Lipid Profile in Portuguese Obese Children and Adolescents

Interaction of Apolipoprotein E Polymorphism With Adiponectin Levels

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Objective: To evaluate how the lipid profile associates with apolipoprotein (apo) E gene polymorphism, plasma adiponectin level, and body mass index (BMI) z score in Portuguese youth.

Design: Transversal cohort study.

Setting: Hospital de São João and Hospital de Crianças Maria Pia, Porto, Portugal, between May 2006 and March 2007.

Participants: One hundred thirty-eight obese children and adolescents (62 boys; mean age, 10.8 years [range, 4-16 years]). Participants were grouped according to (1) apo E polymorphism: presence of the apo E alleles 2 or 4 in E2 (n=11) and E4 (n=31) carriers, respectively, or as E3/E3 (n=94) (carriers of E2/E4 [n=2] were excluded from apo E analysis because they carry both alleles) and (2) BMI z score: group 1, BMI z score less than 2 (n=31); group 2, BMI z score of 2 or more and less than 2.5 (n=65); and group 3, BMI z score of 2.5 or more (n=42).

Main Outcome Measures: Lipid variable comparisons between apo E polymorphism and BMI z score groups and influence of BMI z score and adiponectin level, adjusted for apo E polymorphism, on total cholesterol to high-density lipoprotein cholesterol and apo A-I to apo B ratios.

Results: E4 carriers presented with a worse lipid profile when compared with E2 and E3/E3 carriers. There was also a clear risk of worsening for the group with the highest BMI z score. Apolipoprotein E polymorphism, BMI z score, and adiponectin level were significantly associated with total cholesterol to high-density lipoprotein cholesterol (standardized β coefficient=0.283, 0.354, and −0.292, respectively; P<.001 for all) and apo A-I to apo B ratios.

Conclusion: Our data suggest a more atherogenic lipid profile for some apo E genotypes and for increasing BMI z score, whereas adiponectin level seems to play a protective role.

high-density lipoprotein cholesterol (HDL-C) level. In this way, hyperaponeclemia seems to work as an independent biomarker of the metabolic syndrome.

Apolipoprotein (apo) E plays an important role in atherosclerosis by modifying inflammatory responses, facilitating cholesterol efflux from foam cells, and regulating hepatic uptake of remnant lipoproteins through the low-density lipoprotein (LDL) receptor and LDL receptor–related protein. The E2/E3/E4 apo E polymorphism results from variations in exon 4 at codon positions 112 and 158; E2 has a T allele at both positions 112 and 158; E3 has T and C alleles at positions 112 and 158, respectively; and E4 has C at both positions. These genetic variances create apo E isoforms with the following amino acid differences at positions 112 and 158, respectively: E2: Cys and Cys; E3: Cys and Arg; and E4: Arg and Arg. All combinations of the 2 isoforms are possible: E2/E2, E2/E3, E2/E4, E3/E3, E3/E4, and E4/E4.8,9

For the apo E polymorphism, subjects carrying the E2 and E4 alleles tend to have lower and higher cholesterol levels, respectively, compared with E3/E3 individuals.8 Contradictory results are found in the literature concerning the effect of the apo E allele on TG levels. However, a meta-analysis reported that TG levels were higher in E2 carriers and E3/E4 subjects than in E3/E3 subjects.9 Similar results were found in obese adult and child populations.8-11 The frequencies of the apo E alleles did not seem to differ between normal-weight and obese individuals.11

Although atherosclerosis is a chronic disease that begins early in life, and obesity is an important risk factor for the development of CVD, few studies have addressed CVD markers in obese children and adolescents. Particularly, to date, limited investigations have examined the associations between adiponectin level and the lipid profile in children and adolescents. Also, to our knowledge, no study has assessed the concomitant influence of adiponectin level and apo E genetic polymorphism on the lipid profile in that population. Because atherosclerosis begins early in life, the study of individual differences in the early onset and progression of potential initiating risk factors is important. This is of particular concern in our country because a recent study showed a very high prevalence of overweight/obesity (31.5%) in Portuguese children when compared with other European countries.12

The aim of our work was to evaluate how the lipid profile is influenced by the apo E gene polymorphism, adiponectin plasma levels, and body mass index (BMI) z score in Portuguese obese children and adolescents. We hypothesized that (1) apo E polymorphism and BMI z score would be significantly and independently related to the lipid profile and (2) the effect of the apo E polymorphism on the lipid profile could be influenced by plasma levels of adiponectin.

### METHODS

**SUBJECTS**

The protocol used for all participants was approved by the ethics committees of Hospital de São João and Hospital de Crianças Maria Pia, Porto, Portugal. Obese children and adolescents, aged 4 to 16 years, were identified from medical records at the departments of Pediatrics of Hospital de São João and Hospital de Crianças Maria Pia. All children who met the inclusion criteria were invited to participate. One hundred thirty-eight obese children and adolescents (62 boys and 76 girls) participated in the study after informed and written consent by their parents. The study took place between May 2006 and March 2007.

Obesity was defined as a BMI greater than the 95th percentile for age and sex (calculated as weight in kilograms divided by height in meters squared), according to 2000 Center for Disease Control and Prevention growth charts. Because BMI is not normally distributed, we calculated BMI z score using a calculator based on the 2000 Center for Disease Control and Prevention growth charts. Because we also wanted to study the value of obesity as defined by BMI z score, we divided the obese population into 3 groups: group 1, less than the percentile 97.5, corresponding to a BMI z score less than 2; group 2, between the percentiles 97.5 and 99.5, corresponding to a BMI z score of 2 or more and less than 2.5; and group 3, more than the percentile 99.5 (10% of our obese population), corresponding to a BMI z score of 2.5 or more.

Clinical data regarding the sample population were collected; the development of puberty was clinically assessed in the hospitals, on the basis of Tanner stages, by the pediatrics of our team. The physical examination included the measurement of height, weight, circumferences of waist and hip, Tanner stage assessment, and the presence of skin lesions related to obesity and its comorbidity. The participants were invited to come to the research centers after an overnight fast, and after clinical examination, blood was collected for laboratory analysis. Smokers and subjects with diabetes mellitus, endocrine disorders, hereditary diseases, or inflammatory or infectious diseases or who were undergoing any therapy that could interfere with our results were excluded from the study.

### PROCEDURES AND ASSAYS

**Blood Samples**

Blood samples were obtained on a fasting basis and processed within 2 hours of collection. Blood was obtained by venipuncture in EDTA-containing tubes and in test tubes without anticoagulant. Aliquots of plasma, buffy coat, and serum were made and immediately stored at −80°C until assayed.

**DNA Analysis**

Genomic DNA was extracted from white blood cells (buffy coat) by the proteinase K/salt precipitation method.13,14 Apolipoprotein E genotyping was performed by polymerase chain reaction (PCR)–restriction fragment length polymorphism using the method of Hixson and Vernier,15 with some modifications. A 244–base pair (bp) fragment located in exon 4 of the apo E gene was amplified using oligonucleotide primers that flank positions 112 and 158 in the referred exon (F4: 5'-ACAGAATTCGCCCCGGCCTGGTACAC-3' and F6: 5'- TAAGCTTGCCAGCGGTGC-3'). The PCR reaction was carried out in a thermal cycler (HYBAID TouchDown; Thermo Hybaid, Franklin, Massachusetts) using 1 µL of DNA in a volume of 20 µL containing 1X PCR buffer (HotStarTaq polymerase buffer, with a final concentration of 2.0 mM magnesium chloride; Qiagen, Valencia, California), 1µM of each primer, 10% (volume to volume ratio) dimethyl sulfoxide, 0.2 mM deoxyribonucleotide triphosphate, and 0.5 U of HotStarTaq DNA Polymerase (Qiagen). The PCR conditions were 95°C for 15 minutes followed by 31 cycles at 95°C for 45 seconds, 60°C for 1 minute, and 72°C for 2 minutes and, fi-
High-density lipoprotein cholesterol and LDL cholesterol (LDL-C) levels were measured using enzymatic colorimetric tests after selective separation of high-density lipoprotein and LDL fractions (Direct HDL Cholesterol and Direct LDL Cholesterol; Roche). Apolipoprotein A-I and apo B levels in serum were evaluated by immunoturbidimetric assays (uni-kit apo A-I and B–specific antisera; Roche).

**Plasma Analysis**

Plasma concentration of adiponectin was evaluated by using a standard commercial enzyme-linked immunoassay (Adiponectin; R&D Systems, Minneapolis, Minnesota). Intra-assay and interassay coefficients of variation were lower than 9% and 7%, respectively.

**STATISTICAL ANALYSIS**

Statistical analysis was performed using SPSS software (version 16.0 for Windows; SPSS, Chicago, Illinois). Kolmogorov-Smirnov analysis was used to test if the results were normally distributed. The results normally distributed are presented as mean (SD) and those not normally distributed are presented as median (interquartile range).

Male and female patients were compared using an unpaired t test or Mann-Whitney U test. The distribution of boys and girls with respect to genotypes and other categorical variables was analyzed using the χ² test and Fisher exact test.

Multiple comparisons between groups were performed by 1-way analysis of variance supplemented with the Tukey Honestly Significant Difference post hoc test, after log transformation of the variables (when necessary). Adjustment of statistical differences for confounding factors was performed using analysis of covariance. The strength of the association between the substances was estimated by Pearson correlation coefficient, after log transformation of the variables (when necessary). To evaluate the contribution of the different variables to adiponectin levels, we performed multiple regression analysis, using stepwise selection, with an entry criteria of P < .05.

To evaluate the influence of BMI z score and adiponectin level, adjusted for apo E polymorphism (allele carriers), on both TC: HDL-C and apo A-I:apo B ratios, we performed multiple regression analysis using the 3 variables in the models. Significance was accepted at P < .05.

### RESULTS

The clinical characteristics of the obese children and adolescents (n = 138) are presented in Table 1. The mean age and BMI z score were 10.8 years and 2.30, respectively.

Concerning the comparison between boys and girls (data not shown), no major differences were observed in both clinical and laboratory data, except for waist circumference (mean, boys, 95.4 cm and girls, 90.9 cm; P < .05) and waist to hip ratio (mean, boys, 0.955 and girls, 0.930; P < .005), which were higher in boys, and adiponectin levels (mean, boys, 6.92 mg/L and girls, 8.35 mg/L; P < .05), which were lower for boys. No statistically significant differences were found in the distribution of subjects with respect to apo E genotype between boys and girls.

Apolipoprotein E polymorphism was associated with different lipid and lipoprotein status (Table 2). To evaluate the association of lipid and lipoprotein values with

### Table 1. Clinical Characteristics, Genotype, and Biochemical Data in 138 Obese Children and Adolescents

<table>
<thead>
<tr>
<th>Clinical characteristic</th>
<th>No. (%)</th>
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<tbody>
<tr>
<td>Age, y, mean (SD)</td>
<td>10.8 (3.0)</td>
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<tr>
<td>Tanner stage of puberty, ≥2</td>
<td>69 (50)</td>
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<tr>
<td>Waist, cm, mean (SD)</td>
<td>92.9 (13.4)</td>
</tr>
<tr>
<td>Hip, cm, mean (SD)</td>
<td>98.8 (13.9)</td>
</tr>
<tr>
<td>Waist to hip ratio, mean (SD)</td>
<td>0.941 (0.051)</td>
</tr>
<tr>
<td>BMI, mean (SD)</td>
<td>29.69 (5.34)</td>
</tr>
<tr>
<td>BMI z score, mean (SD)</td>
<td>2.30 (0.45)</td>
</tr>
<tr>
<td>BMI z score &lt; 2</td>
<td>31 (22.5)</td>
</tr>
<tr>
<td>BMI z score ≥ 2</td>
<td>65 (47.1)</td>
</tr>
<tr>
<td>BMI z score ≥ 2.5</td>
<td>42 (30.4)</td>
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<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. (%)</th>
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<tbody>
<tr>
<td>E2/E2</td>
<td>0</td>
</tr>
<tr>
<td>E2/E3</td>
<td>11 (8.0)</td>
</tr>
<tr>
<td>E2/E4</td>
<td>2 (1.4)</td>
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<tr>
<td>E3/E3</td>
<td>94 (68.1)</td>
</tr>
<tr>
<td>E3/E4</td>
<td>28 (20.3)</td>
</tr>
<tr>
<td>E4/E4</td>
<td>3 (2.2)</td>
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<tr>
<th>Biochemical data, median (IQR)</th>
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<tr>
<td>TG level, mg/dL</td>
<td>77.0 (53.0-109.8)</td>
</tr>
<tr>
<td>TC level, mg/dL</td>
<td>161.0 (143.0-181.2)</td>
</tr>
<tr>
<td>HDL-C level, mg/dL</td>
<td>42.0 (35.8-48.0)</td>
</tr>
<tr>
<td>LDL-C level, mg/dL</td>
<td>104.0 (89.0-122.5)</td>
</tr>
<tr>
<td>apo A-I level, mg/dL</td>
<td>118.0 (108.4-128.6)</td>
</tr>
<tr>
<td>apo B level, mg/dL</td>
<td>79.0 (68.0-93.3)</td>
</tr>
<tr>
<td>TC:HDL-C ratio</td>
<td>3.89 (3.29-4.59)</td>
</tr>
<tr>
<td>apo A-I:apo B ratio</td>
<td>1.50 (1.24-1.75)</td>
</tr>
<tr>
<td>Adiponectin level, mg/L</td>
<td>7.92 (5.19-10.99)</td>
</tr>
</tbody>
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Abbreviations: apo, apolipoprotein; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

SI conversion factors: To convert apo A-I and apo B to grams per liter, multiply by 0.01; HDL-C, LDL-C, and TC to millimoles per liter, multiply by 0.0139.
When all participants were considered, no statistically significant correlations were observed between the age of the participants and the lipid values, except for apo A-I level, which was inversely correlated with age ($r = -0.200; P < .02$). The age of the participants was strongly positively correlated with BMI ($r = 0.528; P < .001$).

Adiponectin levels correlated inversely and significantly with age ($r = -0.288; P < .001$), BMI ($r = -0.305; P < .001$), waist to hip ratio ($r = -0.238; P = .005$), TG level ($r = -0.392; P < .001$), and TC:HDL-C ratio ($r = -0.265; P = .002$) and correlated positively and significantly with HDL-C level ($r = 0.267; P = .002$). In the multiple regression analysis, TG level, age, and waist to hip ratio were the only variables that remained statistically associated with adiponectin values ($log \text{ adiponectin level} = 5.683 - 0.354 log \text{ TG level} - 0.023 age - 0.947 waist / hip ratio; standardized \beta \ coefficients = -0.310, -0.261, and -0.186; P < .001, P < .001, and P = .02$, respectively).

Because apo E polymorphism, BMI z score, and adiponectin levels were significantly associated with changes in the TC:HDL-C and apo A-I:apo B ratios (important "atherogenic" ratios), we evaluated the combined effect of apo E genotype with both other factors on such ratios. We observed that the effect of apo E polymorphism on TC:HDL-C and apo A-I:apo B ratios seems to be influenced by BMI z score (Figure 1) and adiponectin values (Figure 2). Indeed, by using multiple regression analysis, and when adjusted for apo E genotype, both BMI z score and adiponectin level remained significantly associated with TC:HDL-C ratio ($log \text{ TC:HDL-C ratio} = 0.245 + 0.063 \text{ apo E polymorphism} + 0.094 \text{ BMI z}$...
score − 6.77 E−6 adiponectin; standardized β coefficients: 0.283, 0.354, and −0.292, respectively; \( P < .001 \) for all) and apo A-I:apo B ratio (log apo A-I:apo B ratio = 2.787 − 0.286 apo E polymorphism − 0.262 BMI z score + 2.62 E−5 adiponectin value; standardized β coefficients: −0.372, −0.284, and 0.327, respectively; \( P < .001 \) for all). For a better visualization of the results (graphically), obese participants were divided on the basis of their BMI z score (Figure 1) and having an adiponectin level lower than or higher than or equal to 7.92 mg/L (cutoff corresponds to the median value for the entire group) (Figure 2).

Figure 1. Effect of apolipoprotein (apo) E polymorphism on total cholesterol (TC) to high-density lipoprotein cholesterol (HDL-C) (A) and apo A-I:apo B (B) ratios, according to body mass index (BMI) z score. Results are presented as mean (95% confidence interval [CI]). The influence of BMI z score, adjusted for apo E polymorphism (allele carriers), on both the TC:HDL-C and apo A-I:apo B ratios was highly significant (\( P < .001 \)) by multiple regression analysis.

Figure 2. Effect of apolipoprotein (apo) E polymorphism on total cholesterol (TC) to high-density lipoprotein cholesterol (HDL-C) (A) and apo A-I:apo B (B) ratios, according to adiponectin level. For a better visualization of the results, we used a cutoff for adiponectin level of 7.92 mg/L, which corresponds to the median value for the entire group. Results are presented as mean (95% confidence interval [CI]). The influence of adiponectin level, adjusted for apo E polymorphism (allele carriers), on both the TC:HDL-C and apo A-I:apo B ratios was highly significant (\( P < .001 \)) by multiple regression analysis.

study, to our knowledge, assessing adiponectin levels in Portuguese obese children and adolescents.

The adiponectin values that we describe in this article are comparable with those found in other populations.\(^{16,17}\) Adiponectin levels were reported to be lower in boys than in girls, and the difference seems to be more striking at postpubertal states.\(^{16,17}\) Moreover, adiponectin levels were inversely related to age and were significantly lower in pubertal compared with prepubertal obese children.\(^{17}\) In our study, we also observed statistically significant differences between boys and girls, with boys presenting with lower adiponectin values. The 2 groups (boys and girls) were matched for age, BMI z score, and puberty stage, and therefore, the analysis of adiponectin level was not affected by these possible confounding factors. A significant inverse correlation between adiponectin levels and age was also observed for both sexes.

Adiponectin levels were lower in nonlean individuals compared with lean individuals\(^{18}\) and it was reported that mean adiponectin values decreased for ev-
ery unit increase in BMI z score. However, in obese subjects, adiponectin plasma concentrations were not correlated with BMI standard deviation score. In the present study, group 3 individuals, defined as obese children and adolescents having the highest BMI z scores (≥2.5), presented with the lowest adiponectin values compared with the other 2 groups, but it was not statistically significant (Table 3). We also found that adiponectin levels correlated inversely with BMI and waist to hip ratio but not with BMI z score.

It was previously reported that adiponectin level is positively correlated with HDL-C level and negatively correlated with TG level in both lean and nonlean adolescents and that these relationships strengthened with increasing adiposity. In the present study, we confirmed these relations and also that adiponectin levels correlated inversely and significantly with the TC:HDL-C ratio. As previously mentioned, adiponectin level was also inversely related to age, BMI, and waist to hip ratio. In the multiple regression analysis, TG level, waist to hip ratio, and age were the only variables that remained statistically associated with adiponectin values.

Regarding the apo E polymorphism, the distribution of subjects with respect to genotype was similar to that found in a previous work involving Portuguese obese children and pregnant women. Thus, and although a control group (nonobese children) was not evaluated in the present study, the frequency of the apo E genotypes is unlikely to be altered in obese children.

The association of the apo E polymorphism with changes in lipid and lipoprotein profiles is highly explored in adults, but not in children, particularly Portuguese children. In our studied population, significant allele effects of apo E genetic variability on plasma lipoprotein and apoprotein levels were observed. The results observed in carriers of E2 and E4 alleles were mainly due to the contribution of E2/E3 and E3/E4 subjects, respectively. E4 carriers presented with the highest LDL-C level, compared with those with the E3/E3 genotype and E2 carriers, in agreement with previous reports in obese children and adults. E4 carriers also presented with the highest TG levels and lower HDL-C and apo A-I levels, although this was not statistically significant. A previous study in pregnant women found no differences in the HDL-C levels between women with different apo E genotypes. However, a meta-analysis reported HDL-C levels to be lower in E3/E4 than in E3/E3 nonpregnant subjects. Furthermore, in the current study, E4 carriers presented with significantly higher TC and apo B levels (P < .05 for both groups); therefore, the TC:HDL-C ratio was significantly higher and apo A-I:apo B ratio significantly lower in this group when compared with the other 2. All these results remained statistically significant after adjustment for confounding factors such as age, sex, Tanner stage, and BMI z score.

A major finding of this study, achieved by performing multiple regression analysis, was that the effect of apo E polymorphism on the TC:HDL-C and apo A-I:apo B ratios seemed to be influenced by BMI z score (Figure 1) and adiponectin level (Figure 2). Individuals presenting with lower adiponectin levels or a higher BMI z score presented with higher TC:HDL-C and lower apo A-I:apo B ratios than those with higher adiponectin levels or lower BMI z scores, irrespective of their apo E polymorphism—carrying nature (Figure 1 and Figure 2). These atherogenic ratios therefore seem to worsen with lower adiponectin levels and higher BMI z scores, modulating early in life the effect of apo E genotype on lipids (ie, in children and adolescents). An article by Wardaningsih et al reported worsening of the lipid profile for E3/E3 children with lower adiponectin levels, especially in boys.

These relations between adiponectin level, apo E polymorphism, BMI z score, and lipid profile that we presented in Figure 1 and Figure 2 may be because adiponectin reduces hepatic release of apo E from hepatocytes and because the hepatic secretion of apo B is also reduced by adiponectin, possibly by a genetic mechanism involving the hepatic nuclear factor-4α.

Considering that the relationships between adiponectin, HDL-C, and TG levels are strengthened with increasing adiposity, heavier adolescents seem to have a greater benefit from high levels of adiponectin than their leaner counterparts. Furthermore, a recent study performed in obese children demonstrated an increase in adiponectin levels due to a significant weight loss over a 1-year period. This may be of particular importance in obese individuals with certain “risk” apo E genotypes, as we demonstrated that the influence of the apo E polymorphism on lipids is influenced by BMI z score and adiponectin levels. Atherosclerosis is a multifactorial disease, involving the interplay of genetic and environmental factors. The improvement of the latter factors, through a healthier lifestyle, seems therefore to be particularly worthy in those obese individuals with a less favorable genetic background.

In conclusion, the lipid profile in obese children and adolescents worsens with increasing adiposity (increasing BMI z score) and the effect of the apo E polymorphism on the TC:HDL-C and apo A-I:apo B ratios is modulated by BMI z score and adiponectin levels. This may be of particular relevance because obese individuals, particularly those with risk apo E genotypes, may benefit from a closer clinical follow-up. Moreover, the implementation of lifestyle modifications (mainly by practicing regular physical activity and eating a healthy diet) should be highly encouraged in such obese children and adolescents.
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REFERENCES


Even when freshly washed and relieved of all obvious confections, children tend to be sticky.

—Fran Lebowitz