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Aerobic fitness modulates the association between APOE genotypes and serum lipemia in adolescents

Maria Fátima Glaner
State University of Londrina, Brazil

Thales Boaventura Rachid Nascimento
Open University of Brazil, Brazil

Otávio de Tôledo Nóbrega
University of Brasília, Brazil

Abstract—The purpose of this study was to analyze the association between APOE alleles and serum lipemia in adolescents with low and adequate aerobic fitness. The sample was comprised of 105 boys and 151 girls (49% and 46% from rural area) of European ancestry, aged 11 to 17 years, and classified according to: 1) APOE genotype: group ε2 (ε2/3+ε2/2), ε3 (ε3/3), and ε4 (ε3/4+ε4/4); 2) aerobic fitness: adequate or low; 3) serum lipemia: elevated total cholesterol (TC), low-density lipoprotein (LDL) and triglycerides, and low high-density lipoprotein (HDL). The results showed that aerobic fitness modulates the association between APOE alleles and serum lipemia in adolescents, suggesting that adequate aerobic fitness levels exert a greater effect of reducing TC and LDL in ε2 carriers, as well as of increasing HDL and reducing triglycerides in ε3 and ε4 carriers.

Keywords: apolipoproteins E, physical endurance, adolescent health, lipoproteins

Resumo—"Aptidão física aeróbica modula a associação entre genótipos da APOE e lipemia sérica em adolescentes." O objetivo deste estudo foi analisar a associação entre os alelos da APOE e a lipemia sérica em adolescentes com baixa e adequada aptidão aeróbia. Amostra: 105 rapazes(49% da área rural) e 151 moças (46% da área rural) descendentes de europeus, com idade de 11 a 17 anos, classificados de acordo com 1) genótipo da APOE: grupo ε2 (ε2/3+ε2/2), ε3 (ε3/3), e ε4 (ε3/4+ε4/4); 2) aptidão aeróbia: adequada ou baixa; 3) lipemia sérica: elevados colesterol total (CT), lipoproteína de baixa densidade (LDL) e triglicérides, e baixa lipoproteína de alta densidade (HDL). Os resultados demonstram que a aptidão aeróbia modula a associação dos alelos da APOE com a lipemia sérica de adolescentes, sugerindo que níveis adequados de aptidão aeróbia têm efeito maior em reduzir CT e LDL elevados nos portadores do alelo ε2, bem como o efeito maior em aumentar HDL e reduzir triglicerídeos naqueles com ε3 e ε4.

Palavras-chave: apolipoproteína E, resistência física, saúde do adolescente, lipoproteínas

Resumen—"La condición física aeróbica modula la asociación entre los genotipos APOE y lipemia sérico en adolescentes." El objetivo fue analizar la asociación entre los alelos APOE y la lipemia sérica en adolescentes con baja y adecuada condición aeróbica. La muestra fue composta de 105 niños (49% de las zonas rurales) y 151 niñas (46% de las zonas rurales) de origen europeo, con edades entre 11-17 años, clasificados de acuerdo con 1) el genotipo APOE: grupo ε2 (ε2 / 3 + ε2 / 2), ε3 (ε3 / 3), y ε4 (ε3 / ε4 + 4/4); 2) la condición aeróbica: adecuada o baja; 3) suero lipémico: colesterol (CT), lipoproteínas de baja densidad (LDL) y los triglicéridos elevados y lipoproteínas de alta densidad (HDL) bajo. Los resultados muestran que la capacidad aeróbica modula la asociación de los alelos APOE con la lipemia de los adolescentes, lo que sugiere que niveles adecuados de capacidad aeróbica tienen mayor efecto en la reducción de CT y LDL elevados en el alelo ε2, y el mayor efecto para aumentar el HDL y la reducción de los triglicéridos en aquellos con ε3 y ε4.

Palabras clave: apolipoproteína E, resistencia física, salud del adolescente, lipoproteínas

Introduction

Low levels of high-density lipoprotein (HDL) and elevated levels of triglycerides, total cholesterol (TC) and low-density lipoprotein (LDL) are risk factors for heart disease in adulthood. In children and adolescents, lipemia is directly related to atherosclerotic processes (Lamotte, Iliescu, Libersa, & Gottrand, 2011) and is inversely associated with physical activity level (Taimela, Lehtimäki, Porkka, Räsänen, & Viikari, 1996). Low serum lipemia is observed in children and adolescents with a high level of aerobic fitness (Kwon, Burns, & Janz, 2010).

The apolipoprotein E (APOE) gene exists in at least three different forms (alleles). The major alleles are called epsilon two (ε2), three (ε3) and four (ε4) and are important modulators of serum lipemia. In general, individuals carrying the ε2 allele present lower serum concentrations of TC and LDL (Alvim *et al.*, 2010; Bernstein *et al.*, 2002; Corella *et al.*, 2001; França, Alves, & Hutz, 2004; Nghiem *et al.*, 2004) whereas the opposite is observed for those carrying ε4 allele (Bernstein *et al.*, 2002; Corella *et al.*, 2001; Medina-Urrutia, Cardoso-Saldaña, Zamora-González, Liria, & Posadas-Romero, 2004; Nascimento *et al.*, 2009). The association between APOE alleles and lipemia seems to be influenced by physical activity levels. Higher concentrations of HDL have been observed in physically active men with ε4 allele when compared to those with ε2 allele. In contrast, in inactive individuals higher concentrations of this lipoprotein were found among those carrying ε2 allele (Corella *et al.*, 2001). In addition, inactive individuals, but not physically active individuals, carrying ε4 allele were found to have higher LDL concentrations than carriers of the other alleles (Pisciotta *et al.*, 2003).

The metabolic responses to physical effort, as well as the capacity to exercise for prolonged periods of time, differ between children, adolescents and adults. Therefore, the observed relationship between APOE gene and physical activity on lipemia in adults cannot be extrapolated to other age groups.

Although aerobic fitness is one of the most important components of health-related physical fitness and is more strongly associated with cardiovascular risk factors than physical activity levels, its interaction on the relationship between the APOE gene and serum lipemia has not been investigated in adolescents. Therefore, the objective of the present study was to analyze the association between APOE alleles and serum lipemia in adolescents with low and adequate aerobic fitness. The hypothesis was that aerobic fitness modulates the association between APOE alleles and serum lipemia in adolescents.

Methods

Study population

In an observational, cross-sectional study, data were collected in 2008 in the municipality of Saudades, State of Santa Catarina, South of Brazil. The municipality has 9,016 inhabitants (Brazilian Institute of Geography and Statistics, 2010) and the human development index is 0.831 (United Nations, 2000). Its population mainly consists of German descendants and a minority of Russian and Italian descendants (Brazilian Institute of Geography and Statistics, 2010). Such a homogeneous sample in terms of ancestry helps preventing biases related to interethnic disparities among populations with different genetic backgrounds.

The adolescents were recruited from two public schools in the municipality, one located in the urban area (elementary and high school) and the other in the rural area (elementary school). All adolescents were invited, and those who agreed to participate in the study answered a recognition questionnaire and a blood sample was collected. The anthropometric measurements and aerobic fitness test were performed during regular school physical education classes. The criteria of exclusion were a) age < 10 and > 17 years; b) failure to complete one or more of the data collections; c) motor limitations that would prevent participation in the physical test; d) use of hypolipemic agents; e) pregnancy. The final sample consisted of 101 boys (49% from the rural area) and 151 girls (46% from the rural area). The study was approved by the local Ethics Committee. All adolescents were volunteers and their legal guardians signed the free informed consent form.

Anthropometric measurements

Body weight was measured with a portable digital scale (Soenhle®) to the nearest 0.1 kg and height was measured with a metric tape fixed to the wall to the nearest 0.1 cm. Triceps and medial calf skinfold thickness was measured with a Lange® caliper. The sum of these skinfolds was used to indicate total body fat. The individuals were classified as having adequate (girls: 16-36 mm; boys: 12-25 mm) and elevated fat (girls: > 36 mm; boys: > 25 mm) (AAHPERD, 1988). The three individuals with body fat below the adequate level were included in the adequate category. Abdominal perimeter was measured with a metric tape 2.5 cm above the umbilical scar. This measure was used as an indicator of abdominal fat, which was classified as normal or elevated (Katzmarzyk *et al.*, 2004).

Aerobic fitness

Aerobic fitness was estimated by the one-mile walking test, with the individuals covering the distance in the shortest time possible. Aerobic fitness was classified as adequate or low according to gender and age (AAHPERD, 1988).

Pubertal stage

Pubertal stage was obtained by self-assessment of pubic hair development in each gender (Tanner, 1962). The volunteers were classified into two groups: pubertal (stages II, III, and IV) and postpubertal (stage V). One adolescent rated as stage I was included in the pubertal group.

Serum lipemia

Venous blood samples were collected in the morning after a 12-14 h fast into vacuum tubes containing clotting accelerator. Serum was separated from the samples and stored under

refrigeration until the time for analysis. Total cholesterol, very low-density lipoprotein (VLDL), HDL and triglycerides were quantified by automated spectrophotometry (Cobas Mira Plus Roche®) using biochemical reagents from Labtest®. LDL concentration was calculated as described elsewhere (Friedewald, Levy, & Fredrickson, 1972). LDL levels were measured directly in individuals who presented triglyceride concentrations > 400 mg/dL. Individuals with LDL and triglycerides < 100 mg/dL, TC < 150 mg/dL and HDL ≥ 45 mg/dL were classified as normal (I Guideline for Preventing Atherosclerosis in Childhood and Adolescence, 2005). Adolescents who presented concentrations outside these ranges were assigned to the elevated group.

Genetic analysis

Genomic DNA was extracted by the salting-out method (Miller, Dykes, & Polesky, 1988) from leukocytes of venous blood samples collected into vacuum tubes containing EDTA . The APOE gene polymorphism was genotyped by multiplex polymerase chain reaction (PCR) in a thermocycler (MJ96+ Biocycler®) (Donohoe, Salomäki, Lehtimäki, Pulkki & Kairisto, 1999). The PCR products were separated by agarose gel electrophoresis and the genotypes were directly identified according to the DNA fragments visualized under ultraviolet light. The adolescents were divided into three genotype groups

Table 1. Characteristics of boys and girls according to APOE allele.

Variable	Mean (SD)			
	Group			Total
	ε2	ε3	ε4	
Boys	(*n* = 7)	(*n* = 69)	(*n* = 25)	(*n* = 101)
Age, years‡	14.4 (2.1)	14.3 (1.7)	14.1 (1.7)	14.3 (1.7)
Height, cm	168.6 (7.9)	164.0 (11.0)	164.4 (10.6)	164.4 (10.7)
Body weight, kg	54.7 (6.6)	57.6 (15.8)	55.3 (11.2)	56.8 (14.3)
TR+CA, mm†	31.6 (17.3)	32.3 (16.7)	30.8 (12.2)	31.8 (15.6)
Adequate TR+CA, n (%)	3 (42.9)	34 (49.3)	7 (28.0)[a]	44 (43.6)
AP, cm‡	69.8 (6.3)	74.5 (10.7)	72.8 (6.4)	73.8 (9.5)
Normal AP, n (%)	4 (57.1)	47 (68.1)	18 (72.0)	69 (68.3)
1-mile time, min‡	7:4 (0:4)	8:1 (1:2)	7:5 (1:0)	8:0 (1:1)
Adequate 1-mile time, n (%)	5 (71.4)	35 (50.7)	16 (64,0)	56 (55.4)
Total cholesterol, mg/dL	131.3 (19.3)[a]	159.5 (27.1)[b]	164.7 (20.5)[b]	158.9 (26.2)
LDL, mg/dL	72.9 (23.3)[a]	99.3 (22.8)[b]	102.4 (21.2)[b]	98.2 (23.3)
HDL, mg/dL†	46.0 (8.4)	46.8 (10.5)	46.1 (12.5)	46.6 (10.8)
Triglycerides, mg/dL†	63.0 (36.8)	69.2 (38.0)	81.3 (40.4)	71.8 (38.6)
Girls	(*n* = 11)	(*n* = 96)	(*n* = 44)	(*n* = 151)
Age, years‡	13.5 (2.2)	14.5 (2.0)	13.8 (2.3)	14.2 (2.1)
Height, cm	156.3 (9.3)[ab]	160.9 (9.0)[a]	156.4 (9.3)[b]	159.3 (9.3)
Body weight, kg	51.3 (13.7)	52.6 (10.9)	50.1 (10.9)	51.8 (11.1)
TR+CA, mm	39.7 (16.8)	40.6 (13.1)	39.8 (12.4)	40.3 (13.1)
Adequate TR+CA, n (%)	4 (36.4)	41 (42.7)	15 (34.1)	60 (39.7)
AP, cm†	71.6 (10.6)	71.2 (8.4)	70.1 (7.3)	70.9 (8.2)
Normal AP, n (%)	5 (45.5)	51 (53.1)	22 (50.0)	78 (51.7)
1-mile time, min‡	9:5 (1:1)	9:5 (1:2)	10:0 (1:2)	9:5 (1:2)
Adequate 1-mile time, n (%)	8 (72.7)	87 (90.6)	36 (81.8)	131 (86.8)
Total cholesterol, mg/dL†	146.8 (15.9)[a]	169.9 (25.7)[b]	182.1 (35.0)[b]	171.8 (29.5)
LDL, mg/dL†	89.5 (20.8)[a]	105.3 (22.5)[ab]	117.3 (31.0)[b]	107.7 (26.1)
HDL, mg/dL	42.0 (7.0)	49.3 (10.0)	49.0 (9.7)	48.7 (9.8)
Triglycerides, mg/dL†	76.5 (38.3)	76.6 (39.2)	80.0 (39.3)	77.6 (38.9)

TR+CA = sum of triceps and medial calf skinfolds; AP = abdominal perimeter; 1-mile time = time in the one-mile walking test; LDL = low-density lipoprotein; HDL = high-density lipoprotein.
Means in the same row followed by different superscript letters differed significantly ($p < .05$).
† Data compared after logarithmic transformation.
‡ Data compared by the Kruskal-Wallis test.

for subsequent analysis: group ε2 (genotypes ε2/3 and ε2/2), group ε3 (genotype ε3/3), and group ε4 (genotypes ε3/4 and ε4/4). Adolescents carrying genotype ε2/4 (n = 8) were only considered in the analysis of allele and genotype frequencies, but ruled out the inferential analyses due to opposing effects that these two alleles exert on serum lipemia.

Statistical analysis

Allele frequencies were obtained by gene counting. Fisher's exact test was used to evaluate the Hardy-Weinberg equilibrium and to compare allele and genotype frequencies between genders. The normality of data distribution was evaluated in each group by the Kolmogorov-Smirnov and Shapiro-Wilk tests. Variables that showed no normal distribution were $\log_{10}$ transformed. Groups of ε2, ε3, and ε4 carriers were compared by one-way analysis of variance (ANOVA), with results expressed in the original scale. If the difference was statistically significant, the Bonferroni *post-hoc* test was applied. Variables showing no normal distribution even after logarithmic transformation were compared by the Kruskal-Wallis test. The frequencies of adequate aerobic fitness, adequate body fat and normal abdominal perimeter were compared by the chi-square test. In view of the high prevalence of the dependent variable (elevated serum lipemia), Poisson regression with robust variance was used. The association between elevated serum lipemia and APOE alleles was evaluated according to aerobic fitness level (adequate and low). For this purpose, the APOE alleles were used as the independent variable and age, gender, pubertal stage, total body fat, and abdominal fat as the controlled variables. Prevalence ratios (PR) and the respective confidence intervals (CI) were estimated. All analyses were carried out using the Statistical Package for the Social Sciences (SPSS), version 15.0. A level of significance of $p < .05$ was adopted for all tests.

Results

Allele and genotype frequencies of the APOE gene

The most prevalent allele was ε3 (78.9%), followed by alleles ε4 (16.1%) and ε2 (5.0%). The genotype distribution followed Hardy-Weinberg equilibrium ($p > .05$). The three most frequent genotypes were ε3/3 (63.5%), ε3/4 (24.2%), and ε2/3 (6.9%), followed by genotypes ε2/4 (3.1%) and ε4/4 (2.3%). There was no difference in allele or genotype frequencies between genders ($p > .05$). Genotype ε2/2 was not detected in this sample.

Characteristics of the sample

Boys carrying ε2 allele presented lower TC and LDL concentrations than those carrying the ε3 and ε4 alleles ($p < .05$). No difference was observed for the other variables ($p > .05$). Girls carrying ε2 allele presented lower TC concentrations than those carrying the ε3 and ε4 alleles ($p < .05$). Lower LDL levels were observed in girls carrying ε2 allele when compared those carrying ε4 allele. In addition, girls carrying ε3 allele were taller than those carrying ε4 allele ($p < .05$). No difference in the other variables was observed among alleles ($p > .05$). Most boys and girls presented a waist circumference (68.3% and 51.7%, respectively) and aerobic fitness (55.4% and 86.8%) within the recommended range. At the same time, only 43.6% of boys and 39.7% of girls had adequate total body fat (Table 1).

Association between APOE alleles and lipemia

Elevated TC levels was more frequent among ε3 (PR, 1.27; 95% CI, 1.08-1.50) and ε4 carriers (PR, 1.32; 95% CI, 1.11-1.56) when compared to ε2 carrier. A higher probability of elevated LDL was observed among ε3 (PR, 1.30; 95% CI, 1.12-1.52) and ε4 (PR, 1.44; 95% CI, 1.23-1.69) carriers than ε2 carriers, and among ε4 (PR, 1.10; 95% CI, 1.01-1.20) than ε3 carriers (Table 2).

In the group with adequate aerobic fitness, the probability of elevated TC was higher among ε3 (PR, 1.39; 95% CI, 1.15-1.69) and ε4 (PR, 1.47; 95% CI, 1.21-1.79) carriers when compared to ε2 carriers. A higher probability of elevated LDL cholesterol was observed among ε3 (PR, 1.30; 95% CI, 1.08-1.57) and ε4 (PR, 1.49; 95% CI, 1.24-1.80) carriers when compared to ε2 carriers, and among ε4 (PR, 1.15; 95% CI, 1.04-1.26) than ε3 carriers. A lower probability of low HDL cholesterol was observed among ε3 (PR, 0.80; 95% CI, 0.67-0.95) and ε4 (PR, 0.81; 95% CI, 0.67-0.98) carriers than ε2 carriers (Table 3). In the group with low aerobic fitness, the probability of elevated triglycerides was higher among ε3 (PR, 1.45; 95% CI, 1.21-1.75) and ε4 (PR, 1.56; 95% CI, 1.24-1.96) carriers when compared to ε2 carriers (Table 3).

Discussion

In the present study, the frequencies of the ε2, ε3 and ε4 alleles (5.0%, 78.9%, and 16.1%, respectively) were similar to those reported for samples of the Swedish (7.0%, 82.0%, and

Table 2. Association between APOE alleles and elevated serum lipemia.

Variable	PR (95%CI)[a]	Variable	PR (95%CI)[a]
Elevated total cholesterol		Elevated LDL	
ε3 *vs* ε2[b]	1.27 (1.08-1.50)*	ε3 *vs* ε2[b]	1.30 (1.12-1.52)*
ε4 *vs* ε2[b]	1.32 (1.11-1.56)*	ε4 *vs* ε2[b]	1.44 (1.23-1.69)*
ε4 *vs* ε3[b]	1.04 (0.97-1.11)	ε4 *vs* ε3[b]	1.10 (1.01-1.20)*
Low HDL		Elevated triglycerides	
ε3 *vs* ε2[b]	0.90 (0.76-1.06)	ε3 *vs* ε2[b]	1.04 (0.87-1.25)
ε4 *vs* ε2[b]	0.91 (0.76-1.08)	ε4 *vs* ε2[b]	1.04 (0.86-1.26)
ε4 *vs* ε3[b]	1.01 (0.92-1.11)	ε4 *vs* ε3[b]	0.99 (0.90-1.10)

PR = prevalence ratio; CI = confidence interval; LDL = low-density lipoprotein; HDL = high-density lipoprotein.
[a] Adjusted for age, gender, pubertal stage, aerobic fitness, body fat, and abdominal perimeter.
[b] Reference allele.
* $p < .05$.

Table 3. Association between APOE alleles and elevated serum lipemia according to aerobic fitness level.

Variables	Prevalence ratio[a] (95% confidence interval)	
	Adequate aerobic fitness (n = 187)	Low aerobic fitness (n = 65)
Elevated total cholesterol		
ε3 *vs* ε2[b]	1.39 (1.15-1.69)*	1.06 (0.85-1.32)
ε4 *vs* ε2[b]	1.47 (1.21-1.79)*	1.00 (0.79-1.27)
ε4 *vs* ε3[b]	1.05 (0.98-1.14)	0.94 (0.79-1.12)
Elevated LDL		
ε3 *vs* ε2[b]	1.30 (1.08-1.57)*	1.32 (0.98-1.80)
ε4 *vs* ε2[b]	1.49 (1.24-1.80)*	1.26 (0.90-1.78)
ε4 *vs* ε3[b]	1.15 (1.04-1.26)*	0.95 (0.79-1.15)
Low HDL		
ε3 *vs* ε2[b]	0.80 (0.67-0.95)*	1.13 (0.85-1.50)
ε4 *vs* ε2[b]	0.81 (0.67-0.98)*	1.15 (0.85-1.58)
ε4 *vs* ε3[b]	1.01 (0.90-1.13)	1.02 (0.84-1.25)
Elevated triglycerides		
ε3 *vs* ε2[b]	0.94 (0.76-1.16)	1.45 (1.21-1.75)*
ε4 *vs* ε2[b]	0.93 (0.74-1.16)	1.56 (1.24-1.96)*
ε4 *vs* ε3[b]	0.99 (0.88-1.10)	1.07 (0.87-1.33)

LDL = low-density lipoprotein; HDL = high-density lipoprotein.
[a] Adjusted for age, gender, pubertal stage, aerobic fitness, body fat, and abdominal perimeter.
[b] Reference allele.
* $p < .05$.

11.0%) (Bernstein *et al.*, 2002) and French (8.0%, 80.0%, and 12.0%) (Marques-Vidal *et al.*,2003) population. Furthermore, no difference in allele or genotype frequency was observed between genders, in agreement with other studies (Correla *et al.*, 2001; França *et al.*, 2004; Nascimento *et al.*, 2009).

The frequencies (Table 1) of adequate total body fat (boys: 43.6%, girls: 39.7%) and aerobic fitness (boys: 55.4%, girls: 86.8%) were similar to those obtained a decade ago for adolescents of the same age group from the same municipality (Glaner, 2002). In the present study, 68.3% of boys and 51.7% of girls had an adequate abdominal perimeter, which was similar to that seen in other developing countries (De Moraes *et al.*, 2011). Considering that aerobic fitness has an inverse relationship with body fat (Kwon, Burns, & Janz, 2010), it was expected that prevalence of high levels of body fat and abdominal perimeter were lower among girls, which did not occur. These findings have been reported in other study (Byrd-Williams *et al.*, 2008), suggesting that, among girls, others factors may be more important determinant to increase adiposity levels, like nutritional ones, because poor eating habits are more prevalent among girls than boys (Neutzling, Assunção, Malcon, Hallal, & Menezes, 2010).

The sum of skinfolds, abdominal perimeter and time in the one-mile walking test did not differ among ε2, ε3 and ε4 carriers (Table 1). These findings, together with the fact that total and abdominal fat (Dai *et al.*, 2009), as well as, aerobic fitness (Kwon *et al.*, 2010) are associated with serum lipemia, suggest that differences in lipemia among alleles are not due the differences in these variables.

In boys and girls, TC concentrations were lower in ε2 carriers when compared to other alleles carriers (Table 1). The same has been reported for children (Nghiem *et al.*, 2004) and other adolescents (França *et al.*, 2004). In addition, the probability of elevated TC was 27% and 32% higher among ε3 and ε4 carriers than ε2 carriers (Table 2). The same has been reported for adults (Alvim *et al.*, 2010), demonstrating that ε2 protects against hypercholesterolemia. Considering the level of aerobic fitness, only adolescents with adequate aerobic fitness benefited from the ε2 lipid-lowering property, resulting in ε3 and ε4 individuals with higher probability (39% to 47%) of elevated TC when compared to ε2 carriers (Table 3). In agreement with this finding, TC concentrations did not differ among inactive adults carrying the different APOE alleles (Hagberg *et al.*, 1999; Thompson *et al.*, 2004). The inverse association between physical activity and TC was found to be stronger among boys with ε2 allele (Taimela *et al.*, 1996). These findings suggest that the protective effect of allele ε2 against elevated TC is context-sensitive, being enhanced by adequate aerobic fitness.

Lower LDL cholesterol concentrations were observed in boys with ε2 alleles when compared to the other groups, whereas girls with ε2 and ε4 alleles exhibited a similar phenotype (Table 1). In agreement with this finding, lower concentrations of this lipoprotein have been demonstrated in children (Nghiem *et al.*, 2004) and other adolescents (França, Alves & Hutz, 2004) with ε2 allele. The probability of elevated LDL cholesterol ranged from 10% to 44% among adolescents carrying ε4 allele compared to the other alleles and was 30% higher in ε3 than ε2 carriers (Table 2). Similar findings have been reported for adults (Alvim *et al.*, 2010), suggesting that allele ε2 protects against an atherogenic lipemic profile. Considering the level of aerobic fitness, the probability of elevated LDL cholesterol was only higher among adolescents with adequate aerobic fitness carrying ε4 allele (15% to 48%) when compared to the other groups, and among those carrying ε3 allele (30%) when compared to those carrying ε2 allele (Table 3). These findings seems to indicate that in adolescents the protective effect of ε2 in relation to ε4 depends on adequate aerobic fitness, in agreement with the study of Pisciotta *et al.* (2003).

HDL cholesterol concentrations did not differ between adolescent boys or girls with the different alleles (Table 1). This finding agrees with other studies involving adolescents (França, Alves & Hutz, 2004; Nascimento *et al.*, 2009), but disagrees with the studies of Medina-Urrutia *et al.* (2004) and Nghiem *et al.* (2004). In addition, the APOE alleles were not associated with low HDL cholesterol (Table 2), as also observed in adults (Alvim *et al.*, 2010). Only adolescents with adequate aerobic fitness carrying the ε3 and ε4 alleles presented a lower probability (20% to 19%) of low HDL cholesterol than ε2 carriers (Table 3). Lower HDL cholesterol concentrations have been observed in physically active men with ε2 allele when compared to those with ε4 allele (Corella *et al.*, 2001). Similar concentrations have been detected

in inactive individuals carrying the different alleles (I Guideline for Preventing Atherosclerosis in Childhood and Adolecence, 2005; Hagberg *et al.*, 1999). As reported for adults with increased physical activity level (Bernstein *et al.*, 2002), these findings suggest a greater increase of HDL cholesterol in adolescents carrying ε4 and ε3 alleles in response to improved aerobic fitness when compared to those with ε2 allele. However, no effect of the interaction between physical activity and the APOE gene on HDL cholesterol has been seen in young people (Taimela *et al.*, 1996). Furthermore, the results of experimental studies on adults comparing HDL cholesterol responses between APOE alleles after an exercise program were not consistent (Hagberg *et al.*, 1999; Leon *et al.*, 2004; Thompson *et al.*, 2004).

As observed for children (Nghiem *et al.*, 2004) and other adolescents (França, Alves, Hutz, 2004; Medina-Urrutia, Cardoso-Saldaña, Zamora-González, Liria, Posadas-Romero, 2004; Nascimento *et al.*, 2009), triglyceride levels did not differ between APOE alleles in either gender (Table 1). This lack of an association with any of the APOE alleles suggests that these alleles do not stimulate an increase of triglycerides, irrespective of other factors (Table 2). However, analysis according to the aerobic fitness levels showed a higher probability (45% to 56%) of hypertriglyceridemia only in adolescents with low aerobic fitness who carry the ε3 and ε4 alleles when compared to those carrying ε2 allele (Table 3). These findings suggest lower triglyceride levels in adolescents with ε4 and ε3 alleles as a result of better aerobic fitness, when compared to those with ε2 allele. However, no difference in triglyceride concentrations among APOE alleles was found in inactive men (Hagberg *et al.*, 1999; Thompson *et al.*, 2004). In children and adolescents, the interaction between physical activity and the APOE gene exerted no effect on triglyceride levels (Taimela *et al.*, 1996). Furthermore, the results of investigations comparing triglyceride responses to physical activity between the different APOE alleles are not consistent, with some studies demonstrating a reduction of triglyceride levels in carriers of alleles ε2, ε3 (Leon *et al.*, 2004) and ε4 (Bernstein *et al.*, 2002), whereas no difference between alleles was reported in another study (Thompson *et al.*, 2004).

The present study has some limitations: 1) the small number of volunteers per alleles, especially for allele ε2, which may have prevented the detection of some associations, and 2) the lack of investigation of eating habits that could have influenced the magnitude and direction of some associations since fat intake is known to influence triglyceride and LDL concentrations in carriers of alleles ε2 and ε4 (Andrade *et al.*, 2010). In contrast, the strengths of this study were the use of a Brazilian sample with the same ethnic background and with similar life habits as a result of the strong cultural tradition of the population in this geographic area; the use of rigorous reference criteria (AAHPERD, 1988) for the classification of aerobic fitness, and the control of variables that could influence serum lipemia (age, pubertal stage, total body fat, and abdominal fat). Another strength of this study was that puberty stage (stage II or III or IV) was observed in most part of the sample (83.8% and 98.1% of the boys and girls, respectively), a fact minimizing the effects of biological differences. The findings suggest that adequate levels of aerobic fitness have a greater effect of reducing TC and LDL in ε2 carriers, as well as of increasing HDL and reducing triglycerides in ε3 and ε4 carriers. To our knowledge, this is the first report demonstrating this association and further studies are needed to elucidate the physiological mechanisms underlying the relationship between aerobic fitness, APOE gene and serum lipemia.

Conclusion

In conclusion, the aerobic fitness modulates the association between APOE alleles and serum lipemia in adolescents by reducing TC and LDL levels in ε2 carriers as well as by increasing HDL and reducing triglycerides in ε3 and ε4 carriers. Therefore, aerobic fitness level was found to be a confounding factor in the analysis of the association between APOE alleles and serum lipemia, and should be taken into account in investigations considering these variables, which may help to interpret some divergent/ambiguous findings. From the public health perspective, policies addressing the improvement and maintenance of adequate levels of aerobic fitness e adiposity should be implemented in childhood and adolescence in order to minimize or prevent the cardiovascular disease.

Recommendations

Study results showed that adolescents with adequate aerobic fitness who carried the ε2 allele have a protective effect against serum lipemia. Thus, this physical quality should be considered in investigation related to APOE gene and serum lipemia, which may help to interpret some divergent/ambiguous findings.

References

Alvim, R.O., Freitas, S.R., Ferreira, N.E., Santos, P.C., Cunha, R.S., Mills, J,G, …Pereira, A.C. (2010). APOE polymorphism is associated with lipid profile, but not with arterial stiffness in the general population. *Lipids in Health and Disease, 8*,128. doi:10.1186/1476-511X-9-128

American Alliance for Health, Physical Education, Recreation and Dance (1988). *Physical best*. Reston, VA: AAHPERD.

Andrade, F.M., Bulhões, A.C., Maluf, S.W., Schuch, J.B., Voigt, F., Lucatelli, J.F., …Hutz, M.H. (2010). The influence of nutrigenetics on the lipid profile: interaction between genes and dietary habits. *Biochemical Genetics*, *48*,342-355. doi:10.1007/s10528-010-9931-6

Bernstein, M.S., Costanza, M.C., James, R.W., Morris, M.A., Cambien, F., Raoux, S., …Morabia, A. (2002). Physical activity may modulate effects of ApoE genotype on lipid profile. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *2*,133-140. doi:10.1161/hq0102.101819

Brazilian Institute of Geography and Statistics (2010). *Censo populacional 2010*. Retrieved from: http://www.censo2010.ibge.gov.br/sinopse/index.php?uf=42&dados=21.

Byrd-Williams, C.E., Shaibi, G.Q., Sun, P., Lane, C.J., Ventura, E.E., Davis, J.N., …Goran, M.I. (2008). Cardiorespiratory fitness predicts changes in adiposity in overweight Hispanic boys. *Obesity (Silver Spring)*, *16*, 1072-1077. doi:10.1038/oby.2008.16

Corella, D., Guillén, M., Sáiz, C., Portolés, O., Sabater, A., Cortina, S., …Ordovas, J.M. (2001). Environmental factors modulate the effect of the APOE genetic polymorphism on plasma lipid concentrations: ecogenetic studies in a Mediterranean Spanish population. *Metabolism*, *50*, 936-944. doi:10.1053/meta.2001.24867

Dai, S., Fulton, J.E., Harrist, R.B., Grunbaum, J.A., Steffen, L.M., & Labarthe, D.R. (2009). Blood lipids in children: age-related patterns and association with body-fat indices: Project HeartBeat!. *A*merican Journal of *Preventive* Medicine, *37*, 56-64. doi:10.1016/j.amepre.2009.04.012

De Moraes, A.C., Fadoni, R.P., Ricardi, L.M., Souza, T.C., Rosaneli, C.F., Nakashima, A.T., & Falcão, M.C. (2011). Prevalence of abdominal obesity in adolescents: a systematic review. *Obesity Reviews, 12*, 69-77. doi:10.1111/j.1467-789X.2010.00753.x

Donohoe, G.G., Salomäki, A., Lehtimäki, T., Pulkki, K., & Kairisto, V. (1999). Rapid identification of apolipoprotein E genotypes by multiplex amplification refractory mutation system PCR and capillary gel electrophoresis. *Clinical Chemistry*, *45*, 143-146. Retrieved from: http://www.clinchem.org/content/45/1/143.full.pdf+html

França, E., Alves, J.G., & Hutz, M.H. (2004). Apolipoprotein E polymorphism and its association with serum lipid levels in Brazilian children. *Human Biology, 76*, 267-275. doi:10.1353/hub.2004.0030

Friedewald, W.T., Levy, R.I., & Fredrickson, D.S. (1972). Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clinical Chemistry*, *18*, 499-502. Retrieved from: http://www.clinchem.org/content/18/6/499.full.pdf+html

Glaner, M.F. (2002). *Physical growth and health-related physical fitness in rural and urban adolescents*. Thesis (Doctorate in Movement Human Science) – Federal University of Santa Maria, Santa Maria. Retrieved from: http://ebookbrowse.com/glaner-tese-pdf-d57376971

Hagberg, J.M., Ferrell, R.E., Katzel, L.I., Dengel, D.R., Sorkin, J.D., & Goldberg, A.P. (1999). Apolipoprotein E genotype and exercise training-induced increases in plasma high-density lipoprotein (HDL)- and HDL2-cholesterol levels in overweight men. *Metabolism, 48*, 943-945. doi:10.1016/S0026-0495(99)90185-3

I Guideline for Preventing Atherosclerosis in Childhood and Adolescence (2005). *Arquivos Brasileiros de Cardiologia, 85*, S3-36. doi.10.1590/S0066-782X2005002500001.

Katzmarzyk, P.T., Srinivasan, S.R., Chen, W., Malina, R.M., Bouchard, C., & Berenson, G.S. (2004). Body mass index, waist circumference, and clustering of cardiovascular disease risk factors in a biracial sample of children and adolescents. *Pediatrics*, *114*, 198-205. doi:10.1542/peds.114.2.e198

Kwon, S., Burns, T.L., & Janz, K. (2010). Associations of cardiorespiratory fitness and fatness with cardiovascular risk factors among adolescents: the NHANES 1999-2002. *Journal of Physical Activity & Health, 7*, 746-753.

Lamotte, C., Iliescu. C., Libersa, C., & Gottrand, F. (2011). Increased intima-media thickness of the carotid artery in childhood: a systematic review of observational studies. *European Journal of Pediatrics*, *170*, 719-729. doi:10.1007/s00431-010-1328-y

Leon, A.S., Togashi, K., Rankinen, T., Després, J.P., Rao, D.C., Skinner, J.S., …Bouchard, C. (2004). Association of apolipoprotein E polymorphism with blood lipids and maximal oxygen uptake in the sedentary state and after exercise training in the HERITAGE family study. *Metabolism*, *53*, 108-116. doi:10.1016/j.metabol.2003.08.013

Marques-Vidal, P., Bongard, V., Ruidavets, J.B., Fauvel, J., Hanaire -Broutin, H., Perret, B., & Ferrièires, J. (2003). Obesity and alcohol modulate the effect of apolipoprotein E polymorphism on lipids and insulin. *Obesity Research*, *11*, 1200-1206. doi:10.1038/oby.2003.165

Medina-Urrutia, A.X., Cardoso-Saldaña, G.C., Zamora-González, J., Liria, Y.K., & Posadas-Romero, C. (2004). Apolipoprotein E polymorphism is related to plasma lipids and apolipoproteins in Mexican adolescents. *Human Biology*, *76*, 605-614. doi:10.1353/hub.2004.0059

Miller, S.A., Dykes, D.D., & Polesky, H.F. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research, 16*,1215. doi:10.1093/nar/16.3.1215

Nascimento, H., Silva, L., Lourenço, P., Weinfurterová, R., Castro, E., Rego, C., …Belo, L. (2009). Lipid profile in Portuguese obese children and adolescents: interaction of apolipoprotein E polymorphism with adiponectin levels. *Archives of Pediatrics and Adolescent Medicine*, *163*, 1030-1036. doi:10.1001/archpediatrics.2009.190

Neutzling, M.B., Assunção, M.C.F., Malcon, M.C., Hallal, P.C., & Menezes, A.M.B. (2010). Food habits of adolescent students from Pelotas, Brazil. *Brazilian Journal of Nutrition*, *23,* 379-388. doi:10.1590/S1415-52732010000300006.

Nghiem, N.T., Ta, T.T., Ohmori, R., Kuroki, M., Nguyen, V.C., Nguyen, T.K., …Kondo, K. (2004). Apolipoprotein E polymorphism in Vietnamese children and its relationship to plasma lipid and lipoprotein levels. *Metabolism*, *53*, 1517-1521. doi:10.1016/j.metabol.2004.06.017

Pisciotta, L., Cantafora, A., Piana, A., Masturzo, P., Cerone, R., Minniti, G., …Bertolini, S. (2003). Physical activity modulates effects of some genetic polymorphisms affecting cardiovascular risk in men aged over 40 years. *Nutrition, Metabolism and Cardiovascular Diseases, 13*, 202-210. doi:10.1016/S0939-4753(03)80012-1

Taimela, S., Lehtimäki, T., Porkka, K.V., Räsänen, L., & Viikari, J.S. (1996). The effect of physical activity on serum total and low-density lipoprotein cholesterol concentrations varies with apolipoprotein E phenotype in male children and young adults: The Cardiovascular Risk in Young Finns Study. *Metabolism, 45*, 797-803. doi:10.1016/S0026-0495(96)90149-3

Tanner, J.M. (1962). *Growth at adolescence*. Oxford, OX: Blackwell Scientific Publications.

Thompson, P.D., Tsongalis, G.J., Seip, R.L., Bilbie, C., Miles, M., Zoeller, R., …Moyna, N. (2004). Apolipoprotein E genotype and changes in serum lipids and maximal oxygen uptake with exercise training. *Metabolism*, *53*, 193-202. doi:10.1016/j.metabol.2003.09.010

United Nations (2000). *Human Development Index, Municipality of Brazil*. Retrieved from: http://www.pnud.org.br/atlas/tabelas/index.php.

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Corresponding author

Maria Fátima Glaner
Rod. Celso Garcia Cid
PR 445, km 380, Campus Universitário, PO Box 10.011
Londrina 86.057-970, PR, Brazil
Phone: 55-43-3367-7738 or 55-43-9676-7738
E-mail: mfglaner@gmail.com.br

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