± SD) for all variables according to gender in 1998 and 2003, respectively. Statistically significant changes over time were found for all the variables.

Table 2 shows the longitudinal relationship between predicted VO_2max and CVD risk factors. In both "crude" and adjusted analyses only VO_2max and BMI showed a statistical significant association, indicating that lower CRF is associated with higher BMI.

With regard, the BMI and the interaction between VO_2max and gender, no statistical significant association was found meaning that the relationship did not differ between genders.

DISCUSSION

The main finding of this study was that regardless of gender there is a negative relationship between CRF and BMI over time. This finding highlights the fact that lower levels of CRF in childhood could influence higher levels of BMI over time. Our data are consistent with some data in the literature, which hypothesizes that low levels of CRF consist in a risk factor for obesity (Lee and Arslanian, 2007).

In our study, we did not find any other statistically significant association of CRF and CVD risk factors. Although a study showed a strong decline in CRF levels over time (Martins et al., 2008) and longitudinal studies covering the time period between childhood and adulthood showed that fitness is an important determinant of CVD risk factors (Ferreira et al., 2005), this relationship continues to be conflicted when referring to pediatric ages (Eisenman et al., 2004). In accordance with our findings, a study evaluating 163 overweight Latino students observed that VO_2max was not correlated with any individual CVD risk factor that would compound the Metabolic Syndrome, after adjusting for age, gender, and body composition (Shaibi et al., 2005). On the other hand, it was shown that high CRF levels during childhood and adolescence were associated with a healthier cardiovascular profile (Mesa et al., 2006).

Nevertheless, it should be considered that there is a possible relationship between obesity (BMI) and the other CVD risk factors. So, the direct effect of low CRF on higher levels of BMI may be indirectly affecting other CVD risk factors over a longer period of time.

Strength of this study is the 5 year follow-up, as difficulties in conducting longitudinal studies are well recognized. Nevertheless, some limitations should be pointed out. First, the small sample size might explain why only one significant relationship was found. Second, CRF was assessed indirectly. Indeed, there are many concerns regarding the use of running tests as an indicator of CRF in young children. Performance in the growing years can be compromised in many children due to their relative immaturity from a biomechanical and energy efficiency perspective (Ebbeling et al., 1992), as well as their motivation, especially in girls. However, the easy administration of shuttle-run test and its common use in large-scale studies makes it a valuable tool for studying CRF in youth. Furthermore, this study could benefit from additional collected data, such as combined behavioral variables and social background characteristics, which could enhance the outcomes.

CONCLUSION

This study showed that lower levels of CRF are associated with higher levels of BMI over time. Therefore, even at young ages the beneficial impact of increasing levels of CRF would be of great clinical and public health relevance.

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5. Main Results and Limitations

The trends in CVD risk factors and aerobic performance in a sample of Portuguese adolescents between 1998 and 2003 were analyzed. The main finding of this study was that both boys and girls showed lower CRF values in the second evaluation and pointed-out that CRF levels in the studied population have been decreasing over time. The data showed that regardless of gender, the percentage of adolescents at risk for CRF (under health zone) was significantly higher ($p \leq 0.05$) in 2003 than in 1998.

These findings are comparable with those of recent studies that highlighted the same decreasing tendency in CRF along the time for both boys and girls (Tomkinson et al., 2007; Huotari et al., 2009). Our results suggest that levels of CRF in our sample from Porto presents the same decreasing tendency observed in several populations. However, it is somewhat difficult to compare studies, considering the variety of methods used and the social characteristics of the different populations.

Our findings also showed that there were no statistical differences in the two cohorts for TC, SBP, DBP and BMI mean values for boys and girls, what might suggest some stability in those variables over time. Moreover, it should be noted that despite no statistical significance was found between the percentage of overweight boys and girls in the two cohorts, the BMI values were higher in the second one.

When evaluating the stability of the indicators of the MS from childhood and adolescence into young adulthood, Katzmarzyk and others (2001) observed a moderate stability in such indicators. Our results seem consistent with this previous finding, although the time point among the two evaluations was more reduced in our study. In addition, a secular trend study with Flemish subjects revealed decreased values for CRF, added to increasing values for weight, BMI and skinfolds (Matton et al., 2007), similarly to our study.

Considering that CRF levels are decreasing and the prevalence of overweight youngsters has been increasing within those five years, we investigated differences between fit-fat groups. It was analyzed if different categories of CRF with regard to obesity status have a different relationship with CVD risk factors in children and adolescents.

As expected, it was observed that BMI was significantly lower and CRF levels were significantly higher ($p \leq 0.05$) in fit children. Concerning the two fit groups, there were statistically significant differences in WC, triglycerides, sum of skinfolds and LDL cholesterol. Regardless of obesity, the fit participants showed a better lipid profile (LDL cholesterol) and WC than their unfit counterparts. Those that were unfit and obese showed significantly worse lipid profile (TC; HDL; LDL) and adiposity indicators (WC and ∑ skinfolds) than their unfit but normal-weight peers. We observed some consensus with data in the literature, which hypothesizes that higher levels of CRF are associated with a better lipid profile even in obese children (Hurting-WennlÖf et al., 2007; Rizzo et al., 2007).

In general, CVD risk factors varied across fit-fat groups, and CRF appeared to attenuate the risk within BMI categories.

Bearing in mind that fit children have a better lipid profile, even being obese, we investigated the relationship between clustered cardiovascular risk factors, CRF and three different indicators of fatness, and analyzed the kind of relationship existent between each of the variables.

Significant relationships between the MRS, fat indicators and fitness were observed. The strongest association was observed when MRS and fitness were related to %Fat (.172 and -.372 respectively). In a cross-sectional study with adult sample aimed to analyse the relationships between obesity markers and 10-year risk of fatal CVD, %FAT was more related with 10-year risk of fatal CVD than obesity markers based on waist/hip ratio, WC or BMI (Marques-Vidal et al., 2009). Our study highlighted that %FAT was better related to MRS than other adiposity indicators even in children and adolescents.

When analysing fitness as a predictor of MRS it was observed that belonging to the unfit category increased the risk of having high MRS (B=.158; $p<0.05$). However, when models were adjusted to each of the indicators of adiposity (BMI, %FAT; WC), the relationship between being fit and MRS was not significant.

The main outcome of this study was that both fitness and fatness were associated with clustering risk factors in children and adolescents. However, the results suggested that adiposity, regardless of the indicator used, has a pivotal role in the relationship between CRF and MRS.

The degree to which an adjustment for adiposity attenuates or modifies the association between CRF and metabolic risk varies across studies. In accordance with our results, data from the European Youth Heart Study (Ekelund et al.,2007; Rizzo et al.,2007) reported that the association between CRF and clustered risk factors is partly mediated or confounded by adiposity. However, the degree of the relationship between CRF and metabolic risk is dependent on the methodology used and how the exposures and the outcomes are measured and expressed (Steele et al., 2008). Therefore, according to

Andersen et al. (2005), two risk factors could be independently associated or could present different steps along the same causal pathway.

Regarding that CRF levels decreased in five years, that fit youngsters have a better lipid profile, even being obese, that both fitness and fatness were associated with clustering risk factors in children and adolescents, and that CRF, when associated to obesity, potentiates the prevalence of MRS in a cross-sectional analysis, we decided to investigate if CRF could predict CVD risk factors in a longitudinal point of view.

Our results highlighted that the longitudinal relationship between CRF and each of the biological risk factors studied was statistically significant only for BMI. These are consistent results with some data in the literature, which hypothesizes that low levels of CRF are a risk factor for obesity (Lee & Arslanian, 2007; Psarra et al., 2005).

Although a recent study suggests that CRF may be an important determinant of changes in adiposity in overweight Hispanic boys but not in girls (Byrd-Williams et al., 2008), our data showed that regardless of gender there is a negative relationship between CRF and BMI. Therefore, our study stresses the fact that low levels of CRF could, over time, influence higher levels of BMI since childhood.

However, the presented results should be interpreted with caution. Firstly, the study is composed of a small sample of subjects, which may confound the results not making it possible to extrapolate the results to other populations.

Secondly, the cross-sectional design of the first three papers does not allow assigning causality, and prevents causal inferences from being drawn.

Third, the indirect CRF measure is a potential weakness of this study. The fact of using the estimated VO_2max in Shuttle Run Test as an indicator of CRF in two papers could lead to the error caused by the use of an estimating equation. Additionally, VO_2max expressed per unit body mass ($ml.kg^{-1}.min^{-1}$), has been criticized (Armstrong & Welsman, 1997). Nevertheless, the number of completed laps used in the other two studies with children and adolescents population who were submitted to changes in weight resulting from the growth process, may accentuate the error caused by the abovementioned points.

Fourth, in the protocol study we do not assess dietary habits or social economic status, which may confound the observed results.

Furthermore, this study could benefit from additional collected data, such as combined behavioural variables and social background characteristics, which could enhance the outcomes.

Despite the cited limitations, the results of the study suggest that promoting CRF plays an important role in the development of CVD risk factors in children and adolescents.

6. Conclusions

Based on the findings of the four different studies that compound the thesis, it seems reasonable to emphasize the following conclusions:

* the trend study revealed a higher percentage of obese adolescents and a higher risk factors clustering between the two time points; a significant marked low CRF level over time in adolescents of both genders;

* regardless fatness, participants with higher CRF levels presented lower prevalence of CVD risk factors;

* there is a significant relationships between clustered CVD risk factors, fatness indicators, especially percentage of fat, and fitness; both fitness and fatness are associated with clustered risk factors by different pathways;

* low levels of CRF are associated with higher levels of BMI over time;

* even at young ages, the beneficial impact of increasing levels of CRF would be of great clinical relevance.

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