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 Euro- pean 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 ofthese variables, a Z-score was computed by age and sex. A metabolic risk score (MRS) was con- structed by summing the Z scores of all individual risk factors. High risk was considered when the individual had 2:1SD 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 Z 9.4; 95%CI:2.6e33.3) followed by the unfit ones with high adherence to the SEADiet (OR Z 6.6; 95% CI: 1.9e22.5) when compared to those who were fit and had higher adherence to SEADiet.

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

KEYWORDS: Metabolic syndrome; Diet; 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 charac- terized 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 [5,7e9]. However, studies examining the combined associations of CRF and diet on cardio-metabolic risk in adolescents are scarce. A better understanding of the combined associa- tions of CRF and diet on cardio-metabolic risk in youth isof 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 eval- uated 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 ± 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 pro- cessed. 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 Corpo- ration, 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 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 summari- zation 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 indi- vidual risk factors. High risk was considered when the individual had 2:1SD 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 sexespecific median of the study's participants as a cut-off value for each of the components, 1 point was given when intake 2: median and 0 points for intakes below median for all items except for wine (::;1 glass/d in women and ::;2 glasses/d in men Z 1 point). In the present study, we adapted this score by attributing 0 points to any wine consumption, since ethanol consump- tion 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 (::;4 points) and high (2:5 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 Le´ger [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 e "fit", and under the healthy zone e "unfit".

Pubertal stage

To determine the pubertal stage (ranging from stage 1e5), 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 vari- ables, 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 $p < 0.05$.

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 Z 9.4; 95%CI:2.6e33.3); 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 Z 6.6; 95% CI: 1.9e22.5). Further analysis to check what would happen if CRF (multiplied per -1) was included in the cluster of metabolic risk regarding the asso- ciation 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 Z 468)	Girls (n Z 273)	Boys (n Z 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, (mU/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] $p < 0.05$, ChieSquare Test.

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

Parameter	Low MRS (*n* Z 408)	High MRS (*n* Z 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 prog- nostic 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 sug- gested that a low CRF during childhood and adolescence is associated with later cardiovascular risk factors, such as obesity, hyperlipidemia, and hypertension [4,23e25]. 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 advo- cated 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 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 12e24 [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 associ- ated with high MRS. Whereas the majority of the adoles- cents 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.

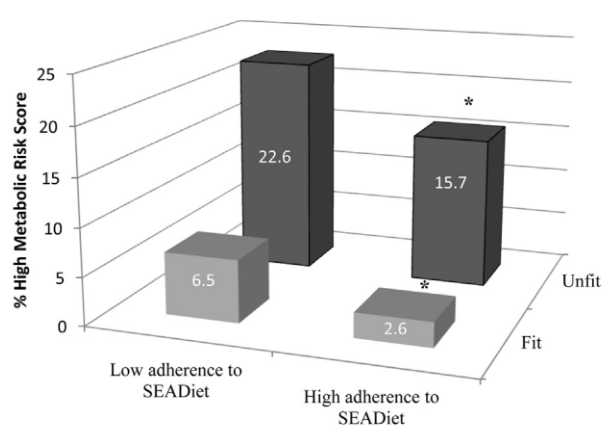

Figure 1 Prevalence of high metabolic risk score across cardiorespiratory fitness and adherence to the Southern Euro- pean Atlantic Diet categories. ❩Significantly different from unfit/fit level ($p < 0.001$).

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 vegeta- bles, 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 produc- tion 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 dys- lipidemia, 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 futurestudies.

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 delete- rious 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 meta- bolic 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

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 þ high adherence to SEADiet	1		1	
Fit þ low adherence to SEADiet	2.6 (0.6e11.1)	0.206	2.3 (0.5e10.1)	0.276
Unfit þ high adherence to SEADiet	6.9 (2.0e23.5)	0.002	6.6 (1.9e22.5)	0.003
Unfit þ low adherence to SEADiet	10.8 (3.2e36.6)	<0.001	9.4 (2.6e33.3)	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 anal- yses 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 chil- dren and adolescents to achieve and maintain healthy CRF levels for cardio-metabolic risk primary prevention purposes.

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