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# Dietary fat and apolipoprotein genotypes modulate plasma lipoprotein levels in Brazilian elderly women

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**Abstract** Studies show that genetic polymorphisms in apolipoproteins, which are in charge of lipid transport, predispose to atherogenic dyslipidemia. This study aimed to investigate the impact of apolipoprotein E, A5, and B genotypes and dietary intake on lipid profile in a sample of elderly women in Brazil. Two hundred and fifty-two women (60 years or older) living in the outskirts of the Brazilian Federal District underwent clinical and laboratory assessments to characterize glycemic and lipidemic variables, and also to exclude confounding factors (smoking, drinking, hormone replacement, cognitive impairment, physical activity). Three-day food records were used to determine usual dietary intake, whereas genotypic evaluations were in accordance to established methodologies. Genotype frequencies were consistent with the Hardy–Weinberg equilibrium. Prior to adjustment, individuals carrying the $\varepsilon 2$ allele showed higher serum levels of triglycerides ($P < 0.05$) and VLDL ($P < 0.005$) compared to $\varepsilon 4$ carriers, whereas LDL levels were considerably elevated in $\varepsilon 4$ compared to $\varepsilon 2$ carriers. In the presence of high intake of total fat or a low ratio of polyunsaturated to saturated fatty acid, $\varepsilon 4$ carriers lost protection against hypertriglyceridemia. There was no association of the apolipoprotein A5 and B genotypes with lipidemic levels independently of the fat intake regimen. Results are suggestive of a dysbetalipoproteinemic-like phenotype in postmenopausal women, with remarkable gene–diet interaction.

**Keywords** Hypertriglyceridemia · Dysbetalipoproteinemia · Genetic polymorphism · Food intake · Fatty acids · Gender · Older adult

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## Introduction

Current evidence demonstrates that several aspects in lipid metabolism are important risk factors for the atherogenic process and, ultimately, for coronary artery disease (CAD). For instance, apolipoprotein E (ApoE) plays an important role in the liver uptake of triglyceride-rich lipoproteins [1], especially the $\varepsilon 2$ and $\varepsilon 4$ variants. These alleles differ from the $\varepsilon 3$ allele, the most common form, by a single nucleotide polymorphism (SNP) that affects ligand binding of the triglyceride-rich lipoprotein to the hepatic low-density lipoprotein receptor (LDLr). Affinity of the $\varepsilon 2$ isoform to its receptor is 100 times lower compared to that of $\varepsilon 3$ and $\varepsilon 4$, resulting in accumulation of very-low-density lipoproteins (VLDL) and of chylomicron remnants in serum [2, 3],

and possibly accounting for the triglyceride-raising effect observed in studies worldwide [4]. Similarly, a body of epidemiological data have linked the ε4 allele to greater risk for coronary heart disease [5], probably resulting from preferential distribution of ApoE4 to triglyceride-rich lipoproteins, accelerating ApoE4 uptake and leading to down-regulation of hepatic LDLr expression and increased LDL-cholesterol levels [1, 6].

Consistent with the notion that some apolipoprotein variations play a major role in triglyceride metabolism, a T/C polymorphism of the promoter region of the ApoA5 gene at position −1131 has been associated with increased CAD risk in multiple ethnic populations [7–9]. Five polymorphisms were found to define three common haplotypes, with the minor ApoA5*2 haplotype being defined by the −1131C allele and associated with lower protein expression, causing 20–30% elevation in plasma triglyceride levels in Caucasians [10]. So far, the role of the ApoA5 gene in the regulation of plasma triglyceride levels has been attributed to its dose-dependent capacity to enhance the lipoprotein lipase (LPL) hydrolysis of VLDL triglycerides mediated by either ApoCII [11] or vascular wall proteoglycans [12].

In addition, other studies demonstrate an association of apoB polymorphisms with high lipid levels and risk for CAD in various populations [13–15]. Among these, four common, yet poorly linked variations, namely the *Ins*/*Del* polymorphism of exon 1, the *Msp*I and *Xba*I SNPs of exon 26, and the hypervariable region at the 3′ end of the gene, have been independently associated with lipid levels [16]. Homozygosity for the allele lacking the *Xba*I site ($X^-$) was found to contribute to CAD in the Brazilian population [17].

Even though lipoprotein concentrations are genetically regulated, dietary fat also greatly contributes to the lipid profile in human biology [18]. Western societies (including Brazil) typically consume diets that are rich in fat, especially saturated fat and cholesterol. Our hypothesis is that levels and composition of dietary fat affect the way apolipoprotein genotypes predispose to dyslipidemic phenotypes. To our knowledge, there have been no previous studies in Brazil concerning the impact of dietary lipids on the association between apolipoprotein genotypes and serum lipids [19, 20]. Since dyslipidemia occurs predominantly in people aged 60 years or older, and the nutrient-base influence has not been studied extensively among the elderly, we examined the effects of diverse apolipoprotein genotypes on lipid profile in a subset of the Elderly Health Promotion Project (EHPP) carried out in Brasília, Brazil. Because factors such as age, sex, physical activity, alcohol use, and smoking affect CAD risk, this report also poses a contribution by standardizing these variables in our sample.

## Materials and methods

### Subjects

Brasília (national capital) is located in the midwest of Brazil. The present cross-sectional analyses were performed using data obtained from 305 apparently healthy female outpatients aged 60 years or older living in the urban outskirts of the Federal District. These women were selected to undergo health screenings and interventions (medical, nutritional and/or pharmacological) as part of a university project (EHPP). After an initial round of assessments of cardiovascular risk factors in this non-probabilistic sample in 2005 [21], clinical data and laboratory results were reassessed [22] and pooled to set up the database used in the present study. Whenever multiple laboratory results were available, only the most recent data obtained immediately before the programed health interventions were considered. This study was approved by the Ethics Committee at the Catholic University of Brasilia and was conducted in accordance with the Helsinki Declaration. Participation was voluntary, and written informed consent was obtained from each participant.

### Clinical procedures

Venous blood samples were collected into an EDTA-containing tube after a 12 h-overnight fast. Laboratory tests included blood glucose, triglycerides (TG), total cholesterol, VLDL, and high-density lipoprotein (HDL). Serum liver function markers—aspartate aminotransferase (AST), alanine aminotransaminase (ALT), and alkaline phosphatase (ALP)—were also measured. All the above tests were performed following routine clinical methods with Boehringer Mannheim (Germany) reagents. LDL levels were determined using Friedewald's formula [23]. During a clinical consultation, blood pressure was measured after at least 10 min of rest in sitting position. The blood pressure value considered for each subject was the mean of three physician-obtained measurements, recorded >3 min apart. To determine body mass index (BMI); weight (kg)/height ($m^2$)], patients were weighed wearing light clothing, and their height was measured without shoes to the nearest millimeter. Body composition (fat and fat-free soft tissue) was determined using dual-energy X-ray absorptiometry (DXA; Lunar DPX-IQ model, software version 4.7e, Lunar Radiation Corp., Madison, WI, USA) according to manufacturer instructions.

Current use of antilipidemic medication was investigated for each patient. Other variables assessed during the clinical interview were smoking habit, alcohol consumption, physical activity, and use of estrogen replacement therapy. Current smokers (having smoked at least 100

cigarettes in their lifetime), drinkers (1 or more doses per week in the past 12 months), regular exercisers (practice of a sport for at least 20 min three or more times per week), and users of estrogen replacement medication were excluded from the present study.

## Diets

The present study evaluated fat consumption in the usual diet. Dietary contents were determined based on 3-day food records (2 weekdays and 1 weekend day), which have been shown to be as reliable as 4- or 7-day food records [24–26]. The dietary record was filled at home after the patient was trained by a clinical dietician to record food intake in terms of number and size of servings. Intake values for each individual patient are thus expressed as the mean intake reported in the 3-day record. To improve reliability of the data, patients were assessed with a validated Brazilian Portuguese version of the Mini-Mental State Examination (MMSE) [27]. Functionally illiterate subjects were excluded from the study, according to the following cut-off points: score <11/30 for illiteracy; <17/30 for individuals with 7 years of formal education; and <25 for individuals with 8 or more years of formal education [28]. To ensure completion of the dietary record, the staff of dieticians provided either personal or telephone assistance. The forms were returned during a clinical interview in which the amounts and types of foods were reviewed. Missing data were obtained and added. Dietary contents were calculated using the Diet-Pro® software, version 4.0 (A.S. Sistemas, Minas Gerais, Brazil), configured for international food tables, and complemented with a table for local food stuffs [29]. Dietary intakes of total protein, carbohydrate, and lipid, as well as of saturated, monounsaturated and polyunsaturated fatty acids (abbreviated SFA, MUFA and PUFA, respectively) were expressed as percentages of total energy and were included in the analysis as continuous variables. To construct categorical variables of fat consumption, intakes were classified into two groups as follows: intakes below (low) or above (high) the mean value of the study population. The polyunsaturated to saturated (P:S) fatty acid ratio was also determined for each patient and included as a categorical variable.

## DNA analysis

Total DNA was isolated from peripheral blood according to standard procedures. ApoE genotypes were analyzed using a modified version of a refractory mutation system (ARMS) for multiplex polymerase chain reaction (PCR) to identify the classical ε2, ε3, and ε4 alleles [30]. In our conditions, PCR products were electrophoresed in 1.6% agarose gels. Each sample was run at least twice, and further checked only if the genotypes yielded by the first two productive analyses were in conflict. For identification of the $X^+/X^-$ ApoB exonic variants, the polymorphic site was PCR-amplified with primers and conditions described by Renges et al. [31]. Enzymatic digestions of the amplified products were carried out at 37°C for 4 h in a total volume of 10 µl, using 0.5 U of the *Xba*I restriction endonuclease.

To evaluate the −1131 T/C promoter polymorphism of the ApoA5 gene, the following procedure was developed: a 468 bp region that encompasses this transition was amplified using a pair of specific primers, 5′-CTTCACTA CAGGTTCCGCAG-3′ (sense) and 5′-GGATGAGCCA CAGTGGAGG-3′ (antisense). Reaction tubes contained 100 ng DNA, 10 mM Tris–HCl pH 8.3, 75 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 40 ng/µl ovalbumin, 2 µM each primer, and 1 unit of *Taq* DNA polymerase (Phoneutria, Minas Gerais, Brazil) in a final volume of 25 µl. After 1 min hot start at 80°C and initial denaturation for 2 min at 94°C, the amplifications were done for 36 cycles of 40s at 94°C, 45s at 60°C, and 50s at 72°C followed by a final 5 min extension at 72°C. Each PCR product was directly sequenced on an ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, USA), using the sense primer. Each sequence obtained was examined using the Staden software package (MRC, Cambridge, UK), and confirmed by visual inspection.

## Statistical analysis

Violation of the Hardy–Weinberg equilibrium was tested using Fisher's exact test. The Kolmogorov–Smirnov test was used to verify normal distribution of data. The analytic strategy for assessing the effect of the genotype and of other variables in the lipid profiles was as follows: because of the intercorrelated nature between dependent variables, multivariate analysis of variance (MANOVA) was used to compare means of anthropometric/clinical/nutritional variables (age, BMI, body composition, blood pressure, liver tests, calorie, and nutrient intake) and of serum variables (serum glucose, total lipids, TG, total cholesterol, VLDL, LDL, and HDL) across genotypes whenever frequencies allowed constitution of more than two genotype groups. Otherwise, the Student *t*-test was applied. To assess gene–diet interactions, multivariate analysis of covariance (MANCOVA) was used to test the effect of dietary variables (whole calorie and total protein, carbohydrate, and lipid intakes) and their components (PUFA, MUFA, and SFA intakes and P:S ratio) on serum lipids according to the genotypes. If this statistical test was significant at the 0.05 level, the dependent variable being influenced was reassessed by univariate MANOVA for differences across genotypes on the low and then on the high intake intervals,

as categorized above. For the non-parametric purpose of evaluating the distribution of antilipidemic drug use between genotypes, the chi-square test was employed.

All analyses were performed with the Statistical Package for the Social Sciences (SPSS) for Windows (version 10.0). A $P$ value $< 0.05$ (two-tailed) was considered significant. Post-hoc comparisons were done using the Scheffé test for pairwise comparisons. Whenever appropriate, data were expressed as means ± standard deviation (SD).

## Results

After applying the exclusion criteria, 252 subjects were eligible for analysis. The prevalence of ε2, ε3, and ε4 alleles was, respectively, 4.4, 87.3, and 8.3%. Of all six expected genotypes, only four were observed: ε2ε3 (8.7%), ε3ε3 (75.0%), ε3ε4 (15.9%), and ε4ε4 (0.4%). Concerning ApoA5 and ApoB polymorphisms, the minor −1131C allele of the former accounted for 9.7% of the gene repertoire, whereas the minor $X^+$ allele of the latter accounted for 33.1%. Genotype frequencies did not deviate from the Hardy–Weinberg equilibrium. Due to the low number of individuals homozygous for the ε4 ApoE and the −1131C ApoA5 alleles, these genotypes were merged into their respective heterozygotic groups to increase statistical power.

The clinical and nutritional characteristics of the subjects analyzed are summarized in Table 1. In brief, multivariate analysis revealed no significant differences between genotypes in age, BMI, body fat or fat-free masses, blood pressure, or liver function markers. Intake of protein, carbohydrate, or fat, and total calories was also similar. Considered as a whole, the study sample demonstrated a remarkable degree of homogeneity regarding basic characteristics and dietary patterns.

When glycemic and fatty serum markers were considered, mean levels of serum TG and VLDL were significantly elevated in subjects carrying the ε2 allele compared to carriers of ε4 (Table 2). It is noteworthy that in this preliminary analysis, serum triglyceride levels were compatible with normotriglyceridemia in most ε3 homozygotes and ε4 carriers, with average dosages below the recommended upper limits (150 mg/dl). Additionally, LDL levels were considerably elevated in ε4 carriers. The observed lipidemic profile is highly suggestive of the dysbetalipoproteinemia phenotype, or type III hyperlipoproteinemia, for which the ε2 allele is recognized as an important risk factor [32]. We did not observe a trend toward association between ApoE genotypes and levels of other serum lipids, or between the ApoA5 and ApoB genotypes with levels of any lipidemic variables.

Because of the possible interaction of apolipoprotein isoforms with variables such as calorie intake and lipid ingestion, differences between genotypes were investigated adjusting for these confounding factors. As described in Methods, MANOVA was used to test the effect of these dietary co-variables (protein, lipid, and carbohydrate intake) and their components (SFA, MUFA, and PUFA intake) on serum lipids according to the genotypes. In this statistical test, total lipid intake was the only variable capable of predicting serum levels of TG ($F = 2.19$; $P = 0.040$), followed by a trend of P:S ratio toward being predictive of serum TG ($F = 0.61$; $P = 0.119$). Using statistical models in which total lipid intake and the P:S ratio were dichotomized according to their corresponding population mean (34.6% of total energy intake for the former and a 0.6:1 ratio for the latter variable), we uncovered an important interaction between ApoE genotypes and serum lipidemic variables: a loss of ε4 protective effect against high TG and VLDL levels in subjects with a high lipid diet or relatively low intake of PUFA (Table 3).

During clinical assessments, 14 (5.6%) women were found to be users of lipid-lowering drugs. Nonetheless, we do not believe that the discrete results we observed were pharmaceutically influenced, since chi-square tests failed to reveal any quantitative variances in the distribution of users and non-users of antilipidemic drugs across apolipotrotein genotypes ($P > 0.05$).

## Discussion

Genetic polymorphisms are a part of the evolutionary process that results from the interaction between the environment and the human genome. Recent changes in diet have upset this equilibrium, potentially influencing the risk of common morbidities such as cardiovascular diseases. Control of these conditions is a major public health concern, and could be achieved by improving our ability to detect disease predisposition early in life and by providing more personalized behavioral recommendations for successful primary prevention.

It is well known that polymorphisms at multiple loci are associated with different aspects of lipid metabolism. However, the connection of these polymorphisms with cardiovascular disease remains elusive because of experimental limitations, the intrinsic complexity of the phenotypes, and the aforementioned interactions with environmental factors.

The present work aimed at posing a contribution by verifying the influence of important apolipoprotein polymorphisms on serum lipid concentrations in a population of elderly women in Brazil in a context controlled for confounding variables and dietary intake. When lipid

**Table 1** MANOVA of clinical and nutritional features of the sample according to apolipoprotein genotypes

| | ApoB | | | ApoA5 | | ApoE | | |
|---|---|---|---|---|---|---|---|---|
| | ($X^-X^-$) CC ($n = 109$) | ($X^+X^-$) TC ($n = 119$) | ($X^+X^+$) TT ($n = 24$) | TT ($n = 207$) | CT + CC ($n = 45$) | ε2/ε3 ($n = 22$) | ε3/ε3 ($n = 189$) | ε3/ε4 + ε4/ε4 ($n = 41$) |
| Age (years) | 67.9 ± 5.8 | 67.8 ± 6.4 | 67.9 ± 5.6 | 67.7 ± 6.2 | 68.6 ± 5.1 | 67.8 ± 6.0 | 68.1 ± 6.2 | 66.8 ± 5.4 |
| BMI (kg/m$^2$) | 27.3 ± 4.9 | 27.7 ± 3.8 | 27.5 ± 2.4 | 27.4 ± 4.4 | 27.8 ± 4.0 | 27.3 ± 3.6 | 27.3 ± 4.0 | 28.1 ± 5.3 |
| Fat tissue (%) | 37.0 ± 7.1 | 38.1 ± 6.0 | 38.1 ± 6.4 | 37.6 ± 6.8 | 37.8 ± 5.8 | 37.6 ± 7.1 | 37.7 ± 6.7 | 37.3 ± 6.0 |
| Soft fat-free tissue (%) | 59.4 ± 6.8 | 58.6 ± 6.0 | 57.9 ± 5.5 | 58.9 ± 6.5 | 58.6 ± 5.4 | 58.1 ± 6.9 | 59.0 ± 6.5 | 58.6 ± 5.1 |
| Systolic blood pressure (mm Hg) | 141.5 ± 27.0 | 140.0 ± 25.9 | 141.0 ± 27.7 | 140.2 ± 26.8 | 143.2 ± 25.0 | 131.4 ± 21.2 | 141.5 ± 26.2 | 142.1 ± 29.3 |
| Diastolic blood pressure (mm Hg) | 82.6 ± 14.7 | 84.5 ± 13.8 | 81.4 ± 17.4 | 83.1 ± 15.4 | 84.6 ± 11.7 | 79.1 ± 11.1 | 84.2 ± 14.8 | 82.1 ± 16.8 |
| Alkaline phosphatase (U/l) | 172.2 ± 65.6 | 188.7 ± 62.9 | 180.5 ± 81.4 | 177.8 ± 66.2 | 195.8 ± 64.3 | 184.6 ± 55.6 | 179.3 ± 68.2 | 186.6 ± 62.7 |
| Aspartate aminotransferase (U/l) | 24.0 ± 6.3 | 25.3 ± 7.7 | 23.6 ± 8.3 | 24.4 ± 6.9 | 25.3 ± 8.4 | 23.3 ± 7.9 | 24.4 ± 7.0 | 26.1 ± 7.9 |
| Alanine aminotransaminase (U/l) | 18.6 ± 8.3 | 21.0 ± 11.1 | 17.7 ± 9.9 | 20.0 ± 9.9 | 18.0 ± 10.1 | 21.0 ± 8.7 | 19.0 ± 9.5 | 22.0 ± 12.0 |
| Calorie intake ($10^3$ kcal) | 2.09 ± 0.51 | 2.06 ± 0.59 | 2.14 ± 0.45 | 2.07 ± 0.53 | 2.13 ± 0.60 | 2.12 ± 0.51 | 2.12 ± 0.54 | 1.89 ± 0.57 |
| Total protein intake (%) | 15.4 ± 3.4 | 14.6 ± 2.8 | 14.9 ± 4.0 | 14.8 ± 3.3 | 15.6 ± 3.0 | 15.9 ± 3.9 | 14.8 ± 3.2 | 15.3 ± 3.1 |
| Total carbohydrate intake (%) | 49.9 ± 6.7 | 51.3 ± 5.7 | 48.7 ± 8.3 | 50.7 ± 6.4 | 49.3 ± 6.4 | 48.8 ± 5.2 | 51.0 ± 6.3 | 48.8 ± 7.4 |
| Total lipid intake (%) | 34.6 ± 4.9 | 34.2 ± 5.1 | 36.4 ± 7.1 | 34.5 ± 5.3 | 34.9 ± 4.6 | 35.3 ± 3.6 | 34.3 ± 5.3 | 34.9 ± 3.6 |
| Polyunsaturated fatty acid intake (%) | 0.99 ± 0.85 | 1.00 ± 0.64 | 0.95 ± 0.67 | 1.01 ± 0.80 | 0.92 ± 0.50 | 1.00 ± 0.94 | 1.05 ± 0.78 | 0.74 ± 0.46 |
| Monounsaturated fatty acid intake (%) | 3.08 ± 2.88 | 2.97 ± 2.08 | 2.74 ± 2.29 | 2.93 ± 2.40 | 3.13 ± 2.03 | 2.24 ± 1.89 | 3.20 ± 2.64 | 2.47 ± 1.55 |
| Saturated fatty acid intake (%) | 2.64 ± 2.40 | 2.63 ± 1.85 | 2.65 ± 2.25 | 2.66 ± 2.30 | 2.72 ± 1.66 | 2.37 ± 2.26 | 2.82 ± 2.40 | 2.20 ± 1.40 |
| Total cholesterol intake (mg) | 135.6 ± 134.4 | 118.3 ± 103.5 | 103.1 ± 97.0 | 120.4 ± 117.0 | 140.7 ± 114.3 | 145.9 ± 144.6 | 131.2 ± 125.6 | 85.5 ± 51.0 |

Data expressed as means ± SD

**Table 2** MANOVA of fasting biochemical variables of the sample according to apolipoprotein genotypes

| | ApoB | | | ApoA5 | | ApoE | | |
|---|---|---|---|---|---|---|---|---|
| | $(X^-X^-)$ CC ($n = 109$) | $(X^+X^-)$ TC ($n = 119$) | $(X^+X^+)$ TT ($n = 24$) | TT ($n = 207$) | CT + CC ($n = 45$) | ε2/ε3 ($n = 22$) | ε3/ε3 ($n = 189$) | ε3/ε4 + ε4/ε4 ($n = 41$) |
| Serum glucose (mg/dl) | 106.1 ± 24.4 | 102.1 ± 15.0 | 98.0 ± 19.6 | 103.7 ± 20.4 | 102.7 ± 16.9 | 104.5 ± 12.9 | 103.0 ± 19.7 | 104.2 ± 21.4 |
| Total serum lipids (mg/dl) | 715.5 ± 158.6 | 743.9 ± 135.4 | 756.8 ± 136.1 | 731.7 ± 148.4 | 738.1 ± 137.3 | 741.7 ± 167.3 | 731.6 ± 145.2 | 733.9 ± 142.8 |
| Serum triglycerides (mg/dl) | 142.3 ± 69.6 | 152.0 ± 63.9 | 149.2 ± 58.9 | 146.0 ± 68.3 | 160.9 ± 72.1 | 182.0 ± 75.7[a] | 147.3 ± 68.7 | 136.3 ± 61.9[a] |
| Total serum cholesterol (mg/dl) | 223.4 ± 44.7 | 231.9 ± 46.6 | 235.6 ± 38.8 | 229.4 ± 44.6 | 224.9 ± 47.6 | 214.7 ± 46.3 | 229.6 ± 44.6 | 231.1 ± 46.7 |
| VLDL cholesterol (mg/dl) | 28.2 ± 13.0 | 29.2 ± 11.3 | 29.8 ± 11.8 | 28.4 ± 12.1 | 31.2 ± 13.6 | 37.0 ± 15.1[b,c] | 28.5 ± 11.8[b] | 26.4 ± 11.3[c] |
| LDL cholesterol (mg/dl) | 134.2 ± 39.7 | 141.9 ± 41.1 | 144.0 ± 34.1 | 140.3 ± 39.6 | 131.9 ± 41.1 | 118.7 ± 42.0[a] | 139.8 ± 37.9 | 145.0 ± 45.6[a] |
| HDL cholesterol (mg/dl) | 59.7 ± 10.2 | 58.5 ± 10.8 | 60.2 ± 11.6 | 59.3 ± 10.2 | 58.4 ± 12.4 | 60.4 ± 10.1 | 59.4 ± 10.9 | 57.4 ± 9.5 |

Data expressed as means ± SD. *P* values from multivariate analyses using the post-hoc Scheffé test

[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.005$

parameters were related to genotypes of ApoB, ApoA5, and ApoE per se, the main result was a more advantageous lipid profile in carriers of the ε4 allele (statistically significant lower TG and VLDL). This finding may be explained by the defective property conferred by ε2, which prevents VLDL remnants from binding to hepatic LDLrs, causing hypertriglyceridemia [33]. Nevertheless, either a high intake of dietary lipids or a low P:S regimen abolished the protective effect of ε4 by considerably raising TG fasting levels. Across ApoE genotypes, the magnitude of the effect of ε4 on TG in individuals with a higher intake of lipids was low by the Cohen convention [34]: $TG_{\varepsilon 2\varepsilon 3}$ versus $_{\varepsilon 4\_}$, $d = 0.4$; $VLDL_{\varepsilon 2\varepsilon 3}$ versus $_{\varepsilon 4\_}$, $d = 0.5$; $LDL_{\varepsilon 2\varepsilon 3}$ versus $_{\varepsilon 4\_}$, $d = 0.4$. On the other hand, the same analysis revealed marked differences between individuals with a lower intake of lipids ($TG_{\varepsilon 2\varepsilon 3}$ versus $_{\varepsilon 4\_}$, $d = 1.3$; $VLDL_{\varepsilon 2\varepsilon 3}$ versus $_{\varepsilon 4\_}$, $d = 1.4$; $LDL_{\varepsilon 2\varepsilon 3}$ versus $_{\varepsilon 4\_}$, $d = 1.0$).

Concerning the observation that ε4 carriers showed a protection against the dysbetalipoproteinemic-like phenotype in the presence of a relatively high polyunsaturated fat intake, the literature provides evidence of a direct effect of PUFA as modulators of apolipoprotein expression. Considering that the peroxisome proliferator-activated receptors (PPARs) are PUFA-activated nuclear transcription factors involved in several aspects of lipid metabolism, including ApoE expression [35], it is plausible that individuals with a usual diet with higher P:S ratio may benefit from enhanced ApoE expression, and therefore from the improved uptake property of ε4-containing VLDL particles and its remnants by the liver.

Given that epidemiological studies implicate elevated plasma triglyceride levels as an independent risk factor for CAD [36, 37], we believe that the present quantitative findings may be of physiological relevance and useful to guide clinical interventions. For instance, the average total lipid intake in the usual diet of the elderly women in this study (34.6%) was found to almost precisely match the maximum (35.0%) recommended lipid intake [38], which shows that a large portion of this population is eligible for nutritional control. Also noteworthy is the fact that except for TG, VLDL, and LDL, no other physiological variables were found to be different across the ApoB and A5 genotypes, in terms of either statistical or actual clinical significance, before or after dichotomization of interacting variables. Nonetheless, our results are in contrast with consensus showing that not only is the dysbetalipoproteinemic phenotype rare among ε2 heterozygotes, it is also associated with hypercholesterolemia and its complications [39]. We do not have a clear explanation for these discrepancies but to consider them context-sensitive. Our findings in such a small sample and in a heterozygotic context may reflect an intensification of the TG-raising phenotype of ε2 carriers probably due to the failure of

**Table 3** Analysis of fasting biochemical variables of the sample according to pattern of fat intake and ApoE genotype

| Diet pattern | Genotype | *n* | Glycemia | Lipids | | | Cholesterol fractions | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | GC (mg/dl) | TL (mg/dl) | TG (mg/dl) | TC (mg/dl) | VLDL (mg/dl) | LDL (mg/dl) | HDL (mg/dl) |
| Low lipid intake | ε2/ε3 | 8 | 97.4 ± 7.9 | 699.8 ± 95.9 | 191.0 ± 64.4[a] | 194.4 ± 34.9 | 38.0 ± 12.8[a] | 101.4 ± 44.2[a,1] | 59.2 ± 10.8 |
| | ε3/ε3 | 97 | 103.7 ± 19.5 | 731.8 ± 155.5 | 147.9 ± 72.1 | 229.4 ± 44.7 | 28.7 ± 12.4 | 129.6 ± 38.4 | 59.9 ± 11.1 |
| | ε3/ε4 + ε4/ε4 | 20 | 85.9 ± 8.1 | 703.4 ± 105.2 | 121.8 ± 42.2[a] | 223.1 ± 31.7 | 23.7 ± 8.0[a] | 139.7 ± 34.4[a] | 54.9 ± 7.2 |
| High lipid intake | ε2/ε3 | 14 | 108.5 ± 13.7 | 765.6 ± 196.3 | 176.8 ± 83.2 | 226.4 ± 49.0 | 36.5 ± 16.7 | 128.6 ± 38.9[1] | 61.1 ± 10.1 |
| | ε3/ε3 | 92 | 101.8 ± 18.6 | 731.4 ± 134.2 | 145.1 ± 60.1 | 229.9 ± 44.7 | 28.2 ± 11.0 | 140.0 ± 37.5 | 58.9 ± 10.8 |
| | ε3/ε4 + ε4/ε4 | 21 | 107.4 ± 22.4 | 762.9 ± 168.7 | 144.8 ± 67.1 | 238.8 ± 57.3 | 29.0 ± 13.4 | 149.9 ± 54.5 | 60.0 ± 10.7 |
| Low P:S diet | ε2/ε3 | 11 | 106.6 ± 23.3 | 687.5 ± 157.4 | 169.6 ± 85.3 | 200.2 ± 36.0 | 35.4 ± 17.3 | 103.2 ± 33.3[2] | 61.5 ± 10.5 |
| | ε3/ε3 | 98 | 102.0 ± 17.5 | 747.8 ± 140.3 | 159.5 ± 77.3 | 232.8 ± 43.8 | 30.2 ± 13.2 | 140.0 ± 37.2 | 59.2 ± 10.7 |
| | ε3/ε4 + ε4/ε4 | 24 | 104.6 ± 22.6 | 715.1 ± 135.4 | 139.5 ± 58.2 | 221.8 ± 45.7 | 27.7 ± 11.7 | 133.2 ± 46.5[3] | 57.0 ± 10.0 |
| High P:S diet | ε2/ε3 | 11 | 106.0 ± 12.4 | 795.8 ± 165.9 | 194.3 ± 66.4[b,c] | 229.3 ± 52.3 | 38.7 ± 13.2[b,c] | 134.3 ± 45.5[2] | 59.4 ± 10.2 |
| | ε3/ε3 | 91 | 103.6 ± 20.7 | 714.2 ± 149.1 | 133.0 ± 51.2[b] | 226.2 ± 45.4 | 26.6 ± 10.2[b] | 139.6 ± 38.8 | 59.6 ± 11.2 |
| | ε3/ε4 + ε4/ε4 | 17 | 103.2 ± 19.1 | 760.4 ± 152.8 | 123.2 ± 53.7[c] | 244.2 ± 46.2 | 24.6 ± 10.7[c] | 161.1 ± 39.8[3] | 58.0 ± 8.9 |

Data expressed as means ± SD. Superscript letters are for *P* values from multivariate analyses using the post-hoc Scheffé test to compare genotypes within the same diet pattern. Superscript numbers are for *P* values from the Student's *t*-test to compare diet patterns within the same genotype

*P:S* polyunsaturated to saturated fatty acid ingestion ratio

Number or [a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.005$

compensatory mechanisms in the aged, especially the well known ability of estrogens to up-regulate ApoE expression [40]. The low levels of endogenous estrogens most likely contribute to diminish ApoE activity, resulting in an augmented net hepatic output of lipoproteins and favoring serum accumulation of VLDL and remnants in postmenopausal women [41]. Therefore, our results are supportive of a dominant inheritance for this secondary hypertriglyceridemia in elderly women. In our case, users of estrogen replacement therapy were not included in the study so as to avoid spurious associations.

Despite the standardization of subjects in terms of sociodemographic characteristics, cognitive performance, and classic CAD risk factors, the present study has limitations. The manifestation of type III hyperlipoproteinemia is greatly enhanced by hypothyroidism. This is explained by activation of the hepatic LDL receptor expression by T3 via a thyroid response element (TRE) in the promoter region of the gene [42]. Unfortunately, the study protocol did not contemplate assessment of thyroid function. Furthermore, a clear-cut diagnosis of dysbetalipoproteinemia could not be established since distribution analysis of lipoprotein particles, including chylomicrons, was not intended. In addition, any apparent disparity in results might be attributed at least in part to confounding factors inherent to the Brazilian scenario, such as the remarkable interethnic variation owing to genetic mix [43], the escalating prevalence of metabolic disorders among the aged [44, 45], and to dietary habits not investigated here.

In sum, the present study suggests the presence of a dysbetalipoproteinemic-like phenotype in postmenopausal women, with remarkable gene–diet interaction. The integration of genetics with environmental complexity into current and future research will drive the field toward the implementation of clinical tools aimed at providing dietary advice optimized for the individual's genome, preferably to be implemented early in life to gain maximum benefit.

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