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Cardiovascular risk factors: associations with physical activity, health-related physical fitness, and diet in Azorean adolescents

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"Em qualquer aventura, o que importa é partir, não é chegar"

Miguel Torga, in *Diário XII*

Para o Gui

Para a minha família

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Resumo

Estudos anteriores sugerem que indivíduos com síndrome metabólica (SM) têm risco aumentado de desenvolverem doenças cardiovasculares (DCV) e diabetes. Atividade física (AF), aptidão física (ApF), nomeadamente a aptidão cardiorrespiratória (ACR) e a dieta são variáveis do estilo de vida que estão fortemente associadas com a SM. Numa perspectiva de saúde pública, é importante entender como as referidas variáveis se relacionam com a SM ou agregação de fatores de risco de DCV em jovens, a fim de se implementar estratégias de prevenção de doenças metabólica e cardiovascular.

Os objetivos da presente tese foram: i) estimar a prevalência da SM e dos seus componentes, bem como, analisar a relação entre a SM e os níveis de ApF numa amostra de adolescentes açorianos (estudo I); ii) avaliar o desempenho de diferentes medidas de adiposidade: índice de massa corporal (IMC), perímetro da cintura (PC) e rácio cintura-altura (RCA) na discriminação do risco metabólico, numa amostra de adolescentes (estudo II); iii) analisar as relações entre fatores risco metabólico e AF e ApF numa uma amostra de adolescentes açorianos (estudo III); e iv) avaliar o impacto das associações combinadas da ACR e da adesão à dieta Atlântica na agregação de fatores de risco metabólico em adolescentes (estudo IV).

Para atingir esses objetivos foram realizados quatro estudos experimentais envolvendo 517 adolescentes Açorianos (estudos I e II), 417 (estudo III) e 468 (estudo IV). Foram recolhidas informações sobre: variáveis antropométricas, análises sanguíneas, pressão arterial, dieta, maturação sexual, estatuto socioeconómico, níveis de AF (pedómetros) e ApF.

As principais conclusões do nosso trabalho mostraram que: i) a prevalência da SM nos adolescente Açorianos foi de 5%; entre os componentes da SM, a obesidade abdominal foi a mais prevalente e o nível aumentado de triglicerídeos foi o componente menos observado; os adolescentes com maiores ACR apresentam menor prevalência da SM e elevada ApF foi preditor positivo da SM; ii) com base nos resultados das curvas ROC, o IMC é a medida de adiposidade com maior poder discriminativo do risco metabólico em comparação com o PC e o RCA, em ambos os sexos; iii) os adolescentes mais ativos (≥ 9423passos/dia) e aqueles que alcançaram a zona saudável nos cinco testes de ApF apresentaram menor probabilidade ter um ou mais fatores de risco metabólico, além disso, verificou-se uma influência positiva dos níveis gerais de ApF sobre cada fator de risco metabólico; iv) os adolescentes com baixa adesão à dieta Atlântica apresentaram maior probabilidade de ter um elevado risco metabólico em comparação com os que apresentaram maior ACR e elevada adesão à dieta, porém, entre os adolescentes com menor ACR, ter alta adesão a um padrão alimentar saudável, confere algum grau de proteção contra um elevado risco metabólico.

Os resultados obtidos, enfatizam a importância de níveis elevados de AF e ACR bem como, a adesão a uma dieta saudável como uma parte integrante de um estilo de vida saudável. Neste âmbito, estratégias futuras para promover um estilo de vida saudável nos adolescentes açorianos devem basear-se numa abordagem multi-fatorial nomeadamente ao nível da AF, ACR e dieta uma vez que estas variáveis parecem interferir nos fatores de risco para DCV, nesta população.

Palavras-chave: FACTORES DE RISCO CARDIOVASCULAR, ACTIVIDADE FÍSICA, PEDÓMETROS, APTIDÃO CARDIORESPIRATÓRIA, DIETA, ADOLESCENTES, AÇORES.

Abstract

Previous research has shown that individuals with metabolic syndrome (MetS) are at an increased risk of cardiovascular diseases (CVD) and diabetes. Physical activity (PA), physical fitness (PF), and diet are lifestyle variables that are strongly associated with MetS. From a public health perspective, it is important to understand the lifestyle correlates of MetS and the clustering of CVD risk factors in youth in order to implement strategies to prevent cardiovascular and metabolic diseases.

The aims of this thesis were: i) to estimate the prevalence of MetS and its components and to analyze the relationship between MetS and overall PF levels in a sample of Azorean adolescents (study I); ii) to evaluate the screening performance of different measures of adiposity: body mass index (BMI), waist circumference (WC), and waist-to-height ratio (WHtR) for high metabolic risk in a sample of adolescents (study II); iio) to analyze the relationships between metabolic risk factors and PA and PF in a sample of Azorean adolescents (study III) and iv) to assess the impact of the combined associations of cardiorespiratory fitness (CRF) and adherence to the Southern European Atlantic Diet (SEADiet) on the clustering of metabolic risk factors in adolescents (study IV).

To accomplish these goals, four experimental studies were conducted involving groups of 517 (study I and II), 417 (study III), and 468 Azorean adolescents (study IV). Anthropometric measurements, blood sampling, blood pressure, dietary intake, pubertal stage, socio-economic status, PA (measured using pedometers), and health-related PF were collected.

The main findings of our experimental work show that i) the prevalence of MetS among Azorean adolescents was 5%; among the components of MetS, abdominal obesity was the most prevalent and hypertriglyceridemia the least observed; fit adolescents had a lower prevalence of MetS than unfit adolescents, and low levels of overall fitness were a positive predictor of MetS; ii) based upon receiver-operating characteristic curve analysis, BMI provides a marginally superior tool for discriminating high metabolic risk score in both sexes, compared with WC and WHtR; iii) adolescents who are more active (≥9423 steps/day) and those who achieve the healthy zone in five fitness tests had lower odds of having one or more metabolic risk factors; additionally, a positive influence of overall PF levels on each metabolic risk factor was observed; iv) adolescents with low adherence to the SEADiet had the highest odds of expressing high metabolic risk scores compared to those who were fit and demonstrated high adherence to the SEADiet; among unfit adolescents, high adherence to a healthy dietary pattern conferred some degree of protection against high metabolic risk score.

The findings presented in this thesis re-emphasize the importance of high PA and health-related PF levels, as well as adherence to a healthy diet as an integral part of a health-enhancing lifestyle. As a result, future strategies to promote a healthy lifestyle among Azorean adolescents should consider a multi-factorial approach to PA, PF, namely CRF levels, and a healthy diet, as these factors seem to affect CVD risk factors and their clustering in this population.

Key-words: CARDIOVASCULAR RISK FACTORS, PHYSICAL ACTIVITY, PEDOMETERS, CARDIORESPIRATORY FITNESS, DIET, ADOLESCENTS, AZORES.

List of Abbreviations

20mSRT	20-m shuttle run test
BMI	Body Mass Index
BP	Blood Pressure
CRF	Cardiorespiratory fitness
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
EYHS	European Youth Heart Study
HDL-c	High-density lipoprotein cholesterol
HOMA	Homeostasis model assessment of insulin resistance
IDF	International Diabetes Federation
IOTF	International Obesity Task Force
LDL-c	Low-density lipoprotein cholesterol
MetS	Metabolic syndrome
NCEP-ATP III	Third Report of the National Cholesterol Education Program's Adult Treatment Panel
NHANES III	Third National Health and Nutrition Examination Survey
PA	Physical activity
PF	Physical fitness
ROC	Receiver operating characteristic curve analysis
SBP	Systolic blood pressure

SEADiet	Southern European Atlantic Diet
TC	Total cholesterol
TG	Triglycerides
VO_{2max}	Maximum oxygen consumption
WC	Waist circumference
WHO	World Health Organization
WHtR	Waist-to-height ratio

List of Publications

I. Moreira, C., Santos, R., Vale, S., Soares-Miranda, L., Marques, A. I., Santos, P. C., Mota. J. (2010). Metabolic Syndrome and Physical Fitness in a sample of Azorean Adolescents. *Metab Syndr Relat Disord, 8*(5), 443-449.

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General Introduction

General Introduction

At the population level, the regular screening of physical activity (PA) and health-related physical fitness (PF) (including body composition) levels should be considered a public health priority, as the lack of PA and/or PF are implicated in the etiology and prevalence of several non-communicable diseases affecting the general health population worldwide such as cardiovascular disease (CVD), diabetes, and cancer, as well as associated risk factors for these diseases such as high blood pressure (BP), raised blood glucose, overweight/obesity, and hyperlipidemia (World Health Organization, 2010) .

Non-communicable diseases are the leading cause of all deaths in the world, and about 30% of these deaths occur before the age of 60 (World Health Organization, 2011). Due to rising rates of CVD, diabetes, and cancer, deaths from these chronic diseases are expected to increase by 17% over the period 2006–2015, accounting for nearly 70% of global deaths by 2030 (Mathers & Loncar, 2006). This burden of premature mortality and disability poses a threat to human, social, and economic development (Alwan et al., 2011). Non-communicable diseases are largely caused by four behavioral risk factors that are pervasive aspects of economic transition, rapid urbanization, and twenty-first century lifestyles: tobacco use, unhealthy diet, insufficient PA, and alcohol abuse (World Health Organization, 2011). These behaviors lead to metabolic/physiological changes that significantly impact public health policies (Beaglehole et al., 2007).

Metabolic syndrome (MetS) comprises a major risk for non-communicable diseases including atherogenic dyslipidemia, abdominal obesity, hypertension, insulin resistance, and prothrombotic and proinflammatory states, leading to increased risk of CVD and type 2 diabetes (Grundy et al., 2004; NCEP-III, 2001), and, therefore, representing a major health risk (Hanson RL, 2002; Isomaa, 2003). The most recognized indicators of CVD risk factors are high-density lipoprotein cholesterol (HDL-c), total cholesterol (TC), triglycerides (TG), total and central body fat, insulin resistance, and BP. These risk factors for

CVDs, traditionally considered to be adult diseases, have started to appear in children and adolescents (Steinberger et al., 2009).

Recent decades have seen a large increase in the number of individuals exhibiting the co-occurrence of adverse cardiovascular and metabolic risk factors. The clustering of CVD risk factors also occurs among youths, especially among those with obesity (Calkins et al., 2004), and low PA and cardiorespiratory fitness (CRF) levels (Andersen et al., 2006; Ekelund et al., 2007; Lobelo et al., 2010; Rizzo et al., 2007; Ruiz et al., 2007a). Furthermore, the clustering of multiple cardiovascular risk factors appears to track from childhood to young adulthood (Bao et al., 1994; Chen et al., 2005; Kelly et al., 2011; Morrison et al., 2008). Hence, considerable attention should be paid to the prevention of chronic disease beginning at an early age, because these diseases and their associated co-morbidities regularly persist from childhood into adolescence, and from adolescence into adulthood (Bao et al., 1994; Chen et al., 2005; Eisenmann et al., 2004; Katzmarzyk et al., 2001; Raitakari et al., 2003; Raitakari et al., 1994).

A consistent body of evidence shows that several anthropometric measures such as body mass index (BMI), waist circumference (WC), and waist-to-height ratio (WHtR) are associated with various obesity-related diseases, including CVD, diabetes, and MetS (Freedman et al., 2007; Kahn et al., 2005a; Lee et al., 2008; McCarthy, 2006).

Puberty, a period of growth and maturation that occurs during adolescence, is initiated by sex steroid hormone signaling, affecting not only physical sexual maturity, but also the reorganization of neural networks that impact behavior (Bramen et al., 2012). Indeed, during puberty changes in body composition (location and quantity of body fat), insulin sensitivity, diet, PA, PF, sedentary behaviour, and psychological health are observed (Alberga et al., 2012). Thus, adolescence is a critical period, because this is when the individual takes control of his/her lifestyle. It is during this period that engagement in PA might contribute to a physically active lifestyle that lasts into adulthood (Malina, 2001b).

The prevalence of physical inactivity in adolescents, in conjunction with the rising prevalence of obesity, is a major threat to health in the twenty-first century (World Health Organization, 2004). Regular PA is reported to be a protective factor against several diseases, mainly obesity, hypertension, type 2 diabetes, (World Health Organization, 2004), and MetS (Lakka & Laaksonen, 2007). Likewise, the lack of PF has also been associated with the development of CVD risk factors in adults (LaMonte et al., 2005) and youth (Ortega et al., 2008b). In fact, high CRF levels are also related to a healthier profile among adolescents (Andersen et al., 2004; Anderssen et al., 2007; Lobelo et al., 2010; Rizzo et al., 2007; Ruiz et al., 2007a). Moreover, adolescents who maintain a high CRF level through early adulthood are less likely to develop MetS in young adulthood (Ferreira et al., 2005). Some studies have shown that the level of PF in adults is conditioned by the level of PF in childhood or adolescence (Hasselstrom et al., 2002; Janz et al., 2000). Indeed, lower levels of both PA and CRF have been associated with a higher clustering of metabolic risk factors in young people (Andersen et al., 2006; Brage et al., 2004; Lobelo et al., 2010; Rizzo et al., 2007).

Diet has been known for many years to play a key role as a risk factor for chronic diseases; therefore it is well established as a determinant of chronic non-communicable diseases (World Health Organization/FAO, 2003). Many studies have shown that adherence to healthy dietary patterns such as the Mediterranean diet or Southern European Atlantic Diet plays a role in the prevention of CVD (Ambrosini et al., 2010; Martinez-Gonzalez et al., 2008; Oliveira et al., 2010; Trichopoulos & Lagiou, 2004).

In this context, this thesis seeks to understand how modifiable factors such as PA, health-related PF (including body composition) namely CRF, and diet may be associated with MetS or the clustering of CVD risk factors in Azorean adolescents. The Azorean Archipelago is composed of nine islands, with a population of 246,746 habitants (Instituto Nacional de Estatística, 2011). The population has European ancestry and the main economic activities include services, agriculture, and fishing. It is classified as one of the outermost territories of the European Union, and consequently is supported by European

Union funds for social and economic development (European Union, 1997). Furthermore, the Azores have some unique social, geographical, and urban design features that differ from the mainland. All of the islands are of volcanic origin, hosting numerous landscapes of virgin forest and green fields. Most of the urban areas are small and located on the coast.

The overall aim of this thesis is to increase the current knowledge about MetS and the clustering of CVD risk factors in adolescence, as well as associations with PA, health-related PF (including body composition), namely CRF, and diet.

The specific objectives on which this thesis is based are the following:

1) To estimate the prevalence of MetS and its components and to analyze the relationship between MetS and overall PF levels in a sample of Azorean adolescents (Study I).
2) To evaluate the screening performance of different measures of adiposity: BMI, WC and WHtR for high metabolic risk in a sample of adolescents (Study II).
3) To analyze the relationships between metabolic risk factors and PA and PF in a sample of Azorean adolescents (Study III).
4) To assess the impact of the combined associations of CRF and adherence to the Southern European Atlantic Diet on the clustering of metabolic risk factors in adolescents (Study IV).

1.Theorical Background

Theoretical Background

There has been a remarkable change in lifestyle worldwide due to industrialization, urbanization, and economic development, with important health consequences for the global population. Despite improved living standards, expanded food availability, and increasing access to services, significant negative consequences have also appeared, such as decreasing PA and inappropriate dietary patterns with a correspondent increase in lifestyle-related CVD, particularly in western countries (World Health Organization/FAO, 2003). CVDs have become a major cause of morbidity and death in developed and developing countries alike. In this context, Portugal is no exception, with CVD accounting for 1.5% of total deaths in 2010 among adolescents aged 15 to 19 years (DGS, 2004).

The literature has clearly shown that risk factors for CVD tend to co-occur within individuals (Jousilahti et al., 1994); usually the metabolic components considered are: disturbances of glucose metabolism, dyslipidemias, central obesity, and elevated BP. It has been speculated that the co-occurrence of these conditions is due to a common underlying process (Liese et al., 1998). Furthermore, the shared features might be responsible for the clustering of these risk factors in the same individual more commonly than can be explained by chance alone (Kelishadi, 2007). A syndrome is defined as a recognizable complex of symptoms and physical or biochemical findings for which a direct cause is not understood; when causal mechanisms are identified, the syndrome becomes a disease (Alberti et al., 2006).

Reaven (1988) described the coexistence of multiple metabolic abnormalities, such as glucose intolerance, hyperinsulinemia, increased TG, decreased HDL-c, and hypertension, as “Syndrome X”, although obesity was not included. Reaven postulated that insulin resistance and consequent compensatory hyperinsulinemia predisposed patients to hypertension, hyperlipidemia, and diabetes, and were thus the essential causes of CVD (Kahn et al., 2005b). One year later, the term “Syndrome X” was renamed by Kaplan (1989) as "The Deadly Quartet" and then renamed to "Insulin Resistance Syndrome” (Haffner et al., 1992). The term “Metabolic Syndrome” is currently well established as the most useful and widely accepted description of this cluster of metabolically related cardiovascular risk factors.

A number of expert groups, in particular the World Health Organization (WHO) (1999), the European Group for the Study of Insulin Resistance (EGIR) (Balkau & Charles, 1999), the Third Report of the National Cholesterol Education Program's Adult Treatment Panel (NCEP-ATP III) (2001), the American Heart Association/National Heart, Lung, and Blood Institute (Grundy, 2005), and the International Diabetes Federation (IDF) (Alberti et al., 2005), have attempted to develop a definition for MetS in adults. The differences between definitions of the syndrome in adults are listed in Table 1. All expert groups agree that the essential elements of the syndrome should include obesity, insulin resistance, dyslipidemia, and hypertension (Alberti et al., 2006). However, the levels set for each component and the combination of components required to diagnose MetS are slightly different in all of these recommendations, affecting the prevalence rates of the syndrome. Thus, it is not possible to make direct comparisons between studies when different definitions were used.

A study of the prevalence of MetS, as defined by NCEP-ATPIII criteria, in a representative sample of the adult United States (U.S.) population identified 23.7% of individuals as having the syndrome (Ford et al., 2002). A population-based study by Meigs et al., (2003) of non-Hispanic Whites and Mexican–American individuals in San Antonio and of individuals participating in the Framingham Offspring Study determined the prevalence of the syndrome, defined by NCEP-ATP III or WHO criteria, to be 24% by either definition. Several studies have shown that individuals diagnosed with MetS demonstrate a higher prevalence of CVD or are at greater risk of developing it (Ford, 2004; Hunt et al., 2004; Isomaa et al., 2001; Lakka et al., 2002). Indeed, epidemiological data shows that MetS is a common and increasing problem worldwide.

Table 1. Definitions of the metabolic syndrome in adults.

	MetS criteria	Abdominal obesity (WC)	Raised triglycerides	Reduced HDL-cholesterol	Raised fasting glucose	Raised blood pressure	
World Health Organization (1999)	Type 2 diabetes, impaired fasting glucose or insulin resistance* plus ≥ 2 components	BMI > 30 kg/m^2 and/or waist:hip ratio >0.9 males >0.85 females	≥150 mg/dL	< 35 mg/dL males < 39 mg/dL females	110-125 mg/dL or OGTT:140-199 mg/dL	Systolic ≥140 mmHg Diastolic ≥90 mmHg	Urinary albumin excretion rate ≥ 20 µg/min or albumin:creatinine ratio ≥ 30 mg/g
EGIR (1999)	Hyperinsulinaemia, top 25% of fasting insulin values plus ≥ 2 components	≥ 94 cm male ≥ 80 cm female	>178 mg/dL or drug treatment	< 39 mg/dL or drug treatment	≥110 mg/dL but non-diabetic	Systolic ≥140 mmHg Diastolic ≥ 90 mm Hg or drug treatment	
NCEP-ATPII (2001)	≥ 3 components	≥ 102 cm male ≥ 88 cm female	≥150 mg/dL or drug treatment	< 40 mg/dL males < 50 mg/dL females or drug treatment	≥110 mg/dL or drug treatment	Systolic ≥130 mmHg Diastolic ≥ 85 mm Hg and/or drug treatment	
American Heart Association/ National Heart, Lung, and Blood Institute (2005)	≥ 3 components	≥ 102 cm male ≥ 88 cm female	≥150 mg/dL or drug treatment	< 40 mg/dL males < 50 mg/dL females or drug treatment	≥100 mg/dl or drug treatment	Systolic≥130 mm Hg Diastolic ≥85 mmHg or drug treatment	
International Diabetes Federation (2005)	WC plus ≥ 2 components	≥ 94 cm europid male ≥ 80 cm europid female	≥150 mg/dL or drug treatment	< 40 mg/dL males < 50 mg/dL females or drug treatment	≥100 mg/dL or previously type 2 diabetes	Systolic ≥130 mmHg Diastolic ≥85 mmHg or drug treatment	

BMI, body mass index; EGIR, European Group for the Study of Insulin Resistance; MetS, metabolic syndrome; NCEP-ATP III, National Cholesterol Education Program's Adult Treatment Panel III; OGTT – Oral glucose tolerance test; WC, waist circumference.

MetS is becoming epidemic around the world. The high prevalence has been reported not only in developed countries (Ford et al., 2002; Qiao, 2006) but also in developing regions (Gelaye et al., 2009). Several hypotheses regarding the etiology of MetS have been postulated in the literature. The prevalence of both MetS and its components is influenced due to differences in genetic background, diet, levels of PA, population age and sex (Cameron et al., 2004; Johnson et al., 2009).

This syndrome was first observed in adults, but more recent investigation has shown that MetS is also evident in children and adolescents (Cook et al., 2003; De Ferranti et al., 2004; Duncan et al., 2004; Ekelund et al., 2009; Ford et al., 2008; Jolliffe & Janssen, 2007; Pirkola et al., 2008).

In adults, although there are several definitions presented in Table 1, MetS is best defined by established cut-off points of abdominal adiposity, elevated BP, dyslipidemia, and hyperglycemia (Alberti et al., 2005; Grundy, 2005; NCEP-III, 2001; World Health Organization 1999). However, there is no consensus regarding the definition of MetS in children and adolescents. Barriers to a consistent definition for children and adolescents include: (i) the use of adult cut-points or (ii) a single set of cut-points for all ages throughout childhood, (iii) the fact that risk factors are not independently distributed in the population but clustered in some individuals, (iv) children do not habitually exhibit CVD, (v) the physiological insulin resistance of puberty, (vi) the lack of central obesity (WC) cut-points linked to obesity morbidity or MetS for children, and (vii) differences in baseline lipid levels among various races (Steinberger et al., 2009). Hence, the criteria used have been variably adapted from adult standards, with the use of sex- and age- dependent values. Moreover, changes in growth and development during childhood and adolescence make it difficult to select a cut-off for risk factors (Noto et al., 2009). Therefore, it is difficult to relate the criteria to a health outcome and to compare results from different studies and establish trends in the prevalence of the syndrome. Nonetheless, the literature contains several studies addressing different proposed cut-off points for MetS in children and adolescents (Cook et al., 2003; Cruz & Goran, 2004; De Ferranti et al., 2004; Goodman et al., 2007; Raitakari et al., 1995). Further details on the different definitions of MetS in children and adolescents are presented in Table 2. For example, Weiss et al., (2004) suggested a BP>95th percentile for age, height, and sex as the cut-point, whereas Cook et al., (2003) suggested the 90th percentile.

In 2007, trying to overcome conflicts arising from different definitions, the International Diabetes Federation (IDF) published a consensus statement on the definition of MetS in children and adolescents (Zimmet et al., 2007). It contains appendices giving age- and sex-specific reference data for WC for European, African, American, and Mexican-American subgroups present in the U.S., derived from the Third Health and Nutrition Examination Survey (NHANES III). This consensus statement is intended to provide a useful and unified tool for the early diagnosis of MetS in order to take preventive measures before the child or adolescent develops type 2 diabetes or CVD (Zimmet et al., 2007).

A recent review reported a proliferation of definitions of MetS; over 40 different definitions were used in studies of children and adolescents (Ford & Li, 2008). Some definitions of the syndrome in children and adolescents are listed in Table 2. Even though prevalence rates vary depending on the diverse criteria used in adolescents, and despite the lack of a uniform definition, several researchers have shown that MetS develops and is prevalent during childhood and adolescence (Cook et al., 2003; De Ferranti et al., 2004; Duncan et al., 2004; Ekelund et al., 2009; Ford et al., 2008; Zimmet et al., 2007).

Table 2. Definitions of metabolic syndrome in children and adolescents.

	MetS criteria	Abdominal obesity (WC)	Raised triglycerides	Reduced HDL-cholesterol	Raised fasting glucose	Raised blood pressure
Raitakari et al.,1995	3 components	--	≥75th percentile	≤25th percentile	--	Blood pressure
Cook et al., 2003	NCEP-ATPII modified for children and adolescents ≥ 3 components	≥90th percentile for age and sex	≥110 mg/dL	≤40 mg/dL	≥110 mg/dL	≥90th percentile for sex, age, and height
Cruz et al., 2004	NCEP-ATPII modified for children and adolescents ≥ 3 components	≥90th percentile for age, sex, and ethnicity	≥90th percentile for age and sex	≤10th percentile for age and sex	≥100 mg/dl or OGTT ≥ 140 mg/dL	≥90th percentile for sex, age, and height
Weiss et al., 2004	NCEP-ATPII and the WHO modified for children and adolescents 3 components	BMI-Z ≥ 2 adjusted for age and sex	≥95th percentile for age, sex, and ethnicity	≤5th percentile for age, sex, and ethnicity	≥140 mg/dL	≥90th percentile for sex, age, and height
Ferranti et al., 2004	NCEP-ATPII modified for children and adolescents ≥ 3 components	≥75th percentile for age and sex	≥100 mg/dL	<50 mg/dL or for 15-19 yrs boys < 45 mg/dL	≥110 mg/dL	≥95th percentile for sex, age, and height
Goodman et al, 2007	AHA modified for children and adolescents ≥ 3 components	≥90th percentile for age, sex, and ethnicity	≥110 mg/dL	≤10th percentile for sex and race	≥100 mg/dL	≥90th percentile for sex, age, and height
IDF, 2007 **Age group** 6 < 10 years		≥90th percentile	MetS should not be diagnosed. Further measurements should be made if there is a family history of the syndrome, type 2 diabetes, dyslipidemia, CVD, hypertension, and/or obesity.			
10 to <16 years	WC plus ≥ 2 components	≥90th percentile or adult cut-off if lower	≥150 mg/dL	< 40 mg/dL	≥100 mg/dL	Systolic ≥130 mm Hg Diastolic ≥ 85mm Hg
≥ 16 Adult criteria		≥94 cm europid male ≥80 cm europid female	≥150 mg/dL	< 40 mg/dL males < 50 mg/dL females	≥100 mg/dL	Systolic ≥130 mm Hg Diastolic ≥ 85mm Hg

AHA, American Heart Association/National Heart, Lung, and Blood Institute; IDF, International Diabetes Federation; MetS, Metabolic Syndrome; NCEP-ATP III, National Cholesterol Education Program's Adult Treatment Panel III; OGTT, oral glucose tolerance; WC, waist circumference; WHO, World Health Organization.

Data from U.S. NHANES III 1988-1994, which was the first U.S. national report on MetS, showed that the prevalence of the syndrome among adolescents aged 12 to 19 years was 4.2% (Cook et al., 2003). In NHANES III 1999-2000 study, the prevalence rate increased to 6.4% (Duncan et al., 2004). Another study using the same data (i.e., NHANES III 1988-1994) but different criteria showed that the prevalence rate was about 10% (De Ferranti et al., 2004). Kranz et al., (2007) using data from NHANES III 1999-2002 also examined the diagnostic patterns of MetS in national representative samples of individuals aged 2 to 18 years. The authors examined three samples: a) children aged 2 to 18 years (n=5,172), b) a subsample of 12 to 18 years old who were not overweight or obese (n=1,064), and c) 12 to 18 years old who were overweight or obese (n=641). The results indicated that the prevalence of MetS was 2%, 0.7%, and 23%, respectively.

The Cardiovascular Risk in Young Finns Study, comprising 1,865 adolescents aged 6 to 18 years, showed that the prevalence of the syndrome was 4% (Raitakari et al., 1995). Later Mattsson et al., (2008) in a prospective cohort study including 2,195 subjects, aged 3-18 years at base-line in 1980, who were re-examined in 1983, 1986, and 2001 reported that the prevalence of MetS was 19% in men and 12% in women, respectively. In the Bogalusa Heart Study the prevalence of MetS was 4% and 3% in white and black children, respectively (Chen et al., 2000). Two years later, a prevalence of 3.6% in children and adolescents aged 8 to 17 years was also reported by researchers from the Bogalusa Heart Study (Srinivasan et al., 2002). Similarly, in another longitudinal study, which comprised 357 individuals from mean age 13 to 19 years enrolled during childhood, aimed to analyze the influence of insulin resistance and obesity in the development of cardiovascular risk, a steady increase was observed in the prevalence of MetS from 3% to 9%, according to NCEP-ATP III definition (Sinaiko et al., 2005).

A school-based study done by Goodman (2004), in North American adolescents using NCEP-ATP III and WHO criteria, found a 4.2% and 8.4% prevalence of MetS, respectively. The authors state that changes in MetS definitions dramatically influence prevalence differences, ranging from 15% to

50% according to which definitions are used. Another study among children and adolescents aged 10 to 18 years from northern Mexico determine the prevalence of MetS according to NCEP-ATP III, American Association of Clinical Endocrinologists, WHO, and EGIR definitions. The prevalence of the syndrome was 6.5%, 7.7%, 4.5%, and 3.8%, respectively (Rodriguez-Moran et al., 2004). More recently, estimates indicate that 2-9.4% of U.S. adolescents have MetS, depending on the definition used (Cook et al., 2008).

Several studies, using IDF's definition of MetS (Zimmet et al., 2007) have been published (Ekelund et al., 2009; Ford et al., 2008; Park et al., 2009; Pirkola et al., 2008). In U.S. adolescents aged 12 to 17 years, the prevalence of MetS was ~4.5% and was relatively stable across the 6-year period: 4.5% for 1999–2000, 4.4–4.5% for 2001–2002, and 3.7–3.9% for 2003–2004 (Ford et al., 2008). Compared with U.S. adolescents, the prevalence estimates are lower in Europe (1.4% *vs* 4.5%) as shown by a large study in 10 and 15 year olds in Denmark, Portugal and Estonia that also used the IDF's definition of MetS (Ekelund et al., 2009). This study reported a MetS prevalence of 0.2% in 10-year-old children and a 1.4% in 15-year-old children (Ekelund et al., 2009). The differences between countries seem to be the result of a higher prevalence of central obesity in U.S. (25.6%) compared to the European counterparts (16.4%) despite using a higher threshold of waist circumference in U.S. (i.e. 94 cm vs 81.8 cm in 15-year-old boys). The prevalence of MetS in Korean adolescents was 2.6% (Park et al., 2009) and in 16-year-old adolescents participating in the Northern Finnish Birth Cohort examined between 2001 and 2002, the prevalence was 2.4% (Pirkola et al., 2008). Recently, Park et al., (2010), reported that the prevalence of MetS in adolescents from the U.S. and Korea was 5.5% and 2.5%, respectively (Park et al., 2010). The overall prevalence of MetS according to 2007 IDF´s criteria in Jordanian children and adolescents aged 7 to 18 years was 2.1%; in individuals aged 10 to 15.9 years the prevalence of the syndrome was 1.4% and between 16 to 18 years old it was 3.6% (Khader et al., 2010).

The prevalence of MetS in children and adolescents differs tremendously worldwide. In table 3 the prevalence data of MetS in different countries is summarized.

Table 3. Prevalence of the metabolic syndrome in children and adolescents worldwide.

	Prevalence rates	Mets definition	Sample size	Study
Balearic Island	5.8%	de Ferranti's criteria	362 12-17 years	(Bibiloni et al., 2011)
China	3.7%	de Ferranti's criteria	2761 15-19 years	(Li et al., 2008)
Jordan	1.4% (aged 10- 15.9) 3.6% (aged 16-18)	IDF's criteria	512 7-18 years	(Khader et al., 2010)
India	4.2%	Cook´s criteria	1083 12-17 years	(Singh et al., 2007)
Iran	14.1%	de Ferranti's criteria	4811 6-18 years	(Kelishadi et al., 2008)
Italy	2.2% 0.5%	Goodman´s criteria IDF's criteria	1629 7-14 years	(Noto et al., 2009)
Korea	6.8% in 1998 9.2% in 2001	Cook´s criteria	1317 in 1998 848 in 2001 12-19 years	(Kim et al., 2007)
Mexico	6.5% 4.5%	NCEP-ATP III criteria WHO criteria	965 10-18 years	(Rodriguez-Moran et al., 2004)
South Africa	6.5% 1.9%	Cook´s criteria IDF's criteria	1272 10-16 years	(Matshaa et al., 2009)
Spain	18%	Cook´s criteria	429 obese 4-18 years	(Lopez-Capape et al., 2006)
Turkey	2.2%	NCEP-ATP III criteria	1385 10-17 years	(Agirbasli et al., 2006)
U.S. NHANES III 1988-1994	4.2%	Cook´s criteria	2430 12-19 years	(Cook et al., 2003)
U.S. NHANES III 1988- 1994	10%	de Ferranti's criteria	1960 12-19 years	(De Ferranti et al., 2004)
U.S. NHANES III 1999-2000	6.4%	Cook´s criteria	991 12-19 years	(Duncan et al., 2004)

IDF, International Diabetes Federation ; MetS, Metabolic Syndrome; NCEP-ATP III, National Cholesterol Education Program's Adult Treatment Panel III; NHANES III, Third National Health and Nutrition Examination Survey.

The use of different definitions can result in enormous differences in the prevalence estimates, as can be seen in Table 3, and these differences should be considered. In this line, the prevalence of each component of MetS also varies according to the definition applied. Nonetheless, some studies have shown that the components of MetS more prevalent were hypertriglyceridemia and low HDL-c, and hyperglycemia and hypertension were the least ones (Cook et al., 2003; Goodman et al., 2004; Kelishadi et al., 2008; Khader et al., 2010).

Since there is still no universally accepted definition of MetS in children and adolescents, some studies have derived a continuous score representing a composite cardio-metabolic risk factor profile or index (Andersen et al., 2006; Anderssen et al., 2007; Brage et al., 2004; Eisenmann et al., 2010; Kelly et al., 2011; Rizzo et al., 2007). Despite the fact that health care providers have historically categorized medical diagnoses as yes/no, MetS may be less informative when a single definition with rigid cut-off points is selected, as opposed to a potential risk score approach (Cook et al., 2008). The computed risk scores represent continuous variables, with lower values indicating a better metabolic profile and higher values indicating a poorer metabolic profile in relation to the sample studied. The rationale for creating a continuous MetS risk score is based on the fact that prevalence rates among children and adolescents are not high; a continuous score is more sensitive and less error prone compared with categorical measures of MetS, and statistical power is increased (Wijndaele et al., 2006). In fact, population studies have shown that the prevalence of MetS in children and adolescents is relatively low (between 3% and 4%) when compared to the adult population (23.7%) (Cruz & Goran, 2004). Moreover, the American Diabetes Association and the European Association for the Study of Diabetes recommended the development of a definition of MetS either including lower and upper cut-off points or using continuous variables in a multivariate score system (Kahn et al., 2005b).

The above-mentioned authors have suggested that the estimation of a metabolic risk score, based on the sum of age- and sex-adjusted Z-scores of several metabolic risk factors, constitutes a valid tool to identify children and adolescents at risk of developing CVD and type 2 diabetes. Indeed, this approach allows the cumulative risk that each of the risk factors provides to be summarized, providing a continuous score encompassing each individual's the overall risk level, and at the same time accounting to some extent for the influence of growth and maturation on cardiovascular risk factors (Lobelo et al., 2010). Other approaches, such as principal component analysis (Katzmarzyk et al., 2001) and centile rankings (Raitakari et al., 1994), have also been used to calculate the score. Besides differences in the statistical approaches, the variables included in the score also vary.

Generally, the key parameters of MetS definition are used to compute a continuous metabolic risk score. These include indicators of body fat as measured by WC, skinfold thickness, or BMI, low HDL-c, elevated TG, elevated BP (systolic, diastolic and/or mean arterial pressure), and indicators of abnormal glucose metabolism such as impaired fasting glucose levels, glucose tolerance measures and homeostasis model assessment of insulin resistance (HOMA) (Eisenmann, 2008).

Obesity is considered one important risk factor for CVD (Eckel & Krauss, 1998). Obesity rates increased worldwide until becoming an important epidemic both in developed and developing countries due to its association with increased morbidity and mortality (World Health Organization, 2000). However, recently, U.S. data reported a possible plateau in the prevalence of overweight and obese youth in the last decade (Ogden et al., 2010). The mainly important factors underlying the obesity epidemic are the energy intake joined with limited energy expenditure (Biro & Wien, 2010).

In children and adolescents the prevalence of overweight and obesity has increased across Europe (World Health Organization, 2004) and is an upsetting public health concern, as overweight and obesity tracks from childhood to adulthood (Yang et al., 2007) and its consequences include increased incidence of MetS and type 2 diabetes in youth and in adulthood (Biro & Wien, 2010). Indeed, in 2003, 23.5% of the Eastern Mediterranean region, 25.5% of European and 10.6% of South East Asian children and adolescents were overweight or obese; these numbers are projected to almost double by 2010 (Kosti & Panagiotakos, 2006). Accordingly with a recent NHANES III data completed in 2007-2008, 12.6% of U.S. adolescents aged 12 to 19 years were found to be obese (Ogden et al., 2010).

In Portugal overweight and obesity among children and adolescents have also reached epidemic proportions. Recently, in a research on the prevalence of overweight and obesity in a representative sample of Portuguese youth (10-18 years old) the authors found a prevalence of overweight and obesity of 17.0 and 4.6% in girls, and 17.7 and 5.8% in boys, respectively, using the International Obesity Task Force (IOTF) cut-offs whereas according to WHO cut-offs resulted in overweight and obesity prevalence scores of 23.1 and 9.6% in girls, and 20.4 and 10.3% in boys, respectively (Sardinha et al., 2011). However, this study only included children and adolescents living in mainland Portugal. Analogous studies in the Portuguese Islands of Azores were lacking. To our best knowledge there is only one study that reported the prevalence of overweight/obesity in Azorean children aged 6 to 10 years (Pereira et al., 2010). The results, using the IOFT criteria, showed that in girls, prevalence of overweight and obesity were 22.8% and 13.2%, and in boys were 17.6% and 12.3%, respectively (Pereira et al., 2010). Regarding the prevalence of overweight/obesity in Azorean adolescents, a recent study in a representative sample of Azorean adolescents aged 15 to 18 years showed a prevalence of overweight and obesity of 24.9 and 6.5% in girls, and 19.3 and 7.7% in boys, respectively, according to the IOTF criteria; whereas the figures for the WHO criteria were 24.9 and 9.8% in girls, and 21.1 and 10.5% in boys, respectively (Santos et al., 2012).

This scenario is particularly alarming due to the increasing risk of multiple co-morbidities (Freedman et al., 1999a; Freedman et al., 2001; Thompson & Wolf, 2001) and because the worldwide obesity epidemic portends the diabetes epidemic as well as its serious health consequences, including CVD risk factors, such as impaired glucose metabolism, increased BP, dyslipidemia (l'Allemand et al., 2008; Kumanyika et al., 2008), and a variety of pro-inflammatory mediators (Kim et al., 2010). The adverse effects of overweight and obesity begin early in life, as insulin resistance, type 2 diabetes, and CVD risk factors are associated with adiposity in children (Nathan & Moran, 2008).

Furthermore, childhood/adolescence fatness is an important predictor of elevated adiposity in adulthood (Biro & Wien, 2010; Dietz, 1998, 2004; Guo et al., 2002), indicating that overweight and obesity track from childhood to adulthood, subjecting individuals to prolonged exposure to cardiovascular and metabolic diseases associated with increased adiposity. In this line, Bao et al., (1997) in the Bogalusa Heart Study found that the offspring of parents with early coronary artery disease were overweight in childhood and developed an adverse cardiovascular risk profile at an increased rate than children whose parents were not overweight.

Surprisingly, a recent systematic review of the literature on childhood obesity and risk of developing components of the MetS in adulthood reported small evidence to support the fact that childhood obesity is an independent risk factor for adult blood lipid status, insulin levels, MetS or type 2 diabetes (Lloyd et al., 2012). However, the nature of the complex relationship between early BMI and adult disease risk was highlighted by the authors.

The emergence of MetS parallels the rising rates of overweight and obesity observed in youth worldwide (Saland, 2007). Indeed, several of the co-morbidities associated with obesity are linked to several abnormal anthropometric and metabolic changes that tend to cluster, increasing the prevalence of MetS (Haslam & James, 2005). Many studies have shown that the prevalence of MetS is higher among the obese (Chang et al., 2004; Cruz et al., 2004; Shaibi & Goran, 2008). For example, Cruz and Goran (2004) demonstrated that MetS was present in 30% of the overweight/obese children. Another study showed that 39% of moderately obese and 50% of severely obese youth had tree or more components of MetS (Weiss et al., 2004). This scenario was also demonstrated in the Bogalusa Heart Study which found that 58% of obese 5 to 10 years olds had at least one of these five cardiovascular risk factors, and 25% had two or more (Freedman et al., 1999a). In NHANES III data, the majority of youth (80%) who were classified as having MetS were also classified as being overweight based on a BMI>95th percentile (Cook et al., 2003). In Bolivian obese children and adolescents MetS was present in 36% (Caceres et al., 2008). It is noteworthy that MetS in adolescents is largely confined to the overweight population and may represent a subgroup of youth who carry a larger risk for CVD and type 2 diabetes later in future, which in turn is likely to create an enormous socio-economic and public health burden for poorer nations in the future (Kelishadi, 2007).

In the literature, there has been an increasing interest over which measure of overweight/obesity best discriminates those individuals who are at increased cardiovascular risk. There are various sophisticated methods to accurately measure body fat, such as computed axial tomography or dual-energy X-ray absorptiometric densitometry; nevertheless, such techniques are not feasible to apply in large epidemiological studies or even in clinical settings because they are complex, time consuming and expensive. Therefore, anthropometry and bioelectrical impedance are the most widely used methods to apply in large samples, and does not require high economic resources. Hence, various anthropometric measures and indices have been proposed for screening overweight/obesity in different populations.

Epidemiologic studies have shown that BMI, the most widely recognised measure of general obesity, defined as the ratio of body weight (kg) to body height (m^2), is a powerful predictor of CVD (Gelber et al., 2008). However, BMI does not distinguish fat from lean tissue or bone (Soto Gonzalez et al., 2007), and, therefore, classifying people as overweight or obese based on their BMI may lead to significant misclassification. Furthermore, BMI is not a suitable method to assess body fat distribution (Brambilla et al., 2006) and it has been suggested that BMI may be a less sensitive indicator of fat in children and adolescents than WC or WHtR (Brambilla et al., 2006; Cattaneo et al., 2010). Actually WC and WHtR measures of central obesity have yielded important insight as well, and they are also significantly associated with the risk of incident CVD events in youth (Maffeis et al., 2001).

According to IDF´s definition of MetS in children and adolescents, WC is a mandatory criterion, which expresses its importance. In fact, the relevance of measuring central adiposity (as measured by WC) is corroborated by some studies in children and adolescents showing that it is an independent predictor of insulin resistance, abnormal lipid levels and BP, all CVD risk factors and components of MetS (Freedman et al., 1999b; Maffeis et al., 2001). Some studies have shown that central adiposity has increased faster than overweight (as measured by BMI) (McCarthy et al., 2001; Moreno et al., 2005). Despite the fact that WC can be measured at different sites, it has been demonstrated that this does not alter its risk prediction (Ross et al., 2008). WC is a simple, effective and inexpensive anthropometric tool to measure abdominal adiposity and related metabolic risks in children of different ethnicities (Brambilla et al., 2006; Kelishadi et al., 2007a; Lee et al., 2006b; Maffeis et al., 2001).

WHtR has also been proposed as a suitable measurement to assess central fatness in children (Savva et al., 2000). Identical to WC, WHtR has been shown to be strongly correlated with abdominal fat measured using imaging techniques (Soto Gonzalez et al., 2007). Correcting WC to height may obviate the need for age-sex and ethnic-related reference values (Ashwell & Hsieh, 2005), while WC requires population-specific cut-off values (World Health Organization, 2000). Particularly, WHtR offers advantages of a simple boundary value which could be used for men and women, and also in all ethnic groups (Ashwell & Hsieh, 2005). Recently, in a systematic review, Browning et al., (2010) showed that the area under the curve in a receiver operating characteristic curve analysis (ROC) provides good evidence that a WHtR of 0.5 is a good boundary value for men and women across many ethnic groups whereas a boundary value of 0.6 was proposed to asses high risk in children.

There is no agreement for which anthropometrical measures of adiposity best predict a role of CVD risk factors. In fact, a recent review has reinforced this idea (Huxley et al., 2010). The authors concluded that there was evidence to indicate that measures of central obesity were more strongly associated with some risk factors compared with BMI, and for cardiovascular outcomes several studies suggested that the magnitude of the relationships between BMI and central obesity with cardiovascular mortality is largely consistent. Thus, because of the consistency in associations between anthropometric measures and cardiovascular risk, there was limited evidence to support the superior discriminatory capability of any of the measures (Huxley et al., 2010). Despite this, studies carried out among children and adolescents in Cyprus (Savva et al., 2000) and Japan (Hara et al., 2002) concluded that both WC and WHtR are better predictors of TC, TG, HDL-c, low-density lipoprotein cholesterol (LDL-c) and systolic and diastolic BP levels than BMI. Conversely, BMI is the most used measure of obesity and has been shown to be extremely effective when used in longitudinal studies (Burns et al., 2009).

Obesity and insulin resistance have been studied in children and adolescents through cross-sectional and prospective studies as they may be the key underlying abnormalities of MetS (Cruz et al., 2004; Liese et al., 1997; Reaven, 1988; Sinaiko et al., 2005). Furthermore, studies on insulin resistance have suggested that it represents an important risk factor for development of type 2 diabetes, MetS and CVD (Cruz et al., 2005; Goran & Gower, 2001; Lee et al., 2006a). Given this scenario, it is important to identify children and adolescents with insulin resistance to design treatment and preventive strategies that are affordable as well as effective, in order to prevent such diseases.

The Cardiovascular Risk in Young Finns Study conducted on 1,865 children and adolescents aged 6 to 18 years reported that fasting insulin at baseline was related to the development of MetS after 6 years of follow-up (Raitakari et al., 1995). An independent effect of insulin resistance on cardiovascular risk in children has also been suggested. For example, a study with Pima Indian children years 5 to 9 reported that fasting insulin was associated with the level of weight gain during the subsequent 9 years of childhood (Odeleye et al., 1997). In the Bogalusa Heart Study the authors observed a strong correlation over an 8-year period of observation between high fasting insulin levels and the development of coronary artery disease in children and young adults (Bao et al., 1996). Similarly, a study in non-diabetic American-Indian aged 5 to 19 years found that features of MetS predict development of type 2 diabetes (Franks et al., 2007). Moreover, the increase of insulin resistance parallels the rise of the risk of MetS in obese children and adolescents, being consistent with findings reported by Weiss et al., (2004). Additionally, preceding studies have demonstrated that insulin resistance in children and adolescents is influenced by several factors, including sex, ethnicity, amount of adiposity, and pubertal stage (Cruz et al., 2005).

It is important to highlight that puberty is associated with rapid and dynamic changes in diverse metabolic systems including body composition, hormonal regulation, as well as changes in insulin resistance (Cruz et al., 2005). Thus, there is a temporary increase in insulin resistance during puberty (Goran & Gower, 2001) with a peak reduction in insulin sensitivity of 25–30% by Tanner stage 3 and complete recovery by pubertal completion (Ball et al., 2006). Likewise, it is known that pubertal girls have lower insulin sensitivity than pubertal boys (Travers et al., 1995). The decrease in insulin sensitivity during puberty is associated with a compensatory increase in insulin secretion (Caprio et al., 1989; Steinberger & Daniels, 2003). In this line, it was postulated that the increase in insulin resistance was due to the great increase of both growth hormone and growth hormone dependent insulin like growth factor I during puberty (Moran et al., 2002; Rosenbloom et al., 1999). Furthermore, the increase in growth hormone during puberty may contribute to insulin resistance via its effect on increasing lipolysis and free fatty acid concentration (Goran & Gower, 2001).

The gold standard test for measuring insulin sensitivity/resistance is the euglycemic-hyperinsulinemic clamp, however, this method is too invasive and costly, and may not be suitable for large epidemiological studies. An easier and less invasive method used to measure insulin resistance and β-cell function is the HOMA. (Wallace et al., 2004). HOMA was calculated as the product of basal glucose and insulin levels divided by 22.5 (Matthews et al., 1985).

The prevalence of type 2 diabetes is increasing in the U.S. and worldwide (Grundy, 2012). This disease also parallels with the increasing prevalence of obesity in children and youth (Cruz et al., 2004; Cruz et al., 2005). In this line, it has been reported that the prevalence of impaired glucose tolerance is remarkably high among overweight children and adolescents (Goran et al., 2004). Data from the NHANES III revealed that the prevalence of type 2 diabetes is 4.1/1000 in adolescents (Fagot-Campagna et al., 2000).

The rising evidence that the risk of type 2 diabetes is already present during childhood and the significant morbidity and mortality associated with it (Katzmarzyk et al., 2004; Rhodes et al., 2011; Weiss et al., 2003), is crucial to identify children at risk. In fact, a recent study has suggested that adolescents and young adults with type 2 diabetes lose approximately 15 years from average remaining life expectancy and may experience severe, chronic complications of type 2 diabetes by the age of 40 (Rhodes et al., 2011). As stated above, an accepted hypothesis describing the pathophysiology of MetS may be insulin resistance. Hence, for both diabetes and MetS, the beneficial approach is lifestyle intervention, especially weight reduction and increase PA levels in order to prevent several diseases (Grundy, 2012).

Dyslipidemia is a commonly recognized risk factor for CVD, and is a component of MetS. It consists mainly of both low HDL-c and high TG levels. Indeed, low HDL-c and high TG levels are strongly associated with insulin resistance, and both are risk factors for coronary heart disease (Steinmetz et al., 2001). Moreover, the relationship between serum lipids and the development of coronary heart disease in children and adolescents is well documented (Berenson et al., 1998).

Epidemiological studies and meta-analysis consistently show that increased levels of TG and low HDL-c are independent risk factors for CVD (Austin et al., 1998; Eberly et al., 2003; Gordon et al., 1989). In fact, the literature suggests that the core of dyslipidemia may be determined in childhood, in conjunction with obesity. Thus, some studies reported associations between childhood obesity and rised TC, LDL-c, TG and low HDL-c concentrations in adulthood, suggesting that lipid concentrations track from childhood to adulthood (Freedman et al., 2001; Reinehr et al., 2005; Webber et al., 1991). Similarly, a study that examined the tracking of clusters of multiple cardio-metabolic risk factors comprising MetS found that the cardio-metabolic risk factors associated with MetS were relatively stable among white children and adolescents in the normal risk category and changes in status were frequent if the risk factor was elevated (Li et al., 2009).

Data from NHANES III 1999-2002 showed that particularly the proportion of adolescents with elevated TG and low HDL-c were at high risk for developing MetS, even in children who were not overweight or obese (Kranz et al., 2007). Nevertheless, data from the Bogalusa Heart Study reported that overweight children have significantly higher levels of TC, LDL-c and TG and lower HDL-c levels than normal weight children (Freedman et al., 1999a).

Another lipid risk factor is the TC/HDL-c ratio. Indeed, several studies reported that the TC/HDL-c ratio is a powerful predictor of CVD risk (NCEP-III, 2002). In fact, a high TC is a marker for atherogenic lipoproteins, whereas a low HDL-c correlates with the multiple risk factors of MetS and probably confers some independent risk (NCEP-III, 2002). Furthermore, results of Physicians' Health Study (Stampfer et al., 1996) concluded that at least higher levels of the TC/HDL-c ratio and additional consideration of TG level improve coronary artery disease prediction.

It is worth considering the fact that some previous studies have shown changes in serum lipids throughout adolescence (Bertrais et al., 2000; Tell et al., 1985). In fact, sexual maturation rather than chronological age seems to explain the decrease in serum TC, LDL-c and HDL-c levels during this period of life, therefore, the assessment of pubertal development may provide additional valuable information when interpreting lipid profile in adolescence (Ruiz et al., 2006b). Thus, measurements in serum lipids should be interpreted with caution due to the influence of pubertal development.

The existing literature has demonstrated that hypertension is detectable in youth and, as in adults, it occurs commonly (Falkner, 2010; Kavey et al., 2010; McNiece et al., 2007; NHBPEP, 2004). Diagnostic criteria for elevated BP in children are based on percentiles derived from population studies of healthy children. BP increase is defined as systolic or diastolic BP at or higher than the 95^{th} percentile based on sex, age, and height percentile, whereas hypertension can be defined as BP persistently higher than that level on 3 or more occasions (Falkner, 2010). In this line, it is important to have in mind that the prevalence of hypertension in children and adolescents depends on several factors such as age, sex, weight, and ethnicity (Lurbe et al., 2010).

Several studies concluded that there is a strong evidence for BP tracking from childhood into adulthood (Chen & Wang, 2008; NHBPEP, 2004). For instance, the Fels Longitudinal Study examined serial data and derived age- and gender-specific BP levels in childhood, which predicted hypertension and MetS later in life (Sun et al., 2007).

It is also known that hypertension and obesity are both common health problems in children and adolescents (Falkner, 2008; Kavey et al., 2010; McNiece et al., 2007; Ogden et al., 2002). In this regard, a school-based survey in the U.S. published in 2004, reported that the overall prevalence of hypertension among adolescents was 4.5%; nevertheless, the prevalence of hypertension was 34% in the group with BMI higher than the 95th percentile (Sorof et al., 2004). In another study, McNiece et al., (2007) also found that the prevalence of hypertension and pre-hypertension associated was over 30% in obese boys and from 23–30% in obese girls, depending on ethnicity.

It is important to stress that both elevated BP and obesity are two components of MetS. Hence, these metabolic risk factors in adolescence may be the clinically recognizable phenotype for MetS in adolescence (Falkner, 2008). Furthermore, children and adolescents with high BP and obesity frequently have other risk factors that are components of MetS (Falkner, 2008). Thus, it is advisable that children and adolescents with hypertension and especially obesity-associated hypertension be evaluated for additionally CVD risk factors. Moreover, early prevention of these metabolic risk factors, including lifestyle changes, needs to be an important focus for policy makers, health care providers, schools, parents, and society as a whole to optimize health outcomes and reduce the excessive burden of CVD.

Cardiovascular and metabolic diseases continue to be a cause for concern worldwide (Lloyd-Jones et al., 2010). Their complex etiology which arises from the interactions of genetic, environmental and behavior factors have been considered to be major contributors in the current epidemic.

The literature has described the beneficial effects of a healthy lifestyle, including regular PA and a healthy dietary pattern, such as the daily consumption of fruits and vegetables, and avoiding smoking, which are main modifiable lifestyle factors and are recommended as the cornerstone of prevention and the first line of treatment for MetS and other co-morbidities in youth (Eckel et al., 2005; Hollenbeck et al., 1988; Pacifico et al., 2011). Thus, there is a crucial need to recognize the importance, efficacy and effectiveness of a healthy lifestyle in the prevention of CVD (Hu et al., 2004).

WHO, in 1994, confirmed that a sedentary lifestyle is an independent risk factor for ischemic heart disease, and individuals with this type of life have nearly twice the risk for ischemic heart disease, or of dying from it, as compared with active ones (Bijnen et al., 1994). In fact, the protective effect of PA on non-communicable diseases has been widely reported in people of all ages (Andersen et al., 2006; Lakka & Laaksonen, 2007; Strong et al., 2005; World Health Organization, 2010). Moreover, it is well established that regular PA improves a number of CVD risk factors (Kohl, 2001) including the prevention and treatment of MetS (Lakka & Laaksonen, 2007) as it reduces all-cause mortality (Lee, 2010). Hence, PA monitoring and promotion should be two basic objectives in public health worldwide (World Health Organization, 2004).

A considerable number of reviews have been published on PA and health in young people (Andersen et al., 2011; Froberg & Andersen, 2005; Must & Tybor, 2005; Ruiz & Ortega, 2009; Strong et al., 2005). Their main findings underlined the evidence-based importance of PA for young people’s health status. Likewise, observational studies have shown that physically active children and adolescents have higher HDL-c (Raitakari et al., 1997), lower TG (Raitakari et al., 1997), lower LDL-c (Craig et al., 1996), lower BMI and body fat (Raitakari et al., 1997), improved insulin sensitivity (Craig et al., 1996; Schmitz et al., 2002) and lower systolic BP (Andersen, 1994). Furthermore, the establishment of healthy patterns of PA during childhood and adolescence is important as the benefits of PA carry over into adulthood, so that an active child is more likely to be a physically active adult (Hallal et al., 2006; Malina, 1996, 2001b). It is, therefore, important to encourage PA in youth, so that when they become adults they continue active.

In this regard, attention should be paid to the assessment of PA. Indeed, different methodologies have been proposed for assessing PA in youth. Some of them subjective, such as direct observation, self-report, and some objective like pedometers, accelerometers and heart rate monitors (Welk, 2002).

Pedometers are motion sensors which are generally worn on the hip that record the number of steps taken. They are simple to use, inexpensive and produce a friendly output (e.g. steps taken, steps/day) (Tudor-Locke & Myers, 2001b). Pedometers are not only useful as measurement tools, but they can also motivate sedentary people to increase their activity level (Tudor-Locke, 2002). Although pedometers are not designed to measure PA intensity, they do provide a feasible and practical means of capturing daily PA volume, especially walking (Tudor-Locke & Myers, 2001a). Moreover, walking is a fundamental behavior in daily life activities (Tudor-Locke & Ham, 2008).

Regarding accelerometers, they are small electronic devices that are generally worn on the hip and which allow detailed data on the frequency, intensity, and duration of activity to be downloaded to a computer for later analysis (Troiano et al., 2008) and provide an estimate of energy expenditure (Bassett, 2000; Ward et al., 2005; Westerterp, 1999). They also provide data to further investigate the dose-response relationship between activity patterns and health and providing evidence to be used in public health recommendations (Reilly et al., 2008). Although accelerometers can offer continuum estimates of PA volume and intensity, their relatively high costs, data management demands, and requisites for personnel time and technical expertise stand as a barrier to widespread implementation outside formal research studies (Tudor-Locke et al., 2009).

Because studies vary substantially in their PA measurement, inconsistent conclusions regarding PA and health outcomes are obvious. For example, data from the Danish Youth and Sport Study reported no association between self-reported PA and MetS in 305 adolescents (Andersen et al., 2004). Similarly, using the PA section of the NHANES III 1999-2002 questionnaire, Pan and Pratt (2008) found no significant relationship between PA and MetS in 4450 adolescents aged 12 to 19 years. On the other hand, McMurray et al., (2008) using a PA validated questionnaire, reported that children who developed MetS as adolescents had 22% lower PA scores than those children who did not develop MetS. Also, a study in 4,811 adolescents aged 6 to 18 years, found a small difference in the overall rate of MetS between tertiles of PA and children with low PA levels, who were 1.6–1.8 times more likely to have MetS (Kelishadi et al., 2007b).

The relationship between PA, MetS on its components has been more consistent when objective measures of PA were used. In fact, several researches have established inverse cross-sectional associations between objectively measured PA with cardio-metabolic risk factors and adiposity in youth (Andersen et al., 2006; Anderssen et al., 2007; Ekelund et al., 2009; Ruiz & Ortega, 2009; Ruiz et al., 2006c; Steele et al., 2008). In this line, a study done with 389 Danish children using an accelerometer-measured PA showed that as PA levels decreased, MetS z-score increased (Brage et al., 2004). In another study of 529 Swedish adolescents aged 9 and 15 years also found an inverse relationship between PA and MetS, particularly in their 15-year-old girls (Rizzo et al., 2007). Data from the European Youth Heart Study (EYHS) measured PA levels with accelerometers in adolescents aged 9 and 15 years and used a z-score classification for the clustering of MetS components. The authors reported a graded association in MetS z-score through all PA percentiles (Andersen et al., 2006; Andersen et al., 2008; Anderssen et al., 2007). Moreover, it was also suggested weak but highly significant associations between objectively measured PA and TC and TG, but no association with HDL-c (Andersen et al., 2006). A recent population-based cohort study conducted in youth from Estonia, Denmark, and Portugal (n=3193) aged 10- and 15-years old, showed that maternal overweight and obesity, low levels of PA, and low CRF independently contributed to MetS in young people and relatively small increases in PA may significantly reduce the risk of MetS in healthy children (Ekelund et al., 2009). Some studies reported that PA is inversely correlated with both markers of insulin resistance (Brage et al., 2004; Rizzo et al., 2007) and indicators of body fat (Ruiz et al., 2006c). The Avon Longitudinal Study of Parents and Children reported that higher levels of PA were associated with lower BP in children (Leary et al., 2008). Conversely, the 2003–2004 U.S. NHANES III study reported an inverse dose-response relation of total and moderate to vigorous

PA with systolic and diastolic BP in adolescents aged 8 to 17 years (Mark & Janssen, 2008). The meta-analysis of Rowlands et al., (2000) states that there is a small to moderate relationship between body fat and PA in children and the size of the relationship depends on the PA measure used. Thus, direct measures of PA movement are the best methods for assessment of the relationship of PA levels with health outcomes.

Another important concept related to health is PF which is also believed to improve several CVD risk factors.

PF is a set of attributes related to a person's ability to perform physical activities that require aerobic fitness, endurance, strength or flexibility and is determined by a combination of regular activity and genetically inherited ability (Caspersen et al., 1985). In adults, there is strong evidence indicating that PF is a powerful predictor of CVD and all-cause morbidity and mortality (Blair et al., 1989; Myers et al., 2002). The lack of PF has also been associated with the development of CVD risk factors in youth, such as lipid disorders, high BP and insulin resistance, among others (Ortega et al., 2008b).

Being one of the five health-related PF components that has been most studied, because it is a health marker across the lifespan (Blair et al., 1996; Ortega et al., 2008b), CRF is a direct marker of physiological status that reflects the overall capacity of the cardiovascular and respiratory systems and the ability to carry out prolonged exercise (Taylor et al., 1955). Different terms have been used to define this PF component: CFR, cardiovascular fitness, cardiorespiratory endurance, aerobic capacity, aerobic fitness, aerobic power, maximal aerobic power, aerobic work capacity, and physical work capacity; all refer to the same concept and are used interchangeably in the literature (Ruiz et al., 2006a).

The maximal rate of oxygen uptake (VO_{2max}) is considered the gold standard for measurement of CRF. VO_{2max} can be measured using direct (laboratory tests) and indirect (field-based tests) methods. The use of direct measures in school settings and in population based studies is limited due to their high cost, necessity of sophisticated instruments, qualified technicians and time constraints (Welk & Meredith, 2008). Field-tests provide a practical alternative since they are time efficient, low in cost and equipment requirements, and can be easily administered to a large number of people simultaneously (Castro-Pinero et al., 2010; Welk & Meredith, 2008).

One of the most common field-tests for assessing CRF among children and adolescents is the 20-m shuttle run test (20mSRT) (Castro-Pinero et al., 2010; Léger et al., 1984; Tomkinson et al., 2003). This test requires participants to run back and forth between two lines set 20m apart. Running speed started at 8.5 km/h and increased by 0.5 km/h each minute, reaching 18.0 km/h at minute 20. Each level was announced on a tape player. The participants were told to keep up with the pace until exhausted. The test was finished when the participant failed to reach the end lines concurrent with the audio signals on two consecutive occasions. Otherwise, the test ended when the subject stopped because of fatigue. Participants were encouraged to keep running as long as possible throughout the course of the test. Number of shuttles performed by each participant was recorded. This test is valid (Shen et al., 1988), reliable (Artero et al., 2011) and feasible (Golay et al., 1988) in young people. However, as the 20mSRT is an indirect method, some error might always be present when estimations of CRF are done.

In adults, several health benefits of CRF have been extensively documented (Blair et al., 1996; Blair et al., 1989; Grundy et al., 2012; Lee et al., 2010). Higher levels of CRF appear to delay all causes of mortality, primarily due to their decrease rates of CVD and cancer (Blair et al., 1989) and provide strong and independent prognostic information about the overall risk of illness and death, especially as this relates to cardiovascular causes (LaMonte & Blair, 2006).

This scenario also occurs in youth. Indeed, research lately suggests that CRF is also an important health marker in young individuals (Andersen et al., 2004; Castillo-Garzon et al., 2007; Ortega et al., 2008a). Cross-sectional studies indicate that high CRF, during childhood and adolescence, is associated with a favorable plasma lipid profile (Mesa et al., 2006), total and central body fat (Ortega et al., 2007), components of MetS (Brage et al., 2004) and novel CVD risk factors (Ruiz et al., 2007c). Longitudinal studies also suggest that higher CRF during these ages is associated with a healthier cardiovascular profile later in life (Hasselstrom et al., 2002; Ruiz et al., 2009; Twisk et al., 2002). The same relationship was noted between CRF and the cluster of metabolic risk factors in young people (Anderssen et al., 2007; Brage et al., 2004; Lobelo et al., 2010). For instance, in a large sample on children and adolescents from three European countries aged 9 or 15 years, CFR was inversely associated with clustered metabolic risk factors independent of country, age and sex (Anderssen et al., 2007). In this line, Ruiz et al., (2007a) with the Swedish and Estonian part of the EYHS, reported an inverse association between CRF and clustered of metabolic risk factors in 9-10-year-old Swedish and Estonian children. Reinforcing this idea, Ortega et al., (2008b) reported that children and adolescents with higher levels of CRF also have a more favorable cardiovascular profile compared to their unfit counterparts.

Despite the vast evidence demonstrating that higher PF is associated with improved health in children and youth (Anderssen et al., 2007; Ekelund et al., 2007; Janssen, 2007; Strong et al., 2005); there is increasing evidence suggesting that the CRF performance of youth is declining (Tomkinson et al., 2003). Furthermore, CRF tends to track moderately from childhood to adulthood (Malina, 1996; Malina, 2001a).

CRF is influenced by several factors, such as age, sex, body fatness, health status, and genetics, however, its main modifiable determinant is habitual PA (Bouchard & Perusse, 1994). It has been suggested that up to 40% of variation in the level of CRF is attributable to genetic factors (Bouchard et al., 1986; Wolfarth et al., 2005). Moreover, longitudinal studies reported that habitual PA explains about 10% of CRF variance across the lifespan (Malina, 1996). In addition to PA, body fatness influences both CVD risk factors and CRF, and it might confound the association between CRF and metabolic health (Steele et al., 2008). Previous studies have shown that CRF is linked to CVD risk factors in youth even after accounting for the role of fatness (Malina, 1996).

Some cross-sectional studies have examined the effects of CRF on CVD risk in adolescents, controlling for adiposity and the results are inconsistent. An adolescent population-based study aged 9 to 10 and 15 to 16 years from three regions of Europe (n=1709) showed significant inverse association between CRF and clustered metabolic risk independently of adiposity (Ekelund et al., 2007). The Aerobics Center Longitudinal Study in 484 adolescents also found that those adolescents with low CFR, regardless of BMI, had higher MetS risk scores; moreover, those adolescents with the highest BMI and lowest CRF had the highest MetS scores (Eisenmann et al., 2007). Kwon et al., (2010) have also demonstrated that a CRF effect on CVD risk factors was significant after controlling for weight status, particularly among boys, in a representative sample of U.S. adolescents (NHANES 1999-2002). Similar results have been found in Spanish adolescents from the *Alimentación y Valoración del Estado Nutricional de los Adolescentes* study that reported associations between increased CRF and a favorable metabolic profile in both overweight and non-overweight adolescents (Mesa et al., 2006). Likewise, DuBose et al., (2007) examined the relationship between MetS and CRF in 375 normal-weight and overweight 7 to 9 years old children and found that BMI was positively and CFR negatively associated with clustered metabolic risk. However, other cross-sectional studies have reported that significant associations between CRF and CVD risk factors were not retained after adjustment for adiposity. For example, a study with children and adolescents aged 9 and 15 years showed that CRF was inversely related to metabolic risk, and body fat had a pivotal role in this relationship (Rizzo et al., 2007). In this line, another study from the Swedish part of EYHS, involving 142 children aged 9 to 10 years, showed that low-grade inflammatory markers were negatively associated with CRF and positively associated with body fat in pre-pubertal children; for most of the variables, the influence of fatness was slightly higher than the influence of CRF (Ruiz et al., 2007b).

Regarding to longitudinal studies only a few have been conducted to examine the independent effects of adiposity and CRF on cardiovascular health. In this line, McMurray et al., (2008) examined a temporal relationship between CRF in childhood and MetS in adolescence among 389 participants, using data from the Cardiovascular Health in Children and Youth Study. Measurements at baseline (7 to 10 years old) and at seven-year follow-up were taken. The study found that adolescents with MetS were six times more likely to be in the lowest tertile of VO2 max at 7 to 10 years old than those without the syndrome.

Studies among children and adolescents often assumed that PA and CRF are strongly associated, but this assumption is not so clear (Ortega et al., 2008b). Indeed, several studies using accelerometers showed that moderate and vigorous PA are associated with high CRF in children and adolescents, but no adjustments were performed for body fat measures (Gutin et al., 2005; Ruiz et al., 2006c). It is generally accepted that 60 min/day of moderate-to-vigorous PA is associated with healthy CRF levels (Strong et al., 2005). More studies have also resulted in similar findings. Ortega et al., (2008c) reported that those adolescents meeting the PA guidelines were more likely to have a healthier CRF level independently of their adiposity status. Data from Sweden found that children who engaged in more than 40 minutes of vigorous PA daily had better CRF than those who accumulated less than 18 minutes per day of vigorous PA (Ruiz et al., 2006c). Recently, data from the Healthy Lifestyle in Europe by Nutrition in Adolescence study in 808 adolescents aged 12.5 to 17.5 years showed that specific doses of light, moderate, vigorous, and moderate to vigorous PA are associated with healthy CRF levels in European adolescents; in addition, the moderate to vigorous PA cut-offs associated with healthy CRF were similar to the current PA recommendations (Martinez-Gomez et al., 2010).

In fact, recent studies using more precise quantification of the exposures suggest that both PA and CRF are separately and independently associated with metabolic risk factors in children, but possibly through different pathways or that CRF is a marker for specific muscle characteristics, for example, muscle fiber-type composition, which may affect metabolic health (Steele et al., 2008). In fact, one pathway could be the insulin-stimulated glucose transport and expression of the insulin-regulated glucose transporter GLUT4 (Daugaard et al., 2000), and the other one suggests that slow-twitch muscle fibers are associated with increased lipid oxidation (Turpeinen et al., 2006). Thus, increasing both PA and CRF in youth is a potential strategy to prevent and reduce the factors that are linked to CVD and also to components of MetS in children and adolescents.

The beneficial effects of lifestyle modification, including regular PA and a healthy dietary pattern, seem to be important, in view of the escalating rates of CVD risk factors in youth. In fact, an increasing amount of research on dietary patterns and health outcomes, including traditional and emerging risk factors for CVD in youth has been published in the last years. Adherence to a healthy dietary pattern has been shown to be inversely associated with MetS and its components, as well as other co-morbidities (Ambrosini et al., 2010; Panagiotakos et al., 2006; Williams et al., 2000).

The Mediterranean dietary pattern is characterized by high consumption of olive oil, fruits and vegetables, and non-refined grains, moderate consumption of dairy products and wine, and low consumption of red and processed meat, cream, and pastries (Trichopoulou, 2001; Trichopoulou et al., 2003; Trichopoulou & Lagiou, 1997). Using these tendencies as guidance, a Mediterranean diet score was constructed based on the intake of food groups (pulses, vegetables, fresh fruits, nuts, whole grains, fish, red and processed meats) as well as on ethanol intake and the ratio of monounsaturated to saturated fat (Trichopoulou et al., 2003).

Recently, another dietary pattern, the Southern European Atlantic Diet (SEADiet) has emerged as a healthy dietary pattern, characterized by a highly palatable diet that is culturally rooted in northern Portugal and Galicia (a region in northwest Spain) in the Atlantic coast, and consisting of a high intake of seasonal legumes, vegetables, potatoes, whole grain bread (either from corn or wheat), and fish, particularly cod, but also including red meat, pork, and dairy products (Oliveira et al., 2010). This dietary pattern has been reported to be protective against CVD outcomes (Oliveira et al., 2010). Therefore, adherence to these dietary patterns might reduce the adverse effects attributed to metabolic risk factors.

Evidence suggests that Mediterranean dietary patterns are associated with reduced risk of death from all causes, including death due to CVD (Mitrou et al., 2007). Indeed, several epidemiological and cross-sectional studies have shown that those living in Mediterranean countries have a longer life expectancy and a lower risk of having certain chronic diseases, including CVD, metabolic disorders, and certain types of cancer (Chrysohoou et al., 2004; Martinez-Gonzalez et al., 2008; Panagiotakos et al., 2004b; Trichopoulos & Lagiou, 2004).

It is important to note that the majority of the literature (Babio et al., 2009a; Babio et al., 2009b; Kastorini et al., 2011) confirms the beneficial effect of adherence to the Mediterranean diet on MetS and its components, although a few studies reported no significant relationship between these two variables. For instance, in a sample of the Greek population, individuals with higher adherence to the Mediterranean diet had a 20% lower odds ratio of having MetS (Panagiotakos et al., 2004a). Conversely, in a cross-sectional study of individuals from the Canary Islands, no significant relationship between adherence to the Mediterranean diet and MetS was found (Alvarez Leon et al., 2006). A recent systematic review and random effects meta-analysis of epidemiological studies and randomized controlled trials reported that the combined effect of prospective studies and clinical trials showed that adherence to the Mediterranean diet was associated with lower MetS prevalence and progression (Kastorini et al., 2011).

Adherence to healthy eating practices such as maintaining a high level of intake of fruit, vegetables, and whole grains has been associated with lower risk of MetS (Wirfalt et al., 2001), while a western dietary pattern, characterized by high intakes of total fat, saturated fat, refined sugars, and sodium, was positively related with individual MetS risk factor components in adolescents (Ambrosini et al., 2010).

In a finding consistent with previous findings in adults, adolescents whose eating patterns included high intakes of fruit and vegetables also exhibited lower prevalence of MetS (Pan & Pratt, 2008). Data from the Bogalusa Heart Study of 1,275 individuals aged 12-24 years (Nicklas et al., 1989), showed that high consumption of fruits and vegetables was associated with higher levels of HDL-c and lower levels of TG and LDL-c, whereas a diet that included high levels of consumption of sugary and fatty foods was associated with higher LDL-c levels (Nicklas et al., 1989). A cohort of overweight Hispanic children showed that an increase in soluble fiber intake through the daily consumption of fruits, vegetables, and beans may improve metabolic health in Latino children (Ventura et al., 2008). In addition, a study in 764 Australian adolescents reported three major dietary patterns: (i) fruit, fish, cereals, and salad, (ii) high fat and sugar, and (iii) vegetables; the authors conclude that adolescents in the upper tertile for the first pattern had significantly lower diastolic BP than those in the lowest tertile, after adjustment for age, sex, and PA (McNaughton et al., 2008a). The Whitehall Study, a study of 7,339 participants, reported that a dietary pattern characterized by high consumption of low-calorie/diet soft drinks, sugar-sweetened beverages, burgers and sausages, crisps and other snacks, onions, and white bread, and low consumption of medium-/high-fiber breakfast cereals, jam, French dressing/vinaigrette, and whole meal bread was associated with insulin resistance. Furthermore, this dietary pattern predicted type 2 diabetes risk after adjustment for a range of confounders (McNaughton et al., 2008b).

Recently, a cross-sectional analysis of 2,128 adolescents aged 12 to 19 years who participated in NHANES III 1999-2002 underlined that higher intake of dietary fiber, but not low intakes of saturated fat or cholesterol, are related to MetS in adolescents (Carlson et al., 2011). In fact, prospective studies are needed to confirm the role of dietary patterns in the development of several non-communicable diseases, such as MetS, CVD, and other co-morbidities.

2.Experimental Work

Study design and sampling

Data for the present study were derived from a longitudinal school-based study, the Azorean Physical Activity and Health Study II, aimed to evaluate PA, PF, overweight/obesity prevalence, quality of life and other health-related factors. The study was carried out in six of the nine Azorean Islands, where 95% of the population live: S. Miguel, Terceira, Faial, Pico, S. Jorge and Graciosa (Instituto Nacional de Estatística, 2011).

All participants in this study were informed of its objectives, and the parent or guardian of each participant provided written informed consent. The study was approved by the Faculty of Sport and the Portuguese Foundation for Science and Technology Ethics Committees; it was conducted in accordance with the World Medical Association's Helsinki Declaration for Human Studies.

The population was selected by means of a proportionate stratified random sampling, taking into account the location (island) and the number of students, by age and sex, in each school. The estimated number of individuals needed was 1,422, but to prevent incomplete data, data collection was made in 1,525 adolescents. Twenty-five individuals aged <14.5 or >18.4 years; and/or having a health condition that did not allow participating in physical education classes; or had missing information on the variables of interest were excluded, resulting in a total of 1,500 participants (892 girls and 698 boys). Data was collected in the fall of 2008 and one year later (mean follow-up length 11.5±2.0 months) 850 individuals were reevaluated. Of the 850 adolescents that participated in the 2009 data collection, 36 were excluded due to lack of information on several variables, and 297 refused to undergo blood sampling measurement. Therefore, the final sample included in this cross-sectional study was comprised of 517 adolescents (297 girls and 220 boys) aged 15 to 18 years (mean age 16.5±1.0) who had complete data on the variables of interest. There were no differences in the variables of interest between individuals who underwent blood collection measurement and those who refused to do so. Power analysis of the final sample was calculated post-hoc being higher than 0.8 for P-value <0.05.

Participants were evaluated during school physical education classes by physical education teachers specially trained for this data collection (anthropometric measures, overall PF and questionnaires). Blood sampling and BP were measured by nurses in a local hospital.

The basic characteristics of the participants and the variables examined in each sub-study are presented in Table 1. A detailed description regarding variables assessment, samples studied and statistical procedures are explain in each paper in the following pages of this thesis.

Table 1. Summary of the characteristics of the sub-studies.

Study	*Sample Size*	*Age*	*Variables studied*	*Statistical analysis*
I. Metabolic Syndrome and Physical Fitness in a sample of Azorean Adolescents	517 297 girls	15-18 years	BMI, WC, BP, HDL-c, TG, glucose, pubertal stage, SES, PF	Independent sample t-test (one tailed), Chi-square test, Binary logistic regression
II. Ability of different measures of adiposity to identify high metabolic risk in adolescents	517 297 girls	15-18 years	BMI, WC, WHtR, HOMA, TC/HDL-C ratio, TG, BP, pubertal stage	Independent sample t- test (one tailed), Linear regression ROC analysis
III. Metabolic Risk Factors, Physical Activity and Physical Fitness in Azorean Adolescents: a cross-sectional study	417 243 girls	15-18 years	BMI, WC, glucose, HDL-c, TG, BP, pubertal stage, SES, dietary intake, PF, PA (pedometers)	Independent sample t- test (one tailed), Ordinal logistic regression
IV. Cardiorespiratory fitness is negatively associated with metabolic risk factors independently of the adherence to a healthy dietary pattern	468 273 girls	15-28 years	BMI, WHtR , HOMA, TC/HDL-C ratio, TG, BP, dietary intake, pubertal stage, SES, smoking status, CRF	Independent sample t- test (one tailed), Chi-square test, Binary logistic regression

BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio; SB, blood pressure; TG, triglycerides; HDL-c, high-density lipoprotein; TC, total cholesterol; SES, socio-economic status; PA, physical activity; PF, physical fitness; CRF, cardiorespiratory fitness.

Paper I

Metabolic Syndrome and Physical Fitness in a sample of Azorean Adolescents

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Metabolic Syndrome and Physical Fitness in a Sample of Azorean Adolescents

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Abstract

Background: Metabolic syndrome, a predecessor of cardiovascular disease and type 2 diabetes, has become prevalent in adolescents. This study aimed to estimate the prevalence of metabolic syndrome and its components and to analyze the relationship between metabolic syndrome and overall physical fitness levels in a sample of Azorean adolescents.

Methods: A cross-sectional school-based study, the Azorean Physical Activity and Health Study II, was conducted on 517 adolescents (297 girls, 220 boys) aged 15–18 years old from the Azorean Islands. Body height, weight, waist circumference, and arterial blood pressure were measured according to standards. Fasting intravenous blood samples were analyzed (high-density lipoprotein cholesterol, triglycerides, and glucose). Physical fitness was assessed using five tests from Fitnessgram Test Battery 8.0: Curl-up, push-up, trunk lift, pacer, and sit-and-reach. Adolescents were then classified as being in the healthy zone or above or under the healthy zone. Metabolic syndrome was defined according to the 2007 International Diabetes Federation's guidelines for adolescents.

Results: The prevalence of metabolic syndrome was 5% (4.7% in girls and 5.5% in boys, $P > 0.05$). Waist circumference was the most prevalent component (32.9%), and hypertriglyceridemia the least (4.4%). Logistic regression analysis showed that after adjusting for pubertal stage and socioeconomic status, unfit adolescents (healthy zone criteria in ≤ 2 tests) were more likely [odds ratio (OR) = 3.414; 95% confidence interval (CI), 1.150–10.129] to be classified as having metabolic syndrome.

Conclusions: The prevalence of metabolic syndrome is high in Azorean adolescents. Unfit adolescents were more likely to have metabolic syndrome than fit adolescents. Improving overall physical fitness levels and abdominal obesity reduction may be important strategies in overcoming this public health problem and its consequences.

Introduction

Metabolic syndrome, defined as a series of risk factors that include atherogenic dyslipidemia, abdominal obesity, hypertension, insulin resistance, and prothrombotic and proinflammatory states, leads to increased risk of cardiovascular disease (CVD) and type 2 diabetes[1,2] and, therefore, represents a major health risk.[3,4] However, despite the abundant research on the subject, no universally accepted definition exists of the metabolic syndrome in children and adolescents; the criteria used have been adapted from adult standards in different ways. In 2007 the International Diabetes Federation (IDF) published a consensus statement on a definition of metabolic syndrome in children and adolescents. This definition was intended to provide a useful and unified tool for the early diagnosis of metabolic syndrome and to take preventive measures before the child or adolescent develops diabetes or CVD.[5]

Body fatness and a sedentary lifestyle, in the setting of a genetic predisposition, are considered the prime etiologic factors of metabolic syndrome.[3,6–8] Indeed obesity[9] and the lack of exercise[10,11] are two modifiable lifestyle factors that have been associated with the metabolic syndrome in adolescents.

The lack of physical fitness has also been associated with the development of CVD risk factors in youth, such as lipid

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disorders, high blood pressure, and insulin resistance, among others.[12] Likewise, some studies have shown that the level of physical fitness in adults is conditioned by the level of physical fitness in childhood or adolescence.[13,14] However, the majority of the studies reported the relationship between physical fitness and metabolic syndrome is confined to cardiorespiratory fitness,[15–17] and, to our knowledge, no studies have been conducted among Portuguese adolescents assessing other health-related fitness outcomes associated with metabolic syndrome. Until now, there have been no data available on Azorean adolescents and the prevalence of metabolic syndrome.

In this context, the aims of this study were to estimate the prevalence of metabolic syndrome using the 2007 IDF criteria and its components, and to analyze the relationship between metabolic syndrome and overall physical fitness levels in a sample of Azorean adolescents.

Subjects and Methods

Study design and sampling

Data for the present study derived from a longitudinal school-based study, the Azorean Physical Activity and Health Study II, aimed to evaluate physical activity, physical fitness, overweight/obesity prevalence, health-related quality of life, and related factors. The study was carried out in six of the nine Azorean Islands, where 95% of the population live[18]—S. Miguel, Terceira, Faial, Pico, S. Jorge, and Graciosa.

All participants in this study were informed about the objectives of the work, and the parent or guardian of each participant provided written informed consent. The study was approved by the faculty and the Portuguese Foundation for Science and Technology ethics committee and was conducted in accordance to the declaration of Helsinki for Human Studies of the World Medical Association.

The population was selected by means of a proportionate stratified random sampling, taking into account the location (island) and the number of students by age and sex in each school. The established number of subjects was 1,422, but the sample was oversized to prevent loss of information and because technically it was necessary to do the fieldwork in complete classrooms. Therefore, data were collected for 1,525 adolescents. Twenty-five subjects aged <14.5 or >18.4 and/or having a health condition that did not allow participating in physical education classes, or had missing information on the variables of interest, were excluded, resulting in a total of 1,500 participants (892 girls and 698 boys).

Baseline data were collected in the fall of 2008, and 850 subjects were reevaluated 1 year later (mean follow-up length 11.5 ± 2.0 months). Of the 850 adolescents that participated in the 2009 data collection, 36 were excluded due to lack of information on several variables, and 297 refused to undergo blood sampling measurement. Therefore, the final sample included in this study was comprised of 517 adolescents (297 girls and 220 boys) aged 15–18 years (mean age 16.5 ± 1.0) who were evaluated in the fall of 2009 and had complete data on the variables of interest. There were no differences in the variables of interest between subjects who underwent blood collection measurement and those who refused to do so (data not shown).

Subjects were evaluated during school physical education classes by physical education teachers specially trained for this data collection (anthropometric measures, physical fitness, and questionnaires). Blood sampling and blood pressure were measured by nurses in a local hospital.

Anthropometric measures

Body height was measured to the nearest millimeter in bare or stocking feet with the adolescent standing upright against a stadiometer (Holtain Ltd., Crymmych, Pembrokeshire, UK). Weight was measured to the nearest 0.10 kg, lightly dressed using a portable electronic weight scale (Tanita Inner Scan BC 532). Body mass index (BMI) was calculated from the ratio of body weight (kg) to body height (m^2). Adolescents were categorized as non-overweight, overweight, and obese, applying the cut-off points suggested by the International Obesity Task Force.[19]

Waist circumference measurement was taken in a standing position, to the nearest 0.1 cm, with a tape measure midway between the lower rib margin and the anterior superior iliac spine at the end of normal expiration.[20]

Blood pressure

Blood pressure (BP) was measured using the Dinamap adult/pediatric vital signs monitors, model BP 8800 (Critikon, Inc., Tampa, Florida). Measurements were taken by nurses, and all adolescents were required to sit and rest for at least 5 min prior to the BP test. Two measurements were taken, after 5 and 10 min of rest. The mean of these two measurements was considered. If the two measurements differed by 2 mmHg or more, a third measure was taken.

Blood sampling

The blood samples were collected from the antecubital vein between 8:00 and 10:00 a.m., in a sitting position after 10 h of fasting. The blood samples were drawn in vacuum tubes gel (Sarstedt) to obtain values of plasmatic high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), and glucose. After resting at room temperature for about 30 min, the samples were centrifuged for 10 min at 3,000 rpm to obtain serum. Samples were divided into aliquots, separated within 30 min, and stored at $-80°C$ until analyzed. The following analyses were measured on a Cobas Integra 400 Plus, (Roche): HDL-C using the precipitation of the apolipoprotein B containing lipoproteins with dextran-magnesium chloride; TG using the glycerokinase-glycerolphosphateoxidase-peroxidase (GK-GPO-POD) method, and glucose using the hexokinase method. The biochemical evaluation of all participants from the different islands was conducted in the same laboratory.

Metabolic syndrome

Metabolic syndrome was defined using the adolescent 2007 IDF criteria.[5] According to this definition, a waist circumference above the 90th percentile for age and sex for adolescents aged <16 years old (or adult cutoff if 90th percentile is lower); and adult cutoff for adolescents aged $\geq$16 years old ($\geq$80 cm for girls and $\geq$94 cm for boys) is a mandatory criterion. For adolescents aged <16 years old, we used the waist circumference percentiles (sex- and age-specific 90th percentiles) for British children,[21] as previously used in Portuguese adolescents.[22] In addition, the presence of two or more of the following components were also required:

TG $\geq$150 mg/dL; HDL-C <40 mg/dL for both genders, except for HDL-C <50 mg/dL for girls age $\geq$16 years; fasting glucose $\geq$100 mg/dL; and systolic blood pressure (SBP) $\geq$130 mmHg or diastolic blood pressure (DBP) $\geq$85 mmHg.

Pubertal stage

To determine the pubertal stage (ranging from stage 1 to 5), each subject was asked to self-assess his/her stage of secondary sex characteristics. Stages of breast development in girls and genital development in boys were evaluated according to the criteria of Tanner and Whitehouse.[23]

Socioeconomic status

The highest level of parental education (in completed years of education) was considered as a proxy of socioeconomic status. Similar procedures had previously been applied in the Portuguese context.[24]

Physical fitness

Health-related components of physical fitness were evaluated using the Fitnessgram Test Battery, version 8.0. The Fitnessgram is included in the physical education curriculum, and the five tests recommended in the Portuguese National Program (curl-up, push-up, trunk lift, pacer, and the modified back saver sit-and-reach) were used in this study. All tests were conducted according to the Fitnessgram measurement procedures.[25]

The pacer test was used to evaluate cardiorespiratory fitness. This test requires participants to run back and forth between two lines set 20 m apart. Running speed started at 8.5 km/h and increased by 0.5 km/h each minute, reaching 18.0 km/h at minute 20. Each level was announced on the tape. The participants were told to keep up with the pacer until exhausted. The test was finished when the participant failed to reach the end lines concurrent with the audio signals on two consecutive occasions. Otherwise, the test ended when the subject stopped because of fatigue. Participants were encouraged to keep running as long as possible throughout the course of the test. Numbers of shuttles performed were recorded.

The curl-up test was used to evaluate abdominal strength and endurance. Participants lie down with knees bent and feet unanchored. Set to a specified pace, adolescents complete as many repetitions as possible to a maximum of 75.

The push-up test was used to evaluate upper body strength and endurance. Participants lower the body to a 90-degree elbow angle and push up. Set to a specified pace, adolescents complete as many repetitions as possible.

The trunk lift test was used to evaluate trunk extensor strength. Participants lie face down and slowly raise their upper body long enough for the tester to measure the distance between the floor and the adolescent's chin, up to a maximum of 30 cm.

The modified back saver sit-and-reach test was used to evaluate the flexibility. Testing one leg at a time, adolescents sit with one knee bent and one leg straight against a box and reach forward. In this study we considered the better of the two results (right and left leg).

According to the results of each test, adolescents were then classified according to the age and sex-specific cut-off points of Fitnessgram criteria, as belonging to the healthy zone or above or under the healthy zone. Adolescents were then classified in three groups: "Fittest" if they had reached the healthy zone or above healthy zone in four or five tests, "fit" if they had reached the healthy zone or above healthy zone in three tests, and "unfit" if they had reached the healthy zone or above healthy zone in two or fewer tests.

Statistical analysis

Descriptive data are presented as means and standard deviation unless otherwise stated. All variables were checked for normality and appropriately transformed if necessary. The HDL-C, TG, SBP, and waist circumference were transformed logarithmically. Independent sample *t*-tests with Bonferroni corrections were performed to compare sexes in continuous variables, and the chi-squared test was used in categorical variables. Binary logistic regression was used to study the relationship between metabolic syndrome and overall physical fitness levels further, adjusting for pubertal stage and socioeconomic status. Data were analyzed with SPSS for Windows (version 17.0). A P value under 0.05 denoted statistical significance.

Results

Descriptive characteristics of the adolescents are shown in Table 1. Boys had higher levels of height, weight, waist circumference, SBP, and glucose than girls ($P < 0.05$ for all), whereas girls had higher values of HDL-C and TG ($P < 0.05$ for both). No differences in age, BMI, and DBP were observed between sexes.

The prevalence of metabolic syndrome and its components according to the 2007 IDF definition is presented in Table 2. The overall prevalence of metabolic syndrome was 5%, and there was no sex difference (girls 4.7%, boys 5.5%, $P > 0.05$). The large waist circumference (32.9%) was the most prevalent component of the metabolic syndrome criteria, followed by high blood pressure (20.1%), and hypertriglyceridemia was the least (4.4%). The prevalence of hyperglycemia, and high blood pressure in boys was significantly higher than in girls, whereas the prevalence of abdominal obesity was significantly higher in girls ($P < 0.001$ for all). The prevalence of metabolic syndrome rose with the increase of BMI: 1.4% in not overweight, 7.6% in overweight, and 34.3% in obese adolescents.

Adolescent's overall physical fitness levels showed that 26.3% were considered unfit. The results in Table 3 show that the prevalence of metabolic syndrome is higher among those under the healthy zone in the push-up and pacer tests ($P < 0.001$ for both).

Logistic regression analyses showed that unfit adolescents were more likely [odds ratio (OR) = 3.414; 95% confidence interval (CI), 1.150–10.129) to be classified as having metabolic syndrome when compared to the fittest ones, after adjusting for pubertal stage and socio-economic status (Table 4).

Discussion

To our knowledge, this is the first study to report the prevalence of metabolic syndrome and to analyze the relationship between metabolic syndrome and overall physical fitness levels in a sample of Azorean adolescents. In this

TABLE 1. DESCRIPTIVE CHARACTERISTICS OF THE ADOLESCENTS

Variables	*Total* (n = *517*)	*Girls* (n = *297*)	*Boys* (n = *220*)
Age, years	16.5 ± 1.0	16.5 ± 1.0	16.5 ± 1.0
Height, cm	165 ± 12.8	160 ± 10.1	172 ± 12.6
Weight, kg	63.1 ± 12.5	58.6 ± 10.0	69.1 ± 12.9*
BMI, kg/m^2	22.9 ± 3.7	22.8 ± 3.6	23.1 ± 4.0
Waist circumference, cm	79.3 ± 10.7	78.3 ± 10.3	80.7 ± 11.1*
SBP, mm Hg	115.2 ± 15.0	111.8 ± 13.6	119.7 ± 15.7*
DBP, mm Hg	66.6 ± 9.5	66.7 ± 10.0	66.3 ± 8.8
HDL-C, mg/dL	55.5 ± 13.3	59.4 ± 12.9	50.3 ± 11.9*
Triglycerides, mg/dL	71.6 ± 37.9	74.5 ± 37.3	67.7 ± 38.4*
Glucose, mg/dL	86.8 ± 9.2	84.6 ± 8.7	89.7 ± 9.1*
BMI (%)			
Non-overweight	70.4%	71.0%	69.5%
Overweight	22.8%	23.2%	22.3%
Obesity	6.8%	5.7%	8.2%

Data are means ± SD, Student *t*-test, with Bonferroni corrections for differences between gender: *$p < 0.05$.
Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; SD, standard deviation.

study, the prevalence of metabolic syndrome was 5%. Within the metabolic syndrome components, abdominal obesity was the most prevalent and hypertriglyceridemia the least observed in our study. Unfit adolescents were more likely to have metabolic syndrome than fit adolescents.

Several studies have established the prevalence of metabolic syndrome during childhood and adolescence,[26–29] however, with different definitions of the syndrome. This fact limits the ability to compare prevalence rates across studies. The first national report of the metabolic syndrome in U.S. adolescents was published in 2003,[30] and it used a definition adapted from the National Cholesteroal Education Program Adult Treatment Panel III (NCEP ATP III) adult criteria. The results of Cook's study indicate that the prevalence of the syndrome among adolescents from ages 12 to 19 was 4.2%. However, current estimates indicate that 2% to 9.4% of United States adolescents have metabolic syndrome.[31] In the developed countries, the overall prevalence of metabolic syndrome in healthy children and adolescents based on the same modified NCEP ATP III criteria is estimated to be between 2.0 and 11.5%.[27]

TABLE 2. PREVALENCE OF METABOLIC SYNDROME AND ITS COMPONENTS ACCORDING TO THE INTERNATIONAL DIABETES FEDERATION 2007 CRITERIA

	Girls (n = *297*)	*Boys* (n = *220*)	*Total* (n = *517*)	p[a]
Components of metabolic syndrome				
Large waist circumference	46.8	14.1	32.9	<0.001
Hypertriglyceridemia	5.1	3.6	4.4	0.441
Low HDL-C	20.9	17.3	19.3	0.305
Hyperglycemia	3.7	13.6	7.9	<0.001
High blood pressure	15.2	26.8	20.1	<0.001
Metabolic syndrome	4.7	5.5	5	0.703
Metabolic syndrome within BMI categories				
Non-overweight	2.4	0	1.4	
Overweight	7.2	8.2	7.6	<0.001
Obesity	23.5	44.4	34.3	

Data are %.
[a]Chi-squared test.
Abbreviations: HDL-C, high-density lipoprotein cholesterol; BMI, body mass index.

The new IDF (2007) pediatric definition of the metabolic syndrome provides a standard that facilitates comparisons on the prevalence of metabolic syndrome across studies. The prevalence of metabolic syndrome in this study is slightly higher than the prevalence previously reported,[22,32–34] which used the same definition of the syndrome. In U.S. adolescents from age 12 to 17, the prevalence of metabolic syndrome was ~4.5% and was relatively stable across the 6-year period: 4.5% for 1999–2000, 4.4%–4.5% for 2001–2002, and 3.7%–3.9% for 2003–2004.[32] The prevalence of metabolic syndrome in Korean adolescents was 2.6%[34] and in 16-year-old adolescents participating in the Northern Finish Birth Cohort examined between 2001 and 2002, the prevalence was 2.4%.[33] Metabolic syndrome was diagnosed in 0.2% of 10-year-old children and in 1.4% of 15-year-old children.[22] Recently Park et al. reported that the prevalence of metabolic syndrome in adolescents from the United States and Korea was 5.5% and 2.5%, respectively.[35] In this last study, the prevalence of metabolic syndrome in U.S. adolescents was slightly higher than our result (5.5% vs. 5%). The prevalence of metabolic syndrome reported in our study is slightly higher than studies mentioned above.

The most prevalent component of the metabolic syndrome in this study was increased waist circumference, followed by high blood pressure; hypertriglyceridemia was the least prevalent. The measure of abdominal obesity and the waist circumference have been consistently identified as better measures of cardiovascular risk than the BMI.[36,37] Data from Bogalusa Heart Study[38] demonstrated significant correlations between waist circumference and cardiovascular disease risk factor levels in children and adolescents. Waist circumference has been shown to be the best predictor of metabolic syndrome in children[39] and in different adult populations.[40] In this study, the prevalence of abdominal obesity in girls was higher than in boys, although the BMI was slightly higher in boys than girls. Similar results were

TABLE 3. RELATIONSHIP BETWEEN METABOLIC SYNDROME AND PHYSICAL FITNESS

	No metabolic syndrome n (%)	*Metabolic syndrome* n (%)	P[a]
Best of sit-and-reach			0.63
Under healthy zone	26 (5.3)	0 (0)	
Healthy zone or above	465 (94.7)	26 (100)	
Curl-up			0.47
Under healthy zone	120 (24.4)	8 (30.8)	
Healthy zone or above	371 (75.6)	16 (69.2	
Push-up			0.001
Under healthy zone	195 (39.7)	19 (73.1)	
Healthy zone or above	296 (60.3)	7 (26.9)	
Pacer			<0.001
Under healthy zone	279 (56.8)	26 (100)	
Healthy zone or above	212 (43.2)	0 (0)	
Trunk lift			0.44
Under healthy zone	227 (46.2)	10 (38.5)	
Healthy zone or above	264 (56.8)	16 (61.5)	
Healthy zone or above in ≤2 or tests	125 (22.5)	11 (42.3)	0.58
Healthy zone or above in 3 tests	166 (33.8)	10 (38.5)	
Healthy zone or above in 4 or 5 tests	200 (40.7)	5 (19.2)	

[a]Chi-squared test.

reported by Park.[35] These differences can be explained by a gender-variable genetic predisposition to the accumulation of visceral fat. Indeed, the gonadarche occurs earlier in girls than boys, reflecting the earlier maturation of the hypothalamic–pituitary–gonadal axis, and waist circumference seems to reflect in girls the increasing fat content, whereas in boys it reflects the predominant lean tissue mass.[41] Moreover, BMI is a measure of excess weight rather than excess body fatness[42] and does not have the capacity to differentiate body fat and lean body mass, or even the distribution of fat. Nevertheless, these findings of an increased waist circumference suggest that Azorean adolescents may be at an increased risk of comorbidities associated with abdominal obesity, including type 2 diabetes.

The emergence of metabolic syndrome parallels the rising rates of overweight and obesity observed in youth worldwide.[43] Indeed, various studies have shown that the prevalence of the syndrome was higher when the subjects were obese.[44–47] Cruz et al. demonstrated that metabolic syndrome was present in 30% of the overweight and obese children.[29] These findings are in agreement with our study showing that metabolic syndrome in adolescents is largely confined to the overweight population and may represent a subgroup of youth who carry a larger risk for CVD and type 2 diabetes later in life.

Fitness has been proposed as a major marker of health status at any age.[48] Our results showed a positive influence of overall physical fitness levels on metabolic syndrome. Fit adolescents had less prevalence of the syndrome than unfit adolescents, and low levels of overall fitness were a positive predictor of metabolic syndrome. Ruiz et al. have shown an inverse association between cardiorespiratory fitness and clustered metabolic risk factors in 9- to 10-year-old Swedish and Estonian children.[49] Reinforcing this idea, Ortega et al.[50] reported that children and adolescents with higher levels of cardiorespiratory fitness also have a more favorable cardiovascular profile compared with their unfit counterparts. Conversely, other studies have shown that high levels of cardiorespiratory fitness and physical activity are associated with a favorable metabolic risk profile.[51,52] On the other hand, evidence suggests that sedentary behavior and low levels of physical activity and cardiorespiratory fitness in youth track into adulthood,[53,54] and may predispose young people to disease later in life.[13] Nevertheless, future cardiovascular risk seems to be more conditioned by the physical fitness attained (especially strength and aerobic capacity) than by the level of physical activity undertaken.[55] Our study is in line with these investigations and extends previous findings by showing that overall fitness capacity and only cardiorespiratory fitness are negatively associated with the presence of the metabolic syndrome.

This study is limited because it consisted of a cross-sectional design, which limits inferences about causality and its direction, as well as the convenient sample. Balancing these limitations are the strengths of this study. This is the first study to estimate the prevalence of the metabolic syndrome in a sample of Azorean adolescents applying the new 2007 IDF pediatric definition. The use of field tests for fitness assessment, which can be administered in school settings while a large number of subjects can be tested simultaneously,

TABLE 4. LOGISTIC REGRESSION ANALYSES PREDICTING THE METABOLIC SYNDROME

	OR (95% CI)	P *value*
Fittest		
Healthy zone or above in ≥4 tests	1	
Fit[a]		
Healthy zone or above in 3 tests	2.293 (0.764–6.887)	0.139
Unfit[a]		
Healthy zone or above in ≤2 tests	3.414 (1.150–10.129)	0.027

[a]Adjusted for pubertal stage and socioeconomic status.
Abbreviations: OR, odds ratios; CI, confidence intervals; 1, reference category.

enhances participant motivation, making it a valuable tool for studying physical fitness in youth.

Conclusions

The results of this study indicate that prevalence of metabolic syndrome in this sample of Azorean adolescents is slightly higher than other studies that used the same definition according to the newly released 2007 IDF definition. Unfit adolescents were more likely to have metabolic syndrome than fit adolescents. These data also showed that a low waist circumference would be potentially important in reducing the prevalence of metabolic syndrome. Therefore, improving overall physical fitness levels and waist circumference reduction may be important strategies to overcome this public health problem and its consequences.

Our results have important public health implications. Identification of the percentage of adolescents who had abdominal obesity and those who were unfit can help identify youths at increased risk of metabolic diseases and can emphasize the importance of promoting healthy lifestyle habits at these ages and maintaining these practices into and throughout adulthood.

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Author Disclosure Statement

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Paper II

Ability of different measures of adiposity to identify high metabolic risk in adolescents

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Research Article

Ability of Different Measures of Adiposity to Identify High Metabolic Risk in Adolescents

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Introduction. This study aimed to evaluate the screening performance of different measures of adiposity: body mass index (BMI), waist circumference (WC), and waist-to-height ratio (WHtR) for high metabolic risk in a sample of adolescents. *Methods*. A cross-sectional school-based study was conducted on 517 adolescents aged 15–18, from the Azorean Islands, Portugal. We measured fasting glucose, insulin, total cholesterol (TC), HDL-cholesterol, triglycerides, and systolic blood pressure. HOMA and TC/HDL-C ratio were calculated. For each of these variables, a Z-score was computed by age and sex. A metabolic risk score (MRS) was constructed by summing the Z-scores of all individual risk factors. High risk was considered when the individual had ≥1SD of this score. Receiver-operating characteristics (ROC) were used. *Results*. Linear regression analyses showed that, after adjusting for age and pubertal stage, all different measures of adiposity are positively and significantly associated with MRS in both sexes, with exception of WHtR for boys. BMI, WC, and WHtR performed well in detecting high MRS, indicated by areas under the curve (AUC), with slightly greater AUC for BMI than for WC and WHtR in both sexes. *Conclusion*. All measures of adiposity were significantly associated with metabolic risk factors in a sample of Portuguese adolescents.

1. Introduction

The prevalence of overweight and obesity is high in most parts of the world, and this is particularly alarming not only for the increasing risk of multiple comorbidities [1], but also due to the tendency of childhood overweight and obesity to track into adulthood [2].

The metabolic syndrome (MetS), a cluster of several cardiovascular disease risk factors, is a complex entity of metabolic disorders that significantly increases the risk of type II diabetes and cardiovascular disease beyond that of its individual components [3]. The emergence of MetS parallels the rising rates of overweight and obesity observed in youth worldwide [4].

A variety of anthropometric indices have been used as a proxy for total and abdominal fat to assess risk for diseases, particularly cardiovascular diseases (CVD) and diabetes [5]. Epidemiologic studies have shown that body mass index (BMI), the most widely recognised measure of obesity, is a powerful predictor of CVD [6]. Nevertheless, waist circumference (WC) and waist-to-height ratio (WHtR) measures of central obesity have yielded important insight as well and also provide information on the risk of CVD [7].

Although different anthropometrical measures of obesity have been proposed, it remains unclear which measures of adiposity best predict the role of metabolic risk factors. The aim of this study was to determine the ability of different measures of adiposity, namely, BMI, WC, and WHtR to discriminate between low/high metabolic risk using receiver-operating characteristic (ROC) curves in a sample of Portuguese adolescents from the Azorean Archipelago.

2. Methods

2.1. Study Design and Sampling. Data for the present study derived from a longitudinal school-based study, the Azorean

Physical Activity and Health Study II, aimed to evaluate physical activity, physical fitness, overweight/obesity prevalence, health-related quality of life, and related factors. Details on the study design and sampling strategy are reported elsewhere [8]. The final sample included in this cross-sectional analysis was comprised of 517 adolescents (297 girls and 220 boys) aged 15 to 18.

All participants in this study were informed about the objectives of the work, and the parent or guardian of each participant provided written informed consent. The study was approved by the faculty and the Portuguese Foundation for Science and Technology ethics committee and conducted in accordance with the Declaration of Helsinki for Human Studies of the World Medical Association.

2.2. Anthropometric Measures. Height was measured to the nearest millimeter in bare or stocking feet with the adolescent standing upright against a stadiometer (Holtain Ltd., Crymmych, Pembrokeshire, UK). Weight was measured to the nearest 0.10 kg, with adolescents lightly dressed using a portable electronic weight scale (Tanita Inner Scan BC 532). BMI was calculated as body weight (kg) divided by body height (m^2). WC measurements were taken as described by Lohman et al. [9]. The waist and height were used to compute the WHtR.

2.3. Pubertal Stage. To determine the pubertal stage (ranging from stage 1 to 5), each subject was asked to self-assess his/her stage of secondary sex characteristics. Stages of breast development in girls and genital development in boys were evaluated according to the criteria of Tanner and Whitehouse [10].

2.4. Blood Sampling. Blood samples were collected from the antecubital vein between 8:00 and 10:00 a.m., in a sitting position after ten hours of fasting. Blood samples were drawn in vacuum tubes gel (Sarstedt) in order to obtain values of plasmatic total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), glucose, and insulin. The following analyses were measured on a Cobas Integra 400 Plus (ROCHE Diagnostics, Indianapolis, Ind, USA): TC, HDL-cholesterol, TG, and glucose. The fasting serum insulin was measured on an Immulite 2000, (Diagnostic Products Corporation, Los Angeles, Calif). The ratio of total cholesterol to HDL-C was calculated as an index of atherogenic lipid profile [11]. The homeostatic model assessment (HOMA), calculated as the product of basal glucose and insulin levels divided by 22.5, was used as a proxy measure of insulin resistance [12]. The biochemical evaluation of all participants from the different islands was conducted in the same laboratory.

2.5. Blood Pressure. Blood pressure (BP) was measured using the Dynamap adult/pediatric vital signs monitors, model BP 8800 (Critikon, Inc., Tampa, Fla, USA). Measurements were taken by trained nurses, and with all adolescents were required to sit and rest for at least five minutes prior to the BP test. The participants were in a seated, relaxed position with their feet resting flat on the ground. Two measurements in the right arm were taken after five and ten minutes of rest. The mean of these two measurements was considered. If the two measurements differed by 2 mmHg or more, a third measure was taken.

2.6. Metabolic Risk Score. Since there is no consensus regarding the establishment of a universal criterion for definition of the MetS in adolescents, we decided to compute a continuous metabolic risk score (MRS) from the following measurements: TC/HDL-C ratio, TG, HOMA, and systolic BP. For each of these variables, a Z-score was computed by age and sex. Then, an MRS was constructed by summing the Z-scores of all individual risk factors. High risk was considered when the individual had ≥1 SD of this score. The score only applies to this study population. A similar Z-score approach has been used previously in children and adolescents [13].

2.7. Statistical Analysis. Comparisons between groups involved Student *t*-test for continuous variables. Linear regression analyses were used to study the relationship between different measures of adiposity and low and high MRS, adjusting for age and pubertal stage. Receiver-operating characteristic (ROC) curve analyses were used to analyse the potential diagnostic accuracy of the different measures of adiposity to discriminate between low and high MRS. The area under the curve (AUC) and 95% confidence interval (CI) were calculated. The AUC represents the ability of the test to correctly classify adolescents having a low and high MRS. The values of AUC range between 1 (perfect test) and 0.5 (worthless test). Data were analyzed using the PASW Statistic v.18 (SPSS, Chicago, Ill, USA) and Med Calc software v.10.4.5 (MedCalc Software, Mariakerke, Belgium). A P value under 0.05 denoted statistical significance.

3. Results

Descriptive characteristics of the adolescents are shown in Table 1. Girls had lower height, weight, WC, systolic BP, glucose, and TC/HDL-C levels and higher TC and HDL-C than boys ($P < 0.05$ for all). All adolescents reported to be in Tanner stage 4 or 5.

Linear regression analyses, adjusted for age and pubertal stage, showed that BMI (girls: $\beta = 0.291$, 95% CI: 0.219–0.364, $P < 0.001$; boys: $\beta = 0.396$, 95% CI: 0.324–0.468, $P < 0.001$), WC (girls: $\beta = 0.086$, 95% CI: 0.060–0.111, $P < 0.001$; boys: $\beta = 0.121$, 95% CI: 0.093–0.150, $P < 0.001$), and WHtR (girls: $\beta = 1.738$, 95% CI: 0.555–2.922, $P = 0.004$; boys: $\beta = 1.220$, 95% CI: −0.209–2.648, $P = 0.09$) were positively and significantly associated with MRS in both sexes, with exception of WHtR for boys.

ROC curve analysis showed that all measures of adiposity performed well on average in identifying high MRS, as indicated by AUC greater than 0.7. The ROC performance of BMI showed a better discriminatory accuracy than WC and WHtR in predicting high MRS in both sexes. In boys, the ROC performance of BMI was slightly better than in

Table 1: Descriptive characteristics of the study sample.

Variables	Total (n = 517)	Girls (n = 297)	Boys (n = 220)
Age, years	16.5 ± 0.9	16.5 ± 1.0	16.4 ± 0.8
Height, cm	165.0 ± 13.6	160.0 ± 11.0	170.2 ± 1.0*
Weight, kg	63.1 ± 12.5	58.6 ± 10.0	69.3 ± 13.1*
BMI, kg/m^2	22.9 ± 3.7	22.7 ± 3.5	22.7±3.5
Waist circumference, cm	79.3 ± 10.7	78.3 ± 10.3	80.7 ± 11.1*
Systolic BP, mmHg	115.2 ± 15.3	111.8 ± 13.8	120.0 ± 16.1*
Diastolic BP, mmHg	66.4 ± 9.4	66.3 ± 10.0	66.5 ± 8.6
Total cholesterol, mg/dL	161.9 ± 32.3	169.3 ± 33.6	151.8 ± 27.5*
HDL-cholesterol, mg/dL	55.6 ± 13.4	59.3 ± 13.0	50.5 ± 12.3*
Triglycerides, mg/dL	70.7 ± 35.1	72.5 ± 34.6	68.1 ± 35.6
Total cholesterol/HDL-C ratio	3.0 ± 0.7	2.9 ± 0.8	3.1 ± 0.7*
Fasting glucose, (mg/dL)	86.8 ± 9.2	84.6 ± 8.7	89.7 ± 9.1*
Fasting insulin, (uU/mL)	9.1 ± 6.0	9.3 ± 5.1	8.7 ± 7.0
HOMA	1.9 ± 1.3	1.9 ± 1.1	1.9 ± 1.6
Metabolic risk score[a]	0.1 ± 3.1	0 ± 3.1	0.1 ± 3.2

Data are means ± standard deviations. *$P < 0.05$ for sex comparisons (one-tailed t-test). BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; [a]obtained by summing individual risk factors (total cholesterol/HDL-C ratio, triglycerides, HOMA, and systolic blood pressure) age- and sex-standardized scores.

girls. The AUC of BMI were significantly different from WC ($P < 0.05$) for the whole sample. In girls, the AUC of WC was significantly different from WHtR ($P < 0.05$).

A BMI of 23.7 kg/m^2 for girls and 27.0 kg/m^2 for boys, a WC of 83 cm for girls and 92 cm for boys, and a WHtR of 0.55 for girls and 0.49 for boys were found to be optimal cutoffs for defining high MRS in this adolescent population (Table 2).

4. Discussion

The main findings of this study suggested that BMI provides a marginally superior tool for discriminating high MRS compared with WC and WHtR, for both sexes. Slightly higher pooled AUC were observed in boys compared to girls, suggesting that discrimination is more precise, on average, in male. Linear regression analyses showed that all different measures of adiposity were positively and significantly associated with MRS in both sexes, with the exception of WHtR for boys.

Jung et al. showed that BMI had the best predictive power to identify metabolic syndrome, its components, and markers for low-grade inflammation [14], which is in agreement with our results. The BMI and WC are widely used to define overweight and obesity across populations [15]. The BMI has been used to predict body composition and health risk [16], whereas WC indicates visceral adipose tissue and can predict health risks in children [17]. Both BMI and WC are simple measures to use and interpret, yet they have some limitations. The BMI does not distinguish fat mass from fat-free mass or between different body fat distributions [18], and for WC, there are currently no agreements about a health-related classification for children and adolescents. Several previous studies considered the 90th percentile as a cut-off point for high WC, whereas other studies consider the 75th or 70th percentile as a cutoff point. Both of these measures are also age and-sex dependent.

The WHtR has been significantly associated with cardiovascular risk factors, due to abdominal obesity both in adults and children [19]. The higher AUC value for WHtR than for WC could be due to the fact that WHtR takes into account differences in body height. Contrary to our findings, some studies have shown that WHtR is better for classifying obesity related to cardiovascular risk than BMI and WC [5, 20]. In girls, the AUC value for WHtR is slightly better than AUC value for WC. Despite the AUC value for BMI was slightly higher than the other two measures in girls, the AUC value for WHtR showed higher sensibility than AUC value for BMI.

There is no agreement for which anthropometrical measures of adiposity best predict a role of unfavorable cardiovascular risk factors. Studies carried out among children and adolescents in Cyprus [21] and Japan [5] concluded that both WC and WHtR are better predictors of TC, TG, HDL-cholesterol, low-density lipoprotein cholesterol, and systolic and diastolic BP levels than BMI. Also, the Bogalusa study reported that WC and WHR were related to adverse levels of TG and HDL-cholesterol, independently of race, sex, age, weight, and height [22]. Conversely, BMI is the measure of obesity most used and has been shown to be extremely effective when used in longitudinal studies [7].

Nevertheless, we were unable to draw cause-effect conclusions and to make observations over the time because of the cross-sectional nature of our data and the multifactorial etiology of high MRS. However, the association between these measures of adiposity and the clustering of metabolic risk factors has not been studied in this population, making it one of the strengths of this

Table 2: Cut-off values, sensitivity, and specificity for the association of different measures of adiposity with metabolic risk score by sex.

	All	Girls	Boys
BMI			
BMI cut-off (kg/m^2)	> 23.6	> 23.7	> 27.0
Sensitivity (%)	79.4	76.3	66.7
Specificity (%)	72.8	71.8	94.7
AUC	0.807 (0.770–0.840) $P < 0.001^{\ddagger}$	0.772 (0.720–0.818) $P < 0.001$	0.852 (0.798–0.896) $P < 0.001$
WC			
WC cut-off (cm)	> 84	> 83	> 92
Sensitivity (%)	66.2	65.8	60.0
Specificity (%)	79.7	75.3	94.7
AUC	0.760 (0.721–0.796) $P < 0.001$	0.714 (0.659–0.764) $P < 0.001^{\dagger}$	0.827 (0.770–0.874), $P < 0.001$
WHtR			
WHtR cut-off	> 0.54	> 0.55	> 0.49
Sensitivity (%)	61.8	60.5	80.0
Specificity (%)	89.5	90.0	80.0
AUC	0.794 (0.756–0.828) $P < 0.001$	0.767 (0.715–0.814) $P < 0.001$	0.834 (0.778– 0.881), $P < 0.001$

AUC: area under the curve; 95% CI in parentheses; ‡AUC significantly different from WC ($P < 0.05$); †AUC significantly different from WHtR ($P < 0.05$).

study. Further studies are needed to confirm or contrast our findings.

5. Conclusion

In conclusion, BMI, WC, and WHtR are all predictors of high MRS. Despite the small differences in the discriminatory capabilities among measures of adiposity, making it difficult to recommend the best measure of obesity, BMI seems to have the best trade-off between sensitivity and specificity to screen for high MRS in both sexes.

Conflict of Interests

All the authors declare not to have any conflict of interests.

Acknowledgments

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Paper III

Metabolic Risk Factors, Physical Activity and Physical Fitness in Azorean Adolescents: a cross-sectional study

Moreira, C., Santos, R., Cazuza, J., Vale, S., Santos, P. C., Soares-Miranda, L., Marques, A. I., Mota, J.

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RESEARCH ARTICLE Open Access

Metabolic risk factors, physical activity and physical fitness in azorean adolescents: a cross-sectional study

Carla Moreira[1*], Rute Santos[1], José Cazuza de Farias Júnior[2], Susana Vale[1], Paula C Santos[1,3], Luísa Soares-Miranda[1], Ana I Marques[1] and Jorge Mota[1]

Abstract

Background: The prevalence of metabolic syndrome has increased over the last few decades in adolescents and has become an important health challenge worldwide. This study analyzed the relationships between metabolic risk factors (MRF) and physical activity (PA) and physical fitness (PF) in a sample of Azorean adolescents.

Methods: A cross-sectional school-based study was conducted on 417 adolescents (243 girls) aged 15-18 from the Azorean Islands, Portugal. Height, weight, waist circumference, fasting glucose, HDL-cholesterol, triglycerides, and blood pressure were measured. A sum of MRF was computed, and adolescents were classified into three groups: no MRF, one MRF and two or more MRF. PA was assessed by a sealed pedometer. PF was assessed using five tests from the Fitnessgram Test Battery. Dietary intake was obtained using a semi-quantitative food frequency questionnaire.

Results: Mean daily steps for girls and boys were 7427 ± 2725 and 7916 ± 3936, respectively. Fifty-nine percent of the adolescents showed at least one MRF and 57.6% were under the healthy zone in the 20 m Shuttle Run Test. Ordinal logistic regression analysis showed that after adjusting for sex, body mass index, socio-economic status and adherence to a Mediterranean diet, adolescents who were in the highest quartile of the pedometer step/counts (≥9423 steps/day) and those who achieved the healthy zone in five tests were less likely to have one or more MRF (OR = 0.56;95%CI:0.33-0.95; OR = 0.55;95%CI:0.31-0.98, respectively).

Conclusions: Daily step counts and PF levels were negatively associated with having one or more MRF among Azorean adolescents. Our findings emphasize the importance of promoting and increasing regular PA and PF to reduce the public health burden of chronic diseases associated with a sedentary lifestyle.

Background

Metabolic Syndrome (MetS) comprises a major risk for chronic disease and is rapidly increasing in prevalence worldwide in association with rising childhood obesity and a sedentary lifestyle [1]. Emerging evidence suggests that children who have clustered MRF are at increased risk for developing cardiovascular diseases and type II diabetes in adulthood [2]. The most recognized cardiovascular disease risk factors are HDL-cholesterol, total cholesterol, triglycerides, total and central body fat, insulin resistance, blood pressure, and cardiorespiratory fitness (CRF). The prevalence of adolescents who are physically inactive, in conjunction with the rising prevalence of obesity, are a major threat to health in the twenty-first century [3]. Thus, adolescence is a critical period, because this is when the individual takes control of his/her lifestyle. It is during this period that engagement in PA might contribute to a physically active lifestyle that lasts into adulthood [4].

Regular PA is reported to be a protective factor against several diseases, such as obesity, hypertension, type II diabetes, [3] and MetS [5]. The use of pedometers to quantify PA has led to the development of guidelines for recommended steps/day for children

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(aged 6-12). Recommendations range from 11,000 to 15,000 steps/day for girls and boys, respectively [6,7]. However, there are no internationally accepted pedometer cut-points for adolescents.

Studies examining the association between CRF and clustered metabolic risk in youth report an inverse relationship, indicating that as the CRF increases, the risk of an unfavorable metabolic risk profile is reduced [8]. Indeed, both lower levels of PA and CRF have been associated with a higher clustering of MRF in young people [9]. Evidence also suggests that sedentary behavior and low levels of PA and CRF in youth continue into adulthood and may predispose young people to disease later in life [10].

Associations between increased CRF and several MRF have been repeatedly found. However, the information about the relationship between the overall PF levels with MRF is scarce. To our knowledge, there is only one study that has compared coronary heart disease risk factors to PA measured objectively by pedometry [11], and, to date, there are no studies that have analyzed the relations between MRF and PA (measured with pedometers) and overall PF levels in adolescents. The present study fills this gap by analyzing these relationships in a sample of Azorean adolescents.

Methods

Study design and sampling

Data for the present study derived from a longitudinal school-based study, the Azorean Physical Activity and Health Study II, aimed to evaluate PA, PF, overweight/obesity prevalence, health related quality of life and related factors beginning in 2008. Study design and sampling are reported elsewhere [12]. For this study we only considered the 517 adolescents with metabolic data evaluated in 2009; of this total, 100 did not have valid pedometer information as described below in the PA section. Therefore, the final sample included in this cross-sectional analysis was comprised of 417 adolescents (243 girls and 174 boys) aged 15 to 18 (mean age 16.5 ± 0.9).

All participants in this study were informed about the objectives of the work, and the parent or guardian of each participant provided written informed consent. The study was approved by the faculty and the Portuguese Foundation for Science and Technology ethics committee and conducted in accordance with the Declaration of Helsinki for Human Studies of the World Medical Association.

Anthropometric Measures

Height was measured to the nearest millimeter in bare or stocking feet with the adolescent standing upright against a stadiometer (Holtain Ltd., Crymmych, Pembrokeshire, UK). Weight was measured to the nearest 0.10 kg, lightly dressed using a portable electronic weight scale (Tanita Inner Scan BC 532). Body mass index (BMI) was calculated as weight/height squared (kg/m^2) and used to define thinness, normal weight, overweight and obese group [13,14]. However, for analytical purposes the thinness and normal weight as well as overweight and obese adolescents were collapsed in two groups, thinness/normal weight and the overweight/obese, respectively. Waist circumference (WC) was measured twice with a metal anthropometric tape midway between the lower rib margin and the iliac crest at the end of normal expiration [15] and the average of the two measures was used for analysis.

Blood Pressure

Blood pressure (BP) was measured using the Dinamap adult/pediatric vital signs monitors, model BP 8800 (Critikon, Inc., Tampa, Florida). Measurements were taken by nurses, all adolescents required to sit and rest for at least five minutes prior to the BP test. The participants were in a seated, relaxed position with their feet resting flat on the ground. Two measurements in the right arm were taken, after five and ten minutes of rest. The mean of these two measurements was considered. If the two measurements differed by 2 mmHg or more, a third measure was taken.

Blood Sampling

Blood samples were collected from the antecubital vein between 8:00 and 10:00 a.m. in a sitting position after ten hours of fasting. The blood samples were drawn in vacuum tubes gel (Sarstedt) in order to obtain values of plasmatic high-density lipoprotein cholesterol (HDL-cholesterol), triglycerides (TG) and glucose. After resting at room temperature for about 30 minutes, the samples were centrifuged for 10 minutes at 3000 rpm to obtain serum. Samples were divided into aliquots, separated within 30 minutes, and stored at -80°C until analyzed. The following analyses were measured on a Cobas Integra 400 Plus, (ROCHE): HDL-cholesterol using the precipitation of the Apolipoprotein B containing lipoproteins with dextran-magnesium-chloride; TG using the Glycerokinase-Glycerolphosphateoxidase-Peroxidase (GK-GPO-POD) method; and glucose using the hexokinase method. The biochemical evaluation of all participants from the different islands was conducted in the same laboratory.

Metabolic Risk Factors

Since there is no consensus regarding the establishment of a universal criterion for the definition of MetS in children and adolescents, it was decided to compute a sum of MRF based on five cut-off points of the MetS

International Diabetes Federation criteria [16] using the following measurements: WC above the 90th percentile for age and sex, for adolescents aged <16 (or adult criteria if the 90th percentile is lower) and for adolescents aged ≥16 (>80 cm for girls and >94 cm for boys); TG≥150 mg/dL; HDL-cholesterol<40 mg/dL for both genders except HDL-cholesterol<50 mg/dL for girls aged ≥16 years; fasting glucose ≥100 mg/dL; and systolic BP≥130 mmHg or diastolic BP≥85 mmHg. For adolescents aged<16, the WC percentiles were used (sex and age-specific 90th percentile) for British children [17] as they had been previously used by Ekelund [18] for Portuguese adolescents. Adolescents were then classified in three groups: no MRF, one MRF and two or more MRF.

Socio-Economic Status

The highest level of parental education (in completed years of education) was considered as a proxy of socio-economic status. Similar procedures had previously been applied in the Portuguese context [19].

Mediterranean Diet Score

Dietary intake was obtained using a semi-quantitative food frequency questionnaire regarding the previous 12 months comprised of 82 food and beverage items [20]. A Mediterranean diet score was adapted from the alternate Mediterranean score [21]. This was constructed based on the intake of group foods (pulses, vegetables, fresh fruits, nuts, whole grains, fish, red and processed meats, ethanol) as well as on the ratio of monounsaturated to saturated fat. Using the sex-specific median of the study's participants as a cut-off value for each of the components, 1 point was given when intake ≥ median and 0 points for intakes < median for all items except for red and processed meats and ethanol (below median = 1 point). If participants met all the characteristics of the Mediterranean diet, they achieved the highest score possible (nine points), reflecting maximum adherence. If they met none of the characteristics, the score was minimum (zero), reflecting no adherence at all. Based on these results, participants were categorized into tertiles (low, medium, and high).

Physical Activity

Physical activity (PA) was assessed objectively using a sealed pedometer (Kenz Lifecorder Plus, Suzuken Co. Ltd, Nagoya, Japan) worn over seven consecutive days. Participants with fewer than three days (two weekdays and one weekend day) of activity recorded were eliminated from the data analysis (74 adolescents) in accordance with previous findings [22]. Another 26 adolescents whose leisure time PA was swimming were also deleted from the analysis, because the pedometer is not capable of detecting water activities. Adolescents were familiarized with the pedometers during a physical education class before the monitoring period. On the first day of monitoring (Monday), adolescents were instructed on pedometer attachment (at the waist), its removal (only during showering, bathing, swimming, or sleeping), and re-attachment each morning before going to school. Pedometer values were taken as the average number of steps/day and weighted according to the ratio of weekdays to weekends. Mean step counts were divided into age-adjusted quartiles.

Physical Fitness

Several studies have shown that in adolescence low CRF is a predictor of cardiovascular disease risk factors [8,23,24]; and therefore, reaching the HZ in the 20mSRT was a mandatory criterion to evaluate the PF levels. Health-related components of PF were evaluated using the Fitnessgram Test Battery 8.0. The Fitnessgram is included in physical education curricula, and the five tests recommended in the Portuguese National Program were used in this study. These activities include curl-ups, push-ups, trunk lifts, a 20 m shuttle run test (20mSRT), and the modified-back-saver-sit-and-reach. The 20mSRT was used to evaluate CFR. The curl-up, push-up and trunk lift tests were used to evaluate strength. The modified-back-saver-sit-and-reach test was used to evaluate flexibility. All tests were conducted according to the Fitnessgram measurement procedures [25]. According to the results of each test, adolescents were classified in two groups according to their ages and sex-specific cut-off points of the Fitnessgram 8.0 criteria, as belonging to the healthy zone (HZ) or above or under the healthy zone (UHZ). Adolescents were classified in different groups: UHZ in the 20mSRT, HZ or above in the 20mSRT plus one test, and continuing consecutively through plus four tests.

Statistical Analysis

Descriptive data are presented as means and standard deviation unless otherwise stated. All variables were checked for normality and appropriately transformed if necessary. The HDL-C, TG, systolic BP, and WC were logarithmically transformed. Independent sample t tests with Bonferroni corrections were performed to compare sexes in continuous variables.

Ordinal logistic regression was used to verify the relationship between MRF (dependent variable in ordinal scale, and it was coded as 0 = no MRF; 1 = one MRF; and 2 = two or more MRF), with PA, and overall PF levels (independent variables). Two models were created for analysis. The first model analyzed the relationship between the MRF and PA, and the second model analyzed the relationship between MRF and overall PF levels. Both models were adjusted for sex, BMI, socio-economic status and adherence to the Mediterranean diet. The assumption of proportional odds for both models was examined using the Brant test (first model: χ^2 = 5.72, p = 0.573; second model: χ^2 = 8.10, p = 0.151). Data were analyzed using the Stata 10.0 software (Stata Corp., College Station, U.S.) and SPSS for Windows (version 17.0). A p value under 0.05 denoted statistical significance.

Results

Descriptive characteristics of the adolescents are shown in Table 1. Boys had higher levels of height, weight, WC, systolic BP and glucose than girls ($p < 0.001$ for all), whereas girls had higher values of HDL-C ($p < 0.001$). No differences in age, BMI, diastolic BP, TG and mean step counts were observed between the sexes ($p > 0.05$). In the overall sample 41.0% had no MRF, 40.3% had one MRF, and 18.7% had two or more MRF. The prevalence of adolescents UHZ in the 20mSRT was 57.6% (72.0% for girls and 37.4% for boys, $p < 0.001$). In the sample 17.5% were categorized in the HZ in five tests (girls 9.1% and boys 29.3%, $p < 0.001$).

Table 1 Descriptive characteristics of the study sample

Variables	Total (n = 417)	Girls (n = 243)	Boys (n = 174)
Age, years	16.5 ± 0.9	16.5 ± 1.0	16.4 ± 0.8
Height, cm	165.0 ± 13.6	160.0 ± 11.0	172.0 ± 1.0*
Weight, kg	63.1 ± 12.5	58.6 ± 10.0	69.3 ± 13.1*
BMI, kg/m^2	22.9 ± 3.7	22.7 ± 3.5	22.7 ± 3.5
Waist circumference, cm	79.3 ± 10.7	78.0 ± 10.3	81.2 ± 11.1*
Systolic BP, mm Hg	115.2 ± 15.3	111.8 ± 13.8	120.0 ± 16.1*
Diastolic BP, mm Hg	66.4 ± 9.4	66.3 ± 10.0	66.5 ± 8.6
HDL-cholesterol, mg/dl	55.6 ± 13.4	59.3 ± 13.0	50.5 ± 12.3*
Triglycerides, mg/dl	70.7 ± 35.1	72.5 ± 34.6	68.1 ± 35.6
Glucose, mg/dl	86.3 ± 8.8	84.5 ± 8.8	88.9 ± 8.3*
Mean step counts	7631 ± 3289	7427 ± 2725	7916 ± 3936
Number of MRF (%, n)			
No risk factors	41.0 (171)	35.8 (87)	48.3 (84)
1 risk factor	40.3 (168)	42.0 (102)	37.9 (66)
2 or more risk factors	18.7 (78)	22.2 (54)	13.8 (24)
Physical Fitness (%, n)			
UHZ 20mSRT	57.6 (240)	72.0 (175)	37.4 (65)
HZ 20mSRT + HZ in 1 test	1.7 (7)	1.6 (4)	1.7 (3)
HZ 20mSRT + HZ in 2 tests	7.7 (32)	6.2 (15)	9.8 (17)
HZ 20mSRT +HZ in 3 tests	15.6 (65)	11.1 (27)	21.8 (38)
HZ 20mSRT + HZ in 4 tests	17.5 (73)	9.1 (22)	29.3 (51)

Values are means (SD). Student's *t* test, with Bonferroni corrections for differences between sex: *p < 0.001; BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; MRF, metabolic risk factors; UHZ, under healthy zone; HZ, healthy zone; 20mSRT, 20 m Shuttle Run Test.

Table 2 Ordinal logistic regression showing estimating results with MRF as dependent variable and pedometer step counts into age-adjusted quartiles as independent variable

Pedometer step quartiles	OR unadjusted (95% CI)	p	OR adjusted[a] (95% CI)	p
1st: ≤5621 steps	1		1	
2nd: 5622 -7403 steps	0.59 (0.35 - 0.98)	0.042	0.65 (0.38 - 1.11)	0.116
3rd: 7404 - 9422 steps	1.22 (0.74 - 2.02)	0.431	1.20 (0.71 - 2.02)	0.490
4th: ≥ 9423 steps	0.52 (0.31 - 0.87)	0.012	0.56 (0.33 - 0.95)	0.032

OR, odds ratios; CI, confidence intervals; 1, reference category; [a] adjusted for sex, body mass index (categories), socio-economic status and adherence to a Mediterranean diet.

Results of the ordinal logistic regression analysis for MRF and PA, adjusted for sex, BMI, socio-economic status and adherence to a Mediterranean diet, are shown in Table 2. Adolescents who were in the highest quartile of the pedometer step counts were less likely to have one or more MRF compared to those in the lowest quartile (OR = 0.56; 95%CI:0.33-0.95).

Similar results were found when an analysis was performed to assess the relationship between the MRF and the overall PF levels. Adolescents who achieved the HZ in five tests (20mSRT plus 4 tests) were less likely to have one or more MRF compared to those who were in the UHZ, after adjusting for sex, BMI, socio-economic status and adherence to a Mediterranean diet (OR = 0.55; 95%CI:0.31-0.98) (Table 3).

Discussion

The main findings from this study indicate that adolescents who are more active (≥9423 steps/day) and those who achieve the HZ in five tests have lower odds for having one or more MRF. Other important findings of the study indicate that 59% of the participants showed at least one MRF and 57.6% were UHZ in the 20mSRT.

Table 3 Ordinal logistic regression showing estimating results with MRF as dependent variable and overall physical fitness levels as independent variable

Overall Physical Fitness levels	OR unadjusted (95% CI)	p	OR adjusted[a] (95% CI)	p
UHZ 20mSRT	1		1	
HZ 20mSRT + HZ in 1 test	0.27 (0.62-1.13)	0.072	0.46 (0.96-2.21)	0.333
HZ 20mSRT + HZ in 2 tests	0.30 (0.15-0.61)	0.001	0.48 (0.23-1.01)	0.054
HZ 20mSRT +HZ in 3 tests	0.42 (0.25-0.71)	0.001	0.73 (0.427-1.28)	0.269
HZ 20mSRT + HZ in 4 tests	0.27 (0.16-0.46)	<0.001	0.55 (0.31-0.98)	0.043

OR, odds ratios; CI, confidence intervals; 1, reference category; UHZ, under healthy zone; HZ, healthy zone; 20mSRT, 20m Shuttle Run Test; [a]adjusted for sex, body mass index (categories), socio-economic status and adherence to a Mediterranean diet.

In this study, the mean daily step counts were 7427 for girls and 7916 for boys. These results are below the ranges of steps/day reported for adolescents by previous authors [6,26]. A three-year follow-up study of adolescents in Sweden showed that the daily mean step for boys was 11,892 and for girls 12,271 [27], while US children took between 11,000 and 13,000 steps/day [28]. The Canadian Physical Activity Levels Among Youth Study reported that Canadian children and youth (aged 5 to 19) take an average of 11,356 steps/day [7]. The ranges of steps/day reported in these studies are much higher than those found for the Azorean adolescents. Some explanations of these differences could be environmental, such as community design as well as other cultural differences.

Given the lack of recommended step counts for the adolescent population, we decided to divide the mean step counts/day into quartiles adjusted to the adolescent's age. In this study, it was observed that the value of the 4th quartile was 9423 steps/day, which is close to the step count cut-off of 10,000 proposed for adults, [29] and as adolescents reach adulthood, they begin to approximate adults in PA patterns.

Though this study has not examined PA intensity, Wild et al. [30] showed that adolescents who reported meeting the recommendations for both moderate and vigorous PA accumulated the most steps/day. Moreover, the highest levels of PA were associated with healthy outcomes. In this study, the finding of very low PA patterns suggests that Azorean adolescents may be at an increased risk for obesity, hypertension, type II diabetes, and coronary heart disease [3]. We found no significant sex differences in PA, although Azorean boys have slightly higher levels of PA compared to girls, which is consistent with the findings reported by Hardman et al. [26].

Reduction in PA is linked to increases in childhood/adolescent obesity [31] and MetS [5]. In the EYHS using a cross-sectional multicenter study of 1732 children and adolescents, Andersen et al. [32] showed that the risk of having clustered risk factors decreased in a dose-gradient manner with increased moderate-to-vigorous PA. In another study with the Danish cohort of 9- to 10-year-old children, PA was also shown to be inversely associated with clustered metabolic risk [9]. However, the total volume of PA necessary for preventing cardiovascular disease risk in adolescents is not clear, and no pedometer guidelines have been set for adolescents.

The lack of PF has also been associated with the development of cardiovascular disease risk factors in youth, such as lipid disorders, high BP and insulin resistance, among others [24]. Our results showed a positive influence of overall PF levels on MRF. Adolescents who are in the HZ in five tests had lower odds of having MRF than those who were UHZ. Some studies have shown that PF levels track from adolescence to adulthood, [10] with moderate to strong coefficients for CRF and strength, respectively [33].

Ruiz et al. have also shown an inverse association between CRF and clustered MRF in 9-10-year-old Swedish and Estonian children [23]. Reinforcing this idea, Ortega et al. [24] reported that children and adolescents with higher levels of CRF also have a more favorable cardiovascular profile compared to their unfit counterparts. Conversely, some studies have shown that high levels of CRF and PA are associated with a favorable metabolic risk profile [32]. Similarly, Ekelund et al. [34] found independent inverse associations of PA and CRF with clustered metabolic risk. However, direct comparisons with our study are difficult because in this study PF was evaluated using five tests from the Fitnessgram Test Battery, while in other studies PF was measured using only the CRF level [8,23,24].

Regarding the relationship between PA and CRF levels with MRF, our study indicated that although Azorean adolescent girls had similar step counts to boys, they had lower CRF levels and had more prevalence of MRF. A partial explanation for these differences could be the fact that boys, in general, are more vigorous in PA [35,36]; therefore, this may to lead to higher CRF levels compared to girls. With this in mind, boys may be more protected in relation to MRS than girls. Although in our study we did not assess PA intensity, it is possible that boys engaged in more vigorous PA than girls, leading to high CRF levels.

The main strength of the current study is that PA was assessed objectively by pedometers, which are a valid and reliable measure. Moreover, walking is one of the most common forms of PA and is easily captured by a pedometer. Their relatively low cost and ease of administration make them attractive for use in field-based PA studies. The use of field tests for PF assessment, which can be administered in school settings where a large number of participants can be tested simultaneously, enhances participant motivation, making it a valuable tool for studying PF in youth. Another aspect to note is the specificity of the place of the study, which was conducted in the Azores Islands. Some studies have been published on Azorean adults [37-39], but in adolescence, the information is scarce [12]. This study is limited because it consisted of a cross-sectional analysis, which limits inferences about causality and its direction. Another limitation of the pedometer it is that it does not provide information about PA intensity, nor does it record activities such as bicycling, swimming and climbing. Moreover, in this study a four sex-and-age-specific BMI categories (thinness, normal weight, overweight and obese) were not analyzed since there were fewer adolescents in the limit categories (thinness and obese), however, in the thinness category none of the adolescents had one or more MRF or had low fitness levels. As evidenced by Bovet et al. [40], there was a trend toward lower performance in lean students, as compared to students with normal weight for all fitness tests.

Conclusion

In conclusion, the results of this study indicate that adolescents who were more active (≥9423 steps/day) and those who achieved the HZ in five tests were less likely to have one or more MRF, after adjusting for sex, BMI, socio-economic status and adherence to a Mediterranean diet. Our findings emphasize the importance of promoting and increasing regular PA and PF to reduce the public health burden of chronic diseases associated with a sedentary lifestyle. Further research is warranted to quantify how much PA and PF are needed to prevent and reduce the risk in those who already have one or more MRF, highlighting potential sex and BMI differences.

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Authors' contributions
CM, RS and JM have made substantial contributions to conception and design of data. CM and RS and have carried out the data collection, statistical analysis, interpretation of data and wrote the manuscript. JCFJ, SV, LSM, AIM and PCS have been involved in drafting the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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Paper IV

Cardiorespiratory fitness is negatively associated with metabolic risk factors independently of the adherence to a healthy dietary pattern.

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Cardiorespiratory fitness is negatively associated with metabolic risk factors independently of the adherence to a healthy dietary pattern

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Abstract *Background and aim:* Cardiorespiratory fitness (CRF) and diet have been involved as significant factors towards the prevention of cardio-metabolic diseases. This study aimed to assess the impact of the combined associations of CRF and adherence to the Southern European Atlantic Diet (SEADiet) on the clustering of metabolic risk factors in adolescents.
Methods and Results: A cross-sectional school-based study was conducted on 468 adolescents aged 15-18, from the Azorean Islands, Portugal. We measured fasting glucose, insulin, total cholesterol (TC), HDL-cholesterol, triglycerides, systolic blood pressure, waits circumference and height. HOMA, TC/HDL-C ratio and waist-to-height ratio were calculated. For each of these variables, a Z-score was computed by age and sex. A metabolic risk score (MRS) was constructed by summing the Z scores of all individual risk factors. High risk was considered when the individual had ≥1SD of this score. CRF was measured with the 20 m-Shuttle-Run-Test. Adherence to SEADiet was assessed with a semi-quantitative food frequency questionnaire. Logistic regression showed that, after adjusting for potential confounders, unfit adolescents with low adherence to SEADiet had the highest odds of having MRS (OR = 9.4; 95%CI:2.6–33.3) followed by the unfit ones with high adherence to the SEADiet (OR = 6.6; 95% CI: 1.9–22.5) when compared to those who were fit and had higher adherence to SEADiet.

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Conclusions: Unfit adolescents showed higher odds of having high MRS, regardless of the adherence to SEADiet suggesting that high CRF may overcome the deleterious effects of low adherence to a healthy dietary pattern in adolescents.

Introduction

The metabolic syndrome (MetS) phenotype, a cluster of several cardiovascular disease risk factors, is a complex entity of metabolic disorders that significantly increases the risk of type II diabetes and cardiovascular disease [1].

As important determinants of a healthy lifestyle, cardiorespiratory fitness (CRF) and diet might influence the development and expression of cardio-metabolic risk. Indeed, healthy levels of CRF has been associated with a lower metabolic risk factors in young people [2,3], and results from longitudinal studies indicate that high CRF in childhood and adolescence is associated with a healthier cardiovascular profile disease later in life [4]. Likewise, there is some evidence of a negative relation between inadequate dietary patterns (i.e. low consumption of vegetables and fruits) and the MetS in adolescents [5].

Recently, the Southern European Atlantic Diet (SEADiet) has emerged as a healthy dietary pattern which is characterized by a highly palatable diet that is culturally rooted in northern Portugal and Galicia (a region in northwest Spain) in the Atlantic coast and consists of a high intake of seasonal legumes, vegetables, potatoes, whole grain bread (either from corn or wheat), fish, particularly cod, but also red meat, pork, and dairy products [6]. This dietary pattern was reported as being protective against cardiovascular disease outcomes [6].

Some studies have analyzed the MetS and its associations with CRF and diet among adolescents, separately [5,7–9]. However, studies examining the combined associations of CRF and diet on cardio-metabolic risk in adolescents are scarce. A better understanding of the combined associations of CRF and diet on cardio-metabolic risk in youth is of great importance because both CRF and diet are main modifiable lifestyle factors and are recommended as the cornerstone of prevention and the first line of treatment for MetS in youth [10]. Furthermore, interventions targeting CRF and diet simultaneously could offer a greater benefit if synergetic effects can be detected in relation to cardio-metabolic risk. Therefore, the purpose of this study was to assess the combined associations of CRF and adherence to a healthy dietary pattern, the SEADiet, on the clustering of metabolic risk factors in a sample of adolescents from the Portuguese Azorean Archipelago.

Methods

Study design and sampling

Data for the present study derived from a longitudinal school-based study, the Azorean Physical Activity and Health Study II, aimed to evaluate physical activity (PA), physical fitness, and prevalence of overweight/obesity, dietary intake, health-related quality of life and related factors. Details on the study design and sampling strategy are reported elsewhere [11]. For this study we only considered the 517 adolescents with metabolic data evaluated in 2009; of these, 49 did not have valid dietary intake information. Therefore, the final sample included in this cross-sectional analysis comprised 468 adolescents (273 girls and 195 boys) aged 15 to 18 (mean age 16.5 $\pm$ 0.9).

Anthropometric measures

Height was measured to the nearest millimeter in bare or stocking feet with the adolescent standing upright against a stadiometer (Holtain Ltd., Crymmych, Pembrokeshire, UK). Weight was measured to the nearest 0.10 kg, with adolescents lightly dressed using a portable electronic weight scale (Tanita Inner Scan BC 532). Body mass index was calculated as weight/height squared (kg/m^2). Waist circumference measurements was taken as described by Lohman [12]. The waist and height were used to compute the waist-to-height ratio.

Blood pressure

Blood pressure (BP) was measured using a Dynamap vital signs monitors, model BP 8800 (Critikon, Inc., Tampa, Florida). Measurements were taken by trained nurses, and all adolescents were required to sit and rest for at least 5 min prior to BP measurement. Participants were in a seated, relaxed position with their feet resting flat on the ground. Two measurements in the right arm were taken, after five and 10 min of rest. The mean of these two measurements was considered. If the two measurements differed by 2 mmHg or more, a third measure was taken.

Blood sampling

Blood samples were collected from the antecubital vein between 8:00 and 10:00 a.m., in a sitting position after 10 h of fasting. Blood samples were processed locally, then stored and shipped to a central laboratory where biochemical evaluation of all study participants was processed. Serum glucose, triglycerides (TG), total cholesterol (TC), and HDL-cholesterol (HDL-C) were determined by colorimetric methods using the Cobas Integra 400 Plus (ROCHE Diagnostics, Indianapolis, IN, USA). The fasting blood insulin was measured using chemiluminescence immunoassay (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA). The ratio of TC to HDL-C was calculated as an index of atherogenic lipid profile. The homeostatic model assessment (HOMA), calculated as the

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product of basal glucose and insulin levels divided by 22.5, was used as a proxy measure of insulin resistance [13].

Metabolic risk score

Since there is no consensus regarding the establishment of a universal criterion for the definition of MetS in children or adolescents and the prevalence rate is not high, some studies [2,14] have derived a continuous score representing a composite cardio-metabolic risk factor profile or index. As some authors have suggested [14], the estimation of a metabolic risk score (MRS) based on the sum of age-and-sex adjusted Z-scores of several metabolic risk factors constitutes a valid tool to identify children and adolescents at risk of developing cardiovascular disease and type II diabetes [3,9]. Indeed, this approach allows the summarization of the cumulative risk that each of the risk factor provides into a continuous score encompassing the overall risk of each individual, and at the same time it accounts to some extent for the influence of growth and maturation on the cardiovascular risk factors [3].

In this study we decided to compute a continuous MRS from the following measurements: TC/HDL-C ratio, TG, HOMA, systolic BP and waist-to-height ratio. For each of these variables, a Z-score was computed by age and sex. MRS was constructed by summing the Z scores of all individual risk factors. High risk was considered when the individual had $\geq$1SD of this score. The score only applies to this study population. A similar z-score approach has been used previously in children and adolescents [14].

Southern European Atlantic Diet score

Dietary intake was obtained using a self-administered, semi-quantitative food frequency questionnaire (FFQ) of the previous 12 months, comprising 82 food items or beverage categories, validated for Portuguese adolescents and adults [15]. Food intake was calculated by weighting one of the nine possibilities of frequency of consumption (from never or less than once per month, to six or more times a day), by the weight of the standard portion size of the food-item. The adherence to the traditional Southern European Diet was assessed by the SEAD adherence score as reported by Oliveira et al. [6]. Briefly, the SEADiet score was originally constructed based on the intake of specific food groups, namely fresh fish (excluding cod), cod, red meat and pork products, dairy products, legumes and vegetables, vegetable soup, potatoes, whole-grain bread and wine. Using the sex—specific median of the study's participants as a cut-off value for each of the components, 1 point was given when intake $\geq$ median and 0 points for intakes below median for all items except for wine ($\leq$1 glass/d in women and $\leq$2 glasses/d in men = 1 point). In the present study, we adapted this score by attributing 0 points to any wine consumption, since ethanol consumption is not recommended for children and adolescents [16]. If participants met all the characteristics of SEADiet, their score was the highest (nine points), reflecting maximum adherence. If they met none of the characteristics, the score was minimum (zero), reflecting no adherence. Based on these results, participants were categorized into two groups: low ($\leq$4 points) and high ($\geq$5 points) adherence accordingly with the sample's median value.

Cardiorespiratory fitness

CRF was measured using the 20 m-Shuttle-Run-Test as previously described by Léger [17]. This test requires participants to run back and forth between two lines set 20 m apart. Running speed started at 8.5 km/h and increased by 0.5 km/h each minute, reaching 18.0 km/h at minute 20. Each level was announced on a tape player. The participants were told to keep up with the pace until exhausted. The test was finished when the participant failed to reach the end lines concurrent with the audio signals on two consecutive occasions. Otherwise, the test ended when the subject stopped because of fatigue. Participants were encouraged to keep running as long as possible throughout the course of the test. Number of shuttles performed by each participant was recorded. Adolescents were then classified in two groups according to the age and sex-specific cut-off points of FITNESSGRAM criteria, as belonging to the healthy zone — "fit", and under the healthy zone — "unfit".

Pubertal stage

To determine the pubertal stage (ranging from stage 1—5), each subject was asked to self-assess his/her stage of secondary sex characteristics. Stage of breast development in girls and genital development in boys were evaluated according to the criteria of Tanner and Whitehouse [18].

Socio-economic status and lifestyle variables

The highest level of parental education (in completed years of education) was considered as a proxy of socio-economic status. Similar procedures had previously been applied in the Portuguese context [19]. Smoking status was self-reported and classified as smoker (including regular and occasional smokers) and non-current smoker (including never and former smokers).

Statistical analysis

Descriptive data are presented as means and standard deviation unless otherwise stated. All variables were checked for normality and appropriately transformed if necessary. Independent sample *t* tests (one tailed) were performed to compare sex differences in continuous variables, and nominal data were analyzed with Chi-square tests. Binary logistic regression models were constructed to verify the relationship between high MRS and the combined associations of CRF and adherence to SEADiet, adjusting for total energy intake, socio-economic status, pubertal stage and smoking status. Participants were divided into four categories according to the CRF level (fit/unfit) and the adherence to SEADiet (high/low). Data were analyzed using the PASW Statistic v.18 (SPSS, Chicago, Illinois, USA). Statistical significance was determined at $p < 0.05$.

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Results

The descriptive characteristics of the study sample are presented in Tables 1 and 2. Girls had lower values of height, weight, waist circumference, TC/HDL-C, systolic BP, glucose and higher TC and HDL-C than boys ($p < 0.05$ for all). The percentage of high adherence to SEADiet was 57.1. No sex differences were found in the adherence to SEADiet. The prevalence of unfit adolescents was 59.2% for the all sample, 72.9% for girls and 40.0% for boys ($p < 0.001$).

When we analyzed the CRF and adherence to SEADiet by MRS the results showed that the majority of adolescents with high MRS were unfit (86.7%, $p < 0.001$) and had lower adherence to SEADiet (55.0%, $p < 0.05$). Conversely when we analyzed the adherence to SEADiet, we observed that the percentage of adolescents with low adherence to SEADiet and high MRS is lower (55%) than the percentage of adolescents who have low MRS and high adherence to SEADiet (58.8%, $p < 0.05$).

Fit adolescents with high adherence to SEADiet showed the lowest prevalence of MRS (2.6%). Conversely, unfit adolescents with low adherence to SEADiet showed the highest prevalence of high MRS (22.6%, $p < 0.001$). Fit adolescents, regardless of their degree of adherence to SEADiet, had lower prevalence of high MRS, compared to their unfit counterparts (Fig. 1).

Results of the binary logistic regression analysis for predicting high MRS by the combination of CRF and adherence to SEADiet, adjusted for potential confounders are shown in Table 3. Unfit adolescents with low adherence to SEADiet had the highest odds of expressing high MRS compared to fit adolescents and with high adherence to SEADiet (OR = 9.4; 95%CI:2.6–33.3); unfit adolescents with high adherence to SEADiet also showed higher odds of expressing high MRS compared to fit adolescents and with high adherence to SEADiet (OR = 6.6; 95% CI: 1.9–22.5). Further analysis to check what would happen if CRF (multiplied per -1) was included in the cluster of metabolic risk regarding the association with diet was performed, however, the results showed no significant associations between the variables.

Discussion

To the best of our knowledge, this is the first study assessing the combined associations of CRF and adherence to a healthy dietary pattern, namely the SEADiet, on the cardio-metabolic risk in a sample of adolescents. Unfit adolescents with low adherence to SEADiet had the highest odds of expressing high MRS compared to those who were fit and with high adherence to SEADiet, independently of total energy intake, socio-economic status, pubertal stage and smoking status. We also found that fit adolescents had lower prevalence of high MRS, and unfit adolescents exhibited higher odds of expressing high MRS, regardless of their degree of adherence (low or high) to SEADiet.

Higher levels of CRF appear to delay all-cause mortality primarily due to decreased rates of cardiovascular disease

Table 1 Descriptive characteristics of the adolescents.

Parameter	All (n = 468)	Girls (n = 273)	Boys (n = 195)
Age (y)	16.5 ± 0.9	16.5 ± 0.9	16.5 ± 0.9
Height (cm)	165.6 ± 8.8	160.4 ± 5.7[b]	172.0 ± 7.0
Weight (kg)	63.1 ± 12.6	58.8 ± 10.2[b]	69.3 ± 13.0
Body mass index (kg/m^2)	22.9 ± 3.8	22.8 ± 3.6	23.1 ± 3.9
Waist circumference (cm)	79.4 ± 10.8	78.3 ± 10.5[b]	80.7 ± 11.2
Waist-to-height ratio	0.48 ± 0.17	0.48 ± 0.06	0.48 ± 0.25
Diastolic blood pressure (mm Hg)	66.9 ± 9.5	67.0 ± 10.1	66.7 ± 8.5
Systolic blood pressure (mm Hg)	115.5 ± 14.9	112.4 ± 13.5[b]	119.7 ± 15.8
Total cholesterol (mg/dl)	162.4 ± 32.4	169.6 ± 33.7[b]	152.3 ± 27.4
HDL-cholesterol (mg/dl)	55.6 ± 13.1	59.4 ± 12.9[b]	50.3 ± 11.6
Triglycerides (mg/dl)	71.3 ± 37.9	74.1 ± 30.0	67.3 ± 37.4
Fasting glucose, (mmol/L)	4.79 ± 0.4	4.69 ± 0.4[b]	4.94 ± 0.4
Fasting insulin, (μU/mL)	9.06 ± 6.1	9.36 ± 5.2	8.62 ± 7.1
HOMA	1.95 ± 1.4	1.97 ± 1.1	1.93 ± 1.6
Total cholesterol/HDL-C ratio	3.03 ± 0.8	2.95 ± 0.8[b]	3.13 ± 0.7
Metabolic risk score[a]	0.02 ± 2.9	0.05 ± 2.7	−0.02 ± 3.1
Adherence to SEADiet (%)			
Low adherence	42.9	44.0	41.5
High adherence	57.1	56.0	58.5
Cardiorespiratory fitness (%)			
Unfit	59.2	72.9[c]	40.0
Fit	40.8	27.1[c]	60.0

Data are means ± standard deviations.
HDL, high-density lipoprotein; SEADiet, Southern European Atlantic Diet.
[a] Obtained by summing individual risk factors (total cholesterol/HDL-C ratio, triglycerides, HOMA, systolic blood pressure and waist-to-height ratio) age-and-sex-standardized scores.
[b] $p < 0.05$ for sex comparisons (one tailed T-Test).
[c] $p < 0.05$, Chi–Square Test.

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Table 2 Cardiorespiratory fitness and adherence to the Southern European Atlantic Diet by metabolic risk score.

Parameter	Low MRS (n = 408)	High MRS (n = 60)	p^a
Cardiorespiratory fitness			
Unfit	55.1	86.7	<0.001
Fit	44.9	13.3	
Adherence to SEADiet			
Low adherence	41.2	55.0	0.043
High adherence	58.8	45.0	

Data are %.
MRS, metabolic risk score: obtained by summing individual risk factors (total cholesterol/HDL-C ratio, triglycerides, HOMA, systolic blood pressure and waist-to-height ratio) age-and-sex-standardized scores.
SEADiet, Southern European Atlantic Diet.
[a] Chi-Square Test.

and cancer [20] and provide strong and independent prognostic information about the overall risk of illness and death. Recent reports suggest that CRF is also an important marker of health in young individuals [8]. Indeed, healthy levels of CRF have been associated with lower metabolic risk in young people [2,3]. Evidence suggests that sedentary behavior, low levels of PA and CRF in youth track into adulthood [21,22]. Several longitudinal studies have suggested that a low CRF during childhood and adolescence is associated with later cardiovascular risk factors, such as obesity, hyperlipidemia, and hypertension [4,23–25]. Our study is in line with these investigations and adds that unfit adolescents had higher prevalence of high MRS, regardless of their degree of adherence to SEADiet. These results seem to support the existence of a strong association between CRF and health-related outcomes in adolescence, which may suggest the significance of including CRF as part of the composite scores to define MetS as has been advocated in some studies [26,27].

Adherence to healthy eating practices such as having high intakes of fruit, vegetables, and whole grains, have been associated with lower risk of MetS [28], while

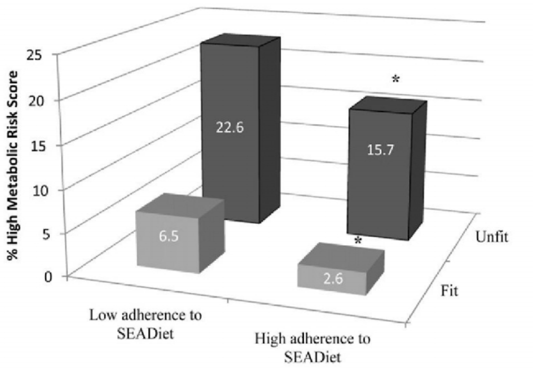

Figure 1 Prevalence of high metabolic risk score across cardiorespiratory fitness and adherence to the Southern European Atlantic Diet categories. *Significantly different from unfit/fit level ($p < 0.001$).

a western dietary pattern was positively related with individual MetS risk factor components in adolescents [9]. Consistent with previous findings from adults, adolescents whose eating patterns included high intakes of fruit and vegetables, also exhibited lower prevalence of MetS [5]. Data from the Bogalusa Heart Study on 1275 individuals aged 12–24 [29], showed that high consumption of fruits and vegetables was associated with higher levels of HDL-C and lower levels of TG and LDL-cholesterol, whereas, high sugary foods, fats and pasta consumption patterns were associated with higher LDL-cholesterol levels [29]. In our study, low adherence to SEADiet was significantly associated with high MRS. Whereas the majority of the adolescents showed adherence to a healthy dietary pattern they also were categorized as unfit. This could explain the lower percentage of adolescents who were fit and had a low MRS when compared to the percentage of adolescents who were unfit and had a low MRS. Moreover unfit adolescents with high adherence to SEADiet had low MRS when compared to unfit adolescents with low adherence to SEADiet, which highlights the importance of exploring both dietary pattern and CRF simultaneously when assessing MRS in adolescents.

Common characteristics in healthy eating patterns usually include limiting intake of salty foods, solid fats, added sugars, and refined grains, and emphasizing the consumption of nutrient-dense foods and beverages, particularly vegetables, fruits, whole grains, fat-free or low-fat milk and milk products, seafood, lean meats and poultry, eggs, beans and peas, and nuts and seeds [30]. Furthermore, in the Azores Islands, the temperate Atlantic climate allows milk production to be heavily based on grazing almost all year round. The milk fat produced from the pasture has long been known to be rich in carotene and unsaturated fatty acids including trans-octadecenoic acids and conjugated isomers of linoleic acid (CLA) [15,31]. CLA has been associated with improving dyslipidemia, insulin sensitivity and the pro-inflammatory state related to obesity and the MetS [21]. The enhanced concentration of CLA in milk fat from dairy products in the Azores may be a significant nutritional characteristic of the SEADiet from this particular Atlantic region, related with positive effects on MRS, but this hypothesis needs to be confirmed in future studies.

When studying the combined associations of CRF and adherence to SEADiet, we observed that unfit adolescents showed higher odds of expressing high MRS regardless of their adherence to SEADiet. This finding suggests that having healthy CRF levels may help overcome the deleterious effects of low adherence to healthy dietary patterns. Diet and PA have different effects on body composition, with both contributing to fat loss. However, only PA increases muscle mass and has a direct effect on metabolic function, which can be associated to a reduced in cardio-metabolic risk profile. Prevention of cardio-metabolic risk via healthy dietary patterns remains essential but future work should also focus on promoting healthy CRF levels.

We noted limitations previously highlighted by others when examining the association between diet and metabolic risk. Evidence about relationships between specific dietary patterns and disease remains typically difficult to understand for a number of reasons, including the difficulty of isolating the effects of nutrients or foods aggregated in

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Table 3 Odds ratio of high metabolic risk score by cardiorespiratory fitness and adherence to the Southern European Atlantic Diet.

Parameter	OR unadjusted (95% CI)	*p*	OR adjusted[a] (95% CI)	*p*
Fit + high adherence to SEADiet	1		1	
Fit + low adherence to SEADiet	2.6 (0.6–11.1)	0.206	2.3 (0.5–10.1)	0.276
Unfit + high adherence to SEADiet	6.9 (2.0–23.5)	0.002	6.6 (1.9–22.5)	0.003
Unfit + low adherence to SEADiet	10.8 (3.2–36.6)	<0.001	9.4 (2.6–33.3)	0.001

SEADiet, Southern European Atlantic Diet.
OR, odds ratios; CI, confidence intervals; 1, reference category.
[a] Adjusted for total energy intake, socio-economic status, pubertal stage and smoking status.

patterns under investigation from the confounding effects of other nutrients, non-nutrient factors and their possible interactions, and the multi-factorial etiology of high MRS. Other limitations should be mentioned. First, the fact that in this study CRF was measured objectively whereas food intake was self-reported could explain our findings of a stronger association for CRF than for SEADiet in relation to high MRS. Second, we are unable to draw cause-effect conclusions because of the cross-sectional nature of our data. In addition, the FFQ relied upon self-reported data from adolescents, and participants may misreport their intake [22]. However, although the FFQ may overestimate total intake, it is a good instrument for ranking intakes [10] as intended in our study and the FFQ used was validated for Portuguese adolescents [15].

Strengths of this study include the novelty of the analyses of combined associations of CRF with adherence to SEADiet on the cardio-metabolic risk of adolescents; our analysis were adjusted for important confounders related to cardio-metabolic risk such as: energy intake, socio-economic status, pubertal stage and smoking status; the use of valid field test for CRF assessment, which can be administered in school settings where a large number of participants can be tested simultaneously, enhancing participant motivation and making it a valuable tool for routinely measuring CRF in youth.

Conclusions

In conclusion, the results of this study indicate that fit adolescents had lower prevalence of high MRS, regardless of their degree of adherence to a healthy dietary pattern, and that among unfit adolescents high adherence to a healthy dietary pattern conferred some degree of protection against high MRS. These findings suggest that in addition to promoting adherence to health dietary patterns such as SEADiet, is also important to help children and adolescents to achieve and maintain healthy CRF levels for cardio-metabolic risk primary prevention purposes.

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3.Overall Discussion

Overall Discussion

The main findings of our experimental work showed that the prevalence of MetS among the Azorean adolescents was slightly higher than the same in other studies that also used the IDF's definition of MetS. Moreover, different measures of adiposity performed well in detecting high metabolic risk score. The results also suggested that PA and health-related PF, namely CRF, are negatively associated with MetS or the clustering of metabolic risk factors in adolescents. Furthermore, the deleterious consequences ascribed to a low adherence to healthy dietary patterns, such as the SEADiet, could be counteracted by having high levels of CRF.

Several different definitions for MetS in children and adolescents have been suggested; the components and cut-offs used to diagnose this syndrome have varied considerably among studies (Cook et al., 2003; De Ferranti et al., 2004). Therefore, the prevalence varies according to the definition used. For example, a study of 1,205 Caucasian overweight children and adolescents aged 4 to 16 years that compared eight different definitions of MetS reported that the prevalence of MetS varied between 6% and 39% depending on the definition used (Reinehr et al., 2007). Recently, a new consensus statement on MetS in children and adolescents has been published (Zimmet et al., 2007). Nevertheless, the accuracy of present MetS definitions for children and adolescents is under debate.

To overcome this question some studies have derived a continuous score representing a composite cardio-metabolic risk factor profile or index (Andersen et al., 2006; Brage et al., 2004; Rizzo et al., 2007). This score includes indicators of body fat, lipid abnormalities, elevated BP, and indicators of abnormal glucose metabolism (Eisenmann, 2008); even so there is no consensus on which of these are best to include in the score. However, some risk factors associated with CVD are not included in the definition of the syndrome. For example, PA and CRF are omitted as underlying risk factors, while obesity is included (Kahn et al., 2005b). In fact, it would be reasonable consider to include PA or CRF as part of the score, as it is difficult to find a rationale for including measures of adiposity but not PA or PF, considering that both indicators are strongly associated with the clustering of other CVD risk factors (Andersen et al., 2011). The existence of a strong association between PA and CRF and health-related outcomes in adolescence, especially in terms of CVD risk factors (Anderssen et al., 2007; Lobelo et al., 2010; Ortega et al., 2008b; Rizzo et al., 2007; Ruiz et al., 2007a), suggests that these factors should be included as part of the composite scores to define MetS, as has been advocated in some studies (Andersen et al., 2011; McMurray & Andersen, 2010).

In study I of this thesis, the prevalence rates of MetS and its components were estimated using the IDF's definition of MetS. The prevalence of MetS was slightly higher than the prevalence previously reported in other studies that used the same definition of the syndrome (Ekelund et al., 2009; Ford et al., 2008; Park et al., 2009; Pirkola et al., 2008).

Childhood obesity is associated with cardiovascular and metabolic disturbances (Saland, 2007). Furthermore, MetS is rapidly increasing in prevalence, with increases in childhood obesity and sedentary lifestyles worldwide (Saland, 2007). Indeed, various studies have shown that the prevalence of the syndrome was higher when the individuals were obese (Chang et al., 2004; Cruz et al., 2004; Shaibi & Goran, 2008). Reinforcing this idea, a systematic review done by Tailor et al., (2010) showed that MetS remains a major worldwide health concern in children and adolescents, particularly amongst the obese. These findings are in agreement with our study showing that MetS in adolescents is largely confined to the overweight population and may represent a subgroup of youth who will carry a larger risk for CVD and type 2 diabetes later in life.

Furthermore, CVD risk factors related to MetS appear to track from childhood to young adulthood (Bao et al., 1994; Chen et al., 2005). A better understanding of the determinants of MetS during adolescence might provide insights into preventive interventions for improving health outcomes during this period of life and reducing the incidence of CVD in adulthood.

Several anthropometric indices such as BMI, WC, and WHtR have been used to predict cardio-metabolic risk factors. Screening measures to be effective must be practical as well as feasible. Along these lines, study II suggested that, for both sexes and based upon ROC analysis, BMI was marginally superior in its ability to discriminate high from low metabolic risk score, compared with WC and WHtR.

Our results were generally consistent with some, though not all, prior studies. For instance, Jung et al., (2010) showed that BMI was the best predictor of MetS, its components and markers for low-grade inflammation among adolescents. Contrary to our findings, some studies have shown that WHtR is more useful in classifying obesity related to cardiovascular risk than BMI and WC (Hara et al., 2002; Kahn et al., 2005a). For example, data from NHANES III showed that WHtR better identifies youth with adverse CVD risk factors (Kahn et al., 2005a). Similarly, some studies have concluded that, compared with BMI, WHtR is more strongly associated with CVD risk factors in adults (Bosy-Westphal et al., 2006; Ho et al., 2003). Reinforcing this idea, a recent systematic review and meta-analysis of studies involving more than 300 000 adults that used ROC curves for assessing the discriminatory power of anthropometric indices in distinguishing adult general cardiovascular outcomes reported the superiority of WHtR over WC and BMI for detecting cardio-metabolic risk factors in both sexes (Ashwell et al., 2012). Nevertheless, Freedman et al., (2007) found that WHtR and BMI showed similar associations with CVD risk factors. Indeed, it has been emphasized that many of the differences between WHtR and BMI are relatively small (Bosy-Westphal et al., 2006).

BMI is the most used measure of general obesity and has been shown to be extremely effective in longitudinal studies (Burns et al., 2009), while WC and WHtR are indicators of central fat distribution (Lee et al., 2008; Zimmet et al., 2007). However, the limitations of all these indices should be considered. BMI does not distinguish fat mass from fat-free mass, nor does it differentiate between types of body fat distributions (Soto Gonzalez et al., 2007). Furthermore, there is currently no agreement on the health-related classification of WC in children and adolescents, and both WC and BMI are age-and-sex-dependent. Regarding WHtR, a simple ratio that does not require specification of sex or age, it might replace or supplement the use of sex- and age-specific measures (Allison et al., 1995).

Although, in our study, measures of general adiposity tended to show closer associations with high metabolic risk scores than did measures of central adiposity, the differences were small, suggesting that these methods were roughly equivalent in their application.

It is well established that regular PA improves a number of CVD risk factors (Kohl, 2001), preventing and facilitating the treatment of MetS (Lakka & Laaksonen, 2007) as it reduces all-cause mortality (Lee, 2010). In addition, a recent review of the relationship between PA and MetS in a pediatric population considered PA the key to the treatment of MetS (Brambilla et al., 2011). However, literature reported that adolescence is commonly characterized by marked declines in PA (Troiano et al., 2008). Nevertheless, evidence also suggests that activity patterns established at an early age have a tendency to follow into adulthood (Malina, 2001b; Telama et al., 1997).

Motion sensors such as accelerometer and pedometers are most appropriate for quantifying PA behaviors in populations (Tudor-Locke & Myers, 2001a). In our study, PA levels were assessed objectively by pedometers, which have been employed in large-scale epidemiological studies (Sequeira et al., 1995); they are also generally considered the most practical alternative for individual and population health promotion efforts (Tudor-Locke et al., 2009). Regardless of their advantages, the main drawback to the use of pedometers is that they do not measure the intensity, duration, or frequency of PA (Tudor-Locke & Myers, 2001b). However, their output correlates highly with that of various other accelerometers (Tudor-Locke et al., 2002).

Despite increasing interest in linking health-related outcomes to pedometer-determined PA, little research has provided evidence on the dose-response relationship between steps/day and specific health outcomes (Tudor-Locke et al., 2008). A meta-analysis reported that pedometer-based interventions increased PA (steps/day) and were effective at decreasing BMI and BP

(Bravata et al., 2007). Likewise, in adults higher volumes of steps/day have been associated with lower levels of insulin (Woolf et al., 2008), glucose (Strath et al., 2007), WC and BMI (Chan et al., 2003), and cardio-metabolic risk factor clustering (Schmidt et al., 2009).

The use of pedometers to quantify PA has led to the development of guidelines for recommended steps/day for health-related outcomes. For children (aged 6-12 years) recommendations range from 11,000 to 15,000 steps/day for boys and girls, respectively (Tudor-Locke et al., 2004). Canada's Physical Activity Guide for children and youth (between the ages of 5 to 19) set a goal of 16,500 steps daily (Cameron et al., 2005). For adults, it has been suggested that accumulating 10,000 steps in a day is appropriate to maintain health (Tudor-Locke & Myers, 2001b). However, there are no international pedometer cut-points defined solely for the adolescent population.

A three-year follow-up study of adolescents in Sweden showed that the daily mean number of steps for boys was 11,892 and for girls 12,271 (Raustorp et al., 2006), while U.S. children took between 11,000 and 13,000 steps/day (Flohr et al., 2006). In our study, it was observed that the average number of steps taken by the highest quartile was 9423 steps/day, which is close to the step count cut-off of 10,000 proposed for adults (Tudor-Locke & Bassett, 2004); as adolescents reach adulthood, they begin to approximate adults in PA patterns.

Though our study did not examine PA intensity, Wild et al., (2004) showed that adolescents who reported meeting the recommendations for both moderate and vigorous PA accumulated the most steps/day. In fact, assessing total PA using steps/day is useful in view of the fact that it considers all domains (transportation, recreation, household, and occupation) and is objectively measured rather than self-reported (Sisson et al., 2010). In this line, Sisson et al., (2010) showed that adults who maintain an active lifestyle by accumulating more steps are likely to have a lower prevalence of MetS and its individual CVD risk factors. Recently, data from the 2005–2006 NHANES III, which included 3,523 individuals aged 20 to 85 years, showed that the accumulation of 8,000 steps/day is a good proxy for 30 minutes of daily moderate-to-vigorous PA, while the accumulation of 7,000 steps/day every day of the week is consistent with obtaining 150 minutes of weekly moderate-to-vigorous PA (Tudor-Locke et al., 2011).

Some literature has recommended that children and adolescents participate in 60 minutes or more of moderate-to-vigorous-intensity PA most days of the week, preferably daily (Centers for Disease Control and Prevention, 2011; Strong et al., 2005). Conversely, data from EYHS, which included 1,732 randomly-selected 9-year-old and 15-year-old school children, suggested that current guidelines could underestimate the activity necessary to prevent the clustering of risk factors in children and, therefore, fixed at 90 minutes the required daily PA level for children and adolescents (Andersen et al., 2006). However, the minimal and optimal amounts of PA required to prevent and treat the clustering of metabolic risk in children are still unknown (Steele et al., 2008).

Higher levels of CRF appear to delay all causes of mortality, primarily due to their ability to decrease rates of CVD and cancer (Blair et al., 1989) and to provide strong and independent prognostic information about the overall risk of illness and death, especially as this relates to CVD (LaMonte & Blair, 2006) and MetS among adults (Lakka et al., 2003). Recent reports have shown similar associations between CRF and clustered metabolic risk factors in U.S (Lobelo et al., 2010) and European (Brage et al., 2004; Ekelund et al., 2007; Ruiz et al., 2007a) pediatric and adolescent populations. Moreover, some studies suggest that CRF is also an important marker of health in young individuals (Castillo-Garzon et al., 2007; Ortega et al., 2008b).

Indeed, lower levels of both PA and CRF have been associated with greater clustering of metabolic risk factors in young people (Andersen et al., 2006; Brage et al., 2004; Ekelund et al., 2007; Steele et al., 2008). Our results (study I, III, and IV) are in line with those studies, and extend existent knowledge to Azorean adolescents. Moreover, evidence suggests that sedentary behavior and low levels of PA and CRF in youth continue into adulthood (Biddle et al., 2010; Froberg & Andersen, 2005; Yang et al., 2007), with moderate to strong coefficients for CRF and strength, respectively (Malina, 2001a). They may also predispose young people to disease later in life (Hasselstrom et al., 2002).

Thus, our findings emphasize the importance of promoting and increasing regular PA and CRF at these ages and maintaining these practices into and throughout adulthood.

Collectively, the current literature agrees fairly consistently that unhealthy eating habits and physical inactivity are two major factors for the development of overweight and obesity as well as their co-morbidities. In fact, a recent systematic review found evidence of both dietary and PA behaviors tracking from childhood to adulthood (Craigie et al., 2011). The authors highlighted that both food choice and PA are modifiable behaviors, and practicing more healthy behaviors in childhood may lead to more healthy adult behaviors, therefore reducing the risk of obesity and of obesity-related disease.

Despite the emphasis on diet in the prevention and treatment of CVD risk factors, the associations between CRF, diet, and MetS in adolescents remain poorly understood. Thus, study IV of this thesis examined the impact of the combined associations of CRF and adherence to SEADiet on the clustering of metabolic risk factors in adolescents. Better understanding of the combined associations of CRF and diet on cardio-metabolic risk in youth is of great importance, because both CRF and diet are modifiable lifestyle factors. Lifestyle modification is recommended as the cornerstone of prevention and the first line of treatment for MetS in youth (Hollenbeck et al., 1988). Furthermore, interventions targeting CRF and diet simultaneously could offer greater benefits if their synergetic effects on cardio-metabolic risk could be detected.

In this line, Pearson et al., (2009) examined the prevalence and clustering patterns of multiple health behaviors among a sample of adolescents in the U.K. The authors conclude that PA and dietary behaviors do not occur in isolation; the potential synergistic effects of multiple dietary and PA behaviors on the risk of chronic conditions and health outcomes are key issues for public health. In the literature, there is evidence of an association between PA and dietary behaviors in adolescents. In this line, Driskell et al., (2008) found an association between low fruit and vegetable consumption and low levels of PA.

The findings of our study are in line with those of these investigations and extend previous knowledge by showing that unfit adolescents with low adherence to SEADiet had the highest odds of obtaining high metabolic risk scores, compared to those who were fit and showed high adherence to SEADiet. Among unfit adolescents, high adherence to a healthy dietary pattern conferred some degree of protection against a high metabolic risk score. Therefore, targeting both dietary patterns and CRF in adolescents offers a potential of increased health benefits.

In summary, the results gathered by this thesis increased our understanding of the associations between PA, health-related PF (namely, CRF), diet and MetS or metabolic risk factors in Azorean adolescents. They provide important knowledge about the correlates of metabolic diseases among Azorean adolescents. Furthermore, they have important implications, since PA and CRF may favorably influence the levels of CVD risk factors in this age group.

4. Conclusions

Conclusions

Based on our findings, it is possible to conclude that:

1. The prevalence of MetS is high in Azorean adolescents. Unfit adolescents are more likely to have MetS than fit adolescents.
2. Although, for both sexes, BMI is marginally superior in its ability to discriminate between high metabolic risk score, compared to WC and WHtR, the differences are minor, suggesting an equivalence of methods.
3. Daily step counts and PF levels are negatively associated with having one or more metabolic risk factors among Azorean adolescents.
4. Fit adolescents have lower rates of high metabolic risk scores, regardless of their degree of adherence to healthy dietary patterns, while among unfit adolescents high adherence to healthy dietary patterns confers some degree of protection against high metabolic risk scores.

The findings presented in this thesis reemphasize the importance of both high PA and CRF levels, as well as the importance of adherence to a healthy diet as an integral part of a health-enhancing lifestyle. As a result, future strategies to promote healthy lifestyles among Azorean adolescents should consider multi factorial approaches to promoting PA, CRF and healthy diets, since these factors seem to affect CVD risk factors and their clustering in this population.

Perspectives for future research

This study revealed diversified associations between PA, CRF, diet and metabolic risk factors. Further research is needed to extend the present findings. In fact, prospective studies must examine the possible long-term relationships between PA and CRF levels in adolescents with metabolic risk factors and disease patterns later in adulthood and also confirm the role of dietary patterns in the development of several non-communicable diseases, such as MetS, CVD and other co-morbidities. Further research on these topics will help identify the target population for CVD prevention efforts, as well as for cardiovascular health promotion programs meant to be implemented in early childhood. Future studies should consider investigating the role of lifestyle-related determinants and their anti-inflammatory functions during adolescence.

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