Original Research Article

Body Fatness and Clustering of Cardiovascular Disease Risk Factors in Portuguese Children and Adolescents

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ABSTRACT
Modifiable cardiovascular risk factors that increase the risk for cardiovascular diseases (CVD) in adult populations have also been observed in pediatric populations. Childhood and adolescence obesity has been strongly implicated in the clustering of risk factors. The aims of the present study were 1) to examine whether clustering of CVD risk factors, either biological risk factors (high blood pressure (HBP), percentage of high fat mass (%HBF), and high total cholesterol (HTC)) and one behavioral/lifestyle risk factor (low physical activity index (LPAI)) exist, and 2) to analyze the relationship between body fatness and the clustering of other risk factors. The cluster of CVD risk factors was determined in 1,533 (8–15 years of age) children, 731 males (age 10.8±2.3 years; weight, 40.6±12.7 kg; height, 143.1±14.1 cm; BMI, 19.4±3.4 kg m−2) and 802 females (age, 11.0±2.4; weight, 41.0±12.4; height, 142.8±13.2; BMI, 19.7±3.5). Sex- and age-specific “high risk” quartiles were formed for each of the biological risk factors and the lifestyle factor. Thus, for blood pressure (high blood pressure, HBP), cholesterol (high cholesterol, HTC), and obesity (high percent of body fat, HBF), the sex- and age-adjusted 4th quartile (4Q) was defined as the “high risk” quartile, while for physical activity the 1st quartile (1Q) was defined as the “high risk” quartile. The majority of children (62% of boys and 62% of girls) at risk of obesity are at risk of another risk factor. In our sample, estimated ORs indicated that, compared with 1Q, the “risk of obesity” children and adolescents were two times as likely (P<0.001) to have two or three risk factors. Our results suggest that children 8–15 years old in the highest quartile of body fatness are an increased risk of having a cluster of other risk factors, namely HBP, HTC, and LPAI. These data provide further evidence that juvenile obesity warrants early intervention because the patterns of unhealthy behavior are formed in adolescence and young adulthood. Am. J. Hum. Biol. 16:556–562, 2004.

Youth obesity has been strongly implicated in the clustering of risk factors for cardiovascular diseases (CVD) (Bao et al., 1994; Chu et al., 1998; Gutin et al., 1997; Twisk et al., 1999) and their tracking into young adulthood (Twisk et al., 1997). The adverse changes associated with overweight and obesity in children and adolescents have been documented in numerous studies and include increased blood pressure, dyslipidemia, elevated glucose, and hyperinsulinemia (Berenson et al., 1993; Gidding, 1995). In fact, most researchers agree that obesity is an important modulator of the metabolic syndrome (DeFronzo and Ferrannini, 1991; Laakso, 1993), which is a clustering of cardiovascular risk factors associated with insulin resistance (Reaven, 1988). Further, it has been reported that the children and adolescents in which risk factors cluster are supposed at high-risk of developing CVD in adult life (Khoury et al., 1980). In addition to these immediate health complications, overweight and obesity during late adolescence appear to be strongly associated with adult mortality from CVD (Hoffmans et al., 1988; Riddoch and Boreham, 2000). In fact, pathological evidence has also linked antemortem
levels of fatness in children with the progression of atherosclerosis in major arteries at autopsy (Berenson et al., 1998; McGill and McMahan, 1998), whereas a longer-term study (Must et al., 1992) has shown that overweight in adolescence was a more powerful predictor of adult morbidity and mortality than overweight in adulthood.

Obesity and associated CVD risk factors are becoming an important public health issue for children and adolescents (Chu et al., 1998); however, there have been few population-based studies to investigate obesity and its association with CVD risk factors in youth. The underlying reasons for the selection of biological risk factors considered in this study (high blood pressure (HBP), percentage of high fat mass (%HBF), and high total cholesterol (HTC)) and one behavioral/lifestyle risk factor (low physical activity index (LPAI)) were the following: 1) they are recognized as some of the most important independent risk factors for CVD; 2) the insufficient knowledge of these risk factors clustering and their manifestation in pediatric years; and 3) they are considered important CVD predictors in adult life (Twisk et al., 1999).

Therefore, the aims of the present study were 1) to examine whether clustering of CVD risk factors, either biological risk factors (HBP, %HBF, and HTC) and one behavioral/lifestyle risk factor (LPAI) exist, and 2) to analyze the relationship between body fatness and the clustering of other risk factors.

SUBJECTS AND METHODS
Sample
This study was conducted in the Porto area, comprising 30 schools (17 primary schools and 13 high schools). All children were apparently healthy and were free of any treatment. All measurements were completed between 9.00 and 11.00 hours. The schools were selected from all districts in a way that at least one school represented each district providing proportional numbers of children from different school types and environments (rural and urban). Children enrolled in these schools were chosen at random from the third to the eighth grades, according to general school system rules (i.e., elementary school, 8–9 years old; middle school, 10–11 years old; and secondary school, older than 12 years old). Therefore, the sample may thus be considered representative of the population from which it was drawn. The Portuguese Ministry for Science and Technology provided permission to conduct this study. Informed written consent was obtained from the children’s parents and individual school principals. To assure confidentiality, each subject’s data were coded and stored on a computer file. An individual feedback result form was sent to parents with the results of their child’s screening.

A total of 2,157 children and adolescents were sampled, of whom 1,533 (71%) and their parents agree to participate in the study. This sample consisted of 731 males and 802 females age 8–15 years. For each child not participating, the form master was asked if the child differed from the rest of the class in any way. Six children who had given consent to participate chose on the day of testing not to take part because of problems with their skin. Five of the nonparticipants were described as being overweight/obese and physically inactive. The rest were all described as normal, with a normal level of physical activity (PA) in comparison with their peers.

In relation to the daily evaluation protocol the participants were first identified through his/her code number and code of the school. Second, blood samples were taken. This was followed by blood pressure measurements. The children were then given breakfast. After that, skinfold thickness was taken followed by stage of maturation determination. Finally, a PA questionnaire was given. The methods of data collection for each of the risk factor variables are further described below.

Blood sampling
Subjects’ capillary blood samples were taken from the earlobe after at least 12 hours fasting in order to obtain values of total plasma cholesterol (TC). The blood samples were drawn in capillary tubes (33 μl, Selzer) coated with lithium heparin and immediately assayed using a Reflotron Analyzer (Boehringer Mannheim, Indianapolis, IN). To decrease the impact of measurement error, the mean of the two measurements was used in all statistical procedures. Elevated levels of TC (HTC) were defined according to the age- and sex-adjusted 4th quartile.

Blood pressure
Blood pressure was measured according to the procedure recommended in the literature (deSwiet et al., 1989; Gillman and Cook,
Systolic (SBP) and diastolic (DBP) blood pressure were measured in the right arm using an automated oscillometric sphygmomanometer (Dinamap, model BP 8800). The subjects were in the sitting position (without crossing their legs), with the right arm at heart level. Three standard pressure cuffs of correct size 9 \times 18; 12 \times 23; 14 \times 28 \text{cm} were used, according to published guidelines for BP assessment in children (Gillman and Cook, 1995). Two measurements were taken after 5 and 10 minutes rest. The mean of these two measurements was used for statistical analysis. If the two measurements differed by 2 mmHg or more the protocol was repeated (two new measurements, which could not exceed 2 mmHg). These methodological considerations are in agreement with several authors (deSwiet et al., 1989; Gillman and Cook, 1995) and were used in a previous study in a population with similar characteristics (Duarte et al., 2000). Elevated levels of SBP and DBP were defined according to the age- and sex-adjusted 4th quartile.

**Anthropometric measures and body composition**

Body height and body weight were determined by standard anthropometric methods. Height was measured to the nearest mm in bare or stocking feet with the child standing upright against a Holtain portable stadiometer. Weight was measured to the nearest 0.10 kg, lightly dressed (underwear and tee-shirt) using a Seca 708 portable digital beam scale. For weight and height the mean of two were used in the analysis. However, if weight and height values exceeded 0.2 kg and 2 mm, respectively, a third measurement was done and the mean of the three values used in the analysis. Body mass index (BMI) (kg/m$^2$) was calculated from the ratio of weight/height$^2$.

A Harpender caliper, with a constant pressure of 10 g/mm, was used to measure skinfold thicknesses at two sites, triceps and subscapular, according to Heyward (1991). Each skinfold was measured twice by a trained technician on the right side of the body. The mean of the two measures was used in the analysis. However, if in these two measurements there was a difference above 5%, a third measure was performed. The final result used the mean of the two or three measurements for each skinfold. For body composition values the model of two compartments was used, fat mass and fat-free mass, according to Lohman (1989). The percentage of body fat ($\%BF$) was estimated from skinfold measurement according to Slaughter et al. (1988) formulas. In these formulas, it is necessary to know the maturational age. Thus, the participants were questioned separately during physical examination. Each subject self-assessed his/her stage of secondary sex characteristics. Stage of breast in females and pubic hair in males was evaluated according to the criteria of Tanner (1962). This method avoids some constraint and is very easy to use (Malina, 1988). Previous study in same population showed a correlation of 0.73 between ratings on two occasions (3-day interval) in a subsample of 50 selected subjects (Mota et al., 2002a). Elevated levels of body fat (HBF) were defined according to the age- and sex-adjusted 4th quartile of %FM.

**Physical activity**

To calculate a physical activity index (PAI), an adapted version of the Weekly Activity Checklist (Sallis et al., 1993a) was applied to the sample. Careful administration procedures were adopted to prepare the children, since recall of PA is a complex cognitive task (Baranowski, 1988). The protocol described by Sallis et al. (1993a) was used. The interviewers looked at the familiarity with vocabulary and language levels of the children. To ensure accuracy of the translation double-checking procedures (translate and back-translate) were carried out and are described elsewhere (Mota and Silva, 1999). The final score was obtained by multiplying the frequency of each activity by the appropriate MET value and summing the products. This results in a score which is technically METs/15 minutes, but is rather considered an arbitrary score (Sallis et al., 1993b), used as PAI. This self-administered PA checklist was validated by Sallis et al. (1993b) and showed significant correlations ($P \leq 0.01$) with both heart rate index ($r = 0.57$) and accelerometer score ($r = 0.30$). More detailed information about the Portuguese validation and reliability data are described elsewhere (Mota et al., 2002b). The values found were consistent with previous studies showing that PA recall by such methods provides reasonably reliable and valid reports of children and adolescent physical activities (Sallis and Saelens, 2000). Levels of PA were defined according the sex- and age-adjusted PAI quartiles, where subjects...
belonging to the first quartile were defined as low level of physical activity (LPAI).

**Statistical analysis**

The data were analyzed using the Statistical Package for the Social Sciences (SPSS, Chicago, IL, v. 10.0) for Windows. Mean and standard deviation were used to describe the sample. Sex- and age-specific “high risk” quartiles were formed for each of the biological risk factors and the lifestyle factor. Thus, for blood pressure (high blood pressure, HBP), cholesterol (HTC), and obesity (high percent of body fat, HBF), the sex- and age-adjusted 4th quartile = highest quartile (4Q) was defined as the “high risk” quartile, while for PA the 1st quartile = lowest quartile (1Q) was defined as the “high risk” quartile. The clustering of CVD risk factors was defined as the number of prevalent conditions in both biological and lifestyle factors. The children were assigned to five categories, 0 (none of the conditions) through 4 (with all the conditions) according to their number of risk factors. The probability of children and adolescents having 0–4 risk factors associated was calculated using the binomial probability formula (Altman, 1991). The expected proportions were: 0.316, 0.422, 0.211, 0.047, and 0.004 for the five groups, respectively. The ratio between observed and expected number of children in each of the risk factor categories was then determined. The differences between observed and expected frequencies were tested with a χ² test. For analysis of the HFM as an independent variable and the clustering of the other CVD factors, both biological (HBP and HTC) and lifestyle (LPAI) clustering was defined as having two or three of those three risk factors. Odds ratios (ORs) and confidence intervals at 95% were calculated using logistic regression analysis to examine whether those at risk of obesity (highest quartile of fatness) were associated with a clustering of CVD risk factors. The alpha was set at 5%.

**RESULTS**

Table 1 shows the descriptive data of the sample. Significant sex differences were found for %BF, TC, and PAI. The number and the proportion of both boys and girls that had 0, 1, 2, 3, or 4 risk factors are presented in Table 2. The risk factor clustering was analyzed by comparing the observed number of subjects with a certain number of risk factors and the expected number calculated from a random distribution with 95% confidence interval. The percentages of observed and expected risk factors were similar. The proportion of children with three or four risk factors is under the expected number. The relation of “risk of obesity” (%FM 4Q) to adverse risk factors clustering (having 2RF or 3RF) is shown in Table 3. The results show that HFM-4Q had the highest percentage

**TABLE 1. Mean (X) and standard deviation (SD) of age, weight, height, body mass index (BMI), blood pressure (BP), total cholesterol (TC), percentage of body fat (%BF), and physical activity index (PAI) for males and females**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Males (n = 731)</th>
<th>Females (n = 802)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SD</td>
</tr>
<tr>
<td>Age (years)</td>
<td>10.8</td>
<td>2.3</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>40.6</td>
<td>12.7</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>143.1</td>
<td>14.1</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>19.4</td>
<td>3.4</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>118.1</td>
<td>10.2</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>61.4</td>
<td>9.9</td>
</tr>
<tr>
<td>TC (mmol/L)</td>
<td>4.2</td>
<td>0.7</td>
</tr>
<tr>
<td>BF (%)</td>
<td>18.3</td>
<td>8.0</td>
</tr>
<tr>
<td>PAI</td>
<td>1473.9</td>
<td>881.2</td>
</tr>
</tbody>
</table>

*P < 0.05; **P < 0.001.

**TABLE 2. Number of children observed (n) and expected (expe %) percentage (from a random distribution) with 0 risk factors (0 RF), 1 risk factor (1 RF), 2 risk factors (2 RF), 3 risk factors (3 RF), and 4 risk factors (4 RF) in males and females**

<table>
<thead>
<tr>
<th></th>
<th>0 RF</th>
<th>1 RF</th>
<th>2 RF</th>
<th>3 RF</th>
<th>4 RF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Males</td>
<td>323</td>
<td>42.9</td>
<td>285</td>
<td>37.8</td>
<td>121</td>
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<tr>
<td>Females</td>
<td>339</td>
<td>41.5</td>
<td>316</td>
<td>38.7</td>
<td>120</td>
</tr>
<tr>
<td>expe %</td>
<td>31.6</td>
<td>42.2</td>
<td>21.1</td>
<td>4.7</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**TABLE 3. Logistic regression in accordance to percentage of body fat (%BF) quartiles, adjusted to age and sex, for two and/or three risk factors (2–3 RF)**

<table>
<thead>
<tr>
<th>%BF</th>
<th>2–3 RF (%)</th>
<th>Odds ratio</th>
<th>Confidence interval</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st quartile</td>
<td>(9.3)</td>
<td>1.0</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>2nd quartile</td>
<td>(12.7)</td>
<td>1.4</td>
<td>0.9–2.3</td>
<td>0.16</td>
</tr>
<tr>
<td>3rd quartile</td>
<td>(13.2)</td>
<td>1.5</td>
<td>0.9–2.4</td>
<td>0.11</td>
</tr>
<tr>
<td>4th quartile</td>
<td>(17.1)</td>
<td>2.0</td>
<td>1.3–3.2</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>
DISCUSSION

This study reports a clustering of CVD risk factors in a large cross-sectional school-based survey of CVD risk factors in children and adolescents. Because there is no clear definition of hypertension, hypercholesterolemia, obesity, or physical inactivity in children and adolescents, our approach used the definition of "risk" quartiles. This approach has the limitation that the dichotomization (i.e., the definition of "risk quartiles") leads to a loss of power and there is a possibility of (nondifferential) misclassification, which can lead to bias towards the null. It should be kept in mind that the "risk groups" are based on arbitrary cut points, based on the observed distributions rather than on objective fixed cut-off points. Despite this limitation the "risk-groups" approach is interesting because the "risk groups" are the target population for prevention of disease and, therefore, prior identification of individuals at high risk allows for earlier intervention. The use of age- and sex-adjusted quartiles with the aim of defining a high CVD risk group is common in the literature (Christensen et al., 1980; Dennison et al., 1990; Kilkens et al., 1999; Raitakari et al., 1994).

While the level of each individual risk factor may be influenced by many factors, including genetics and lifestyle, aggregation of risk factors may indicate a common underlying causative factor.

The mean values for anthropometric measurements (weight, height, BMI) were near the average population values and CVD risk factor values are likely in the same range of sex- and age-specific percentiles of our population (Duarte et al., 2000; Guerra et al., 2002) and agree with other epidemiological studies (Maffeis et al., 1993; Toselli et al., 1997).

In this cross-sectional survey of 1,533 children and adolescents, we found an association between those in the highest quartile of body fatness and several CVD risk factors. Our results show that a majority (62%) of children and adolescents in the HFM-4Q had a clustering of two and/or three CVD risk factors, similar to other surveys. For instance, Becque et al. (1988) documented that 80% of obese adolescents have elevated SBP or DBP and 97% of these adolescents had four or more risk factors. In fact, our findings are in accordance with the literature that reported obese children and adolescents having higher rates of HBP, dyslipidemia, and hyperglycemia than their nonobese counterparts (Berenson et al., 1995; Khoury et al., 1980; Smoak et al., 1987).

Girls had a higher percentage of three and four risk factors compared to boys. An interesting question of this study was whether more participants than expected by chance had a higher number of risk factors for CVD. The clustering of risk factors we studied are similar to those found in the metabolic syndrome. Our results reveal that our sample had a better percentage of observed risk factor compared to the percentage of expected risk factors from a random distribution. Moreover, our findings clearly showed that more than half (57% boys and 58% girls) of the sample had at least one risk factor, which provides additional information for primary prevention strategies targeting youth.

The clustering of CVD risk factors in a random sample of Danish children and adolescents was studied by Andersen et al. (2002). In their study, five CVD risk factors (ratio HDL-C/TC, plasma triglyceride, plasma insulin, sum of four skinfolds, and SBP) were selected to assess the degree of clustering. Similar to our study, the risk was defined in each gender and age group as the lowest quartile of HDL-C and the top quartile of any of the other risk factors. The authors concluded that clustering of risk factors for the metabolic syndrome was found in children and adolescents. Low levels of physical fitness and high skinfolds in these individuals indicated that lifestyle factors such as PA and diet might influence the development of these unhealthy risk profiles.

The findings of our study also showed that those in the highest quartile of body fatness still had more risk factors than lean children. Therefore, our results suggest that the clustering of CVD risk factors may begin early in obese youth. Obesity and associated CVD risk factors are now a global problem and unless steps are taken to reduce obesity, rates of CVD and obesity-related mortality may continue to rise. Many authors pointed out that because obesity is related to clustering of CVD risk factors, screening for these risk factors among obese children and adolescents should be considered in national guidelines designed...
for the detection and prevention of CVD (Rifai et al., 1996).

CONCLUSIONS

This study adds data to the literature on childhood and adolescent obesity. Our results suggest that children 8–15 years old in the highest quartile of body fatness are at increased risk of having a cluster of other risk factors, namely, high blood pressure, high total cholesterol, and low physical activity index. These data provide further evidence that juvenile obesity warrants early intervention because the patterns of unhealthy behavior in childhood and adolescence are formed in adolescence and young adulthood.

LITERATURE CITED


